Inter-compartmental behavior of persistent organic pollutants in aquatic environment

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DECLARATION

I, hereby declare that the investigation presented in the thesis has been carried out by me. The work is original and has not been submitted earlier as a whole or in part for a degree/diploma at this or any other Institution / University.

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List of Publications arising from the thesis

Journals

1. M. Tiwari, S.K. Sahu and G. G. Pandit, (2016). Distribution and ecotoxicological concerns of persistent organic pollutants in sediment from creek ecosystem. Journal of Environmental Science and Health, Part B 51 (9), 616–621.

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3. M. Tiwari, S.K. Sahu and G. G. Pandit, (2017) Distribution of PAHs in different compartment of creek ecosystem: Ecotoxicological concern and human health risk. Environmental Toxicology and Pharmacology 50, 58–66.

Conferences/Symposia

1. M. Tiwari, R.C. Bhangare, P.Y. Ajmal, S. Maity, S. K. Sahu, G. G. Pandit, Persistent Organic Pollutants (POPs) in fly ash collected from coal fired power plants across India", 19th National Symposium on Environment (NSE-19) 11-13 Dec. 2014 at Kottayam, Kerala page 71-72.

2. M. Tiwari, T. D. Rathod, R.C. Bhangare, P.Y. Ajmal, S. K. Sahu and G. G. Pandit, Distribution of Phthalic Acid Esters (PAEs) in estuarine sediments from Trans Thane Creek Mumbai., Paper presented at National Conference on Environmental Monitoring, Assessment and pollution control (EMAPCO-2015). Held at SIES Nerul, in Dec 2015.

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4. M. Tiwari, S. K. Sahu, T. D. Rathod, R. C. Bhangare, P. Y. Ajmal, G. G. Pandit Chronology assessment of sediment core from Kongsfjorden, Arctic using 210 Po/210 Pb dating techniques. NUCAR 2017.

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Dedicated to All Environmentalist

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Chapter 4- RESULTS AND DISCUSSION

LIST OF ABBREVIATIONS

BCF- Bio Concentration Factor

BPA- Bisphenol-A

DDD- 1-chloro-4-[2,2-dichloro-1-(4-chlorophenyl) ethyl] benzene

DDE- 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene

DDT- 1,1'-(2,2,2-Trichloroethane-1,1-diyl)bis(4-chlorobenzene)

DI- Daily Intake

E1- Estrone

E2-17-β-Estradiol

ECD- Electron Capture Detector

EDCs- Endocrine Disrupting Chemicals

EDI- Estimated Daily-dietary Intake

EE2-17-α-Ethynylestradiol

EEF- Estradiol Equivalency Factor

EEQ- Estradiol Equivalent Concentration

ER-L- Effects Range-Low

ER-M- Effects Range-Median

FF- Fugacity Fraction

FSeQG- Federal Sediment Quality Guidelines

GC- Gas Chromatography

HCH- Hexachlorocyclohexane

HPLC- High Performance Liquid Chromatography

IAEA- International Atomic Energy Agency

ILCR- Incremental Life-time Cancer Risk

IUPAC- International Union of Pure and Applied Chemistry

LODs- Limits of Detection

LOQs- Limits of Quantification

MEC- Measured Environmental Concentration

MS- Mass Spectrometry

NP- Nonylphenols

OCPs- Organochlorine Pesticides

OP- Octylephenol

PAEs- Phthalic Acid Esters

PAHs- Polycyclic Aromatic Hydrocarbons

PBDEs-Polybrominated Diphenyl Ethers

PCBs- Polychlorinated Biphenyl

PEL- Probable Effect Level

POPs- Persistent Organic Pollutants

SIM- Single Ion Monitoring

SQGs- sediment quality guidelines

SRMs- Standard Reference Materials

TEFs- Toxicity Equivalency Factors

TEL- Threshold Effect Level

TOC- Total Organic Carbon

US EPA- United States Environmental Protection Agency

PAHs Compounds

NAP-Naphthalene, **ACY-**Acenaphthylene, **ACE**-Acenaphthene, **FLU-**Fluorene, **PHEN**-Phenanthrene, **ANT**-Anthracene, **FLUO-** Fluoranthene, **PYR**- Pyrene, **BaA**- Benzo (a) Anthracene, **CHY**- Chrysene, **BbF**- Benzo (b) Fluoranthene, **BkF**- Benzo (k) Fluoranthene, **BaP-** Benzo (a) Pyrene, **DBA**- Dibenz (a,h) anthracene, **BghiP**- Benzo (ghi) Perylene, **IND**-Indeno(1,2,3-cd) Pyrene.

PAEs Compounds

BBP- Benzyl butyl phthalate, **DBEP-** Bis(2-n-butoxyethyl) phthalate, **DEEP**- Bis(2-ethoxyethyl) phthalate, **DEHP-** Bis(2-ethylhexyl) phthalate, **DMEP**- Bis(2-methoxyethyl) phthalate, **BMPP**- Bis(4-methyl-2-pentyl) phthalate, **DBP**- Di-n-butylphthalate, **DEP**-Diethylphthalate, **DNHP**- Di-n-hexyl phthalate, **DMP**- Dimethylphthalate, **DNP**- Di-nonyl phthalate, **DNOP**- Di-n-octyl phthalate, **DNPP**- Dipentylphthalate, **DCP-** Dicyclohexyl phthalate, **DIBP-** diisobutyl phthalate.

SYNOPSIS

Preamble

Persistent organic pollutants (POPs) are a group of diverse chemicals that are persistent in the environment; having long half-life in different environmental matrices such as soils, sediments, air and biota. POPs are lipophilic, and have tendency to enter the gas phase under environmental temperatures; are subject to long range transport. These compounds are globally distributed and even found in the pristine environment such as arctic where they have never been used. The combination of their resistance to metabolism and lipophilicity makes them subject to bioaccumulation and transport through food chains (bio-magnification). Animal and human studies link a wide variety of health problems to exposure to POPs, such as reproductive abnormalities, birth defects, immune system dysfunction, neurological defects and cancer.

These pollutants have received intense international attention in recent year because of their ubiquity, recalcitrance, high bioaccumulation potential and harmful biological effects. Under Stockholm Convention on POPs, use and production of 12 chlorinated chemical substances have been banned or severely restricted. These chemical include organochlorines pesticides viz. dichlorodiphenyl-trichloroethane (DDT), chlordane, toxaphene, dieldrin, aldrin, endrin, heptachlor, mirex, industrial chemicals such as polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and other byproducts dioxins and furans (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, PCDD/Fs) which having no known commercial use. These have been often referred as 'legacy' POPs because of their long history of use and release into the environment. Studies on the levels of POPs in the global environments indicate that emission sources of a number of legacy POPs in the last 20 years have shifted from industrialized countries to developing countries in tropical and subtropical regions including India. Organochlorine pesticides used in agriculture and pest control, industrial chemicals like PCBs present in capacitors and transformers. There are legacy POPs that have been around for decades and have either been banned or strictly regulated, but are still found in the environment;

and there are emerging POPs that are either not yet or are very newly regulated e.g. phthalates, BPA, PBDEs etc. Phthalates (phthalic acid esters; PAEs), BPA as plasticizer in variety of plastic materials, PBDE, which are used as flame retardants in consumer products are the main POPs with significant sources in developing countries. There are also unintentionally produced compounds such as polycyclic aromatic hydrocarbons (PAHs), dioxin and furans.

As there is scarcity of data of POPs in environmental matrices specially from the developing countries like India, it is needed to monitor these chemicals in the environment for their distribution, inter-compartmental behaviour, and health risk. This thesis describes status quo levels of various POPs (legacy and emerging) in Thane Creek area, Mumbai, India. Methodology was optimized for identification and quantification of POPs in different matrices using chromatographic techniques. Distribution of these chemicals monitored in different creek compartments viz. seawater, sediment and biota of the marine ecosystem. Data of POPs in creek environment were statistically treated, reported and compare with other studies from other part of world. Data were also compared with guideline values recommended by national and international bodies/regulatory authorities for their ecotoxicological concerns. Human health risk, in terms of integrated lifetime cancer risk (ILCR) was also calculated for POPs exposure via marine food consumption from study area. This study will provide current levels of POPs and their toxicological concerns in Thane creek area, which may be helpful to policymakers to take suitable action for their reduction in environment.

The thesis is divided into the following five chapters, which elaborates the work done during entire study period.

1. Introduction
2. Literature review
3. Materials and Methods
4. Results and Discussion

4.1 Legacy POPs in creek environment their fate and environmental and human risk.

4.2 Endocrine disrupting chemicals (EDCs) and their estrogenic potential in creek ecosystem.

4.3 Inter-compartmental behavior of polycyclic aromatic hydrocarbons (PAHs) in Thane creek and their ecotoxicological concerns.

4.4 Poly brominated diphenyl ethers (PBDEs) in marine sediment and their chronological assessment and source contribution.

5. Summary and Conclusions

The contents of each chapter are explained in brief in the following sections.

Chapter-1: Introduction

This chapter starts with a definition of persistent organic pollutants (POPs) as indicated by Stockholm convention **[1]**. Legacy and emerging persistent organic pollutants were discussed subsequently. Brief introduction to each category of pollutants viz. organochlorine pesticide (OCPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), endocrine disrupting chemicals (EDCs) especially phthalates and bisphenol A, and polycyclic aromatic hydrocarbon (PAHs) are mentioned **[2-7]**. Physiochemical properties of these chemicals were also described in this chapter.

This chapter also contains an overview of the study area which includes the principal discharges to the Thane Creek, water flow, nearby industries, demography and tidal action in the creek. Additionally, main objectives of the present study were also incorporated in this introduction chapter such as, distribution of POPs in different compartment of creek environment, comparison of levels of POPs in samples with guideline values recommended by national and international statuary bodies, inter compartmental behavior of POPs, bio-concentration and fugacity fraction calculations, intake of POPs to human being through consumption of biota and associated carcinogenic risk due to POPs.

Chapter 2-Literature review

This chapter mainly discuss about the various studies on organic pollutants that were carried out in recent past from different part of world with focus on aquatic environment. Distribution of emerging POPs such as phthalates, and PBDEs in environment of were also mentioned. This chapter also deals with environmental toxicity of these contaminants and also their impacts to human beings. Significance of chromatographic and hyphenated techniques for identification and quantification of persistent organic compounds in different environmental matrices is also detailed in this chapter.

Chapter 3- Materials and Methods

This chapter deals with description of sampling locations, sampling methodology, chemical processing/extraction, instrumental analysis, quality control and quality assurance measures used in the present study. Various marine samples such as sweater, sediments, and biota (fish and crabs) were collected from different locations across the Thane creek, Mumbai. Sediment samples were extracted using a sonication assist technique, and varying combinations of organic solvents were used for different class of organic pollutants. Multilayer columns were used for cleanup of extract prior to instrumental analysis. Biota samples were also processed and extracted in similar manner for identification and quantification of organic contaminants. Both liquid and gas chromatographic techniques are employed for analysis. Pollutants such as OCPs, PCBs, PBDEs, PAEs, and PAHs were analyzed using gas chromatography coupled with electron capture detector (ECD) or mass spectrometer (MS). Ultra-high performance liquid chromatography (UHPLC) coupled with a diode array detector (DAD) was used for analysis of endocrine disrupting chemicals. Details of chromatographic system and parameter used in analysis were also include in this chapter. Quality control and quality assurance measure were

also discussed in this chapter, which includes use of field and laboratory blanks, calibration using external standards, determining the recovery for analytical procedure, analysis of standard or certified reference materials (SRMs/CRMs). For identification of chemicals by mass spectrometry, NIST mass fragmentation library was used in addition to retention time.

Chapter – 4: Results and Discussion

4.1 Distribution of POPs in creek environment, their inter-compartmental behavior and ecotoxicological concern.

This chapter discuss the spatial distribution of OCPs and PCBs in sediment and their ecotoxicological concern. Average concentrations (ng g^{-1}) of α-HCH (hexachlorocyclohexane), β-HCH, γ-HCH, DDT, DDD, and DDE in sediments (dry weight) was monitored at 10 different locations across Thane creek. Total DDT concentration, which is the sum of concentrations of DDT and its major metabolites, i.e. DDD and DDE, was found to range from 3.14 to 6.74 ng g^{-1} with an average value of 4.6 ng g^{-1} dry weight of sediments. Concentrations of DDT were found comparable with sediment reported in literature from different part of world **[8,9]**. OCPs concentrations were positively correlated with organic carbon contents in surface sediment samples. In seawater samples from Thane creek, the mean concentrations of total DDT, α-HCH, β-HCH, and γ-HCH were 4.45, 1.12, 1.23 and 1.9 ng L^{-1} respectively. The fugacity fraction (FF) values for DDT and its metabolites were less than 0.5, indicates these chemicals have tendency to accumulate in sediment from seawater, while reverse was observed for HCH isomers (FF > 0.5). The bio-concentration factor for these organochlorine pesticide was found in range of 1500 to 7500 in fish samples via seawater.

Total nine PCBs were analyzed in sediment samples collected from the Thane creek, most of these are major constituent of technical mixture of PCBs. Order of the analyzed PCB congeners' concentration in sediment was found as CB-138 > CB-153 > CB-180 > CB-101 >

CB-77 > CB-126 > CB-52 > CB-169 > CB-194. Hexa-chlorinated biphenyls are contributing more than half with around 53% to total PCBs, while octa-chlorinated biphenyls were found to be contributing least with an average value of 3% in sediment. The sediment quality guidelines (SQG) declared by the US Environmental Protection Agency (USEPA) and the Canadian Council of Ministers of the Environment (CCME) were used to assess the potential ecotoxicological impact of analyzed organic contaminants in the surface sediments of Thane creek.

4.2 Endocrine disrupting chemicals and their estrogenic potential in creek environment

This chapter describes, distribution and estrogenic potential of endocrine disrupting chemicals (EDCs) in estuarine sediments from Mumbai, India. EDCs monitored in sediment are 14 phthalic acid esters, BPA, estrone (E1), 17β-estradiol (E2), 17α-ethinylestradiol (EE2), 4-para-nonylphenol (NP) and 4-tert-Octylphenol (OP). The order of abundance of phthalate esters (PAEs) in surface sediments sample are as DBP > DIBP > DMPP > DNOP > DEP > DBEP > DNP > DNPP > DMP > DEHP > DEEP > DMEP > DNHP ≈ BBP. It was found that the spatial distribution of PAEs generally followed the distribution pattern of TOC. Concentrations of BPA in sediment are found within range of values reported in earlier literature **[10]**. The spatial distribution of BPA in sediment found in very similar as for PAEs. Order of average occurrence of EDCs in sediment samples observed in this study is nonylphenol > octylphenol > 17α-ethinylestradiol > estrone > 17β-estradiol. The concentrations of individual EDCs in sediments were determined using chromatography technique, while the EEFs were obtained from scrutinizing most recent values reported in the literature **[11].**

Abundance of Phthalates in each media was discussed and compared with literature value. Di-n-butylphthalate (DBP) was most abundant compound among analyzed PAEs in all tested aquatic media. In situ bio-concentration factors (BCFs) were calculated of all monitored

phthalates in fish and crabs and compared with log Kow values. Risk Quotient (RQ) and estradiol equivalent concentration (EEQs) were calculated to evaluate ecological risk and estrogenic potential of seawater in terms of DBP and DEHP. The average values of total PAEs daily intake were calculated as 58.1 ± 13.6 and 79.6 ± 19.6 (µg/kg-bw/day) for fish and crab respectively to an adult population.

4.3 Polycyclic aromatic hydrocarbons (PAHs) in Thane creek, inter-compartmental behavior and their ecotoxicological concerns.

In this chapter levels of polycyclic aromatic hydrocarbons in sediment, seawater, fish and crab samples from Thane creek, India were reported. Seasonal variation and ring number wise distribution of PAHs were also described. This chapter also describes the ring number wise distribution of PAHs in all compartment. PAHs concentration were compared with sediments quality guidelines viz. ERL-ERM, TEL-PEL indexes for finding ecotoxicological risk on marine organism.

The carcinogenic risk of PAHs in food is often expressed by its BaP equivalent concentration (BaPeq) and is calculated from the concentrations of individual PAHs and their toxicity equivalency factors (TEFs). Toxicity equivalency factors (TEFs) of PAHs were relative toxicity of PAHs with respect to BaP. BaPeq concentrations were calculated of fish and crab samples. The calculated BaPeq concentrations were found as 3.12 ± 1.1,3.9 ± 0.47 and 43.3 ± 27.3 ng g^{-1} (wet weight) in lizard fish, bombay duck and crabs respectively averaged over monitoring period. Those values are much higher than the recommended BaPeq of PAHs (0.67 ng g^{-1}, ww) suggested by USEPA (2000) for human fish consumption [**12**]. Fugacity fraction of PAHs were calculated for water sediment exchange of PAHs. Bio-concentration factors were also calculated for individual PAHs in fish and crab samples.

4.4 Poly brominated diphenyl ethers (PBDEs) in marine sediment and their chronological assessment and source contribution.

This chapter incorporates the measured concentration of 15 PBDEs congeners in grab and sediment core from five locations across Thane creek for spatial and temporal distribution. PBDE sediment concentrations were comparable to the studies carried out in different parts of the world. The order PBDEs abundance in grab sediments was found as BDE-209 > BDE-47 > BDE-28 > BDE-99 > BDE-100 > BDE-154 > BDE-190 > BDE-183 > BDE-66 > BDE-138 > BDE-77 > BDE-153 > BDE-85 > BDE-75 > BDE-71. Most of BDEs have peaked concentration at the depth of 10-15, 30-40 and 50 cm of depth, which are corresponding to years 2003-2008, 1988-1994 and 1980 respectively.

Age of sediment core slices were determined using Pb^{210} dating technique. To check the contribution of different homologues of PBDEs in sediments, analyzed chemicals were classified in 6 groups base on their degree of bromination, from the most brominated homologue (DecaBDE) to the least (TriBDE). This chapter also describes a least square method for finding the contribution of commercial pentaBDE, octaBDE and decaBDE technical mixture to the sediment. Levels of PBDEs in sediment from the Thane creek were compare with the Federal Sediment Quality Guidelines (FSeQG) which are intended to protect sediment dwelling animals as well as pelagic animals which bioaccumulate PBDEs from sediments.

Chapter 5: Summary and Conclusions

This chapter summarizes the results and overall conclusion of the present study. The summery and few major conclusions drawn from the study are given below.

In present study monitoring of persistent organic pollutants was carried out in aquatic environment of creek system. The study gives levels of organic pollutants in variety of matrices such as seawater, sediments and biota. Spatial and temporal variation of many organic pollutants was also studied with respect to their load in sediment. For the analysis of POPs in various matrices chromatographic techniques such as GC-MS/ECD, HPLC-UV/PDA were

optimized. Data of POPs in different matrices were compared with studies carried out other parts of globe. Data were also treated statistically for finding significance variation with season and representation purposed such as mean, standard deviation etc. Levels of POPs were compare with various environmental quality guidelines to assess their potential threat to marine organisms. Various toxicological indices such as BaP_{eq}, EEQ Concentration, etc. were also calculated for addressing their ecotoxicological concerns. Incremental lifetime cancer risk (ILCR) was calculated for exposure of selected POPs via consumption of marine food e.g. fish and crab. Source contribution and consumption pattern of some POPs was also incorporated in the thesis.

Few major conclusions that can be drawn from the study are given below.

- Spatial distribution of OCPs and PCBs reveals their high concentration at the wastewater receiving point of the creek compared to other locations. Concentrations of OCPs and PCBs in grab sediments was found to decline to large extent compared with core sediments from earlier studies [**13**]. This indicates that the use of OCPs and PCBs around the Thane creek area declined over the decades.
- PCB profile in grab sediments was found to be different from core in earlier studies. This indicates that the sources of PCBs in the Thane creek are diffused and decline over time. Concentrations of PCBs in sediment samples were found to be within sediment quality guidelines given by USEPA and CCME.
- PAEs higher concentrations in samples were explained as; the advent of huge quantities of pollutants from rivers/outflow containing a large amount of urban runoff and industrial discharge across the Thane creek. BCFs values were not found well correlated to the log K_{ow} values of phthalates for both fish and crab samples.

- RQ values for DBP and DEHP in sweater indicates that these compounds had high ecological risk. Total EEQs values of DBP and DEHP suggests there are no threat of estrogenic activity to marine organism in seawater, which are less than 1 ng-E2/L.
- 17α-ethinylestradiol (EE2) was main contributor to EEQs in sediment in terms of estrogenic potential, followed by estrone (E1) and 17β-estradiol (E2); which indicates those compounds should be the priority EDCs concerns in Thane Creek sediment.
- Winter season was found more favoring PAHs accumulation in sediment and marine organism compare to summer. Ring number wise distribution of PAHs revealed that, in seawater and fishes low molecular weight PAHs (2+3-ring PAHs) were dominant compare to high molecular weight PAHs (5+6-ring PAHs), while just opposite was found in cases of sediment and crab samples.
- Contribution of commercial penta-BDE (f_P), octa-BDE (f_O), and deca-BDE (f_D) to the profile found in sediments collected across Thane creek were in the proportion of their worldwide consumption. Levels of all measured PBDEs in sediment met with guideline values except for the penta-BDE (total, BDE-99 and BDE-100) at few locations.
- Assuming that there will be no future use/ emission to environment of POPs; levels were observed to be declining exponentially in sediments and estimated they may found below detection levels in upcoming decades.

Monitoring data of POPs in different matrices were helpful in understanding the fate of these chemicals, estimating threat to human and environment from their exposure, also in source apportionment of mentioned pollutants. Data reported in this study can also serve as baseline and will be useful in future to estimate their trend over time. In response to the continuing discovery of the persistent, bio accumulative properties, and toxicity of POPs, regional, national and international policies ban the intentional production of these chemicals. However, the levels of some of these banned compounds in environment are

hovering that could still be problematic rather than dwindling. Present study also indicates that effluent water treatment facilities surrounding study area are not efficient to remove these organic contaminants and there is build-up of these chemical in creek environment. Organic pollutants in marine consumables were found in significant concentration, and may cause adverse human health effect, and therefore people consuming marine product from study area should exercise caution.

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Chapter-1

INTRODUCTION

1.1. Persistent Organic Pollutants

Persistent organic pollutants (POPs) are a group of diverse chemicals that are persistent in the environment; having long half-life in different environmental matrices such as soils, sediments, air and biota. POPs are lipophilic, and have tendency to enter the gas phase under environmental temperatures; are subject to long range transport. These compounds are globally distributed and even found in the pristine environment such as arctic where they have never been used. The combination of their resistance to metabolism and lipophilicity makes them subject to bioaccumulation and transport through food chains (bio-magnification). Animal and human studies link a wide variety of health problems to exposure to POPs, such as reproductive abnormalities, birth defects, immune system dysfunction, neurological defects and cancer **[14]**. According to Stockholm Convention, Persistent Organic Pollutants (POPs) are organic chemical substances, that is, they are carbon-based. They possess a particular combination of physical and chemical properties such that, once released into the environment, they:

- Remain intact for exceptionally long periods of time (many years);
- Become widely distributed throughout the environment as a result of natural processes involving soil, water and, most notably, air;
- Accumulate in the fatty tissue of living organisms including humans, and are found at higher concentrations at higher levels in the food chain; and
- Are toxic to both humans and wildlife.

As a result of releases to the environment over the past several decades due to human activities, POPs are now widely distributed over large regions and, in some cases, they are found around the globe. This extensive contamination of environmental media and living organisms includes

many foodstuffs and has resulted in the sustained exposure of many species, including humans, for periods of time that span generations, resulting in both acute and chronic toxic effects [**15]**. In addition, POPs concentrate in living organisms through another process called bioaccumulation. Though not soluble in water, POPs are readily absorbed in fatty tissue, where concentrations can become magnified by up to 70,000 times the background levels. Fish, predatory birds, mammals, and humans are high up the food chain and so absorb the greatest concentrations. As a result of these two processes, POPs can be found in people and animals living in regions such as the Arctic, thousands of kilometers from any major POPs source [**16**]. Specific effects of POPs can include cancer, allergies and hypersensitivity, damage to the central and peripheral nervous systems, reproductive disorders, and disruption of the immune system. Some POPs are also considered to be endocrine disrupters, which, by altering the hormonal system, can damage the reproductive and immune systems of exposed individuals as well as their offspring; they can also have developmental and carcinogenic effects [**17, 18**]. These pollutants have received intense international attention in recent year because of their ubiquity, recalcitrance, high bioaccumulation potential and harmful biological effects. Under Stockholm Convention on POPs, use and production of 12 chlorinated chemical substances have been banned or severely restricted. These chemical include organochlorines pesticides viz. dichlorodiphenyl-trichloroethane (DDT), chlordane, toxaphene, dieldrin, aldrin, endrin, heptachlor, mirex, industrial chemicals such as polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and other byproducts dioxins and furans (polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, PCDD/Fs) which having no known commercial use [**15, 19**]. These have been often referred as 'legacy' POPs because of their long history of use and release into the environment. Studies on the levels of POPs in the global environments indicate that emission sources of a number of legacy POPs in the last 20 years have shifted from industrialized countries to developing countries in tropical and subtropical regions

including India. Organochlorine pesticides used in agriculture and pest control, industrial chemicals like PCBs present in capacitors and transformers, phthalates (phthalic acid esters; PAEs), BPA as plasticizer in variety of plastic materials, unintentionally produced compounds such as polycyclic aromatic hydrocarbons (PAHs), dioxin and furans, and PBDE, which are used as flame retardants in consumer products are the main POPs with significant sources in developing countries [**20**].

Because of their hydrophobic nature, upon reaching aquatic environments, POPs are very quickly sequestered by organic-rich particles and delivered to the bottom sediments where they are ultimately buried. Sediments tend to integrate contaminants over long periods of time and are often the best matrix for assessing spatial and temporal concentrations of hydrophobic organic contaminants. Sediments are thought to act as the ultimate 'sink' of POPs, and sediment samples have been analyzed extensively in developing countries to understand historical and recent level of pollution, especially along coastal areas affected by industrial developments **[1]**.

1.1.1 Organochlorine Pesticides (OCPs)

Over the past few decades there has been a steady increase in the use of many pesticides for agricultural and disease control proposes in developing countries including India. India has promulgated several laws for the control of such chemicals, in practice, there has been little control over their production and uses. DDTs and HCHs were two intensively used insecticide in Indian context especially late 1980s [**21**]. A brief introduction on these pesticides is mentioned as following:

DDT was widely used during World War II to protect soldiers and civilians from malaria, typhus, and other diseases spread by insects. After the war, DDT continued to be used to control disease, and it was sprayed on a variety of agricultural crops, especially cotton. DDT continues to be applied against mosquitoes in several countries to control malaria. Its stability, its

persistence (as much as 50% can remain in the soil 10-15 years after application), and its widespread use have meant that DDT residues can be found everywhere; residual DDT has even been detected in the Arctic **[22]**.

Perhaps the best known toxic effect of DDT is egg-shell thinning among birds, especially birds of prey. Its impact on bird populations led to bans in many countries during the 1970s. Although its use had been banned in many countries, it has been detected in food from all over the world. Although residues in domestic animals have declined steadily over the last two decades, food-borne DDT remains the greatest source of exposure for the general population. The short-term acute effects of DDT on humans are limited, but long-term exposures have been associated with chronic health effects. DDT has been detected in breast milk, raising serious concerns about infant health [**23**].

Lindane is the common name for the gamma isomer of hexachlorocyclohexane (HCH). Technical HCH is an isomeric mixture that contains mainly five forms, namely alpha-, beta-, gamma-, delta- and epsilon-HCH. Lindane has been used as a broad-spectrum insecticide for seed and soil treatment, foliar applications, tree and wood treatment and against parasites in both veterinary and human applications [**24**]. The production of Lindane has decreased rapidly in the last few years and only few countries are still known to produce Lindane. Lindane is persistent, bio concentrates, bioaccumulate in the food chain rapidly [**25**]. There is evidence for long-range transport and toxic effects (immunotoxicity, reproductive and developmental effects) in laboratory animals and aquatic organisms. Alternatives for Lindane are generally available, except for use as a human health pharmaceutical to control head lice and scabies. Regulations on the production, use and monitoring of Lindane already exist in several countries [**15**]. The chemical structure and general physicochemical properties of these compounds were represented in **Fig 1.1** and **Table 1.1** respectively.

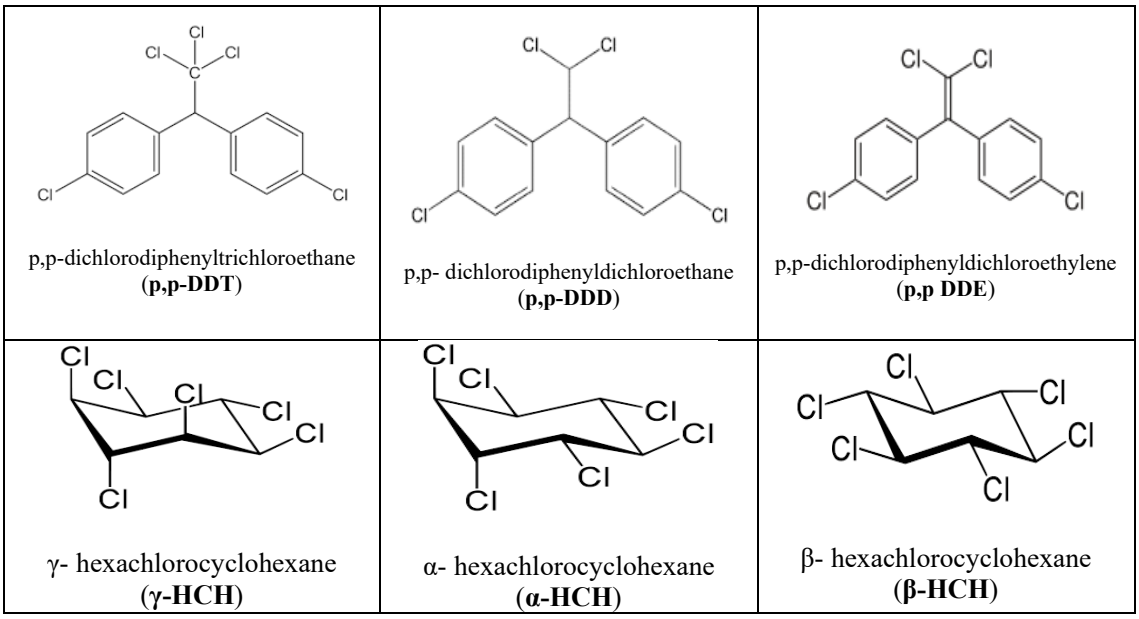


Fig 1.1 Chemical structure of DDT compounds and HCH isomers.

Table 1.1 Physicochemical properties of DDT compounds and HCH isomers [**26, 27**].

Compound	**Chemical formula**	**Molar mass (g mol^{-1})**	**Boiling Point (^{o}C)**	**Solubility (mg/L) in water at 25^{o}C**	**Log K_{ow}**
DDT	$C_{14}H_9Cl_5$	354.48	260	0.025	6.91
DDD	$C_{14}H_{10}Cl_4$	320.04	350	0.09	6.02
DDE	$C_{14}H_8Cl_4$	318.02	316	0.065	4.7
α-HCH	$C_6H_6Cl_6$	290.83	288	69.5	3.82
β-HCH	$C_6H_6Cl_6$	290.83	283	34.8	3.78
γ-HCH	$C_6H_6Cl_6$	290.83	323.4	7.3	3.72

Use of pesticides in India began in 1948 when DDT was imported for malaria control and BHC (benzenehexachloride) for locust control. India started pesticide production with manufacturing plant for DDT and BHC, HCH in the year 1952. In 1958, India was producing over 5000 metric tonnes of pesticides. DDT had been used in agriculture for decades until it was restricted in 1989, but 6,000 tonnes of DDT are still produced annually for the eradication

of mosquitoes and other pests. After it restricted DDT, the government began encouraging the use of other POPs that were potentially even more harmful, such endosulfan (later banned in 2011) and then Lindane (restricted in 2012). Rather than acknowledge that the makeup of all POPs render them intrinsically harmful, the government seems to be promoting different POPs in turn until each is found to have tangible toxic effects [**353, 354**]. Use of technical grade HCH began in 1943, and the total global consumption was estimated to be as high as 6.0 million tonnes with maximum annual usage at 334,400 tonnes in 1981. There is large technical grade HCH consumption for agriculture and public health sectors in India. Maximum annual usage reached 57,000 t in the latter 1980s. In,1990 the government of India banned technical HCH usage on vegetable, fruit, and oilseed crops and for preservation of grains, but continued to allow its use for public health protection and on certain food crops at around 20,000 t annually. It was reported that the India Government has taken a decision to phase out a production of 30,000 tonnes of HCH per annum [**355, 356**].

1.1.2 Polychlorinated Biphenyles (PCBs)

PCBs are used in industry as heat exchange fluids, in electric transformers and capacitors, and as additives in paint, carbonless copy paper, and plastics. Of the 209 different types of PCBs congeners, 13 exhibit a dioxin-like toxicity. Their persistence in the environment corresponds to the degree of chlorination, and half-lives can vary from 10 days to one-and-a-half years. PCBs are toxic to fish, killing them at higher doses and causing spawning failures at lower doses **[28]**. Research also links PCBs to reproductive failure and suppression of the immune system in various wild animals, such as seals and mink. Large numbers of people have been exposed to PCBs through food contamination. Consumption of PCB-contaminated rice oil in Japan in 1968 and in Taiwan in 1979 caused pigmentation of nails and mucous membranes and swelling of the eyelids, along with fatigue, nausea, and vomiting. Due to the persistence of PCBs in their mothers' bodies, children born up to seven years after the Taiwan incident showed

developmental delays and behavioral problems. Similarly, children of mothers who ate large amounts of contaminated fish from Lake Michigan showed poorer short-term memory function. PCBs also suppress the human immune system and are listed as probable human carcinogens [**29, 30, 31**]. Chemical structure of polychlorinated biphenyls investigated in present study are shown in **Fig 1.2**, and their physicochemical properties of are summarized in **Table 1.2**.

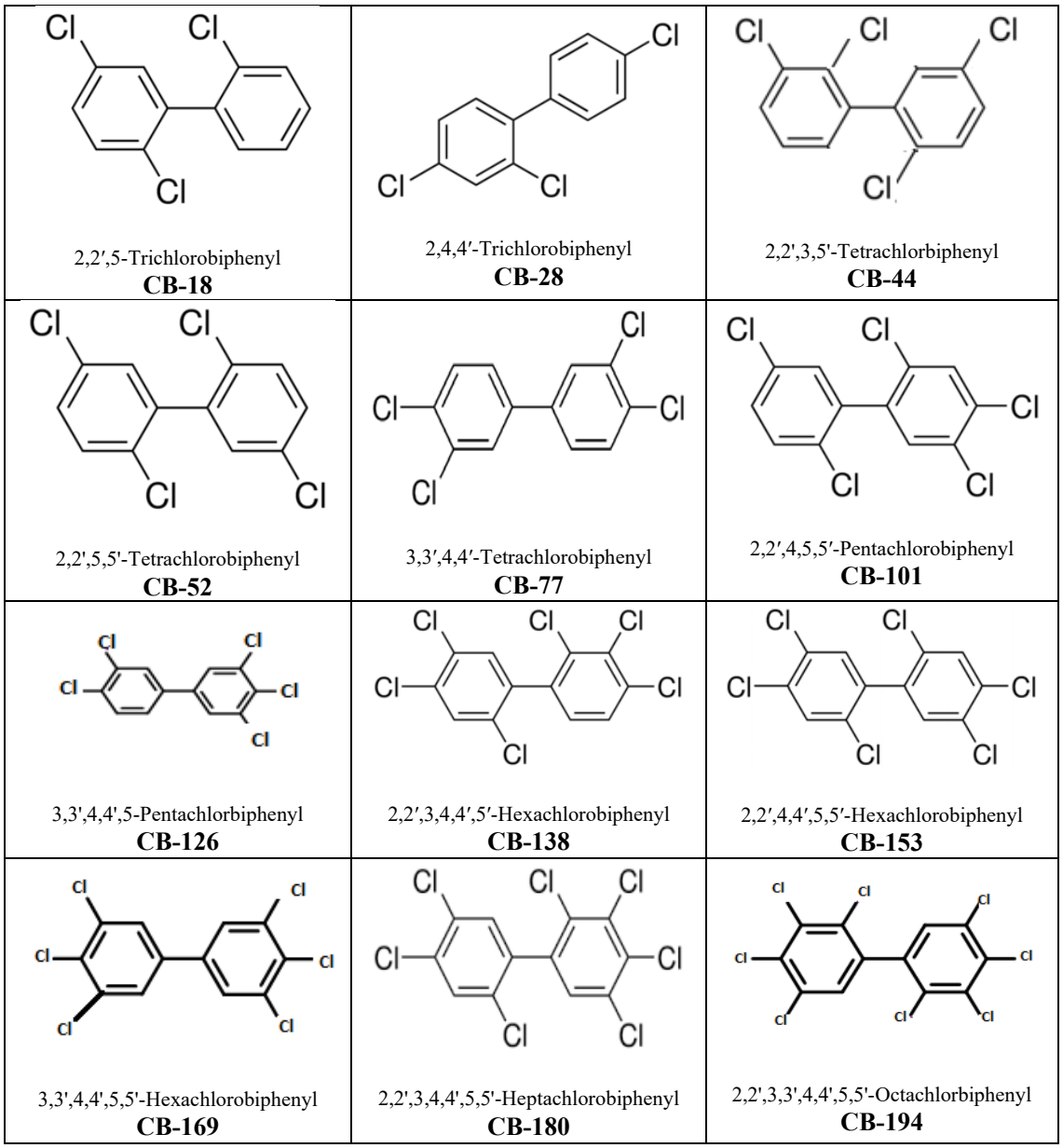


Fig 1.2 Chemical structure of selected polychlorinated biphenyl congeners.

Table 1.2 Physicochemical properties of select polychlorinated biphenyl congeners [**26, 27**].

Compound	Chemical formula	Molar mass (g mol^{-1})	Boiling Point (^oC)	Solubility (µg/L) in water at 25^oC	Log K_{ow}
CB-18	$C_{12}H_7Cl_3$	257.54	338	407	5.24
CB-28	$C_{12}H_7Cl_3$	257.54	338	266	5.67
CB-44	$C_{12}H_6Cl_4$	291.98	359.5	121	5.75
CB-52	$C_{12}H_6Cl_4$	291.98	359.5	41	5.84
CB-77	$C_{12}H_6Cl_4$	291.98	360	17.4	6.36
CB-101	$C_{12}H_5Cl_5$	326.43	378.2	13.3	6.38
CB-126	$C_{12}H_5Cl_5$	326.43	378.2	9.3	6.89
CB-138	$C_{12}H_4Cl_6$	360.87	400	15.9	6.83
CB-153	$C_{12}H_4Cl_6$	360.87	396.9	2.7	6.92
CB-169	$C_{12}H_4Cl_6$	360.87	396.9	2.5	7.42
CB-180	$C_{12}H_3Cl_7$	395.32	415.6	0.2	7.36
CB-194	$C_{12}H_2Cl_8$	429.76	434.3	0.1	7.8

1.2 Endocrine Disrupting Chemicals

Increasing public and scientific awareness on possible human and environmental health effects of endocrine disrupting chemicals (EDCs) can be attributed to alarming evidence from scientific studies indicating abnormalities in the reproductive system of aquatic species, wildlife, and humans as a result of very low exposure (ng L^{-1}) concentrations [**32-35**]. Endocrine disrupting chemicals (EDCs) are responsible for inappropriate development and they alter the hormonal and homeostatic systems of organism. EDCs is group of a wide range of chemicals, most of them are introduced into the environment by anthropogenic activities. Steroid estrogens, which composed natural and synthetic ones, are most potent EDCs and their estrogenic effects have been observed in laboratory studies at very low concentrations. Natural

(e.g. 17β–estradiol [E2] and estrone [E1]) and synthetic estrogens (e.g. mestranol and 17α-ethinylestradiol [EE2], active formulation of oral contraceptives) enter environment predominantly through sewage discharge after they have been excreted. Phenolic compounds, such as alkylphenols (APs) and bisphenol-A (BPA), are known as xenoestrogens because they are also suspected to influence the hormonal system of aquatic organisms. The main pathways for phenolic EDCs into environment are domestic and industrial wastewater discharges. Steroid estrogens and xenoestrogenic phenols have been detected in a variety of waters in earlier studies [**36- 39]** Phthalates (PAEs), Bisphenol A (BPA) and other EDCs in aquatic environment are studied in present study.

1.2.1 Phthalic Acid Esters (PAEs)

Phthalates or phthalate esters are esters of phthalic acid, mainly used as plasticizers (substances added to plastics to increase their flexibility, transparency and durability). They are used primarily to soften polyvinyl chloride (PVC) [**40]**. PVC is a widely used material, including extensive use in toys and other children's products such as chewy teethers, soft figures and inflatable toys. Phthalic acid esters (PAEs) are used man-made chemical released in the environment and human exposure is mainly through the diet. As the phthalate plasticizers are not covalently bound to PVC, they can leach, migrate or evaporate into the environment and as a result have become ubiquitously contaminants. Phthalates are commonly used to provide flexibility to rigid polymers and di-n-butyl phthalate (DBP) and bis (2-ethylhexyl) phthalate (DEHP) are the most used chemicals [**41]**. Phthalic acid esters are not soluble in pure water as they are hydrophobic in nature. However, they may be soluble by interaction with fulvic and humic acids or become adsorbed onto particulate matter. Slow environmental degradation of phthalates by photolysis or hydrolysis results in half-live values in the order of years. Phthalates, which make up 10%–40% of the total weight of a toy, have been under scrutiny because of their potential health effects, particularly on reproductive development [**40,42]**.

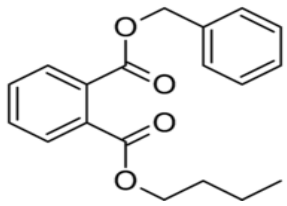
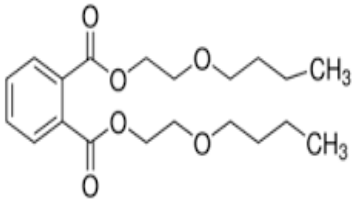
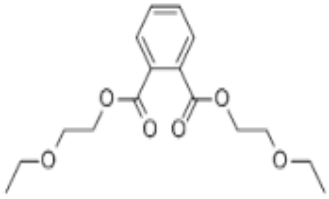
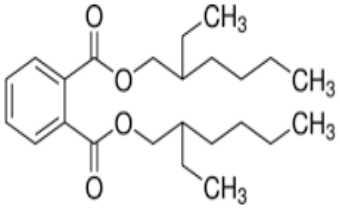
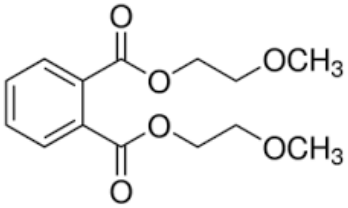
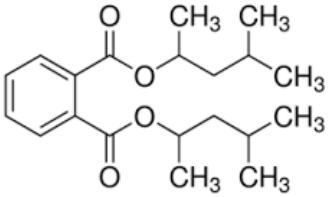
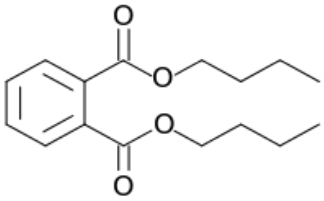
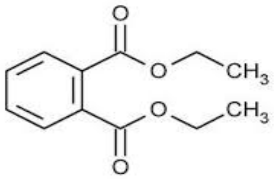
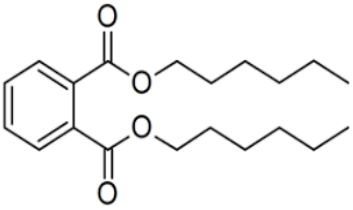
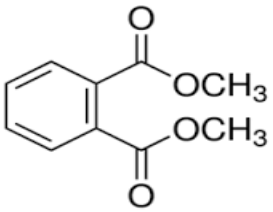
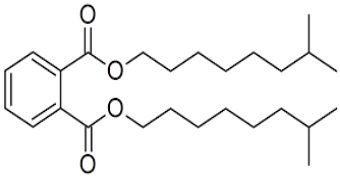
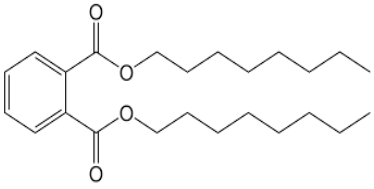
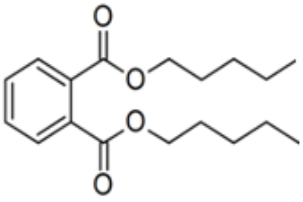
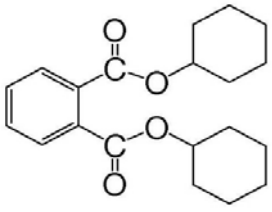
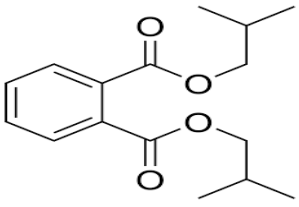
 Benzyl butyl phthalate **[BBP]**	 Bis(2-n-butoxyethyl) phthalate **[DBEP]**	 Bis(2-ethoxyethyl) phthalate **[DEEP]**
 Bis(2-ethylhexyl) phthalate **[DEHP]**	 Bis(2-methoxyethyl) phthalate **[DMEP]**	 Bis(4-methyl-2-pentyl) phthalate **[BMPP]**
 Di-n-butylphthalate **[DBP]**	 Diethylphthalate **[DEP]**	 Di-n-hexyl phthalate **[DNHP]**
 Dimethylphthalate **[DMP]**	 Di-nonyl phthalate **[DNP]**	 Di-n-octyl phthalate **[DNOP]**
 Dipentylphthalate **[DNPP]**	 Dicyclohexyl phthalate **[DCP]**	 Diisobutyl phthalate **[DIBP]**

Fig 1.3 Chemical structure of 15 EPA priority phthalates analyzed in this study.

Table 1.3 Physicochemical properties of 15 EPA priority phthalic acid esters (PAEs) [**26, 27**].

Compound	Chemical formula	Molar mass (g mol^{-1})	Boiling Point (^oC)	Solubility (mg L^{-1}) in water	Log K_{ow}
BBP	$C_{19}H_{20}O_4$	312.4	370	3.8	4.7
DBEP	$C_{20}H_{30}O_6$	366.4	270	300	4.06
DEEP	$C_{16}H_{22}O_6$	310.34	383	4	8.39
DEHP	$C_{24}H_{38}O_4$	390.6	386	2.49×10^{-3}	7.73
DMEP	$C_{14}H_{18}O_6$	282.29	340	8500	1.11
BMPP	$C_{20}H_{30}O_4$	334.45	-	-	5.5
DBP	$C_{16}H_{22}O_4$	278.34	340	11.2	4.5
DEP	$C_{12}H_{14}O_4$	222.2	298	591	2.54
DNHP	$C_{20}H_{30}O_4$	334.4	345	0.159	6.0
DMP	$C_{10}H_{10}O_4$	194.2	284	5220	1.61
DNP	$C_{26}H_{42}O_4$	418.6	380	3.08×10^{-4}	8.6
DNOP	$C_{24}H_{38}O_4$	390.56	390	2.49×10^{-3}	7.73
DNPP	$C_{18}H_{26}O_4$	306.4	342	1.3	5.12
DCP	$C_{20}H_{26}O_4$	330.42	-	4	6.2
DIBP	$C_{16}H_{22}O_4$	278.35	320	6.2	4.1

Phthalate Esters Mixture consist of 15 EPA priority phthalates namely Benzyl butyl phthalate [BBP], Bis(2-n-butoxyethyl) phthalate [DBEP], Bis(2-ethoxyethyl) phthalate [DEEP], Bis(2-ethylhexyl) phthalate [DEHP], Bis(2-methoxyethyl) phthalate [DMEP], Bis(4-methyl-2-pentyl) phthalate [BMPP], Di-n-butylphthalate [DBP], Diethylphthalate [DEP], Di-n-hexyl phthalate [DNHP], Dimethylphthalate [DMP], Di-nonyl phthalate [DNP], Di-n-octyl phthalate [DNOP], Dipentylphthalate [DNPP], dicyclohexyl phthalate [DCP] and diisobutyl phthalate

[DIBP] were analyzed in aquatic system in this study. Chemical structure of these PAEs are shown in **Fig 1.3**, and their physical and chemical properties are represented in **Table 1.3.** Globally, more than 18 billion pounds of phthalates are used each year and well above 2 million tons of DEHP alone are produced annually worldwide [**357**]. In India, data on phthalate production is unavailable, however phthalic anhydride (the major raw material of phthalates) production during 2012–2013 was 225,262 metric tons [**139**]. Bisphenol A (BPA; 4,4′-isopropylidenediphenol) is produced at over 5 million tons annually (in 2015), and the demand for this chemical has been growing steadily over the past few decades [**358**]. Asian countries, especially South Korea, China, and Japan, account for a major share of BPA production globally. BPA is used as a monomer in the production of polycarbonate plastics and epoxy resins. Because of BPA's diverse uses in consumer products, its exposure to humans is widespread. Several studies have reported on the exposure of humans to BPA [**359-361**].

1.2.2 Bisphenol-A (BPA)

Bisphenol A (BPA) is used as a monomer for the production of polycarbonate (PC) plastics, epoxy resins and flame retardants; which are used as coatings on cans, as powder paints, as additives in thermal paper, in dental fillings and as antioxidants in plastics. Leaching of BPA from PC tubes to water increases with higher temperature and exposure time, though in river water BPA rapidly degrades with aerobic conditions [**43**]. It is reported that BPA degrades under aerobic conditions but not under anaerobic conditions in marine sediments. The release of BPA into the environment is possible during manufacturing processes and by leaching from final products [**44]**. Most of these estrogen-like chemicals are widely used persistent organic compounds, which are ubiquitous in the environment and in biological samples. The BPA at environmentally relevant concentrations could produce potential harm to fish reproduction through alternations of sex steroidogenesis and vitellogenin (VTG) induction [**45]**. A study

showed decrease of androgens, which involve in spermatogenesis and sperm maturation in males exposed to BPA at environmentally relevant concentration. Alternations of androgen synthesis lead to decrease of sperm motility and velocity, probably via disruption of sperm maturation. Also, BPA at high environmentally relevant concentration can induce VTG in fish and exhibit estrogenic mode of action [**46]**.

1.2.3 Other Endocrine Disrupting Chemicals (EDCs)

Natural and synthetic estrogens can induce endocrine disrupting effects in the aquatic organisms, in both male and female hormonal system, or even stimulation of feminization or hermaphroditism in fish at very low concentrations [**47]**. As limitations associated with bacterial application as pollution tracers, human and animal sterols are currently being used as indicators of anthropogenic contamination in environmental compartments. Together with plant sterols, they are applied for distinguishing between sources of pollution based on their ratios [**48]**. Marine organisms or terrestrial plants can synthesise these molecules; alternatively, they can be produced by human activities and reach the oceans through various streams such as rivers, continental runoffs, and atmospheric deposition as well as sewage and petroleum inputs [**49]**. Once, these chemicals transported by water streams and deposited in sediments where they are often preserved. Sterols are typically used to distinguish aquatic and terrestrial organic matter (OM) contributions from ‘‘biogenic’’ sources and indicate faecal material present in sewage inputs to coastal areas. Several species of zooplankton, phytoplankton, and higher plants are the primary sources of “biogenic” sterols. Conversely, mammals are the primary source of ‘‘faecal’’ sterols in estuary sediments [**50]**. To know fate of EDCs in sediments is critical for their environmental exposure and risk assessment. 17-β-Estradiol, Estrone, 17-α-Ethynylestradiol, 4-para-Nonylphenol, and 4-tert-Octylphenol were analyzed in aquatic environment in this study. Chemical structure of BPA and other endocrine disrupting

chemicals (EDCs) are depicted in **Fig 1.4** and their physicochemical properties were presented in **Table 1.4.**

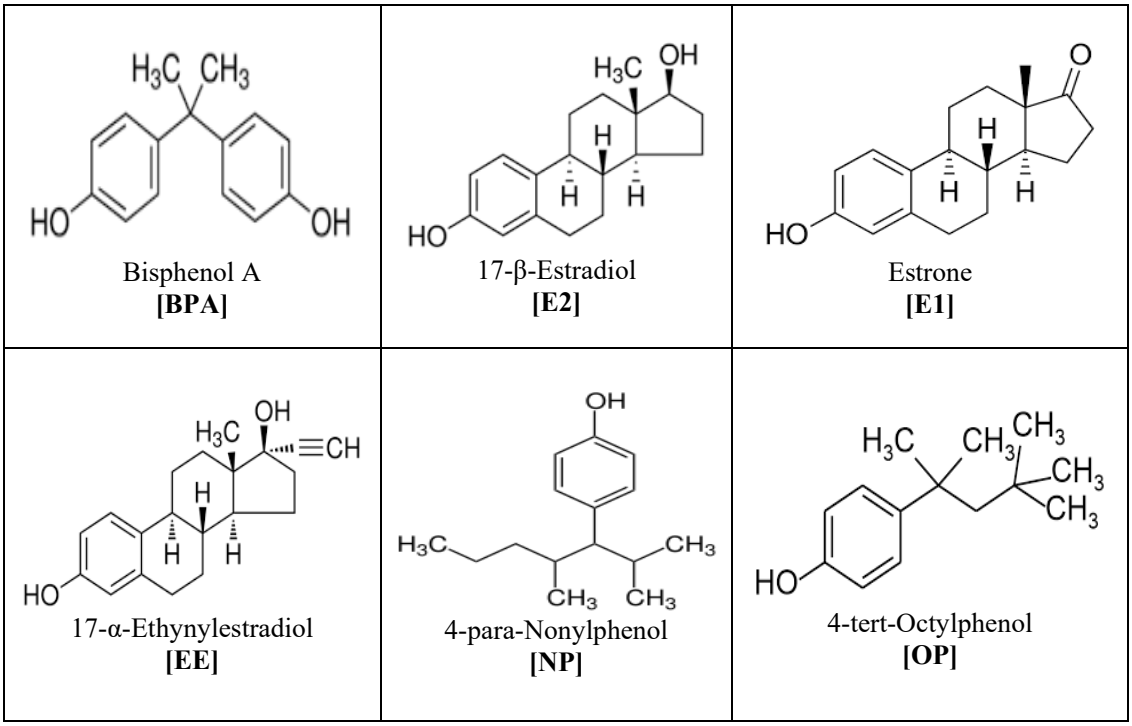


Fig 1.4 Chemical structure of BPA and other endocrine disrupting chemicals (EDCs).

Table 1.4 Physicochemical properties of BPA and other endocrine disrupting chemicals [**26, 27**].

Compound	Chemical formula	Molar mass (g mol^{-1})	Boiling Point (^oC)	Solubility (mg L^{-1}) in water	Log K_{ow}
BPA	$C_{15}H_{16}O_2$	228.29	360.5	120	3.32
E2	$C_{18}H_{24}O_2$	272.38	445.9	3.7	4.01
E1	$C_{18}H_{22}O_2$	270.37	445.2	12.42	3.13
EE	$C_{20}H_{24}O_2$	296.4	457.2	11.3	3.67
NP	$C_{15}H_{24}O$	220.35	293-297	7	5.76
OP	$C_{14}H_{22}O$	206.32	282.3	4.82	5.28

1.3 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) contains two or higher number of aromatic rings, and they are produced by high-temperature reaction, such as pyrolysis of fossil fuels and incomplete combustion of organic materials [**51, 52]**. Many PAHs are having mutagenic and carcinogenic properties, they form one of the most important group of environmental pollutants, sixteen of them are on the US EPA list of priority pollutants (although not of all them are mutagenic and carcinogenic), and have attracted attention of environmental scientist and policy makers for several decades. PAHs enter the environment primarily through anthropogenic activities such as combustion of fossil fuels, various industrial use of fuel, biomass burning for cooking and heating purposes, waste incineration, and oil **[53; 54]**.

PAH toxicity is highly depends on its chemical structure, and even PAH isomers may vary from non-toxic to extremely toxic. The toxicity of PAHs is also associated enzymatic biotransformation, and organisms that have poor bio-transformation capacity (e.g., blue mussels) are less vulnerable to PAH hazards. In contrast, fish can metabolize PAHs to form reactive metabolites, which subsequently can bond covalently as adducts to cellular macromolecules such as nucleic acids and proteins. As fish do not have a highly developed DNA repair system, this may lead to many forms of lesions and adverse conditions in cells and in the organism [**55, 56]**. Humans are exposed to polycyclic aromatic hydrocarbons primarily through food consumption. Once PAHs ingested, can be absorbed by the human body and may cause cancers and decreased fecundity, among other health problems [**57]**. Besides ecological consequences, seafood safety is an issue of concern in every oil spill incident. Commercial and recreational fisheries and subsistence seafood use could potentially be affected as a consequence of the fauna and flora exposure to oil. In order to guarantee public health, restrictions or closure of seafood harvesting might be necessary [**58, 59]**.

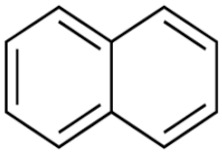
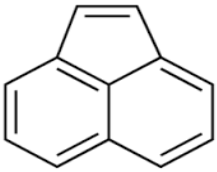
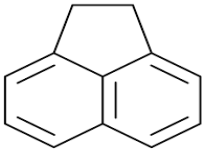
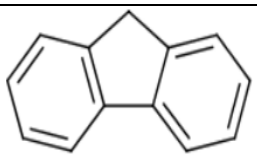
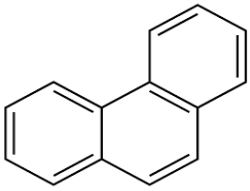
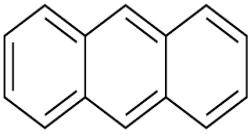
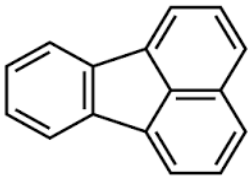
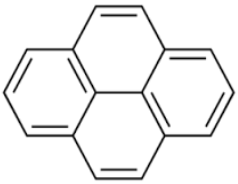
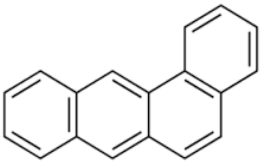
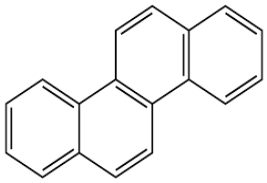
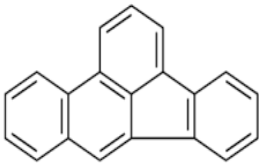
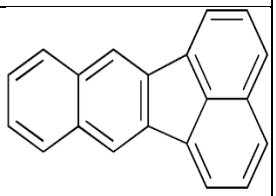
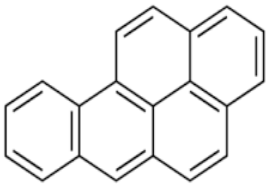
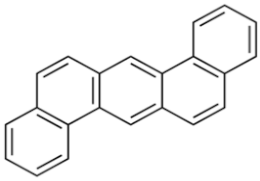
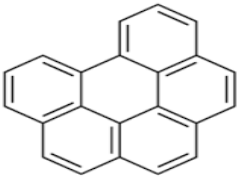
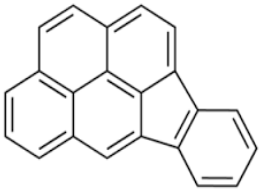
 Naphthalene **[NAP]**	 Acenaphthylene **[ACY]**	 Acenaphthene **[ACE]**	 Fluorene **[FLU]**
 Phenanthrene **[PHEN]**	 Anthracene **[ANT]**	 Fluoranthene **[FLUO]**	 Pyrene **[PYR]**
 Benzo(a)Anthracene **[BaA]**	 Chrysene **[CHY]**	 Benzo(b)Fluoranthene **[BbF]**	 Benzo(k)Fluoranthene **[BkF]**
 Benzo (a) Pyrene **[BaP]**	 Dibenz(a,h)anthracene **[DBA]**	 Benzo (ghi) Perylene **[BghiP]**	 Indeno(1,2,3-cd) Pyrene **[IND]**

Fig 1.5 Chemical structures of 16 EPA priority polycyclic aromatic hydrocarbons (PAHs).

In this study, 16 US EPA priority PAHs viz. Naphthalene (NAP), Acenaphthylene (ACY), Acenaphthene (ACE), Fluorene (FLU), Phenanthrene (PHEN), Anthracene (ANT), Fluoranthene (FLUO), Pyrene (PYR), Benzo (a) Anthracene (BaA), Chrysene (CHY), Benzo

(b) Fluoranthene (BbF), Benzo (k) Fluoranthene (BkF), Benzo (a) Pyrene (BaP), Dibenz (a,h) anthracene (DBA), Benzo (ghi) Perylene (BghiP), and Indeno(1,2,3-cd) Pyrene (IND) were analyzed in different compartment of environment. Chemical structures of all PAHs studied in marine environment were represented in **Fig 1.5,** and their physicochemical properties are mentioned in **Table 1.5.**

Table 1.5 Physicochemical properties of 16 EPA priority polycyclic aromatic hydrocarbons [**26, 27**].

Compound	Chemical formula	Molar mass (g mol^{-1})	Boiling Point (^oC)	Solubility (mg L^{-1}) in water	Log K_{ow}
NAP	$C_{10}H_8$	128.17	218	31	3.3
ACY	$C_{12}H_8$	152.19	280	9	3.93
ACE	$C_{12}H_{10}$	154.21	277.5	4	3.92
FLU	$C_{13}H_{10}$	166.22	294	1.7	4.18
PHEN	$C_{14}H_{10}$	178.23	340	1.15	4.46
ANT	$C_{14}H_{10}$	178.23	342	1.29	4.45
FLUO	$C_{16}H_{10}$	202.25	384	0.20-0.26	5.16
PYR	$C_{16}H_{10}$	202.25	404	0.135	4.88
BaA	$C_{18}H_{12}$	228.29	437.6	9.4×10^{-3}	5.76
CHY	$C_{18}H_{12}$	228.29	448	2.0×10^{-3}	5.73
BbF	$C_{20}H_{12}$	252.32	481	1.5×10^{-3}	5.78
BkF	$C_{20}H_{12}$	252.32	480	8.0×10^{-4}	6.11
BaP	$C_{20}H_{12}$	252.32	495	1.62×10^{-3}	6.13
DBA	$C_{22}H_{14}$	278.35	524	2.49×10^{-3}	6.5
BghiP	$C_{22}H_{12}$	276.34	550	2.6×10^{-4}	6.63
IND	$C_{22}H_{12}$	276.34	536	1.9×10^{-4}	6.7

1.4 Polybrominated diphenyl ethers (PBDEs)

A group of synthetic compounds, polybrominated diphenyl ethers (PBDEs) are type of brominated flame retardants (BFRs). These chemicals have been most widely used in a multitude of products including televisions, computers, textiles, furniture upholstery etc. for more than four decades as flame retardants [**60, 61]**. PBDEs significantly reduce fire hazards in polymeric substances by releasing bromine atoms which capture OH and H radicals formed during combustion at a temperature 50 ^{o}C below ignition temperature of the polymer matrix [**62]**. PBDEs have been produced and used in three commercial mixtures: decabromodiphenyl ether (deca-BDE) octabromodiphenyl ether (octa-BDE), and pentabromodiphenyl ether (penta-BDE). The penta- and octa-BDEs are listed as persistent organic pollutants (POPs) recently, due to environmental and human health concerns these chemicals are banned from production in United States and Europe [**63, 64]**. Among PBDEs, a popular commercial penta-brominated diphenyl ether (PBDE) flame retardant mixture (DE-71) has been extensively used for many years. 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) were the major components of DE-71 commercial mixture [**65, 66]**.

In the large production volumes of brominated diphenyl, tetra- and penta-brominated congeners predominate in biota; and BDE-47 was the most abundant PBDE congener [**67- 69]**. The annual global production of PBDEs last decade of 20th century had increased from 40000 t (1992) to 67000 t (2001) [**61]**. It is estimated that United States and Canada, approximately 46000, 25000, and 380000 t of commercial penta-BDE, octa-BDE, and deca-BDE, respectively, will be used in products in the between 1970 and 2020 [**70]**. In Asian countries, the major commercial product was deca-BDE. The volume of production of commercial penta-BDE, octa-BDE, and deca-BDE were estimated to be 150, 1500, and 23000 t, respectively, in year 2001 for Asian countries [**71]**. Commercial mixture of penta-BDE is highly persistent in the environment, bio accumulative and has a high potential for long-range environmental

transport. These chemicals have been detected in humans in all regions. There is evidence of its potential for toxic effects in wildlife, including mammals.

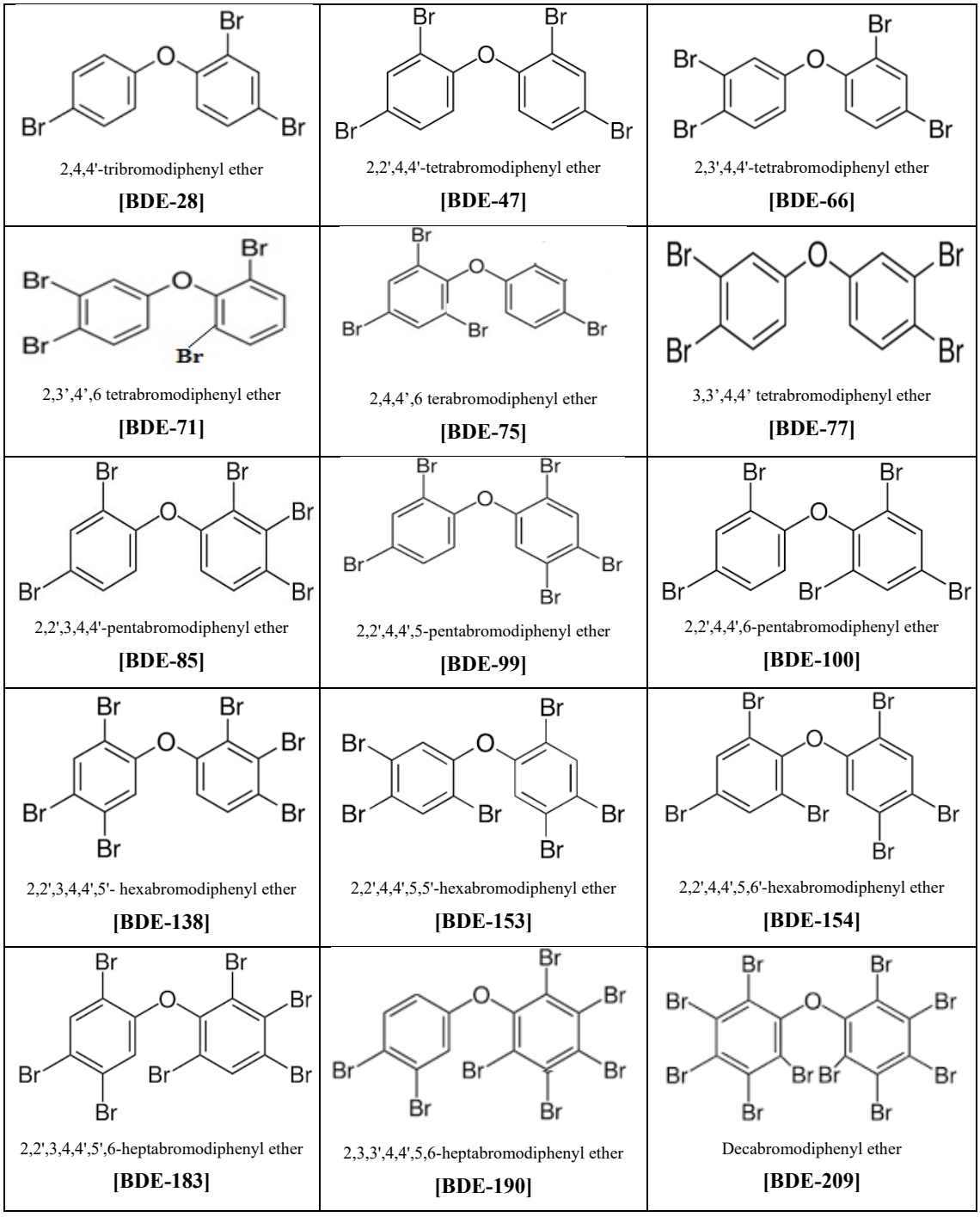


Fig 1.6 Chemical structure of select polybrominated biphenyl ethers (PBDEs) congeners.

Alternatives are available and used to replace these substances in many countries, although they might also have adverse effects on human health and the environment. The identification

and also handling of equipment and wastes containing brominated diphenyl ethers is considered a challenge. PBDEs may act as an endocrine disruptor and can cause thyroid hormone disruption, alter neurodevelopment and interfere with reproductive systems [**72]**. Chemical structure and physicochemical properties of polybrominated biphenyl esters (PBDEs) congeners are shown in **Fig 1.6** and **Table 1.6** respectively.

Table 1.6 Physicochemical properties of select polybrominated biphenyl ethers congeners [**26, 27,73-75**]

Compound	**Chemical formula**	**Molar mass (g mol^{-1})**	**Boiling Point (^{o}C)**	**Solubility (µg L^{-1}) in water**	**Log K_{ow}**
BDE-28	$C_{12}H_7Br_3O$	406.89	346	70	5.7
BDE-47	$C_{12}H_6Br_4O$	485.79	366	15	6.7
BDE-66	$C_{12}H_6Br_4O$	485.79	366	18	6.7
BDE-71	$C_{12}H_6Br_4O$	485.79	402	1	6.7
BDE-75	$C_{12}H_6Br_4O$	485.79	391	1	6.7
BDE-77	$C_{12}H_6Br_4O$	485.79	434	6	7.6
BDE-85	$C_{12}H_5Br_5O$	564.69	339	6	7.37
BDE-99	$C_{12}H_5Br_5O$	564.69	391	9	7.13
BDE-100	$C_{12}H_5Br_5O$	564.69	393	40	7.24
BDE-138	$C_{12}H_4Br_6O$	643.58	399	<1	7.06
BDE-153	$C_{12}H_4Br_6O$	643.58	422	1	7.9
BDE-154	$C_{12}H_4Br_6O$	643.58	424	1	7.82
BDE-183	$C_{12}H_3Br_7O$	722.48	461	2	8.27
BDE-190	$C_{12}H_3Br_7O$	722.48	450	<1	7.5
BDE-209	$C_{12}Br_{10}O$	959.17	530	< 1	9.6

1.5 Inter-compartmental behavior of POPs

Once POPs released to the marine environment are bioavailable to fish via the food chain, as waterborne compounds, and from contaminated sediment [**76, 77**]. Of these three possible

routes, uptake of dissolved POPs from seawater to the gills is considered to be the most significant [**78**]. During the metabolism of some POP compounds, reactive intermediates are formed that may bind to macromolecules such as DNA to produce covalently bonded adducts. The metabolism of POPs is believed to be an essential factor in the development of various hepatic diseases, including neoplasia and liver tumors, and it is assumed that formation of DNA adducts is a necessary step toward the development of cancer [**79**].

The potential threat of adverse effect, organic contaminants pose has resulted in many years of monitoring their concentrations in water, sediment, and biota. Their absorption from water by fish may be regarded as a simple partition process between water and lipids within the organism. Consequently, the larger and more hydrophobic organic contaminants are expected to be more efficiently absorbed than the smaller and less hydrophobic compounds **[80, 81**]. Another important factor that significantly alters the uptake of POPs is the level of particulate organic material and dissolved organic matter (DOM) in the water. Increased levels of particulate organic material and DOM reduce the bioavailability of POPs, due to association of POPs to the organic matter. High molecular weight PAHs are more affected than the low MW PAHs, as low MW PAHs are more water-soluble compounds [**82**].

It is well known that organisms can have elevated concentrations of organic contaminants with respect to concentrations of these substances in the environment they inhabit. In this context, `organisms' can include plants, invertebrate and vertebrate animals including fish, mammals, reptiles, and birds. `Environment' includes the air or water the organisms respire, the air, water, soil, and sediment in which they dwell and may contact intimately, and the food which they consume [**83]**. Bio concentration in fish involves the uptake of contaminant by absorption from the water only (usually under laboratory conditions), can occur via the respiratory surface and/or the skin, and results in the chemical concentration in an aquatic organism being greater than that in water. The bio concentration factor (BCF) is defined as the ratio of the chemical

concentration in an organism C_B, to the total chemical concentration in the water C_{WT}, or to C_{WD}, the freely dissolved chemical concentration in water and is shown in equation (1).

$$BCF = \frac{C_B}{C_{WT}} \; or \; \frac{C_B}{C_{WD}} \qquad (1)$$

The use of C_{WD} is preferred because it only takes into account the fraction of the chemical in the water that is biologically available for uptake [**83]**. Not all the contaminant presents in water (C_{WT}), is available to uptake for dwelling organism. Some is sequestered in sorbed (absorbed or adsorbed) form in dissolved and particulate organic matter. There is thus a 'bioavailable' fraction which is often equated to the truly dissolved fraction i. e. C_{WD}. The dissolved fraction can only be defined operationally in terms of specific filter pore size such as 0.4 μm. In reality, much sorbed material passes through such filters and is only apparently dissolved [83, 350].

There is another fate of these pollutants in aquatic environment except from the discussed earlier i.e. bioconcentration in the living organism. Sediment is the dominant compartment of aquatic environment, where most of pollutants get accumulate. The surface water-sediment exchange is driven by the fugacity difference between water and surface sediment. The fugacity fraction (ff) is used to assess equilibrium status of a chemical between two interacting phases, in this case water– sediment exchange can be described as equation (2).

$$ff = \frac{C_S}{(C_S + C_W \varphi_{OC} K_{OC})} \qquad (2)$$

where C_S is chemical concentration in sediment, in the unit of ng g^{-1} dw, C_W is chemical concentration in water, in the unit of ng mL^{-1}, Organic carbon fraction (ϕ_{OC}) for each sediment sample can be calculated by assuming ($\phi_{OC} = \phi_{OM}$ x 0.55). K_{OC} (in mL g^{-1}) is the organic carbon–water partition coefficient [**84]**. The K_{OC} defined here as "organic carbon-water partition coefficient" is calculated on the basis of n-octanol-water partitioning coefficient as ($\log K_{OC} = 0.81 \log K_{OW} + 0.1$ [352]) not as (C_s/C_w).

If we define ‘‘in situ organic carbon–water partition coefficient’’ (K’oc) following equation (3).

$$K'oc = \frac{C_s}{C_w \varphi_{oc}} \quad (3)$$

And fugacity fraction can be rewritten as equation (4).

$$ff = \frac{K'_{oc}}{(K'_{oc}+K_{oc})} == \frac{1}{\left(1+\frac{K_{oc}}{K'_{oc}}\right)} \quad (4)$$

when K'_{oc} is equal to K_{oc}, ff = 0.5, indicating sediment–water equilibrium and no net exchange. When K'_{oc} is bigger than K_{oc}, ff > 0.5, which indicates a net flux from sediment to water, while when K'_{oc} is smaller than K_{oc}, ff < 0.5, indicating a net flux from water to sediment [**85, 86**].

1.6 Study area, knowledge gap and objectives

Thane creek is situated on the west coast of India, opening it mouth to Arabian sea. Thane creek gets exposed to large Mumbai Metropolitan Region with a population of 20 million, which incorporates areas of Thane, Navi Mumbai, Vasai-Virar, Bhiwandi and Panvel, spread in Mumbai's adjoining districts of Thane and Raigad and considered highly vulnerable to their industrial discharge and urban runoff. Major urban discharge channels into the creek from the west side of the creek and industrial discharge from eastern side i.e., Navi Mumbai [**226]**. Thane creek receives sewage from open drains and partially treated effluents from Colaba and secondary treated effluent from Ghatkopar and Bhandup wastewater treatment facilities (WWTFs) located in west side [**301**]. The Thane–Belapur region is one of the largest industrial regions in India also situated on the east side of the creek. In 2006, it had 1136 industries generating industrial effluent [**227]**; 639 in 1994 produced 100 tons of solid waste, 80 % of it being either acidic or alkaline, with 5 tons of waste containing halogens making it difficult to treat. The bulk of this waste, along with municipal solid waste, polluted water bodies in the vicinity. The region has chemical, textile, bulk-drug manufacturing plants, and IT parks [**228]**.

These all factor makes Thane creek a choice of legacy and emerging POPs distribution study for present thesis.

As there is scarcity of data of POPs in environmental matrices especially from the developing countries like India, it is needed to monitor these chemicals in the environment for their distribution, fate in aquatic environment and human health risk assessment studies. Although there are scattered data are available for legacy POPs in different environmental matrices, but very few for emerging POPs e.g. PAEs, BPA, PBDEs etc. The major objectives of present thesis are summarised as following:

- To monitor various POPs (legacy and emerging) in different environmental compartments viz. seawater, sediment and biota from Thane Creek, Mumbai, India.
- Optimization of analytical methods for identification and quantification of POPs and other organic compounds of concern in different marine environmental matrices, which includes hyphenated chromatographic techniques such as high performance liquid chromatography (HPLC) and gas chromatography – mass spectrometry (GC-MS) techniques.
- Data interpretation using various statistical tools and inter-comparison of data with studies from India and other part of world. Comparison of data with environmental quality guideline values recommended by national and international bodies/regulatory authorities for their ecotoxicological concerns.
- Estimation of inter-compartmental behaviour of POPs and other organic contaminants in terms of bioconcentration factor (transfer of pollutants from seawater to organisms) and fugacity fraction (seawater sediment exchange).
- Evaluation of toxicological concerns by calculating various toxicological indices such as B[a]Peq concentration of PAHs, estradiol equivalent concentration (EEQ) for estrogenic potential of EDCs. Human health risk assessment, in terms of incremental

lifetime cancer risk (ILCR), daily intake (DI) for certain POPs exposure via marine food consumption from study area.

Overall objective of the study to provide current levels of POPs and their toxicological concerns in Thane creek area, which may be helpful to policymakers to take action which may lead to their reduction in environment or minimise risk associated with them. To achieve the objectives a work plan of the study is sketched and represented in **Fig 1.7**.

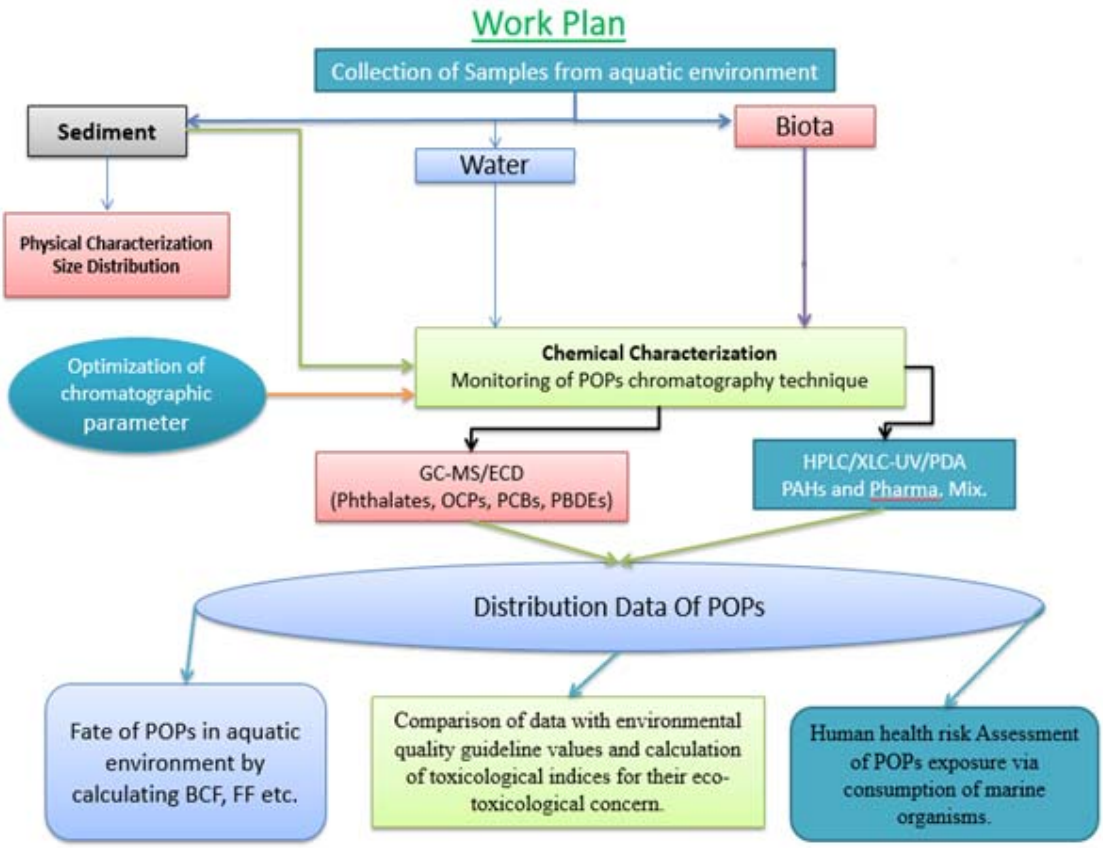


Fig 1.7 Work plan of the present thesis.

Chapter 2
LITERATURE REVIEW

2.1. Organochlorine pesticides in aquatic environment

The status quo of marine pollution by POPs is of significant paramount in India from the view point of the enormous and uncontrolled use of pesticides by farmers and health workers. Ever increasing domestic wastes accompanying with its subtropical climate with high temperature and heavy rains makes feasible to global transport of these chemicals from point sources. There are number of rivers along the east and west coast of India through which a large number of pollutants are being carried into the marine ecosystem afterword causing a great concern on the quality of the coastal marine environment. India has a long coast line of about 7000 km. Considerable number of monitoring works on POPs could be seen in the 1980s and the 1990s from the Indian estuaries and coastal environment after which the attention of environmentalists working on Indian samples have diverted to the biotic material [**87]**.

Tanabe and Tatsukawa (1980) observed the presence of all the classical organochlorines chemicals (HCHs, DDTs and PCBs) in air and water samples collected from Arabian Sea, Bay of Bengal and Indian Ocean in various cruises during 1975-1975. High concentration of ∑DDT and ∑HCH in the air and surface waters off the western coast of India in the Arabian Sea than in the Bay of Bengal were observed [**88**]. The follow up studies by the same researchers in the eastern Indian ocean, western and northern Pacific and Antarctic oceans **[89-91]** showed that the concentration of HCH in the northern hemisphere were higher than in the southern hemisphere depending largely on their extensive use in the Asian continent. In fact, FAO (1979) reported that the consumption of technical grade HCH in India during 1975- 1977 was 77,000 million tones extraordinarily high in comparison with that of other nearby countries [**92**]. This has been substantiated by the finding of high concentrations of HCH residue in the western coast of India in the closely following years [**88, 93]**.

Organochlorine pesticide residues are prime constituents of the chemical pollutants ubiquitously present in global marine environment. These chemicals are potentially hazardous to living beings as they have tendency to bioaccumulation in the lipid component of biological species and persistent. It is cognizant that a major fraction of pesticide residue advent the oceans via agricultural runoff, atmospheric transport and sewage discharge [**94]**. In India, DDT and HCH were used extensively till 1990s both for agriculture and vector control purposes. It is estimated that about 25000 MT of chlorinated pesticides were used annually in India and DDT accounted for 40% of this group [**95]**.

Sarkar (1994) has reported that conformers of HCH, dieldrin, aldrin, and PCBs occur in water of different regions of Indian ocean and surrounding seas with remarkable variation, likewise in the occurrence of DDT between the open and coastal sea waters. PCBs were found to be relatively in higher amount in the surface waters of southwest Indian ocean than the eastern Indian ocean, which is explained as the larger input of these chemicals from the African coast [**96**]. Pandit et al., (2001) reports organochlorine pesticide in sediment and fish collected from the east and west coasts of India. HCH conformer and DDT and its metabolites (DDD and DDE) are the major compounds in the samples. Although, the vast quantity of application, HCH and DDT levels in fish in India were lower than those in mild temperature countries suggesting a lower accumulation in tropical fish, which could be due to rapid volatilization and biotransformation these insecticides in tropical environment. The predominance of α- and β-HCH reflect the use of technical grade HCH in India. From the study it was attributed that high temperature in the tropics also enhances the elimination rate of chemicals in fish, as the biological half-lives of semi-volatile compounds such as DDT are less at high temperature [**97**]. A more comprehensive study was carried out by Pandit et al., (2002) coastal marine environment of Mumbai, India [**98**]. In that study, OCPs in sediment, water and biota samples from coastal marine environment of Mumbai were measured for evaluating their distribution

in different environmental compartment. HCH isomers, DDT and its metabolites (DDE) were identified in all the samples from the study area. Ratios of DDT to DDE were found high in seawater samples, which attributes to the presence of a significant source of DDT in that monitoring area. The contribution of γ-isomer was almost 55% to the total HCH, which explained as high affinity of the γ-isomer towards the sediment. The occurrence of OCPs in fish obtained from this region were found to be lower than the levels of organochlorines in fish in temperate regions.

A multimedia POPs distribution study is reported by Senthilkumar et al. (2011), which reveals magnitude of OCP concentrations increased in the order of sediments < green mussel < earthworm < frog < lizard < fish < bird egg < bats < birds' tissues. Bio magnification features of OCs were also reported in resident and migrant birds to evaluate the exposure levels of these chemicals in wintering grounds of migrant birds. Accumulation of DDTs in migratory birds during wintering in India may be of concern due to the great bio magnification potential of DDTs. Eggs of some resident species contained noticeable concentrations of OCs. Concentrations of OCs in three species of bats analyzed in this study were lower than that found in passerine birds [**369**].

Similarly, in a more recent study, Babu Rajendran et al., (2004) reported HCHs and DDT concentrations in two seawater samples from Chennai, and sediment samples from six stations in the southeast coast of India, along the coastal line of Bay of Bengal. They observed that the water samples had higher levels of HCHs than DDTs, but the sediment samples near the major cities along this coast showed a reverse trend [**99**]. Persistent organic pollutant (POP) concentrations in air across several Indian agricultural regions was also reported for 2006-2007 in literature. In which, passive samplers comprising polyurethane foam (PUF) disks were deployed on a quarterly basis at seven stations in agricultural regions, one urban site and one background site. The project was called as Global Atmospheric Passive Sampling (GAPS)

Network. analytes were detected with relatively high concentrations in air (mean for 2006 and 2007, pg m^{-3}): a- and g-hexachlorocyclohexane (HCH) (292 and 812, respectively); endosulfan I and II (2770 and 902, respectively); p,p'-DDE and p,p'-DDT (247 and 931, respectively) [**368**]. Significantly high levels of food contamination with HCH, DDT, aldrin, and dieldrin were reported throughout India, for 1992. Dairy products and livestock meat were confounding the prime sources of human dietary exposure to these chemicals. Concentrations of these organochlorine compounds in a few dairy products were above the maximum residue limits (MRLs) set forth by the FAO/WHO as well as the Ministry of Health of the Indian government [**370**].

A recent study by Lohmann et al., (2012), using a passive sampler they enabled to detect DDT and its transformation products across the tropical Atlantic, indicating net deposition. They also observed, there were clear differences between the southern and northern hemisphere apparent in terms of atmospheric concentrations. Monitoring data revealed moving southern to the northern hemisphere air, concentrations of organochlorine pesticides increased several-fold. It was also reported in their study, for large swaths of the tropical Atlantic Ocean, organochlorine pesticide dissolved concentrations varied much longitudinally, probably due to efficient mixing by ocean currents. In selected samples, dissolved concentrations reflected the influence of river plumes and major ocean currents far away from the continents [**100**].

A current study from Babitonga Bay Brazil, which has been under pressure from anthropogenic activities coexisting with a natural area of Atlantic rainforest and mangrove systems reports the concentration of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) to evaluate the contamination status and the determine possible pollution sources in the estuary **[101]**. Researcher have found the ΣDDT (sum of DDT, DDE and DDD concentrations) was the predominant OCP group. They also report exceptionally high concentration of p,p′-DDT near São Francisco harbour exceeded

SQG limits indicating highly toxic conditions in the area, attributed to a recent contamination from some local input. Their results suggest strongly anthropogenic impact in specific sites of this estuary **[101]**. Studies were also reports OPCs and PCBs in wildlife form India, such as concentrations of OC pesticides and PCBs in Irrawaddy dolphins, which are lower than the concentrations reported for coastal and riverine dolphins collected in Asia [**364, 371].** Concentrations of PCBs (including non-ortho coplanar congeners), DDTs, HCHs, HCB, aldrin, dieldrin, heptachlor, heptachlor epoxide and chlordane were reported in river dolphins from the Ganges, India. Residue levels of DDTs were the highest followed by PCBs and HCHs. Noticeable amounts of toxic non-ortho coplanar PCBs were also detected in the blubber **[367].** Persistent organochlorines such as DDT and its metabolites, HCH isomers, chlordane compounds (CHLs), hexachlorobenzene (HCB), and polychlorinated biphenyls (PCBs) were reported in whole-body homogenates of resident and migratory birds collected from South India. Organochlorine contamination pattern in birds, was found to be varied depending on their migratory behavior. Resident birds contained relatively greater concentrations of HCHs (14–8,800 ng g^{-1} wet wt) than DDTs and PCBs concentrations **[366].**

Numerous methods have been published over the past 30 years related to specific analytical techniques for the determination of OCPs in food and environmental matrices. Laboratory standard operating procedures (SOPs) are available from the National Environmental Methods Index in the USA and the Japan Environment Agency, the National Oceanographic and Atmospheric Administration in the USA and the US Food and Drug Administration [**102, 103]**. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance was reviewed by Muir and Sverko (2006) **[104]**. That review includes sampling, sample processing, preparation and instrumental aspect of OCPs and PCBs qualification in details. Useful information may also be available from the international committee for exploration of the sea **[105]**. Access to modern capillary gas chromatography (GC) equipment

with either electron capture or low-resolution mass spectrometry (MS) detection to separate and quantify OCP/PCBs is essential. However, screening of samples, especially in areas of known use of OCPs or PCBs, could be accomplished with bioanalytical methods such as specific commercially available enzyme-linked immunoabsorbent assays and thus this topic is also reviewed. New analytical techniques such two-dimensional GC (2D-GC) and "fast GC" using GC–ECD may be well-suited for broader use in routine PCB/OCP analysis in the near future given their relatively low costs and ability to provide high-resolution separations of PCB/OCPs. Procedures with low environmental impact (SPME, microscale, low solvent use, etc.) are increasingly being used and may be particularly suited to developing countries [**104**]. Fused silica open tubular capillary columns, generally coated with nonpolar or medium-polarity chemically bonded liquid phases are almost universally used for GC separation of PCBs and OCPs [**106]**.

Determination of several classes of pesticides is usually carried out by gas chromatography (GC) or high performance liquid chromatography (HPLC), depending on their polarity, volatility and the risk of decomposition at high temperature. For GC separations electron capture detector (ECD) is popular for the quantification of OCPs and PCBs residues [**107]**. Alternatively, mass spectrometric detector (MS) is a universal detector employed not only for the quantification but for the identification of the majority of pesticides in complex matrix samples. In the full-scan MS method, all ions produced in the MS could be employed in confirmation and quantitation of the target analyte, allowing high confidence in the results [**108]**. However, the detection limits of MS detectors are above from those obtainable using ECD and NPD. So far, the improvement of pre-concentration methods is absolutely necessary to reach good sensitivity using MS detectors [**107]**.

In Indian context, most of the data available are on the two classical organochlorine pesticides, DDT and HCH and some sporadic reports on PCBs, BTs, cyclodines and some other

organophosphorus pesticides in the coastal environmental samples. Nevertheless, some recent works showed the presence of other compounds like PBDEs, BTs, dioxins and related compounds in the Indian terrestrial and aquatic animals, necessitating the need for a survey of the aquatic occurrences for these and many other chemical in India [**87]**.

2.2. Polychlorinated biphenyls in aquatic environment

PCBs are ubiquitous in the environment due to their atmospheric transport in spite of the fact that they are not very volatile. PCBs cycle among sediment, water, air and soil compartment of environment. PCBs were commercially produced as complex mixtures (Aroclor), which have been used for a variety of applications in various industries. There has been considerable interest in the study of transport and fate of PCBs in the environment as to the resistance these hydrophobic compounds to chemical and biological degradation, their toxic effects to aquatic life and their status as a probable human carcinogen [**109]**.

Once these compounds reaches to the aquatic environment, PCBs readily adsorb to particles and then incorporate into sediment for their low water solubility and hydrophobic nature. Accumulation of PCBs in sediment depends on sediment type, organic matter content etc. Sediments with high organic carbon content and a smaller particle size accumulate more pollutants compared to coarser sandy sediments. The deposition of these particles in sea can lead to an accumulation of PCBs in the sediment. Sediments can serve as sorbents or concentrator for various inorganic and organic chemicals **[110].**

Sahu et al., (2009), reports the depth profile of accumulated PCBs in sediments from Thane Creek of Mumbai, India. Researcher collected the sediment core samples using a gravity corer, and analyzed for different PCB congeners using gas chromatography. They observed, the vertical distribution of PCBs in sediment reflecting the geochemical history including changes due to anthropogenic releases into the system. They also report surface sediments has indicated

downward trend for these compounds, revealing slow phase out of PCBs. Overall, the contribution of different congeners towards the total PCB content in the whole sediment core was in the order CB-28 > CB-52 > CB-44 > CB-180 > CB-101 > CB -126 > CB-18 > CB-138 > CB-153 [**111**].

Another study by Mai et al., (2005), report PCB congeners concentration of three dated sediment cores collected from the Pearl River Delta of southern China. That study concludes as, although production and use of PCBs have been banned or highly restricted in China since the early 1980s, the fluxes of total PCBs continued to increase in the Pearl River Delta sediments. Further reports, concurrent increase of PCB fluxes and gross domestic product per capita in the region from 1980 to 1997, and a decline of agricultural land use was evident at the same time. Finally, sharp rise of PCB fluxes in the recent sediments were attributed to large-scale land transform since the early 1980s as well as emissions from the PCB-containing electrical equipment [**112**].

Apart from sediment, PCBs were also found in fish samples few of the studies are incorporated here. Xia et al., (2012) reports thirty-six PCB congeners in the fishes, of which 11 congeners were dioxin-like PCBs. Authors also mentioned total PCB concentrations in the fish were at the low end of the global range, and claimed the smaller usage and shorter consumption history of PCBs in China. PCBs CB-18, CB-29, CB-52, CB-66, CB-101, CB-104, CB-138, CB-153, CB-180 and CB-194 were the major constituents found in the fish samples. Among the species investigated by the researchers, significantly higher concentrations of total PCBs were reported in croakers than in pomfrets, which may be attributed to their different feeding and living habits. Another study from eastern coastal area of China claims, spatial distribution showed that the concentrations of target contaminants in bass from south fisheries were in general much lower than those from north fisheries in China [**113**]. Based on the maximum allowable fish assumption rate, it could cause human health risk **[114]**. In India fish samples were investigated

for PCBs content and values were comparable with other part of globe [**115- 118]**. Isomer-specific concentrations of polychlorinated biphenyls (PCBs) including highly toxic non-, mono- and di-ortho coplanar congeners were also reported in resident, migratory birds and bat collected from south India. Among 11 different species, total PCB concentrations were in the range of 80–2000 ng g^{-1} (wet wt) in birds and 190–330 ng g^{-1} (wet wt) in bat were reported [**365**].

PCBs were also investigated worldwide in seawater samples [**119- 122]**. Air−water exchange gradients of selected polychlorinated biphenyl (PCB) congeners across a large section of the tropical Atlantic suggests net volatilization of PCBs to the atmosphere. Study from Atlantic Ocean reveals, dissolved concentrations of PCBs 28, 52, 101, and 118 are increasing [**100]**.

The toxicity of the individual PCB congeners depends on the chlorine substitution pattern. Coplanar PCBs, especially the non-(PCB 77, 81, 126 and 169) and mono-ortho PCBs (PCB 105, 114, 118, 123, 156, 157, 167 and 189) are the most toxic congeners. These non- and mono-ortho PCBs share a structural similarity and common toxic mechanism with the most toxic dioxin compound (i.e., 2,3,7,8-tetrachlorodibenzo-p-dioxin; 2,3,7,8-TCDD) [**123, 124]**.

The production and/or usage of PCBs have been banned or restricted since the early 1970s, and under the 2001 Stockholm Convention, PCBs are classified as persistent organic pollutants (POPs), and are subject to international restrictions on their production and use [**15]**. Although the concentrations of PCBs in aquatic environment have decreased dramatically since peaking in the 1970s [**125, 126]**, they continue to bio-accumulate in organisms and be categorized as major global contaminants**.** Jonsson et al. (2003**)** predicted that human exposure to PCBs is expected to continue for decades and more and this was attributed to the very long global environmental mean residence times of these pollutants [**127**].

2.3. Phthalates in aquatic environment

Phthalic acid esters (PAEs) or phthalates are ubiquitous in the environment due to their widespread application. Their presence has attracted considerable attention due to their potential impacts on ecosystem functioning and on public health, so their quantification has become a necessity [**128]**. A detail account on the environmental fate of phthalate esters was reviewed by Staples et al., (1997), and Net at al., (2015a) **[4, 129]**. In brief, biodegradation is considered to be the major loss mechanism of phthalates in surface water, and sediments. Primary degradation half-lives in surface and marine waters range from <1 day to 2 weeks and in soils from <1 week to several months. Longer half-lives may occur in anaerobic, oligotrophic, or cold environments. Numerous experiments have shown that the bioaccumulation of phthalate esters in the aquatic and terrestrial food chain was limited by biotransformation, which increases with increasing trophic level. That review also provides the logical first step in elucidating multimedia exposure to phthalate esters [**4]**.

Phthalic acid esters are not soluble in pure water as they are hydrophobic in nature. However, they may be soluble by interaction with fulvic and humic acids or become adsorbed onto particulate matter. Slow environmental degradation of phthalates by photolysis or hydrolysis results in half-live values in the order of years. However, several bacteria, freshwater invertebrates and fish may degrade them, completely or in part, in aerobic conditions. Distribution of phthalates in an aquatic environment was mainly affected by their physical–chemical properties and natural degradation **[130]**. Phthalates in freshwater were considered as short-term inputs, with half-lives of a few days or weeks. Therefore, sediment is the final sink of phthalates, and it may play an intermediate role in phthalate conversion from environmental media to biological organisms in an aquatic environment **[131]**.

PAEs exhibits an eight order of magnitude increase in octanol-water partition coefficients (Kow) as alkyl chain length increases from 1 to 13 carbons, and a detail on their physio-chemical properties were described elsewhere **[4]**. Numerous processes such as atmospheric

deposition, leaching, drainage, and WWTP output are the main contributor of PAEs in aquatic system. Among the large variety of phthalates, DMP, DEP, DiBP, DMEP, DnBP, BBP DEHP, and DnOP are most frequently detected in surface water. Generally, studies focused on the six PAEs listed as priority substances which are the most toxic and also the predominant PAEs in the environment [**129]**. Zheng et al., (2014) measured the concentration of 15 PAEs in water both in dissolved phase and associated with suspended solids matter (SSM). The Σ6PAEs represents 64.8 and 66.9% of the Σ15PAEs respectively in dissolved phase and associated with SSM [**132**]. Marine and coastal environment present low level of PAEs compared to freshwater [**129]**.

The phthalates have low water solubility (0.04–0.4 mg L^{-1}) and, when released into the aquatic environment, they tend to adsorb strongly on suspended particles and sediments **[133]**. Concentrations of phthalates in sediment are affected by different physiochemical parameter, such as oxygen supply in the water, temperature and pH. It has been reported that half-lives of PAEs with an anaerobic condition were 3–10 times higher compare to aerobic environment in sediment [1**34, 135]**.

Several studies on the distribution and contamination level of PAEs in sediments have been reported worldwide, which reveal that polluted sediments are adversely affecting the ecosystem [**133, 134**, **136, 137**]. Distribution of different phthalates in bed sediments of Gomti River was reported by Srivastava et al., (2010) [**138**]. Researchers have collected samples from rural, semi-urban, urban, and industrial locations throughout the course of Gomti River, reveals that the PAEs ware ubiquitous in the sediment of Gomti River. It was observed, that all obtained values are under described environmental risk limits for DBP and DEHP, respectively. Another study on phthalate in water and sediments of the Kaveri River, India was carried by Selvaraj et al., (2015) [**139**]. In that study environmental levels of phthalate esters were determined and ecotoxicological risk assessments were performed. DEHP and di-n-octyl phthalate levels in

water were found to pose little threat to sensitive organisms in the riverine ecosystem. In case of sediment, the DEHP concentration was well above the USEPA sediment guideline value.

PAEs are lipophilic chemicals with log K_{OW} can be up to 12.06, which indicate that they have strong ability to accumulate into organisms. Hydrophobicity of PAEs increase with increase of carbon chain length. Fish species were reported to accumulate large variety of PAEs both parent and metabolite products including monoalkyl phthalate esters (MPEs). PAEs and their metabolites were detected from the top of food chain (plankton, algae) to predator organisms (fish, marine mammals) [**4, 140, 141]**. Plankton and shellfish can accumulate individual PAE from not detected (nd) level to few hundreds ng/g [**142]**. For freshwater ecosystem, the concentrations of individual PAE detected in fish species were in the range of nd to few hundreds $\mu g\ g^{-1}$. Two order of magnitude lower were reported for marine fish [**140, 141, 143]**. Bioaccumulation/Bio-concentration factors (BAF or BCF) have been reported for phthalates. A BCF or BAF >1000 indicates a high capacity for the species to accumulate or concentrate the pollutant. Fishs have been reported to concentrate PAEs at significant level with total BCF of 57, 117, 45−663, 11−900, 207, and 2668−2125 mL/g/wet respectively for DMP, DEP, BBP, DEHP, DOP, and DDP [**4, 144]**.

Few phthalates like DEHP is capable of reducing sperm production, motility and velocity in goldfish following a monthly exposure. Significant decreases in 11-ketotestosterone and luteinizing hormone levels were observed following 15–30 d of exposure. Earlier study also suggest that DEHP-reduced sperm quality is due to DEHP effects of testicular and pituitary hormonal functions [**35]**. These compounds are classified as priority pollutants and endocrine disrupting compounds by the US Environmental Protection Agency (EPA) and other governmental agencies. High priority has been posed on understanding their fate in aquatic ecosystems such as coastal areas [**145, 146]**. Various extraction procedures as well as gas/liquid chromatography and mass spectrometry detection techniques are found as suitable for reliable

detection of such compounds. However, PAEs are ubiquitous in the laboratory environment including ambient air, reagents, sampling equipment, and various analytical devices, that induces difficult analysis of real samples with a low PAE background. Therefore, accurate PAE analysis in environmental matrices is a challenging task. A comprehensive review on sampling, sample extraction/pretreatment and detection for quantifying PAEs in different environmental matrices (air, water, sludge, sediment and soil) have been reviewed by Net et al., (2015) **[128]**. An overview of mass spectrometric methods used for the determination of endocrine disrupting compounds (EDCs) including phthalates in environmental samples was reviewed by Petrovic et al., (2002). Various aspects of current LC–MS and GC–MS methodology, including sample preparation, are discussed also discussed in literature [**147]**.

2.4. BPA and other EDCs in aquatic environment

BPA is used as an intermediate (binding, plasticizing, and hardening) in plastics, paints/lacquers, binding materials, and filling materials. It is also used as an additive for flame-retardants, brake fluids, and thermal papers. About 95 % of BPA produced in industry is used to make plastics, in particular polycarbonate resins (71 %) and epoxy resins (29 %) [**148]**. BPA is listed as an endocrine disrupter. It has been proven to have estrogenic activity even at concentrations below 1 μg m^{-3} [**149]**. Estrogenic compounds can have deleterious effects on living organisms because they can disrupt natural hormone balance in both men and women. The effects of exposure to BPA can be particularly harmful to fetus, infants, and young children, because of lack of feedback regulating the activity, synthesis, and elimination of hormones [**149, 150]**. The acute toxicity of BPA is relatively low. In subacute toxicity studies, a marked reduction in the rate of body weight increase was observed in treated animals [**150]**. There is limited evidence for carcinogenicity in animals; according to the international agency for research on cancer (IARC) classification, BPA belongs to group 3 (“not classifiable as to its carcinogenicity to humans”) [**151]**. Asian countries, especially South Korea, China, and Ja-

pan, account for a major share of BPA production globally. BPA is used as a monomer in the production of polycarbonate plastics and epoxy resins. Because of BPA's diverse uses in consumer products, its exposure to humans is wide spread. Several studies have reported on the exposure of humans to BPA [**359**]. BPA was also reported at a concentration in the range of several tens to several hundred so nano grams per liter in most of the rivers and some of the highest concentrations (54–1950ng/L) were found in rivers in Chennai, India [**363**]. A recent study reports Bisphenol analogues (BPs) in influents and effluents plant in India as 98.0 and 9.6 ng L^{-1} respectively, indicating 10-time removal of these contaminants but still remaining in significant amount in effluent waste water [**375**].

Alkylphenol polyethoxylates (APEs) are widely used as nonionic surfactants in a large variety of industrial and commercial applications [**152]**. These surfactants are manufactured by sequential ethylene oxide addition to a hydrophobic alkylphenol; the most common alkylphenols used for this application are 4-nonylphenol, 4-NP and 4-tert-octylphenol, 4-t-OP. Nonylphenol ethoxylates (NPEs) account for about 80–90% in the APEs big annual production [**153**].

Nonylphenol (NP) is a term used to refer to a wide group of isomeric compounds ($C_{15}H_{24}O$) consisting of a nine-carbon alkyl chain bond to a phenol ring. The NP isomers most produced and measured in the environment is 4-NP. NP is used as a formulant in pesticides, as a lubricating oil additive, as a catalyst in epoxy resins curing, at industrial laundries and, in the past, to produce nonylphenol ethoxylates (NPEs) for consumer products (e.g., surfactants, detergents, wetting agents, dispersants, defoamers, de-inkers, antistatic agents) {**154]**. NP is an estrogen agonist [**155]**. It is highly irritating and corrosive to skin and eyes, but it does not have significant skin-sensitizing potential. The acute (oral and dermal) toxicity is low. NP is highly toxic to fish, aquatic invertebrates, and aquatic plants [**156]**. A more detail review of Bisphenol

A and NP on their environmental distribution and toxicity was done recently by Careghini et al., (2015) **[157]**.

Octylphenol is one of the most potent alkylphenols with respect to endocrine disruption [**158, 159]**. Although numerous investigations have been performed in recent years on the estrogenic impact of octylphenol, e.g., on stimulation of the prolactin gene [**160]**, disruption of the rat estrous cyclicity [**161]**, and the increase of vitellogenin levels in the plasma of medaka [**162]** and brown trout [**163]**, little attention has been given to the impact of chronic exposure of octylphenol in aquatic organisms. David et al., (2009) has reported a detail reviews on alkylphenols (NP and OP) in marine environments and on their distribution monitoring strategies and detection considerations [**164**]. A HPLC base method for determination of NP and OP in water samples is described by Cruceru et al., (2012) **[165]**.

The EDCs particularly found to be of concern for the aquatic wildlife are those originated by the discharge of either treated or untreated urban runoff and industrial wastewaters. Pharmaceuticals and personal care products as well as their metabolites were also reported in domestic STPs (wastewater influent, effluent, and sludge) and in raw domestic sewage collected in open sewerage channels in residential areas in India [**372- 374**]. The identified most potent EDCs contained in these effluents are the natural and synthetic steroid estrogens, such as 17β-estradiol (E2), estrone (E1), and 17α-ethinylestradiol (EE2), although less potentnon-steroidal chemicals such as alkylphenols and bisphenol-A (BPA) are widely encountered at significant concentration levels [**166]**. A few earlier studies have associated exposure to endocrine-disrupting chemicals (EDCs) with childhood obesity. Urinary concentrations of selected EDCs were also reported higher in obese children than in non-obese children, independent to age, sex, family income, parent education, physical activity, and urinary creatinine. Urinary concentrations of several EDCs were higher in Indian children than the concentrations reported for children in the USA and China [**362**].

Chemical analysis is essential for determining the identity and concentration of individual EDCs in the environment. As additive effects of the estrogenic activity of EDCs mixtures have been proved, the total estrogenic potential of environmental samples can be quantitatively evaluated in terms of EEQ (estradiol equivalent concentration), provided that individual concentrations of most active compounds are known [**46, 167]**. The EEQ is the sum of the concentrations of individual EDCs after normalization on E2 by means of estradiol equivalency factors (EEFs). The EEF is the quotient of $EC_{50}E2/ EC_{50}$ compound and is conventionally set to 1 for E2. These factors cover a very wide range of values, from 2.7 for diethylstilbestrol (DES) to 1.1×10^{-7} for benzophenone (BP) [**167- 169**].

Natural and synthetic estrogens can induce endocrine disrupting effects in the aquatic organisms, in both male and female hormonal system [**170, 171]**, or even stimulation of feminization or hermaphroditism in fish at very low concentrations [**172- 174**]. As limitations associated with bacteria application as pollution tracers, human and animal sterols are currently being used as indicators of anthropogenic contamination in environmental compartments. Together with plant sterols, they are applied for distinguishing between sources of pollution based on their ratios [**175- 177]**. Marine organisms or terrestrial plants can synthesise these molecules; alternatively, they can be produced by human activities and reach the oceans through routes such as rivers, continental runoffs, and atmospheric deposition as well as sewage and petroleum inputs. Once, these chemicals transported by currents and deposited in sediments where they are often preserved. Sterols are typically used to distinguish aquatic and terrestrial organic matter (OM) contributions from ‘‘biogenic’’ sources and indicate faecal material present in sewage inputs to coastal areas [**178- 180]**. Several species of zooplankton, phytoplankton, and higher plants are the primary sources of “biogenic” sterols. Conversely, mammals are the primary source of ‘‘faecal’’ sterols in estuary sediments [**49, 181, 182]**. To

know fate of EDCs in sediments is critical for their environmental exposure and risk assessment [**183, 184].**

2.5. PAHs in aquatic environment

PAHs inputs to the coastal marine environment are primarily from two sources: (a) the movement of water containing dissolved and particulate constituents derived from watersheds; and (b) atmospheric deposition of both in precipitation and dry deposition from air sheds of the of the coastal ocean. PAHs have been observed to be most concentrated in estuaries and coastal environments near urban centers, where inputs from the watersheds and airsheds are most localized. The major sources of PAHs to the coastal marine environment include urban runoff, wastewater effluent, industrial outfalls, atmospheric deposition, and spills and leaks during the transport and production of fossil fuels [**185]**.

PAHs in water partition between dissolved and particulate fractions, depending upon the solubility of the individual PAHs and the availability of binding substrates such as suspended particulates. There is gradient in which offshore concentration of PAHs are lowest, followed by inshore, and lastly, the sea surface microlayer (SSM). For sample collected at the same time and in the same place, the SSM values are over a factor of 10 as larger as the bulk seawater [**186**]. As PAHs are hydrophobic nature, they rapidly tend to stick with particulate/organic matter in aquatic environments [**187, 188]**. Sediments represent the most important reservoir of PAHs in the marine environment. For that reason, sediments are handy in environmental assessment of aquatic ecosystems and can represent a useful tool for monitoring inputs of PAH in coastal ecosystem. PAHs accumulation in coastal sediments is both due to anthropogenic and natural emissions. In particular, PAHs from pyrolysis processes are more strongly associated to sediments and much more resistant to microbial degradation than PAHs of petrogenic origin [**189, 190]**. Due to the exposure time to industrial effluents, sediments are valid

for long-term studies. Sediments may contain a high level of pollutants ready to pass on to the food chain or be mobilized by anthropogenic or natural means [**191]**.

In the seawater most PAH tend to absorb to particles and get deposited to the underlying sediments [**192]**. Degradation of PAH in sediments is slow, for the higher molecular weight PAH and when sediments are anaerobic [**193, 194]**. PAHs are of concern in the aquatic environment as the lower molecular weight PAH may be toxic to aquatic organisms. Some of the higher molecular weight PAH produces carcinogenic metabolites, and PAH concentrations in sediments have been linked with liver neoplasms and other abnormalities in bottom-dwelling fish [**195**].

The high levels of PAH contamination in aquatic environment also linked with adverse biological effects for flatfishes [**196]**. Many studies have shown that PAHs are toxic to fish and other aquatic organisms [**197- 201]**. PAH toxicity is highly depends on its chemical structure, and even PAH isomers may vary from non-toxic to extremely toxic. The toxicity of PAHs is also associated enzymatic biotransformation, and organisms that have poor bio-transformation capacity (e.g., blue mussels) are less vulnerable to PAH hazards. In contrast, fish can metabolize PAHs to form reactive metabolites, which subsequently can bond covalently as adducts to cellular macromolecules such as nucleic acids and proteins. As fish do not have a highly developed DNA repair system, this may lead to many forms of lesions and adverse conditions in cells and in the organism **[55, 56, 202]**. Humans are exposed to polycyclic aromatic hydrocarbons primarily through food consumption. Once PAHs ingested, can be absorbed by the human body and may cause cancers and decreased fecundity, among other health problems [**203]**. Besides ecological consequences, seafood safety is an issue of concern in every oil spill incident. Commercial and recreational fisheries and subsistence seafood use could potentially be affected as a consequence of the fauna and flora exposure to oil. In order

to guarantee public health, restrictions or closure of seafood harvesting might be necessary [**58, 59]**.

The chromatographic separation of the PAHs is performed either by GC with mass spectrometric (MS) detection operated in selected ion monitoring (SIM) mode, or by HPLC-FLD. Besides the methods devised by the US EPA, standard procedures were also published by ISO. ISO standard 13877:1998 describes a method for the determination of PAHs in soil by HPLC, whereas the very recently published standard ISO 18287:2006 specifies a method for the determination of PAHs in soil by GC-MS [**204, 205]**.

Analytical protocols for the determination of PAHs in air and water have also been developed. GC and HPLC methods were published in 1989 in "The Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air" and revised in 1996 **[206]**. For the analysis of PAHs in water, several methods exist, even focusing on different numbers of analytes [**207]**. A more comprehensive overview of sample preparation and analytical techniques for determination of polyaromantic hydrocarbons in solid samples e.g. soil is given by Khan et al., (2005) [**208**]. Analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples using gas chromatographic (GC) is also a critically reviewed by Poster et al., (2006) [**209**]. Authors claims, in contemporary analysis of environmental samples, gas chromatography (GC), rather than liquid chromatography (LC), is often the preferred approach for separation, identification, and quantification of PAHs, largely because GC generally affords greater selectivity, resolution, and sensitivity than LC. In that article reviewers also describe modern-day GC and state-of-the-art GC techniques used for the determination of PAHs in environmental samples. GC separations of PAHs on a variety of capillary columns ware examined, and the properties and uses of selected mass spectrometric (MS) techniques ware presented.

2.6. PBDEs in aquatic environment

Polybrominated diphenyl ethers (PBDEs), a group of brominated flame retardants (BFRs) that have been most widely used in electronic equipment, textiles, and other materials [**210, 211]**. Having a history of more than three decades for their production and use, nowadays, PBDEs include aliphatic, cycloaliphatic, aromatic series and other variant. PBDEs have the largest consumption in halogen flame retardants in the world currently [**212]**. Wide use of products containing technical PBDE, substances were found to be potentially hazardous to the environment, and human beings [**213]**. Among all the environmental compartment, sediments are claimed as the significant and final sink. A large variety of lipophilic persistent organic pollutants (POPs) including PBDEs accumulate and transform in sediments [**214]**. PBDEs in sediments can pose an ecological risk to aquatic biota. Accumulated PBDEs in sediments bio-accumulate abundantly in benthic organisms and their secondary consumers through the aquatic food web [**215]**.

Owing to the hydrophobic nature of PBDEs, accumulation of PBDEs in sediment and biota of aquatic environments was regarded as a serious environmental problem around the globe. Since sediment makes up layer by layer over many years' process known as sedimentation, the different layers carry the information of pollution loading into these waters over the time. Air deposition (dry or wet) can be one of the major sources of many Persistent Bio-accumulative Toxics (PBTs) contaminants including PBDEs, although emissions from known point sources have been greatly decline. As PBDEs has widespread use, they can get into gaseous phase from various products can be a source in the air with even more significant amounts than from the point sources. Areas where air deposition is the major pathway of input, chronological assessment using sediment cores provide insight to atmospheric deposition pattern [**216, 217]**. The variations in levels of PBDEs in sediments may result from the differences of their historical discharge and accumulation among different areas. BDE-209 was dominance in sediments confirmed the fact that commercial deca-BDE mixtures accounted for most of the

brominated flame-retardant production in nearby areas. The major source of PBDEs in sediment samples was associated with the prevalent use of technical deca-BDE, and degradation of high brominated BDEs contributed to the lower brominated BDEs in sediments [**213]**.

Incineration of discarded commercial products especially electronic gazettes being one of the major sources for PBDEs, sewage and sludge runoffs also contribute to the inventory [**217, 218].** Biodegradation was considered to be a possible mechanism of eliminating PBDEs from the environment and could be effectively excluded by microorganisms under anaerobic conditions [**66, 219, 220]**. PBDEs once accumulated in sediments is of concern as they can potentially bioaccumulate, and transfer up the food web. On consumption of sea food by humans, they may act as an endocrine disruptor and can cause thyroid hormone disruption, alter neurodevelopment and interfere with reproductive systems [**221]**.

Measurement techniques for PBDEs has been reviewed by Covaci et al., (2007) **[222]**. In that review, authors have included sample pretreatment, extraction, clean-up and fractionation, injection techniques, chromatographic separation, detection methods, quality control and method validation are discussed. Mass spectrometry (MS) is almost universally applied within BFR analysis, although the type of instrumentation and the mode of ionization vary. For the PBDEs, GC-MS is routinely applied, with both low and high resolution MS instruments in use. However, seeing the degradation problems that are sometimes experienced for certain congeners, more insight was generated regarding this issue and the methodology has been further optimized. Taking BDE-209 as the most extreme example, with a log Kow~9, due to its instability, photosensitivity and its ability to bind strongly to surface, including laboratory glass ware, determination of this congener is problematic. Achieving clean blanks, and avoiding cross-contamination due to incomplete cleaned glassware are key, as is optimizing the GC condition. Protocols suitable for the determination of BDE209 also have been

developed [**223]**. Bjorklund et al. (2004) have investigated of the influence of the gas chromatographic separation system on the determination of polybrominated diphenyl ethers (PBDEs) [**224**]. Capillary columns, retention gaps and press-fit connectors, as well as different injection techniques have been evaluated with respect to yield and repeatability by the authors. Another technique that has been applied for the analysis of BDEs is comprehensive two dimensional gas chromatography (GC × GC) [**225]**.

All though legacy POPs are banned from agricultural use in India, they are still in scattered use e.g. mosquito control. As these contaminants has got longer half-life in environmental matrices (persistent) they are ubiquitously present in global environment in lower concentration after their peak use in 1970-80s in agricultural sector. As on one side there is concern of POPs in environments, one the other side there in hazard of emerging POPs as PAEs, BPA, PBDEs etc. There are challenges also in their analysis in environmental matrices as they are present in ultra-trace levels. Sophisticated analytical instrumentation such as gas chromatography mass spectrometry (GC-MS), and specialized trainings are desired for accurate and reliable monitoring of these contaminants in environment. Special precautions are required in sample processing and extraction as many laboratory glass ware can give falls peak of contaminants e.g. Phthalates, BPA. Rapidly growing population, urbanization may impose additional stress on use of emerging POPs viz. phthalates for plasticizers, BPA for polycarbonate, PBDEs as fire retardant and so on. Marine ecosystems are ultimate sink of these contaminants particularly for coastal city like Mumbai. So monitoring these contaminants in creek environments may help understanding their inter-compartmental behavior, fate, human and environmental toxicity.

Chapter 3

MATERIALS AND METHODS

3.1 Study Area

Mumbai (commercial capital of India) is a heavily populated industrial city on the west coast of India. The 26-km-long Thane Creek, which covers a ground area of 1690 ha, separates the island city from the mainland on the east [226]. In extreme north, the creek also receives outlet from the Ulhas River. Extensive development provides a large amount of flux into the Thane creek. Fine particulate flux makes up mud flats. The Mumbai harbor in the west and New Nava Sheva port in the east, which handle more than 30 million tons of goods per year, in addition contribute to pollutants in the creek by way of leakage, spill, and corrosion. Being landlocked, with very few fresh water inlets and a large amount of sewage and industrial effluent discharges, the creek is relatively stagnant. In addition, atmospheric fallout from chimneys, stack, and vehicle exhaust also contributes to the pollutants' load in the creek. Hence, the Thane creek is a receptor for various pollutants from a number of sources, and a good fraction of these are immobilized into sediments. The Thane creek is also exposed to diurnal tides as well as seasonal dilution that make sorption characteristics of sediment very complex [**229]**. During the south-west monsoon period there is also an influx of approximately 1.0 –1.5 × 10^6 m^3 of fresh water from the river per tidal cycle, affecting the circulation pattern considerably. The Ulhas water flows in to Thane Creek only when there is high flood during the monsoon months (June–September). Average rainfall in this area is about 250 cm $year^{-1}$ [**226]**. Map of study area and key pollution contributor to the Thane Creek are shown in **Fig 3.1.1**.

The creek supported diverse life forms around 1960 -1980 and earlier. A few decades back heavy industrialization and consequent urbanization have occurred along both the banks of the creek. The growing pollution in the creek has resulted in significantly low dissolved oxygen,

high nutrients, siltation, declined fishery and biodiversity especially in the upstream part of the creek where pollution is higher. In the lower stretches of the creek the pollutants get diluted hence it supports relatively higher diversity. The creek supports good diversity of mangroves and birds including Flamingos [**230]**.

Fig 3.1.1 Map showing the study area (Thane creek, enclosed with red line) and surrounding major anthropogenic activities (dumping yard, power stations, industrial area, etc.).

Thane Creek in also Mumbai's one of the three sites of the conservation and sustainable management of existing and potential Coastal and Marine Protected Areas (CMPA) Project in Maharashtra. The Government of Maharashtra (Revenue & Forest Department) issued a notification, declaring the northern part of Thane Creek as a Wildlife Sanctuary under Sec. 18 of the Wildlife Protection Act, 1972. This sanctuary, named "Thane Creek Flamingo Sanctuary" is Maharashtra's second marine sanctuary, after Malvan. It is spread over an area

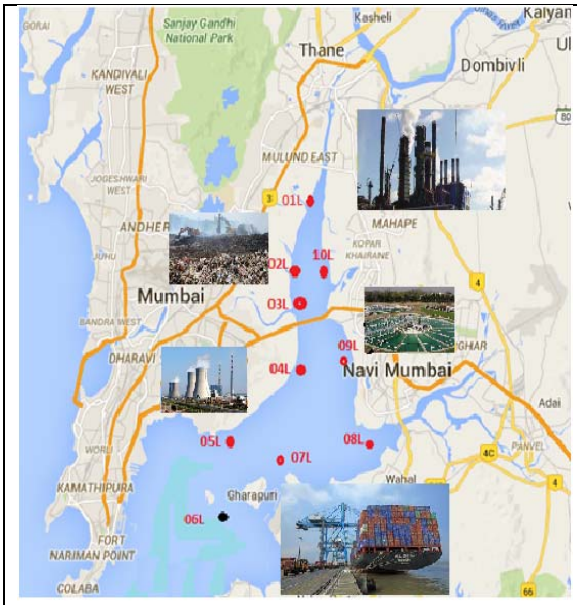
of 1690 hectares, which includes 896 ha of mangroves and 794 ha of adjacent water body. The 'Mumbai Mangrove Conservation Unit' under the Mangrove Cell will be responsible for the management of the sanctuary [**231]**.

Fig 3.1.2 Biodiversity (polychaetes, fish types, zooplankton and gastropods) across Thane creek (adapted from Athalye, 2013 [**230**]).

Besides supporting a large congregation of flamingos, the area is a refuge for several resident and migratory birds. In all, about 200 species of birds have been reported from this area, which include the globally threatened species like the Greater Spotted Eagle (IUCN Category-Vulnerable) and others like Osprey (listed in Schedule I of Wildlife Protection Act). Other bird species include the Pied Avocet, Western Reef Heron, Black-headed Ibis, Common Redshank, Marsh Sandpiper, Common Greenshank, Curlew Sandpiper, Brown-headed Gull, Whiskered Tern, Gull-billed Tern, Caspian Tern, Little Tern, White Bellied Sea Eagle, Eurasian Marsh Harrier etc. 'Birdlife International' has already declared Thane Creek as an Important Bird Area (IBA) [**231]**. Pictorial representation of biodiversity (polychaetes, fish types, zooplankton and gastropods) across Thane creek is shown in **Fig 3.1.2.**

3.2 Sampling

Sediment, seawater and biota samples were collected from the study area, i.e. Thane creek Mumbai. The surface sediments (top 0–5 cm) were collected in May 2014 (summer) using a Van Veen grab sampler made of stainless steel from 10 different locations across Thane creek in triplicates (n=3). Five core sediment samples were also collected using a gravity corer of length 1 meter approximately in triplicates (**Fig 3.2.1**). Precautions were taken during sediment sampling to ensure minimum disturbance of the sediment–water interface, and cross-contamination of the samples. Sediment samples were collected in sealed polythene bags as well as in glass containers. The collected sediment samples were pooled, homogenized, freeze-dried and sieved through a 400 mesh size sieve. Subsequently, samples were kept at -20 ^{O}C in refrigerator prior to analysis for POPs. Total 30 seawater samples were collected from Thane creek at 10 locations i.e. 3 sample from each location. Seawater samples were also collected during the same period in clean plastic and glass containers.

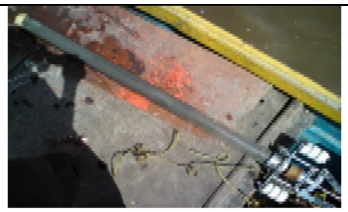


Sampling locations of surface sediment

Sampling locations of core sediment

Fig 3.2.1 Map showing the sediment collection locations (01L to 10L) for surface and core samples (C1 to C5) respectively across Thane creek.

van veen sampler



Sediment core sampler

Seawater Container

Bombay Duck Fish

Lizard Fish

Crab

Fig 3.2.2 Sediment sampling tools and marine environmental samples collected across Thane creek.

Then seawater samples were filtered through 0.45 μm pore size filter paper to remove the suspended solids. Consumables marine species viz. Lizard Fish, Bombay Duck and crab samples were also collected from study area with help of local fishermen. The collected marine organism samples were freeze dried, pooled, ground and homogenized and kept at −20◦C in refrigerator prior to analysis for organic contaminants. **Fig 3.2.2** shows the sediment sampling tools and samples collected from the study area. All the samples were collected in same sampling period i.e. May 2014.

3.3 Sample preparation and analysis of OCPs/PCBs

3.3.1 Sample extraction

Freeze dried sediment samples (2 ± 0.02 g) were weighed into amber glass extraction vessels. 30 mL of HPLC grade n-Hexane was added, and extracted in an ultra-sonication bath (PCI Analytics Pvt. Ltd) for 40 minute and then filtered. The extracts were treated with concentrated 1 mL electronic grade H_2SO_4 to remove interfering organic matter. The extracts were then washed with de-ionized water three times to remove traces of acid. After H_2SO_4 treatment, extracts were treated with elemental Hg to remove sulfur compounds [**111]**. The extracts were collected in a 250 mL round-bottomed flask and rotary evaporated to 1 mL. Extraction procedure is also summarized in **Fig. 3.3.1**. For both OCPs and PCBs analysis same extraction procedure has been adopted.

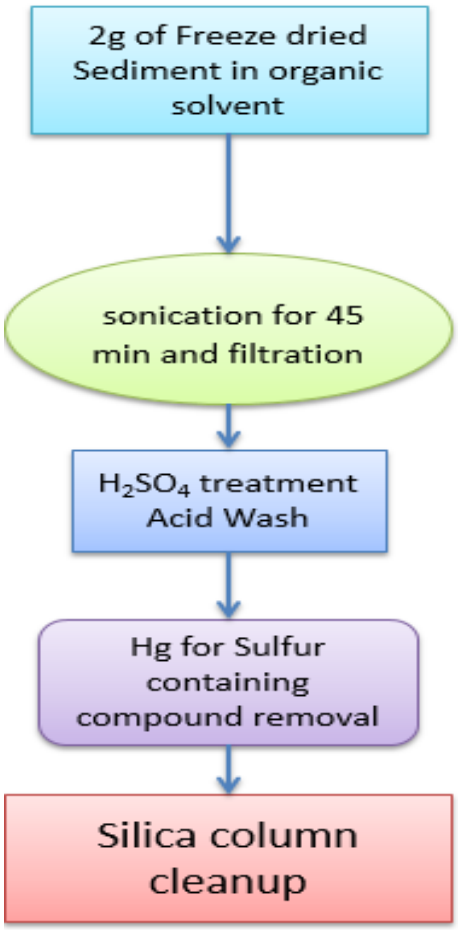


Fig 3.3.1 Stepwise extraction procedure for DDT compounds, HCH isomers and PCBs congeners from sediment.

Liquid–liquid extraction technique was employed to seawater samples for the determination of OCPs/PCBs levels. In general, the EPA protocols, with certain modifications, were used for the analysis [**232]**. Around 500 mL of the water sample was filtered using a 0.45-µm Whatman glass fiber paper filter, treated with 6 g of sodium chloride and extracted thrice with 50 ml of dichloromethane (DCM). The combined extracts were filtered and concentrated in a vacuum rotary evaporator. The solution obtained was filtered through a pinch of sodium sulfate to remove moisture and evaporated to dryness, finally make up with hexane, up to 5 ml as DCM is highly volatile for GC injection.

For PCBs analysis, the fish and crab samples were homogenized and saponified by 1 M KOH in ethanol for 24 h at room temperature. The saponified solution was transferred to hexane. After treatment with concentrated sulfuric acid, the samples were rinsed with water and dehydrated with anhydrous sodium sulfate. More detail on the method is described elsewhere [**233]**. For the determination of OCPs, 10 g homogenized biota samples were extracted in ultrasonic bath with 75 mL dichloromethane for 45 min, twice. An aliquot of the extract obtained by concentrating the extract was transferred to 150 ml hexane. After treatment with concentrated sulfuric acid, the samples were rinsed with water and dehydrated with anhydrous sodium sulfate.

3.3.2 Cleanup of sample extracts

As sediment matrices are complex in nature, it was necessary to do a cleanup to remove interfering substances from sediment extracts in order to improve compound separation in chromatography. The silica gel and alumina were heated overnight in an oven at 130 ^{o}C (>18 h) and cooled to room temperature. A 5-mL graduated glass pipette is packed in order with sorbent phase (alumina, alumina: silica (3:1), alumina: silica (1: 1), alumina: silica (1: 3) 1 g of each), all flanked top and bottom by a bed of glass wool baked at 500 ^{o}C for 3 h. The columns were conditioned with methanol and the sediment extracts were quantitatively transferred to the columns using a Pasteur pipette. The samples were then eluted with 20-mL of hexane and the eluent blown down to 0.1 mL under a gentle stream of ultra-pure N_2 [**234**]. For OCP and PCB analysis, biota samples extracts were concentrated, subjected to cleanup by silica gel (2 g) column prior to above mentioned method for cleanup of sediment.

3.3.3 Instrumental Analysis

Quantitative analysis of OCPs and PCBs was carried out using gas chromatography (GC The GC system (GC-17A, Shimadzu, Japan) was equipped with DB-5 capillary column (0.25 mm id × 60m in length, 0.5 µm film thickness, J&W Scientific) and an Electron Capture Detector

(ECD). Analytical parameters used in GC system are as; injector temperature 250 ^{o}C, injection volume was 1 µL, the column oven temperature was maintained at 55 ^{o}C for 0.5 min, then ramped at 35 ^{o}C min^{-1} to 230 ^{o}C, held for 10 min, 5 ^{o}C min^{-1} to 280 ^{o}C, the final temperature (280 ^{o}C) was then held for 13 min in order to ensure full elution of the sample from the GC column,), ECD was kept at 300 ^{o}C, and nitrogen was used as carrier gas. All the reported OCPs and PCBs were separated and eluted in a single run under described parameters. A chromatogram of sample and standard mixture is also represented in Fig 3.3.3. Limit of detection, percentage recovery and retention time (t_R (min)) for OCPs and PCBs congeners were shown in Table 3.1. LOQ values were considered two times of LODs i.e. LOQ ≥ 2 LOD

Fig 3.3.2 Gas Chromatography- Mass Spectrometry (GC-MS) system used for identification of contaminants in marine environmental samples.

Peak identification for 20% of the total sediments sample was also confirmed using mass spectrometry technique i.e. GC-MS (QP2010 ultra, Shimadzu, Japan). The GC–MS system equipped with a Multi-Purpose auto-sampler (MPS-GERSTEL, Germany) auto sampler and a

Restek (Columbia, MD) Rxi®-5Sil MS column (60 m, 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas, with a column flow rate of 1mL min^{-1}. All analyses incorporated split-less injection and electron impact ionization. The interface temperature between the GC and the MS was maintained at 280 ºC. The oven temperature programing was similar to as discussed for GC. The scan method was used for MS detection, m/z value ranging from 35 to 500. Peak identifications were carried out using NIST mass library for both OCPs and PCBs. Image of chromatographic system used in analysis is presented in **Fig 3.3.2**.

Table 3.1 Limit of detection, % recovery and retention time (t_R (min)) for selected OCPs and PCB congeners using GC-ECD.

Compound	LOD (pg g^{-1}), % recovery, t_R (min)	Compound	LOD (pg g^{-1}), % recovery, t_R (min)
α-HCH	500, 82, 18.23	CB-101	10, 88, 27.63
β-HCH	550, 85, 18.77	CB-126	30, 87, 37.39
γ-HCH	400, 90, 19.26	CB-138	20, 85, 34.81
DDT	100, 92, 34.32	CB-153	10, 84, 32.66
DDD	250, 83, 31.65	CB-169	50, 83, 41.27
DDE	300, 87, 29.09	CB-180	65, 91, 39.27
CB-52	65 , 90, 23.39	CB-194	125, 92, 46.34
CB-77	85, 87, 29.90		

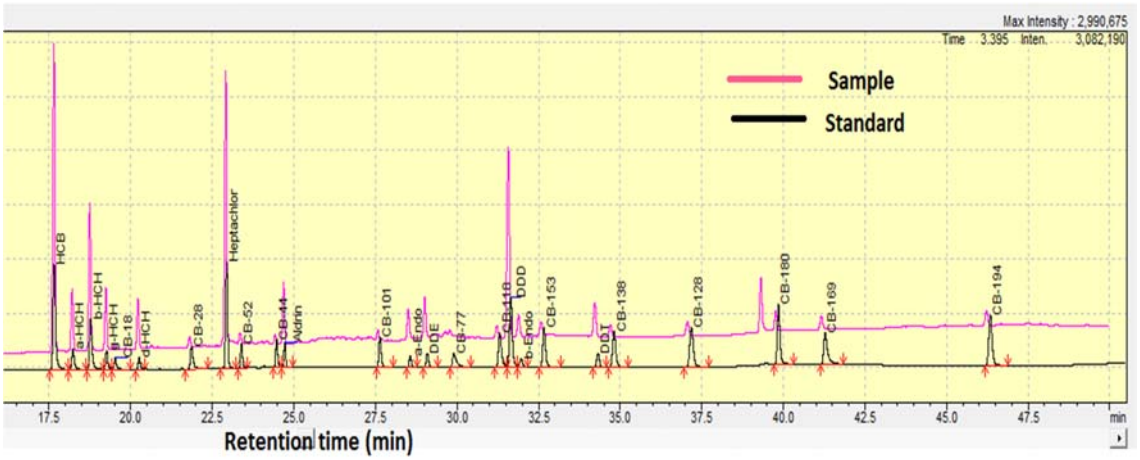


Fig 3.3.3 Sample and standard chromatogram for DDT compounds, HCH isomers and select PCBs congeners mixture for surface sediment sample.

3.3.4 Quality control and quality assurance

Standards for α-HCH, β-HCH, γ-HCH, DDT, DDE, DDD, CB18(2,2',5-trichlorobiphenyl), CB28 (2,4,4'-trichlorobiphenyl), CB 44 (2,2',3,5'-tetrachlorobiphenyl), CB 77 (3,3',4,4'-tetrachlorobiphenyles), CB 52 (2,4',5,5'-tetrachlorobi-phenyl), CB 101 (2,2',4,5',5-pentachlorobiphenyl), CB 138 (2,2',3,4,4',5-hexachlorobiphenyl), CB 153 (2,2',4,4',5,5'-hexachlorobiphenyl), CB 169 (3,3',4,4',5,5'-hexachlorobiphenyl), CB 180 (2,2', 3',4,4',5,5'-heptachlorobiphenyl), CB 194 (2,2',3,3',4,4',5,5'-octachlorobiphenyl) were purchased from Accu Standards, USA. Standards were prepared in the range of 20 μg L^{-1}. For identification, the retention data was obtained by analyzing the individual standard. Good linearity was found for the range of 10–100 μg L^{-1} for both OCPs and PCBs. Procedural blanks were also analyzed to check background contamination.

Few samples of seawater, sediment and biota were spiked with the mixture of external standard containing OCPs and PCBs, to quantify how much of each could be recovered from the matrix. The recoveries for the OCPs and PCBs were in the range of 82–96%. The quality assurance of the measurements polychlorinated biphenyl in sediment samples was ensured by analysis of standard reference material IAEA-408 Marine sediment. The quality assurance of measurements of organochlorine pesticides in sediment samples was assessed through analysis of the Standard Reference Material 'IAEA-417' Marine sediment sample. IAEA-435 Tuna homogenate was used for quality assurance of organic contaminants (OCPs/PCBs) in biota samples. Certified and measured values of selected POPs in marine sediment (IAEA-417) and Tuna homogenate (IAEA-435) are represented in Table 3.2 and Table 3.3 respectively. The accuracy of the measurement was within ±5% of certified values for both reference materials. For seawater samples, recovery was evaluated by extracting spiked water samples at a 10 μg L^{-1} concentration.

Table 3.2 Certified and measured values of selected POPs in IAEA-417 marine sediment.

Compound	Certified value (ng g^{-1})	Measured Value (ng g^{-1})	Compound	Certified Values(ng g^{-1})	Measured value (ng g^{-1})
α-HCH	-	-	CB-101	42.0	40.8
β-HCH	-	-	CB-126	-	-
γ-HCH	0.54	0.52	CB-138	45.0	43.9
DDT	19.0	17.9	CB-153	39.0	
DDD	21.0	20.55	CB-169	-	-
DDE	14.0	13.65	CB-180	16.0	16.2
CB-52	17.0	17.1	CB-194	2.7	2.6
CB-77	-	-			

Table 3.3 Certified and measured values of selected POPs in IAEA-435 Tuna homogenate.

Compound	Certified value	Measured value	Compound	Certified Values	Measured value
α-HCH	0.76	0.77	CB-101	23	22.6
β-HCH	1.3	1.2	CB-126	-	-
γ-HCH	1.1	0.98	CB-138	70	70.2
DDT	18	17.2	CB-153	81	80.5
DDD	12	12.1	CB-169	-	-
DDE	91	90.5	CB-180	32	32.2
CB-52	4.4	4.2	CB-194	-	-
CB-77	-	-			

3.4 Sample preparation and analysis of phthalates and other EDCs

3.4.1 Extraction of PAEs from marine sample

For analysis of phthalates, dry sediments (2 g, each) were ultrasonically extracted with 30 mL dichloromethane (DCM)–Acetone (2:1) for 1 h. Sample was then centrifuged and extract was separated. Further, extraction of residue sample was performed with DCM as extracting solvent according to the method developed by Tronczynski et al. (2005) [**235**]. Sulfur containing compounds in the sediment extracts was removed by addition of mercury (Hg) to the extracts. The sediment extracts were concentrated, solvent-exchanged to hexane, and were purified and fractioned on a silica column to eliminate organic interferences. High purity nitrogen was employed as the purge gas. Extracts were then combined and subjected to a purification step

described elsewhere to remove the interferences **[128]**. Each sediment sample was concentrated to 250 µL in hexane in a vial for analysis.

Seawater samples were filtered through 0.45 µm pore size filter paper, 1 L of sample was transferred to a separating glass funnel. For the isolation of phthalates from seawater liquid–liquid extraction method has been used. Dichloromethane (3 × 50 mL) was employed as extracting solvent [**236]**. The combined extracts of seawater were passed through anhydrous sodium sulfate column to remove any water as impurity. Further extracts were concentrated to 1 mL under a gentle stream of nitrogen. Freeze dried biota samples (10 g) were powdered and introduced into centrifuge tubes where they were treated with 6M NaOH (10 mL) at 30 ◦C for 18 h, in the absence of light, and under stirring condition. Afterword, the alkaline phase was extracted with n-hexane (3 × 15 ml) in an ultrasonic bath. Further the extracts of biota samples were concentrated to 0.5 mL using rotary evaporator (Buchi, Rotavapor® R-300). Clean-up was done on a multilayer chromatographic column with 8 g deactivated alumina (5% water, and at top) and 8 g deactivated silica gel (5% water, at bottom). After adding the extract (0.5 mL) at the top of multilayer column, three fractions were collected i.e. 20 mL of n-hexane, 20 mL of n-hexane–methylene chloride (90:10) and 40 mL of n -hexane–methylene chloride (80:20). Fractions eluted with 40 mL of n-hexane–methylene chloride (80:20) was used for PAEs analysis in present study [**237**].

3.4.2 Extraction procedure for other EDCs

The extraction procedure for sterols and BPA from sediment was as follows: 2 g of the freeze dried sediment sample was extracted with 40 mL of methanol in the ultrasonic bath (PCI India) for 30 min. Sample was then centrifuged for 10 min at 4000 rpm and extract was separated. Extraction was repeated two more times. The resulting extract was evaporated to the volume of 1 mL and transferred onto silica gel (3 g, on bottom) /anhydrous sodium sulphate (1 g, on

top) clean up cartridge plugged with glass wool at top and bottom. In clean up cartridge elution of analyte was performed using 10 mL of methanol. Final extracts were filtered through 0.45 µm PVDF filter paper, volume is reduced to 1 mL using a rotatory evaporator and analyzed. A sample extract cleanup method has been used similarly as describe elsewhere [**238]**.

3.4.3 Instrumental Analysis

Phthalates compounds in marine samples extract were analyzed with gas chromatograph (Shimadzu, GC-17A) equipped with a RXi-5SIL MS (length 60m, Dia 0.25mm, thickness 0.25 µm) phase fused-silica capillary column ((low-polarity phase; Crossbond 1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane) and a Shimadzu, quadrupole mass detector model GCMS-QP2010 ultra. The mass spectrometer was operated in electron ionization (70 eV) and selected ion monitoring (SIM) mode. Mass used for quantification was 149 (m/z) for most of the phthalates and different combination of m/z ranging from 41 to 293 were used for qualifier mass in SIM mod. GC oven temperature for PAEs started at 100°C (1.5 min.) and ramped to 280 °C at 25 °C min^{-1} (hold time 6 min.), again ramped to 320 °C at 10 °C min^{-1} and maintained for 15 min. Injection of 1 µL sample was performed using Shimadzu auto sampler (AOC-20i). The instrument was equipped with a split/splittless injector with glass wool injection port liner; split mod was used at a split ratio of 10. Injector port temperature was set to 270^0C. Total flow 27 mL min^{-1} was used during analysis. The carrier gas was helium and a flow rate 1.0 mL min^{-1} (with linear velocity of 26.3 cm s^{-1}) through column was maintained during analysis.

Fig 3.4.1 UHPLC setup for analysis of BPA and sterols.

Analysis of BPA and sterols were carried out using ultra high performance liquid chromatography (Jasco X-LC). It consists of binary pump (Jasco X-LC 3185PU) and auto sampler (Jasco X-LC 3159AS), multi wavelength detector (Jasco MD-2015plus). Jasco LC-NETII/ADC was used for data processing and ChormNAV as acquisition software (**Fig 3.4.1**). Chromatography system was operated in gradient mode with two solvents viz. acetonitrile and 1% phosphoric acid (H_3PO_4). Their composition varied in gradient mode from 10: 90 (V/V) to 90:10 (V/V) while the flow rate was maintained constant during elution i.e. 0.2 mL min^{-1}. X-PressPak V-C18 column (2.0 mm i.d., 50 mm L, 2 µm) was used for separation of target compounds. The total run time for analysis was 12 minute and injection volume was 5µL. Phthalates free polyethylene bags were used for sample collection and storage. These polyethylene bags may have BPA as plasticizer, lab and field blank polythene bags were analyzed for BPA leaching to sediment and concentration were below detection limits.

3.4.4 Quality control and quality assurance

Steroid and mixed pharmaceuticals standard each 200 µg L^{-1} (Acetonitril) was purchased from RESTEK. EPA Method 8061A Phthalate Esters Mixture 1000 µg mL^{-1}, Hexane/Acetone (80:20), was also purchased from RESTEK. Steroid standard contents of Bisphenol A, 17-β-Estradiol, Estrone, 17-α-Ethynylestradiol, 4-para-Nonylphenol, 4-tert-Octylphenol. Phthalate esters mixture consist of 15 EPA priority phthalates namely Benzyl butyl phthalate [BBP], Bis(2-n-butoxyethyl) phthalate [DBEP], Bis(2-ethoxyethyl) phthalate [DEEP], Bis(2-ethylhexyl) phthalate [DEHP], Bis(2-methoxyethyl) phthalate [DMEP], Bis(4-methyl-2-pentyl) phthalate [BMPP], Di-n-butylphthalate [DBP], Diethylphthalate [DEP], Di-n-hexyl phthalate [DNHP], Dimethylphthalate [DMP], Di-nonyl phthalate [DNP], Di-n-octyl phthalate [DNOP], Dipentylphthalate [DNPP], dicyclohexyl phthalate [DCP] and diisobutyl phthalate [DIBP]. Different dilutions standards have been prepared for both groups of chemicals for calibration curve which is used for quantification. Various steps have been taken for quality assurance of PAEs analysis. All laboratory glassware utilized in this study were soaked in a K_2CrO_7 / H_2SO_4 mixture for 12 h before they were washed ultrapure water, and were baked at 450 °C for 5 h before use, to avoid PAE contamination. Also all the glass wares were rinse with different organic solvents acetone, dichloromethane, and n-hexane. All the solvents used in extraction, purification and dilution were also analyzed for phthalate contamination levels. To abjure phthalates contamination of samples in the experimental process (Sampling, extraction, purification etc.), wherever possible all plastic containers were avoided. Field blank, instrument blank, extraction solvent, and silica gel used for cleanup, and laboratory glassware were analyzed for background contribution. Different approach for minimizing system contamination of PAEs has been adopted described elsewhere [**242, 243, 376]**. Blank levels of PAEs were subtracted from measured concentration of sediment for reporting and calculation of estradiol equivalent concentrations in this study.

The detection limits (LOD) were estimated as 3 σ (three times the background noise) (IUPAC criterion) [**244]**. LOD values were estimated in single ion monitoring (SIM) mode for phthalates as they are analyzed in GC-MS system, while for others using PDA detector (maximum absorbance). The procedure was checked for recovery efficiencies by analyzing spiked samples, the average recoveries ranged from 71% to 94% with a precision of 1.9 - 7.8% (20 ng g^{-1}), spiking level and limit of detection vary between 0.03 and 0.1 ng g^{-1} for analysed EDCs in sediment matrix (**Table 3.4**). The analytical procedure was also checked for recovery by analyzing phthalate spiked seawater and biota samples, the mean recoveries of chemicals varied between 70% and 95% for with a precision of 2.9 - 7.4%. As this study does not involve humans or experimental animals, no institutional or national guidelines were required for the protection of human subjects and animal welfare.

Table 3.4 Limits of detection (ng g^{-1}) and mean recovery (for 20 ng g^{-1}, spiking level) for EDCs in sediment (dry weight).

Compounds	LOD (ng g^{-1}), Mean recovery (%)	Compounds	LOD (ng g^{-1}), Mean recovery (%)
Bisphenol A	0.10, 75 %	Benzyl butyl phthalate	0.05, 84.5%
17-β-Estradiol	0.20, 71 %	Bis(2-n-butoxyethyl) phthalate	0.10, 79.1%
Estrone	1.00, 78%	Bis(2-ethoxyethyl) phthalate	0.10, 83.5%
17-α-Ethynylestradiol	0.20, 71%	Bis(2-ethylhexyl) phthalate	0.05, 87.4%
4-para-Nonylphenol	0.50, 85%	Bis(2-methoxyethyl) phthalate	0.10, 77.1%
4-tert-Octylphenol	0.10, 84%	Bis(4-methyl-2-pentyl) phthalate	0.10, 84.8%
Di-nonyl phthalate	0.10, 90.6%	Di-n-butylphthalate	0.05, 90.5%
Di-n-octyl phthalate	0.10, 79.2%	Diethylphthalate	0.10, 78.5%
Dipentyl phthalate	0.05, 79.1%	Di-n-hexyl phthalate	0.05, 92.5%
Dicyclohexyl phthalate	0.05, 94%	Dimethylphthalate	0.01, 91.4%
Diisobutyl phthalate	0.05, 87.5%		

3.5 Analysis of PAHs in marine environmental samples

3.5.1 Sample extraction

The procedure used for extraction of PAHs from sediment samples was as follows: 3 g of the freeze dried, homogenized sediment sample was extracted with 80 mL of mixture of hexane and acetone (1: 1, v/v) in the ultrasonic bath (PCI Analytics Pvt. Ltd., India) for 30 min. Extraction was repeated for three more time. Samples were then centrifuged for 10 min at 4000

rpm and extracts were separated. About 1 g activated copper was added for desulphurization. The resulting extract were filtered and evaporated to the volume of 1 ml and transferred onto alumina (2 g, at bottom) / silica gel (2 g, at middle) / anhydrous sodium sulphate (1 g, on top) chromatographic column for clean-up. Analyte were eluted in 25 ml n-hexane from clean-up column. All extracts were filtered through 0.45 μm PVDF filter, and volume was reduced to 1 mL using rotatory evaporator and analyzed. A sample extract cleanup method has been adopted in this study similarly as describe elsewhere [**245]**.

For seawater samples liquid-liquid extraction methodology has been applied, in this procedure 1 L of seawater samples were extracted with 100 mL DCM in a separatory funnel with agitation followed by a 1 h setting time on separatory funnel shaker (MRC LAB, VD-12-2S). Extraction was repeated thrice, followed by DCM collection and rotatory evaporation to 1 mL (Rotavapor® R-300; Buchi). Fish and crab samples were also extracted for PAHs analysis. 5 g of freeze dried, edible part of organisms was extracted ultrasonically using 200 mL of DCM / hexane (1: 1, v/v) mixed solvent. Than extracts were filtered and evaporated up to 1 mL of extract remains. The 1 mL extracts were purified using gel permeation chromatography (GPC) (with 1:1 hexane/DCM as the mobile phase) and silica gel (4 g) column chromatography. Gel permeation chromatography is a size-exclusion clean-up procedure that readily separates high molecular weight interferents from sample extracts. The procedure uses organic solvents and a porous hydrophobic gel (primarily a cross-linked divinylbenzene-styrene copolymer) that readily separates large molecular weight molecules from the smaller molecular weight analytes of interest. The extract was rotary evaporated to 2 mL, then solvent-exchanged into isooctane and was reduced to 1 mL under nitrogen evaporation prior to analysis. The procedure of PAHs extractions were same as recommended by National Laboratory for Environmental Testing (NLET), Environment Canada [**86, 246]**.

3.5.2 Instrumental Analysis

Identification and quantification of PAHs were carried out using gas chromatography - mass spectrometry (GC-MS, Shimadzu QP 2010 ultra) technique in sediment, seawater and marine organism samples. The GC–MS system equipped with a Multi-Purpose Sampler (MPS-GERSTEL) auto sampler and a Restek (Columbia, MD) Rxi®-5Sil MS column (60 m, 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas, with a column flow rate of 1mL min^{-1}. The GC and MS interface temperature was maintained at 280 ºC. A sample volume of 1 µL was injected in the split less mode, and the injector temperature was maintained at 280 °C. The oven temperature was cycled according to the following program: 70 °C (2 min), 15 °C min^{-1} to 180 °C (2 min), 5 °C min^{-1} to 260 °C (2 min), and 3 °C min^{-1} to 300 °C (6 min). GC-MS analysis was conducted using the electron impact ionization mode at 70 eV. The ion source was operated at 200 °C [**247]**. The scan method was used for MS detection, m/z value ranging from 35 to 500. Peak identifications were carried out using NIST mass library for PAHs. External standards of PAHs mixture in different concentration ranges from 100 pg μL^{-1} to 100 ng μL^{-1} were used for quantification.

3.5.3 Quality control and quality assurance

Several dilutions corresponding to 0.1-100 ng absolute of synthetic standard mixture of Naphthalene (NAP), Acenaphthylene (ACY), Acenaphthene (ACE), Fluorene (FLU), Phenanthrene (PHEN), Anthracene (ANT), Fluoranthene (FLUO), Pyrene (PYR), Benzo (a) Anthracene (BaA), Chrysene (CHY), Benzo (b) Fluoranthene (BbF), Benzo (k) Fluoranthene (BkF), Benzo (a) Pyrene (BaP), Dibenz (a,h) anthracene (DBA), Benzo (ghi) Perylene (BghiP) Indeno(1,2,3-cd) Pyrene (IND), (purchased from Supelco, Belle-fonte, USA) were used for determining the retention data and for studying the linearity of the MS detector. The limits of detection (LODs) and limits of quantification (LOQs) for each PAHs were calculated as the concentrations at which the signal-to-noise ratios were 3 and 10, respectively. Limit of

detection were in range of 10- 100 pg g^{-1} for sediment and 1-10 ng L^{-1} for sea water. The quality assurance of the measurements PAHs in sediment samples was ensured by analysis of standard reference material IAEA-408 Marine sediment. The quality assurance of measurements of PAHs in marine organism samples was assessed through analysis of the certified reference material 'IAEA-435' Tuna homogenate. The accuracy of the measurement was within ±5% of certified values for both reference materials. Spiked PAHs samples were used for analytical recovery of seawater samples. The concentrations of the PAHs were not detected in the field blank samples**.** The average recoveries obtained from the GC–MS analysis of the PAH-spiked seawater samples ranged from 98.9% to111%.

3.6 Analysis of PBDEs in sediment core

3.6.1 Dating of core sediment

Core sediment samples was also used for determining the sedimentation rate by measuring the specific activities of ^{210}Pb using the polonium distillation procedure [**248, 249]**. The ground and homogenized sediments samples were subjected to radiochemical separation for ^{210}Po activity concentration measurement, by acid digestion followed by reduction of interfering Fe(III) by ascorbic acid treatment. Silver disk were submerged into the extracted solutions and were kept at a temperature of about 85 ^{o}C for 7 h with continuous stirring; dried under infra-red lamp and their activities were determined by alpha spectrometry [**250]**. Activities of ^{210}Po along with the ^{209}Po tracer were measured by alpha spectrometry on a silicon surface barrier detector connected to a multichannel analyzer (Alpha Spectrometer System, PAS-01-4, 25% efficiency, and 22 keV resolution for 5.49 MeV alpha particle). The spike tracer efficiency was obtained as 70-90%. The quality assurance of measurements was assessed through analysis of the Standard Reference Material IAEA-135 Marine Sediment sample. Counting time was set as 60,000 s for each sample.

3.6.2 Extraction for PBDEs

2 g of freeze dried sediment sample from each slice was accurately weighed and transferred to 50 mL stoppered conical flasks. The samples were agitated ultrasonically using 40 ml of n-hexane: Acetone, 3:1 mixture for 60 minutes for extraction, and repeated. The combined extracts were evaporated up to 2 mL, and 2 mL of concentrated H_2SO_4 was added to remove fat and organic polymers from the extracts. After centrifugation, the hexane layer was removed, and the sulfuric acid residue was washed twice with 2 mL of hexane [**249]**. The extracts were filtered and treated with elemental Hg for removal or sulfur containing organic compounds. The volume of the extracts was then reduced to 0.5 ml and then loaded on a multilayer column for further clean-up. Grab samples were also processed using the same procedure.

3.6.3 PBDE clean-up procedure

Sediment extracts were subjected to column cleanup prior to analysis. The column was prepared from the top with 2 g anhydrous sodium sulphate followed by 10 g of Florisil (activated by heating at 650 ^{O}C for 16 hours) and 10 g of silica gel 60 (activated by heating at 150 ^{O}C for 24 hours then deactivated by adding 0.5 mL of de-ionized water). 10 mL of 8:1:1 n-hexane: acetone: dichloromethane, mixture was used to wash and prepare the column. The samples were loaded at the top of column and eluted using 50 ml n-hexane followed by 50 ml 1:1 n-hexane: dichloromethane mixture. The two eluent fractions were pooled together and evaporated under a gentle stream of pure nitrogen. The extracts thus prepared were re-dissolved into 200µl of n-hexane and then injected into the gas chromatograph.

3.6.4 Analysis of PBDEs using Gas Chromatograph

Synthetic standards for 15 PBDE congeners viz., BDE-28 (2,4,4’ tribromodiphenyl ether), BDE-47 (2,2’,4,4’ tetrabromodiphenyl ether), BDE-66 (2,3’4,4’ tetrabromodiphenyl ether), BDE-71 (2,3’,4’,6 tetrabromodiphenyl ether), BDE-75 (2,4,4’,6 terabromodiphenyl ether), BDE-77 (3,3’,4,4’ tetrabromodiphenyl ether), BDE-85 (2,2’,3,4,4’ pentabromodiphenyl ether),

BDE-99 (2,2',4,4',5 pentabromodiphenyl ether), BDE-100 (2,2'4,4',6 pentabromodiphenyl ether), BDE-138 (2,2'3,4'4',5' Hexabromodiphenyl ether) BDE-153 (2,2',4,4',5,5' Hexabromodiphenyl ether), BDE-154 (2,2'4,4'5,6' Hexabromodiphenyl ether), BDE-183 (2,2',3,4,4',5',6 Heptabromodiphenyl ether), BDE-190 (2,3,3',4,4',5,6 Heptabromodiphenyl ether) and BDE-209 (2,2',3,3',4,4',5,5',6,6' Decabromodiphenyl) were purchased from AccuStandards, USA. Standards were prepared in the range of 100 ng mL^{-1}. A gas chromatograph (GC-17A, Shimadzu, Japan) equipped with an electron capture detector (ECD-17, Shimadzu, Japan) was used for analysis. A 60m x 0.25mm id capillary column (DB-5, Agilent Technologies, USA) was used for the separation of PBDE congeners except Decabromobiphenyl (BDE-209). A DB-5, 15 m x 0.25 mm i.d capillary column was used for the analysis of BDE-209. The analytical parameters optimized for PBDE separation and quantification are detailed in **Table 3.5**. Due to possibility the thermal breakdown of BDE 209 in the column, the parameters set for the decabromodiphenyl analysis were different from that of the rest of the PBDE congeners.

Table 3.5 Analytical conditions optimized for PBDE congeners analysis using GC-ECD.

Analytical parameters for analysis of PBDEs except BDE-209	
Analytical Column	DB-5, 60 m × 0.25 mm i.d., 0.5 µm film thickness.
Detector	ECD at 325◦C
Carrier gas	Nitrogen
Injector	315°C, Split/Splitless @ split ratio 5
Column oven temp profile	45°C hold for 0.5 min, @35°C per min. upto 230°C, hold for 9 min, 5°C per min. upto 280°C, hold for 13 min., @10°C per min. to 310°C, hold for 13 min., @10°C per min. to 325°C, hold for 44 min.
Analytical parameters for analysis of BDE-209	
Column	DB-5, 15 m x 0.25 mm i.d, 0.5 µm film thickness
Detector	280°C
Carrier gas	Nitrogen
Injector	Cool-on-Column Split injection, split ratio - 5
Column oven temp profile	100°C hold for 1 min, @25°C upto 325°C, hold at 325°C for 10 min.

Identification of PBDEs in 20% of sediment samples was confirmed using GC-MS. In brief, analysis of PBDEs was carried out on Shimadzu GC-MS QP2010 ultra system, connected with a Rtx-5MS—Low-Bleed GC-MS Columns (15 m × 0.25 mm × 0.10 μm, RESTEK). The flow rate of helium was kept constant at 1.5 mL min^{-1}. A 1 μL sample was injected in splitless mode with the injector temperature maintained at 250 ºC. The oven temperature program was: 110 ºC for 0.5 min, 4.5 125 ºC min^{-1} to 220 ºC, 15 ºC min^{-1} to 280 ºC, 5.0 ºC min^{-1} to 310 ºC and held for 3.0 min. Target ions were monitored in the electron capture negative ionization mode with the ion source temperature of 150 ºC. For separation of BDE-153 and BDE-154, a longer column 60 m was used and GC-MS program used is described elsewhere [**249]**.

Table 3.6 Full names, abbreviations, homologue groups, monitoring ions (m/z), logK_{OA} (at 25 ºC) and MDLs for PBDEs congeners in sediment (dry weight).

PBDE Congeners	**Short name**	**Homologue**	**m/z**	**logK_{OA}**	**MDLs (pg/g)**
2,4,4'-tribromodiphenyl ether	BDE-28	TriBDE	79/81	9.31	0.84
2,2',4,4'-tetrabromodiphenyl ether	BDE-47	TetraBDE	79/81	10.54	0.71
2,3',4,4'-tetrabromodiphenyl ether	BDE-66	TetraBDE	79/81	10.83	0.84
2,3',4',6 tetrabromodiphenyl ether	BDE-71	TetraBDE	79/81	10.20	0.86
2,4,4',6 terabromodiphenyl ether	BDE-75	TetraBDE	79/81	10.42	0.64
3,3',4,4' tetrabromodiphenyl ether	BDE-77	TetraBDE	79/81	10.87	0.87
2,2',3,4,4'-pentabromodiphenyl ether	BDE-85	PentaBDE	79/81	11.67	1.34
2,2',4,4',5-pentabromodiphenyl ether	BDE-99	PentaBDE	79/81	11.32	0.99
2,2',4,4',6-pentabromodiphenyl ether	BDE-100	PentaBDE	79/81	11.14	0.68
2,2',3,4,4',5'- hexabromodiphenyl ether	BDE-138	HexaBDE	79/81	13.27	1.73
2,2',4,4',5,5'-hexabromodiphenyl ether	BDE-153	HexaBDE	79/81	11.83	1.16
2,2',4,4',5,6'-hexabromodiphenyl ether	BDE-154	HexaBDE	79/81	11.93	1.05
2,2',3,4,4',5',6-heptabromodiphenyl ether	BDE-183	HeptaBDE	79/81	11.97	1.40
2,3,3',4,4',5,6-heptabromodiphenyl ether	BDE-190	HeptaBDE	79/81	14.56	5.85
Decabromodiphenyl ether	BDE-209	DecaBDE	484.5/ 486.5	18.42	2.25

Summary of the full names, abbreviations, homologue groups, monitoring ions (m/z), logK_{OA} (at 25 ºC) and MDLs for PBDEs in sediment (dry weight) are represented in **Table 3.6.**

3.6.5 Quality Control and Quality Assurance

The retention times were obtained for individual congeners by single standard injections and the data obtained was utilized for identification of congeners in the sample. The detector response was found to be linear or the range of 10 ng to 100 µg mL^{-1}. Calibration curves were prepared to calculate the concentrations. Blank samples (by using anhydrous sodium sulfate) were prepared in the same manner as the sediment samples and analyzed concurrently with the field samples to measure interference and laboratory contamination. Solvent and procedural blanks were run intermittent for every batch of 10 samples. For PBDEs congeners, only values more than three times of the instrument signal to noise ratio (S/N) were considered as true peak; otherwise neglected. PBDEs in the blank were negligible with the levels being < 3 % of the concentrations in sediment samples. The recoveries of individual congeners were found to be in the range of 76–95% for spiked blank sample.

Chapter 4

RESULTS AND DISCUSSION

4.1 Distribution of POPs in creek environment, their inter-compartmental behavior and ecotoxicological concern.

In the present section distribution data of OCPs and PCBs are reported in sediment, seawater and biota from Thane creek area. Further results are compared with literatures for all matrices. Bioconcentration factor (BCFs) and fugacity fractions are calculated for these contaminants to evaluate their inter-compartmental behavior in creek environments. Finally, their ecotoxicological concerns are described by comparing their levels with environmental quality guidelines.

4.1.1 Distribution of organochlorine pesticide (OCPs) in sediment

Concentrations of OCPs and selected metabolites were analysed in surface sediments collected from 10 different locations in across Thane creek. Average concentrations (ng g^{-1}) of α-HCH, β-HCH, γ-HCH, DDT, DDD and DDE in sediments (dry weight) collected from different locations are represented in **Fig. 4.1.1**. Total DDT concentration which is sum of DDT and its major metabolite i.e. DDD and DDE concentrations was found to range from 3.14 to 6.74 ng g^{-1} with average value of 4.6 ng g^{-1} dry weight of sediments. Concentrations of DDT were found comparable with sediment from Brazil reported by Miranda et al. (2008) **[8]**. Similarly, total HCH which is sum all three major conformer α-HCH, β-HCH and γ-HCH was found to range from 4.21 to 24.66 ng g^{-1} with average value of 12.5 ng g^{-1} across Thane creek. Percentage contributions of α-HCH, β-HCH and γ-HCH were founds to be in the ranges of 25 - 38 %, 10-18% and 46 - 57 % respectively of the total HCH measured across Thane creek in surface sediment samples. Percent contribution of DDT in total DDT was found maximum with range of 42-60%, while percentage contributions of DDD and DDE were in ranges of 5-15% 32-44 % respectively. Higher concentration of HCH compare to DDT may be due to

higher use HCH compared to DDT, or may be faster environmental degradation of DDT. Concentrations of OCPs were found high at location 3, 8 and 10 compared to other locations. The probable reason for this observation is likely the urban and industrial run off discharge to near to these locations. Also, the organic carbon content in sediment samples from these locations were founds to be higher relative to other locations which reveal the OCPs concentrations are associated with organic carbon contents in surface sediment samples.

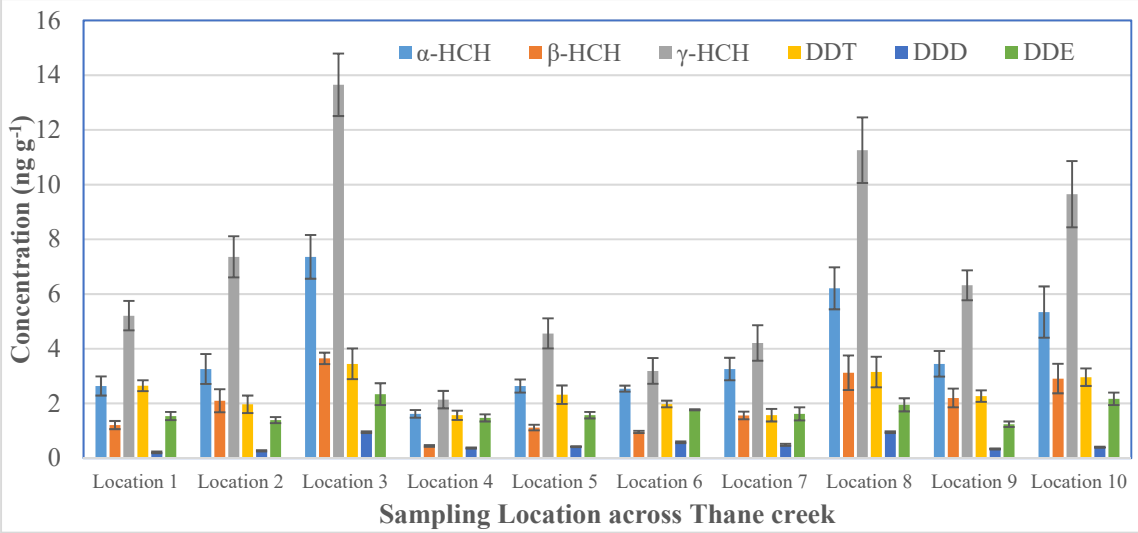


Fig 4.1.1 Concentration (ng g^{-1}) of DDT compounds and HCHs isomers (μ (mean) ± 1σ (SD), n=3 (number of sample)) in grab sediment samples at different locations across Thane creek.

Data observed in present study are quite comparable to other published in Asian countries [**9, 251- 253**]. A recent study on chronological assessment of organochlorine pesticide in sediment for this site suggests maximum concentration in the late 70's. Concentrations of Total-DDT were highest in the 1974-1978 slice sediments (124.1 ng g^{-1} d.w.). The production and use of pesticides were at their peak in this period especially in India during the first phase of the green revolution. Hence although the use of DDT was banned in several of the western countries, the sediments in Thane creek showed their maximum concentration in the same period. Total DDT concentrations (DDTs) continuously decreased up to the surface sediments with two small peaks in the mid 80's and 90's. This may be due to the application of DDT in vector control

during that period. The peak is observed in the year 1970, this is the period when widespread use of DDT in agricultural pest control had just begun in India [**13]**.

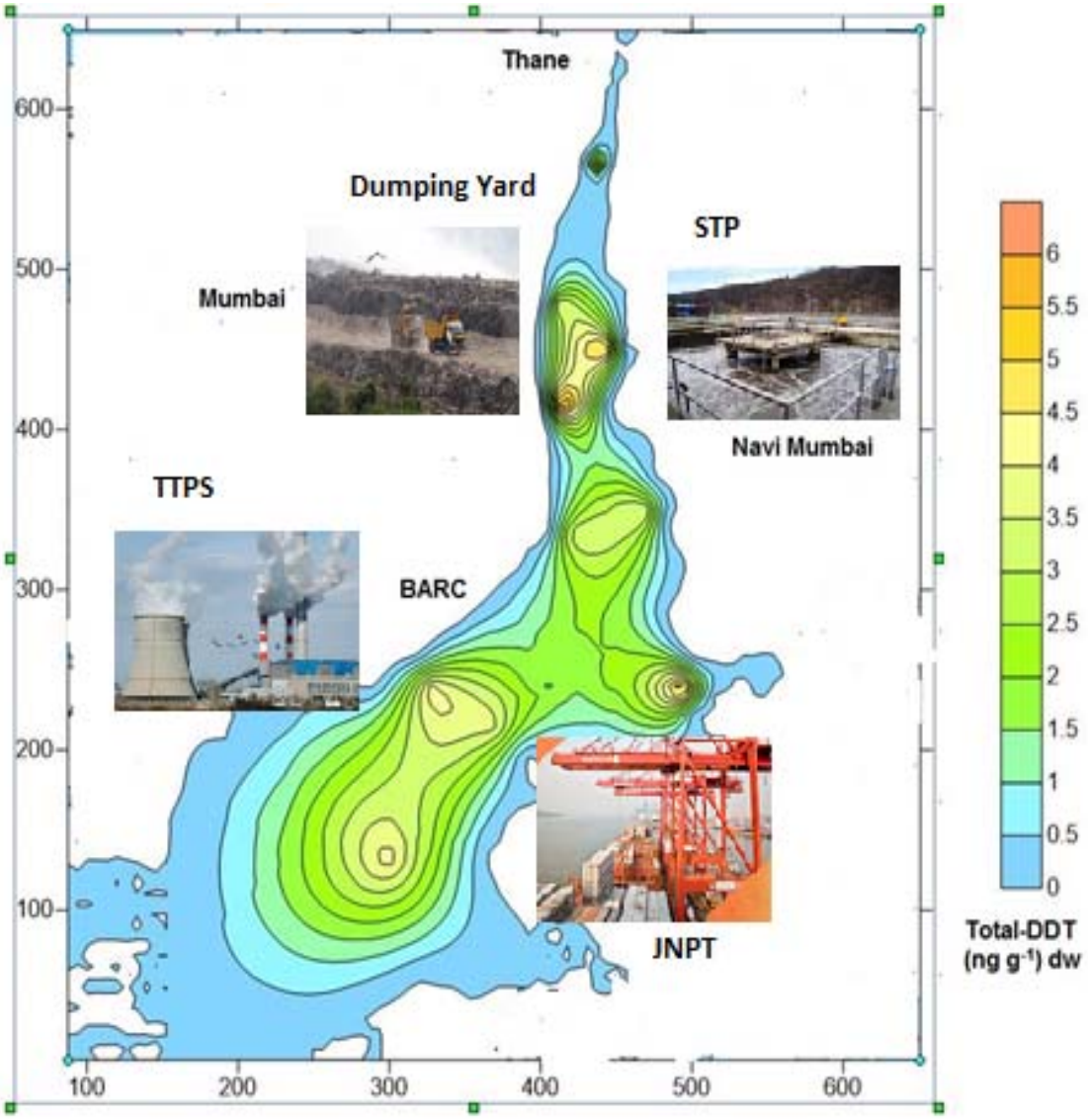


Fig 4.1.2 Spatial distribution of total DDT in sediments across Thane creek and major pollution sources around study area.

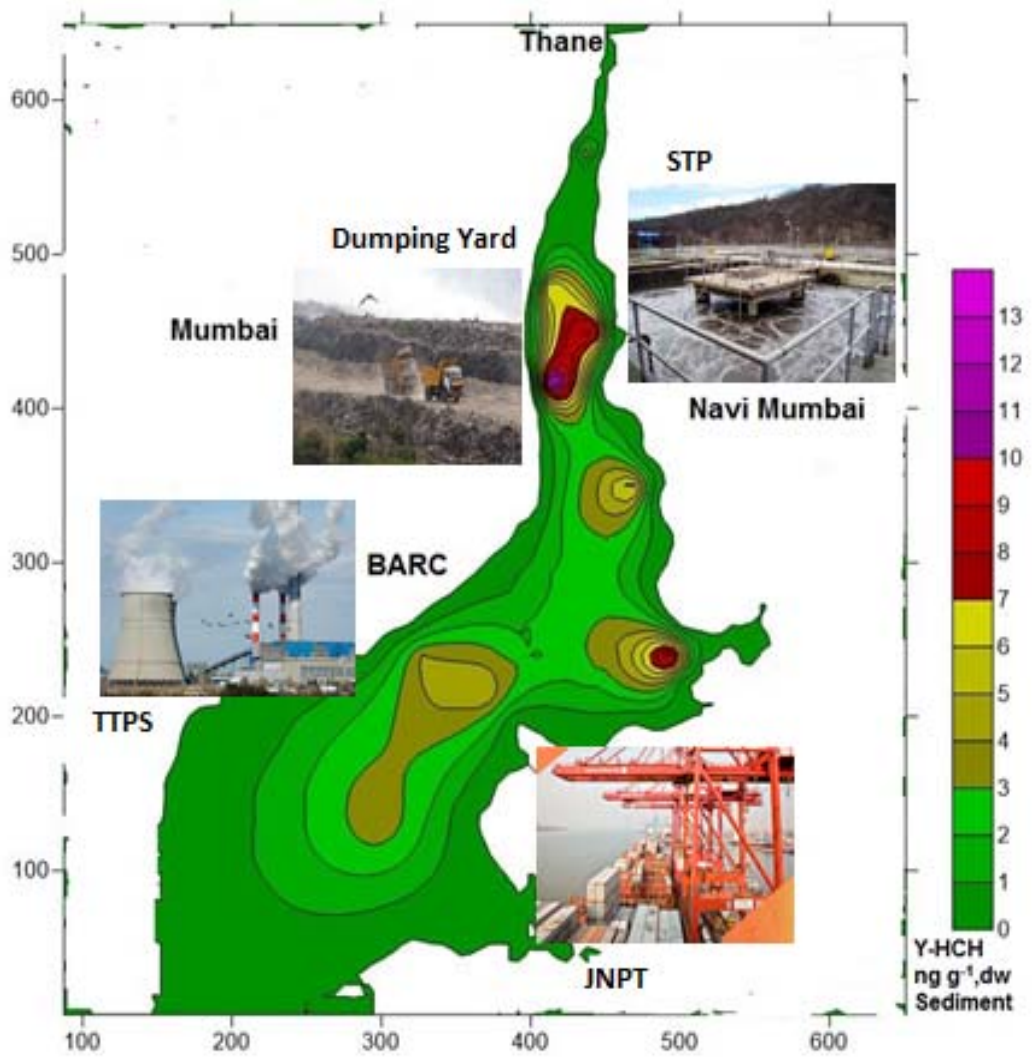


Fig 4.1.3 Spatial distribution of Lindane (γ-HCH) in sediments across Thane creek and major pollution sources around study area.

Spatial distribution of total DDT (DDT and its metabolite) and Lindane (γ-HCH) in sediments across Thane creek and major pollution sources around are shown in **Fig 4.1.2** and **Fig 4.1.3** respectively. Spatial distributions of these chemical are indicating their higher accumulation in sediments near dumping yard and waste water receiving points in creek.

4.1.2 Distribution of polychlorinated biphenyls (PCBs) in sediment

Total nine numbers of PCBs were analysed in sediment samples collected from Thane creek, most of them are major constituent of technical mixture of PCBs. Mean concentration of total

PCBs was found to be 2.9 ± 0.5 ng g^{-1} (dry weight) in grab sediment samples across Thane creek. Location wise PCBs distributions in grab sediment samples were shown in **Fig. 4.1.4**. High concentration of total PCBs in sediments samples were found at Location 3, 8 and 10 compared to other monitored locations. Order of analysed PCBs congener's concentration in sediment was founds as CB-138 > CB-153 > CB-180 > CB-101 > CB-77 > CB-126 > CB-52 > CB-169 > CB-194. CB-138 was most abundant congener with mean concentration value of 0.73 ± 0.5 ng g^{-1} across study area.

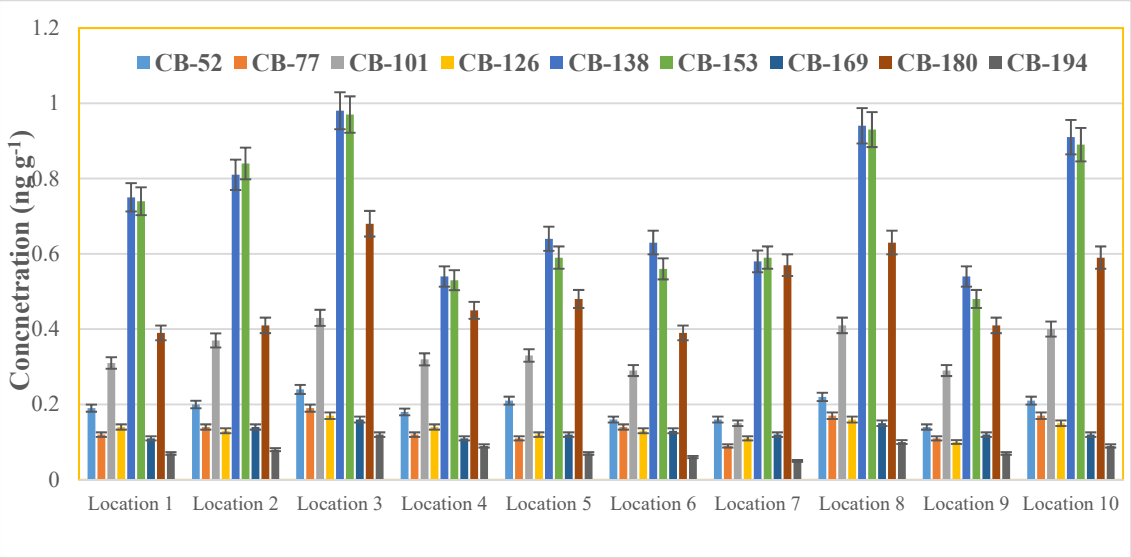


Fig 4.1.4 Distributions of Polychlorinated biphenyls congeners in garb sediment samples at different locations of Thane creek (μ (mean) ± 1σ (SD), n=3 (number of sample)).

PCBs analysed in sediments can be classified on the basis of degree of chlorination i.e. tetra chlorinated (CB-52 and CB-77), penta chlorinated (CB-101 and CB-126), hexa chlorinated (CB-138, CB-169 and CB-153), hepta chlorinated (CB-180) and octa chlorinated (CB-194). Percentage contributions of different degree of chlorinated PCBs are represented in **Fig. 4.1.5.** Hexa chlorinated biphenyls were contributing more than half around 53% to total PCBs, and it was observed, that octa-chlorinated biphenyls were contributing least with average value of 3% of total PCBs.

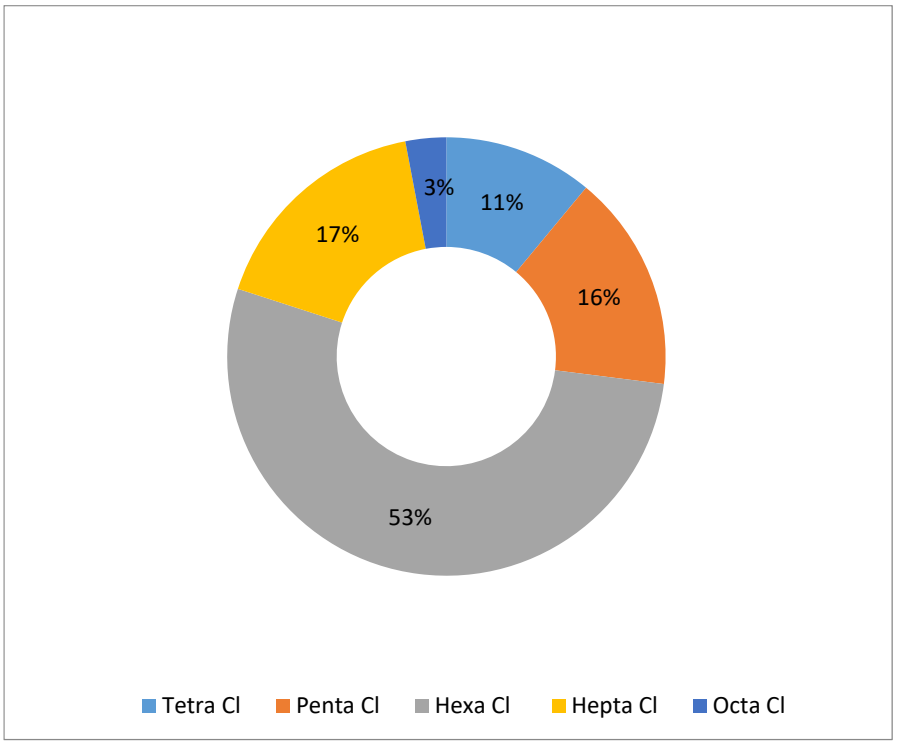


Fig. 4.1.5. Average percentage contributions of different degree of polychlorinated biphenyls congeners across Thane creek in grab sediment matrix.

Earlier study on core sediment samples from Thane creek indicate high concentration of PCBs. PCBs content in the whole sediment core was in the order CB28 > CB52 > CB44 > CB180 > CB101 > CB 126 > CB18 > CB138 > CB153 which is different from the current study. The concentration and percent contribution of different congeners varies at different depths. A sharp distinctive change suggests input from source whereas gradual change indicates weathering. The percent contribution of different congeners is a very good indicator of the processes like degradation, diffusion, accumulation, etc., which drives the distribution of these congeners at different depths. When PCBs are stored in sediments for a prolonged period of time, they are subjected to biodegradation by bacteria **[111]**. Spatial distribution of polychlorinated biphenyls in grab sediments across Thane creek are represented in **Fig. 4.1.6**, and were found following similar trend of distribution as of OCPs.

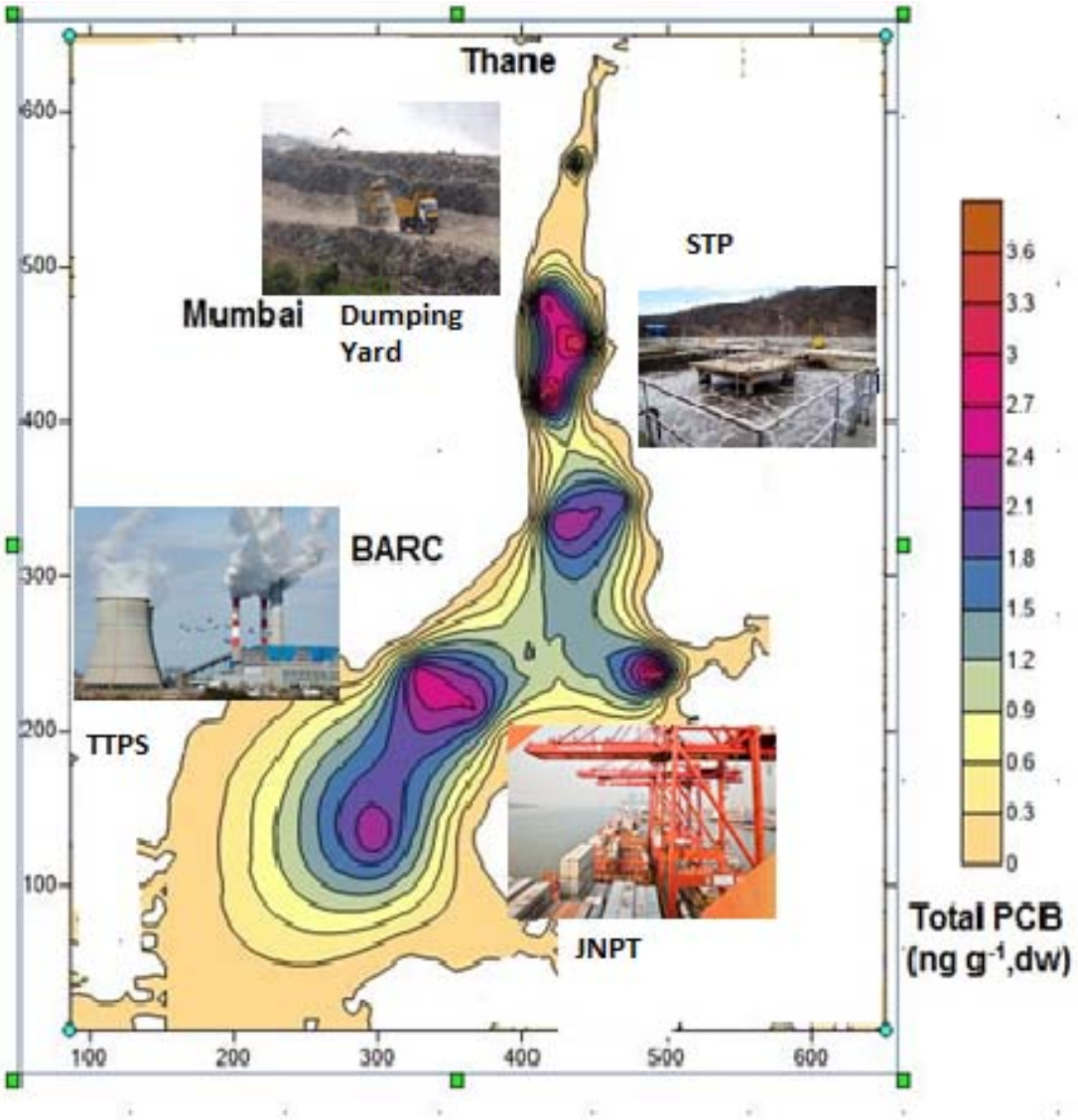


Fig. 4.1.6. Spatial distribution of select polychlorinated biphenyls congeners in sediments across Thane creek and major anthropogenic activities around the area.

A comparison of OCPs and PCBs levels in sediments from the study area with other studies from different parts across the globe is shown in **Table 4.1.1**. ∑DDTs includes DDT and its metabolite DDE and DDD, while ∑HCH comprises of α-, β-, and γ-HCH conformers. Concentration of these contaminants were in range with other costal environment specially from India Babu Rajendran et al. (2004) **[99],** Guzzella et al. (2005) **[254],** Barakat et al. (2002) **[255],** Hong et al. (1995) **[256],** Zhoue et al. (2008) **[257],** Dai et al. (2011) **[122],** Mohmmed

et al. (2011) [**258**]. Although, occurrence of OCPs and PCBs was slightly lower than sediments from Indian river Ganga [**259, 377]**.

Table 4.1.1 Comparison of DDT compounds, HCHs isomers and PCBs congeners (in sediments) with literature, all values are in ng g^{-1}, dry weight.

Locations	∑DDTs	∑HCHs	∑PCBs	References
Thane Creek, India	3.41 – 6.74	4.21 - 24.66	2.26 - 3.94	This Study
Bay of Bengal, India	0.04 - 4.79	0.17 - 1.56	0.02 - 6.57	Babu Rajendran et al., (2004)
Hugli Estuary, India	0.18–1.93	0.11–0.4	0.18–2.33	Guzzella et al. (2005)
Alexandria Harbour, Egypt	<0.25 - 885	0.25–6.0	0.9 – 1210	Barakat et al., (2002)
Masan Bay, Korea	0.27 – 89.2	0.02–0.59	2.48–75	Hong et al. (2003)
Ganga River, India	32 - 3101	18.89 - 392.60	-	Singh et al., (2012)
Qiantang River, China	4.47-95.77	9.23-120.2	-	Zhou et al., (2008)
Xiamen harbour, China	4.5-311	0.14–1.12	0.05–7.2	Hong et al. (1995)
Baiyangdian Lake North China, China	0.91–6.48	1.75–5.70	5.96–29.61	Dai et al., (2011)
South-eastern Port of Spain, Spain	6.1–29	0.7–1.8	62 - 601	Mohammed et al., (2011)

4.1.3 OCPs and PCBs in sweater and biota samples

The concentrations of OCPs and PCBs were also measured in seawater and fish samples collected from Thane creek, apart from sediments. **Table 4.1.2** summarizes the analytical results of these contamination in seawater and fish samples. In sediment, DDT was most abundant contaminants in fish and sea water. Contaminates levels in seawater samples were observed three order of magnitude less than fish samples. The abundance of PCBs congener in seawater was observed as CB101 > CB52 > CB138 > CB153 > CB194 > CB180 > CB77, while CB126 and CB169 were not detected. Likewise, the abundance of PCBs congers in fish

samples were as CB52 > CB138 > CB153 > CB101 > CB180 > CB194 > CB77, and CB126 was not detected in any sample.

Table 4.1.2 Concentrations of DDT compounds, HCHs isomers and PCBs in seawater (ng L^{-1}) and fish (ng g^{-1}, dry weight) samples from Thane creek (n = 30).

Chemicals	Seawater (ng L^{-1})	Fish (ng g^{-1}) dw
	Range (Min, Max), Mean	Range (Min, Max), Mean
α-HCH	0.1-3.1, 1.12	0.71-4.26, 1.72
β-HCH	0.1-5.23, 1.23	0.9-3.21, 1.91
γ-HCH	0.2-6.32, 1.9	1.4-23.5, 6.64
DDT	1.32-9.71, 3.12	10.1-23.1, 12.2
DDD	0.12-1.11, 0.56	2.1-5.4, 4.21
DDE	0.2-1.1, 0.77	0.74-5.95, 4.25
CB-52	10.5-34.2, 27	131-2000, 1300
CB-77	4.1-16.2, 10	40-210, 131.3
CB-101	20.7-72.1, 40.5	450-1100, 810
CB-126	-, nd	27-82.5, 53
CB-138	11.2-33.5, 25.8	740-1100, 940
CB-153	7.5-26.4, 19.2	660-1200, 890.6
CB-169	-, nd	-, nd
CB-180	6.4-13.6, 10.5	160-310, 240
CB-194	4.2-17.5, 12.2	110-172.5, 145

A comparison of OCPs and PCBs concentration in seawater with the studies in recent past from water bodies of different location across glob was made, and is shown in **Table 4.1.3**. DDT, DDD, DDE, Lindane, ∑DDT, ∑ HCH, and ∑PCBs are the chemicals compared. The levels of OCPs in Indian river water e.g. Ganga and Yamuna studied by Singh et al. (2012), Aleem and Malik, (2005) and Singh et al. (2004) were either in range or slightly higher than the observed in present study [**259, 262, 263]**. OCPs concentration in seawater were quite similar as of Chinese water bodies, while the PCBs levels found in this study were three order of magnitude less compare to Baiyangdian lake from north China reported by Zhou et al. (2008) and Dai et al. (2011) [**122, 257]**. Comparing the levels of OCPs in seawater between present study and from Egyptian Mediterranean Coast studied by Shreadah et al. (2014), it was reveals that levels in Thane creek were on the lower side of the range observed at that site [**264]**.

Table 4.1.3 Comparison of DDT compounds, HCHs isomers and PCBs in seawater with literature, concentrations are in ng L^{-1} except for PCB (pg L^{-1})**.**

Location	DDT	Lindane	DDE	DDD	∑ HCH	∑DDT	∑ PCBs	References
Thane creek India	6.6 ± 2.3	1.9± 0.4	0.77 ± 0.12	0.56 ± 0.07	7.94	4.25	145.2	This study
Yamuna River India	8.8 ± 1.9	-	1.6 ± 0.26	-	-	-	-	Aleem and Malik (2005)
Ganga River India	3.3 - 5.3	ND-0.56	-	0.88-2.41	-	-	-	Singh et al., (2004)
Ganga River India	-	ND-74.04		ND-112	-	-	-	Singh et al., (2012)
Qiantang River, China	1.0 -11.8	1.76 - 4.0	0.8 - 5.12	0.18-2.08	1.58-16.03	14.9-57.2	-	Zhou et al., (2008)
Baiyangdian Lake North China	1.2 ± 0.3	1.08 ± 0.84	2.16 ± 2.14	2.16 ± 1.0	3.13–10.60	4.0 -20.6	19 –131 ng L^{-1}	Dai et al., (2011)
Egyptian Mediterranean Coast, Egypt	ND- 972	ND- 798	ND- 1247	ND-195	ND- 767	ND - 2547	-	Shreadah et al., (2014)
Baltic Sea, Sweden	0.05 - 0.24	-	-	-	0.35 ±0.13	0.14±0.05	0.98 ± 0.44	Sapot (2004)
Atlantic seawater		ND - 0.009			0-0.057		0.56-3.47	Lohmann et al., (2012)

Concentration of ∑DDT, ∑ HCH, and ∑PCBs in seawater were observed significantly higher compare to seawater from Baltic sea, Sweden reported by Spota, (2004) [**265**]. Lohmann et al., (2012) reported levels of these chemical ware quiet low in Atlantic seawater compare to levels observed in present study for Thane creek Mumbai [**100]**.

Similarly, a comparison for levels of OCPs and PCBs in fish from study area and literature was also made and is represented in **Table 4.1.4**. The concentration of ∑DDT and ∑ HCH in fish from Thane creek were two and three degree of magnitude lower than of the Bay of Bengal and Cauvery river of India, respectively reported by Das et al. (2002) and Patil et al. (2015) **[266, 267]**. Comparing with the study from Scheldt eastury Netherlands by Voorspoels et al. (2004), it was found that levels of ∑DDT and ∑ HCH in fish from Thane creek were higher than reported values, while ∑PCBs levels were relatively lower [**268**]. The levels of these persistent organic pollutants in fish samples was very similar to reported in different studies from China by Li et al. (2008), Yang et al. (2006) and Pan et al. (2016) [**114, 233, 269**]. The

concentrations of ∑DDT and ∑PCBs compound in muscle samples of salmon from Atlantic reported by Svendsen et al. (2007) were higher than the fish samples analysed in this study, while concentration of ∑HCH was less [**270**]. Very similar range of these contaminants was also reported in fish samples from Chenab river Pakistan reported by Eqani et al. (2013) [**271**].

Table 4.1.4 Comparison of DDT compounds, HCHs isomers and PCBs congeners in fish samples with literature, concentrations are in ng g^{-1} wet weight.

Location	∑DDTs (ng g^{-1})	∑HCHs (ng g^{-1})	∑PCBs (ng g^{-1})	References
Thane Creek, India	12.94 – 34.45	3.01 – 30.9	0.5 - 6.1	This Study
Bay of Bengal, India	397 - 716	71.3 – 101.2	-	Das et al. (2002)
Kaveri (Cauvery) River, India	2,805	228	-	Patil et al., (2015)
Scheldt Estuary, Netherlands	1.49 - 14.1	0.28 – 0.9	23-120	Voorspoels et al., (2004)
Gaobeidian Lake, China	15.4 - 25.4	2.99 - 7.01	6.43 – 7.9	Li, et al 2008
Dalian, China	0.81 – 212.1	0.11 – 3.81	1.11 – 8.04	Yang et al., (2006)
Eastern coastal area of China, China	0.44–1.74	2.84–106.11	1.02–2.2	Pan et al., (2016)
North Atlantic (Salmon)	141.2	4.5	114.2	Svendsen et al., (2007)
River Chenab, Pakistan	8.83 - 190	2.5 - 8.6	3.1–93.7	Eqani et al., (2013)

4.1.4 Ecotoxicological concerns of OCPs and PCBs

The sediment quality guidelines (SQG) declared by the USEPA and Canadian Council of Ministers of the Environment (CCME) were used to assess the potential ecotoxicological impacts of analysed organic contaminants i.e. OCPs/PCBs in the surface sediments of Thane creek [**154, 260**]. Effects range-low (ER-L) and effects range-median (ER-M) values are used to find potential impacts of contaminants in sediments, whereas ERL values correspond to the lower 10 percentiles and ERM values to median values, when the chemical concentration of a contaminant in marine sediments are sorted according to the degree of their effects levels.

Table 4.1.5. Minimum, maximum and average concentrations of major organochlorine contaminants in ng g^{-1}, and corresponding sediment quality criteria.

	Cmin	Cmax	Caverage	ER-L*	Samples Above ER-L	+ER-M	Samples above ER-M	£TEL	Samples above TEL	¥PEL	Samples above PEL
ΣPCBs	2.26	3.94	2.94	22.7	None	180	None	21.55	None	188.79	None
DDT	1.57	3.45	2.39	1	All	7	None	1.19	All	4.77	None
DDD	0.21	0.95	0.50	2	None	20	None	1.22	None	7.81	None
DDE	1.24	2.34	1.71	2.2	10%	27	None	2.07	20%	374.17	None
Σ DDT	3.41	6.74	4.59	1.58	All	46.1	None	3.89	60%	51.7	None
γ HCH	2.14	13.65	6.76	-	-	-	-	0.32	All	0.99	All

* Effects range-low value(ER-L), +Effects range-median value (EM-L), £Threshold effects level (TEL), ¥ Probable effects level (PEL)

ERL were calculated using existing toxicity data compiled from completed toxicity assays with varying endpoints, including effects on commonly tested organisms, particularly at sensitive life stages. The process is considered a "weight of evidence approach", in which results are based on a large database of previously conducted studies. The studies used included synoptically collected sediment chemical analyses and toxicity effects data and values are developed by National Oceanic and Atmospheric Administration (NOAA) and United States Environmental Protection Agency (EPA). ER-L represents the value at which toxicity may begin to be observed in sensitive marine species, whereas ER-M represents the concentration below which adverse effects are expected to occur only rarely. The threshold effect level (TEL) and the probable effect level (PEL) are used as the criterion for the prediction of toxicity, and corresponds to a level above which adverse effects are frequently expected [**252, 261**]. TEL values were also based on weight of evidence approach and developed by Canadian Council of Ministers of the Environment (CCME). Minimum, maximum and average values of OCPs and corresponding sediment quality guideline indices were represented in **Table 4.1.5**.

The ΣPCB concentration was found to be below all discussed sediment quality guidelines i.e. ER-L, ER-M, TEL and PEL. For Σ DDT the levels in locations higher than the ER-L value but

below than the ER-M value suggesting to an intermediate ranking of sediment toxicity across Thane creek. 60% of samples were found to be above TEL, while none of the sample exceeding PEL value of Σ DDT. γ-HCH (Lindane) concentration in all samples were founds to exceeding TEL and PEl values significantly. So Lindane can be a potential threat to marine organism of Thane creek.

4.1.5 Inter-compartmental behavior of OCPs and PCBs

The bio concentration factor (BCF) were calculated for OCPs and PCB in fish samples collected across Thane creek. The average BCF values for DDT and its metabolites (DDD, DDE), and HCH conformers are shown in **Fig. 4.1.7** with standard deviation. The mean BCFs values for these contaminants ranged from 1535 (α-HCH) to 7517 (DDD), and their order was as α-HCH < β-HCH < γ-HCH < DDT < DDE < DDD. The bio concentration factor (BCF) is defined as the ratio of the chemical concentration in an organism C_B, to the total chemical concentration in the water $C_{WT.}$ Concentration of contaminants in biota samples and water samples first averaged and then the ratio was taken for Thane creek.

The values of BCF of HCHs conformers were less compare to DDT and its metabolite, this may be due to their low octanol–water partition coefficients (log K_{ow} ~4) [**272]**. These values of BCF indicate that these compounds have significant potential to bio concentrate as a theoretical value of BCF < 250 indicates a low potential for bio concentration [**273]**. The BCF values were in same order of magnitude but slightly higher side reported by Eqani et al. (2013) for fish samples at Chenab river to these contaminants, according to that study BCF varied between 695–1254 for herbivorous fish and 950–1562 for carnivorous fish [**271**]. In present study, the high value of BCFs for DDD can be explain as due to the metabolisation of parent DDT that can take part in DDD bio concentration in the fish tissues.

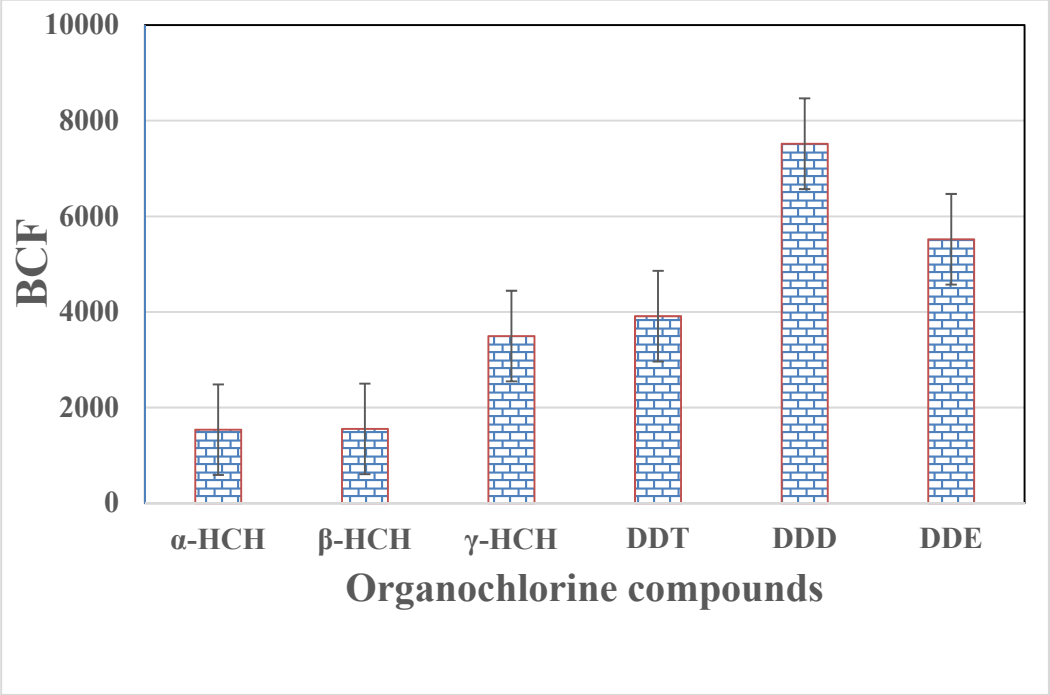


Fig 4.1.7. Bio concentration factor (mean ± standard deviation) for DDT compounds, HCHs isomers in fish samples collected across Thane creek.

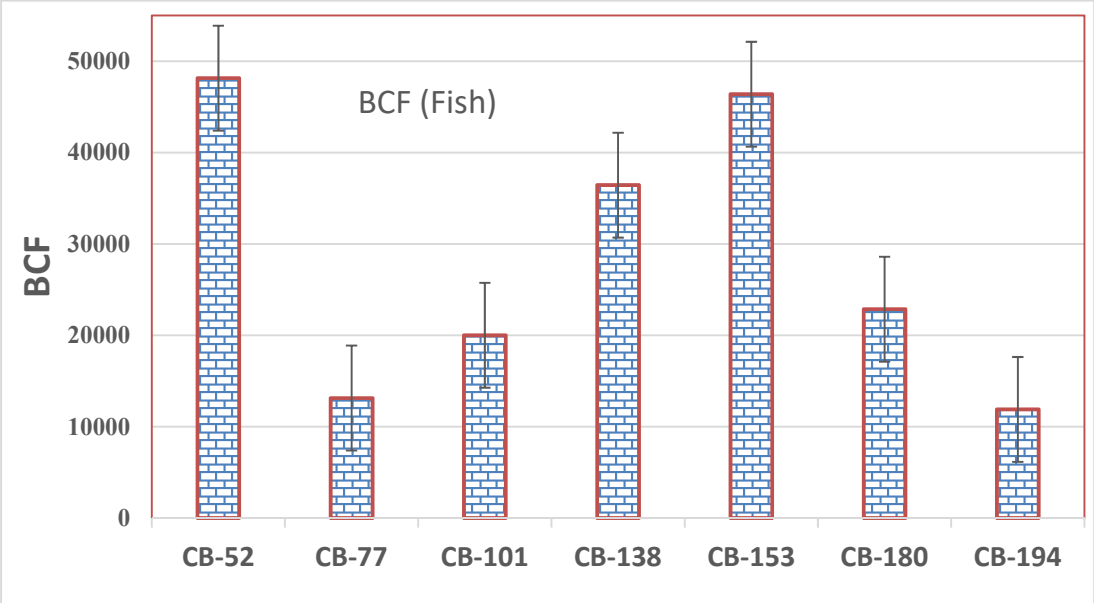


Fig 4.1.8. Bio concentration factor (mean ± standard deviation) for PCB congeners in fish samples collected across Thane creek.

Likewise, the BCF values for PCB congeners were also calculated for fish samples and represented in **Fig 4.1.8**. BCF value was found highest for CB52 and least for CB194, among

analysed PCB congeners with mean value of 4.8×10^4 and 1.1×10^4 respectively. The order of BCFs values for PCBs congeners for fish samples was found as CB194 < CB77 < CB101 < CB180 < CB138 < CB153 < CB52.

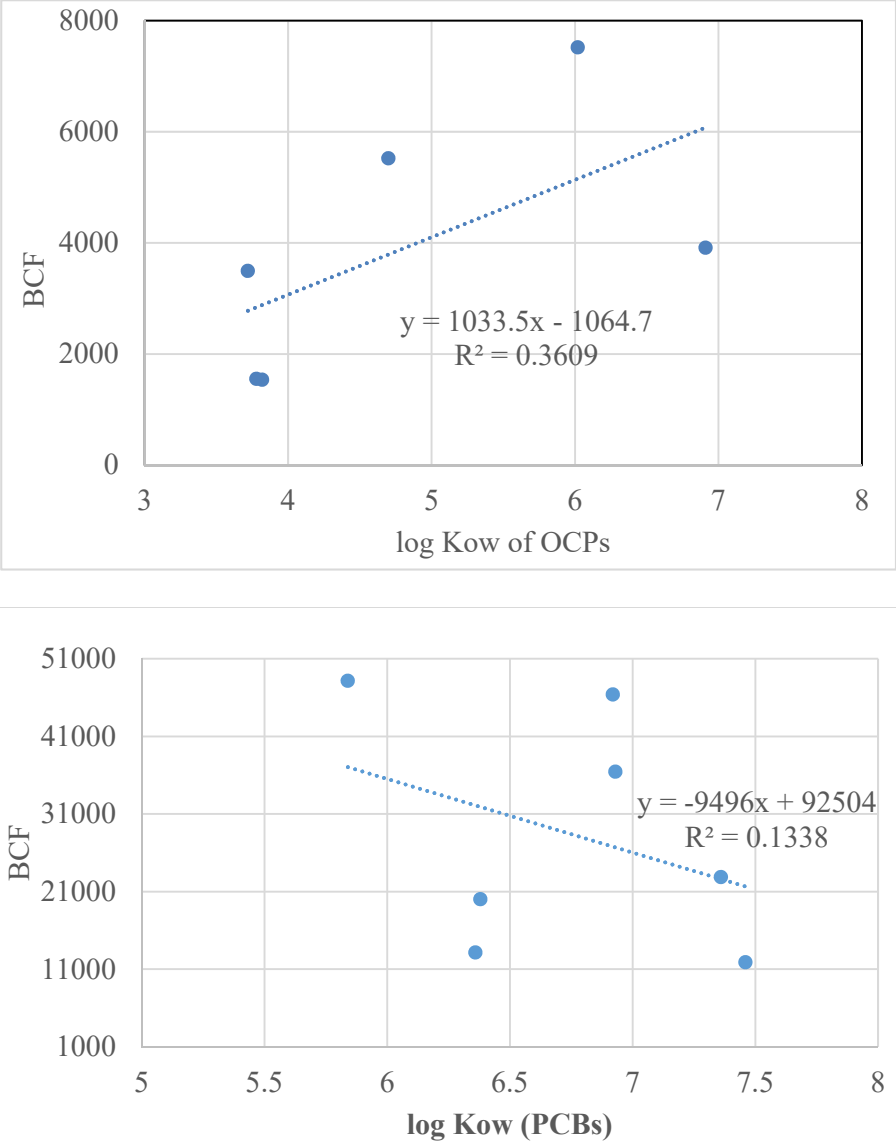


Fig. 4.1.9. Plots of log Kow v/s Bioconcentration Factor (BCF) in fish for select OCPs and PCBs.

The BCF values obtained for PCBs in fish samples were very similar to study from river Emin in southern Sweden except for CB52 [**274**]. It was observed by Bremle et al. (1995) BCFs of low chlorinated PCBs domains were less than these of highly chlorinated [**274**]. However,

above a certain degree of chlorination the BCF was reduced. If we exclude CB52, the bell shape of BCFs was observed in present study. High levels of BCF for CB52 may be due dechlorination of highly chlorinated PCBs metabolisms in fish.

To check the dependency of BCF values of contaminants and their octanol-water partitioning coefficient (log Kow) data were plotted and shown in **Fig 4.1.9**. For both group of contaminants were found poorly correlated (R^2 values are 0.36 and 0.13 for OCPs and PCBs respectively), which indicated there are more possible factors such as bioavailability, metabolism etc. which determined the bioconcentration of these chemical. To evaluate the seawater – sediment exchange of OCPs and PCBs, K'_{OC} and fugacity fraction (ff) were calculated. The fugacity fraction (ff) values for OCPs are represented in **Fig 4.1.10** using a radar plot.

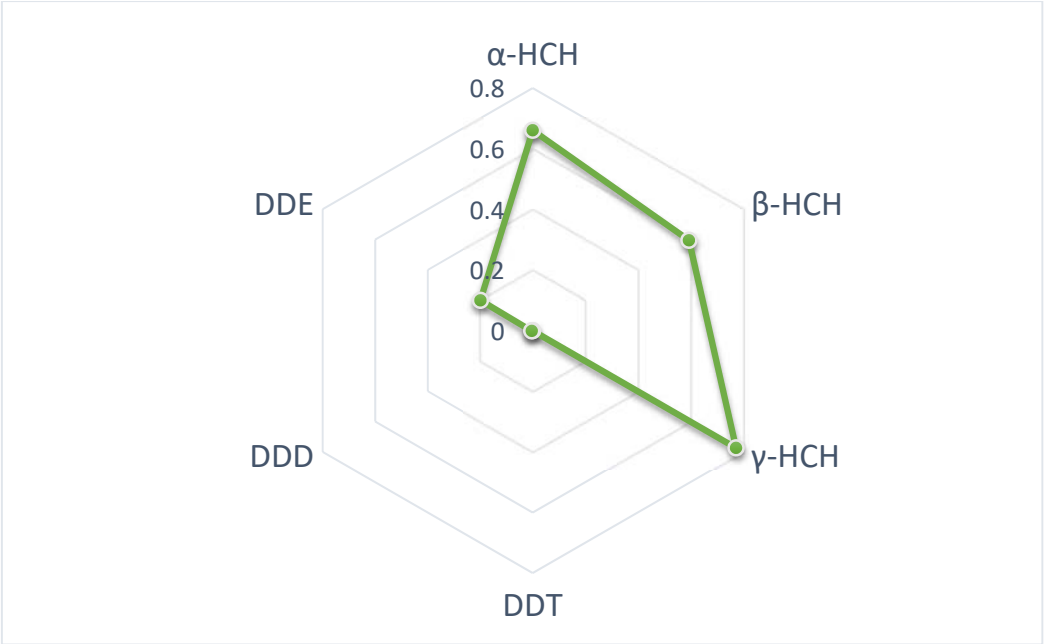


Fig. 4.1.10 Fugacity fraction (ff) between seawater and sediment in Thane creek area for DDT compounds, HCHs isomers.

For DDT and its metabolites, the ff values were found below than 0.5, indicates these chemical have tendency (net flux) to accumulate in sediments from seawater, while this was not same for HCH conformers (ff > 0.5). The fugacity fraction suggests that there is net flux of α-, β-, γ-

HCH from sediment to seawater. Therefore, sediment can act as source of HCH conformers in aquatic system present study area.

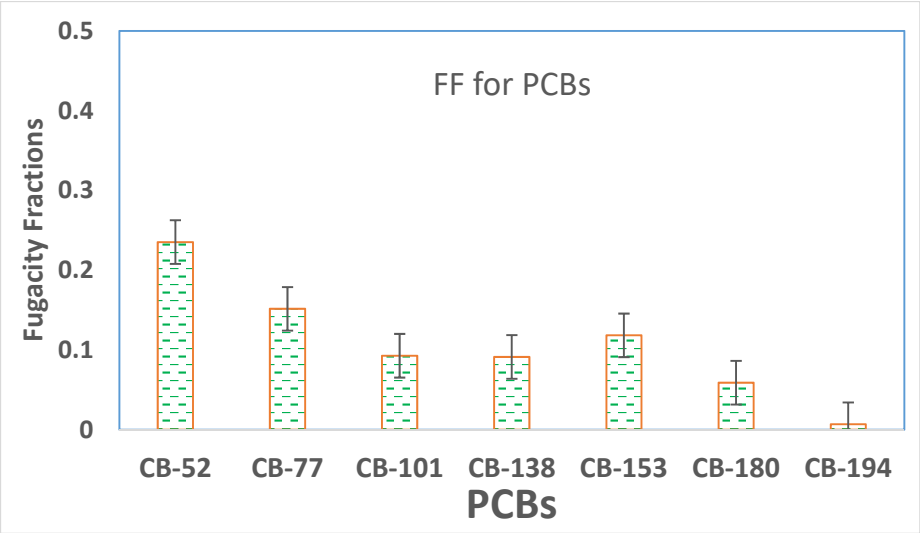
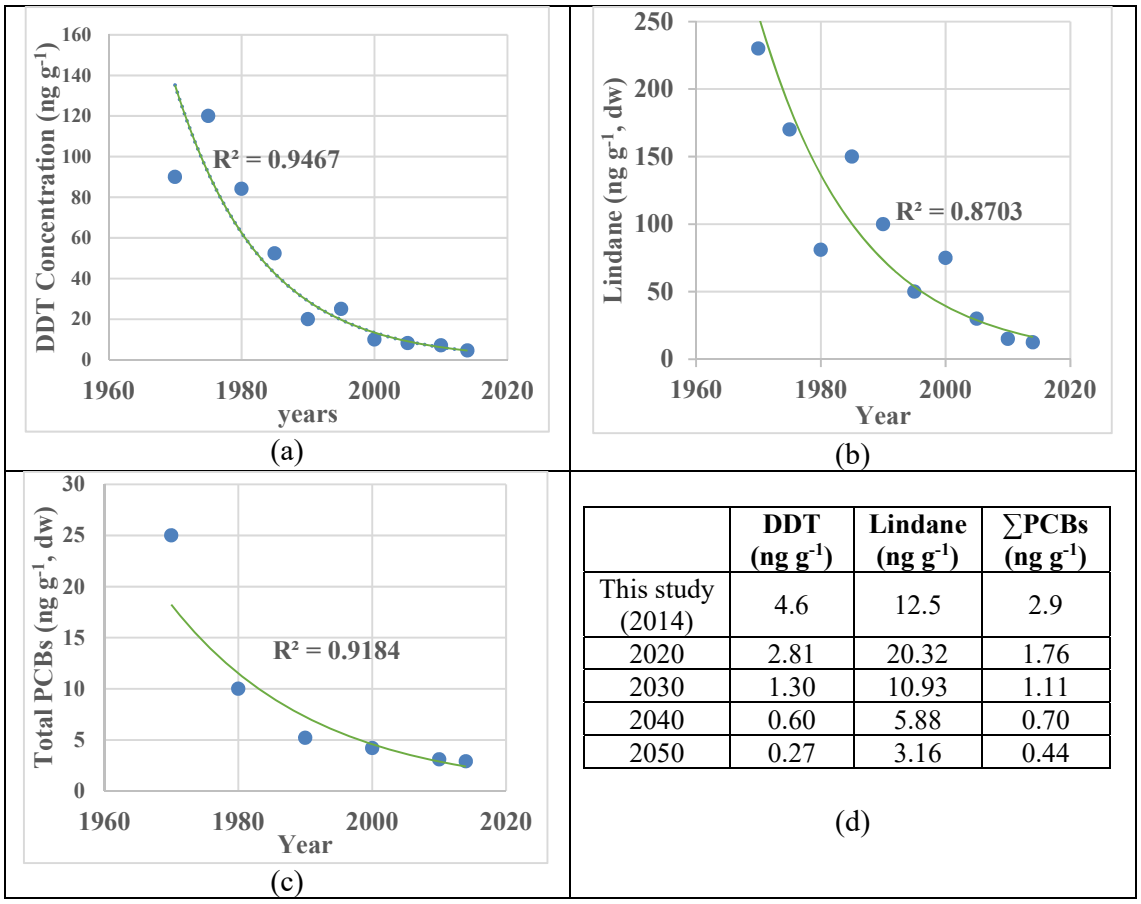


Fig. 4.1.11 Fugacity fraction (ff) between seawater and sediment in Thane creek area for PCBs congeners.

The values of ff for PCBs congeners are shown in Fig **4.1.11**. The ff < 0.5, for PCBs congeners indicating their net flux from seawater to sediment. Higher log K_{ow} values of PCBs may be attributing to such observation.

4.1.6 Future Trends of POPs in sediments

Taking chronological data of OCPs and PCBs in sediment core from Thane creek area since 1970s [**13, 111]**, the levels of these contaminants for year 2014 were added from this study and plotted (**Fig 4.1.12 a), b) c)**). Assuming, there is no future use of these chemical in surrounding area and degradation/bio mixing process were continuing in sediments over the upcoming decade as previously.



	DDT (ng g^{-1})	Lindane (ng g^{-1})	∑PCBs (ng g^{-1})
This study (2014)	4.6	12.5	2.9
2020	2.81	20.32	1.76
2030	1.30	10.93	1.11
2040	0.60	5.88	0.70
2050	0.27	3.16	0.44

(d)

Fig 4.1.12 Historical record of contaminants in core sediment a) DDT, b) Lindane c) PCBs and d) Predicted contaminants levels for upcoming decades in sediment of Thane creek.

Data were extrapolated using their regression equation, and predicted values are represented in **Fig 4.1.12 (d)**. It is observed that there is significant reduction of their abundance in sediment for upcoming decades. Although there are number of limitation for considering such prediction as mentioned previously.

4.2 Endocrine disrupting chemicals and their estrogenic potential in creek environment

Total 15 phthalates compounds were analyzed in sediment, seawater and biota samples from study area. Subsequently, spatial distribution of phthalates in sediments was described. Inter-compartmental behavior and ecotoxicological concerns of phthalates for Thane creek area is

also presented. Subsequently, Bisphenol A and other endocrine disrupting chemicals are reported in sediments, followed by calculation of estrogenic potential.

4.2.1 Phthalate levels in sediment

The concentrations of phthalates in sediment samples collected from locations across Thane creek are shown in **Fig. 4.2.1**. Average total phthalates (fourteen) concentrations varied from 364.8 to 914.2 ng g^{-1} with average value of 656.7 ng g^{-1} in surface sediment samples. Occurrence of PAEs was high at locations L2, L4 and L5; and low at L1 and L6 relative to other locations. Location with high PAEs concentrations ware near the urban runoff receiving points. L1 location is near the freshwater receiving point and L6 is far from shore as compare to other locations which are the probable cause of less PAEs abundant with respect to other sampling points. Dicyclohexyl phthalate was not present in levels of quantification in any surface sediment samples analysed in this study.

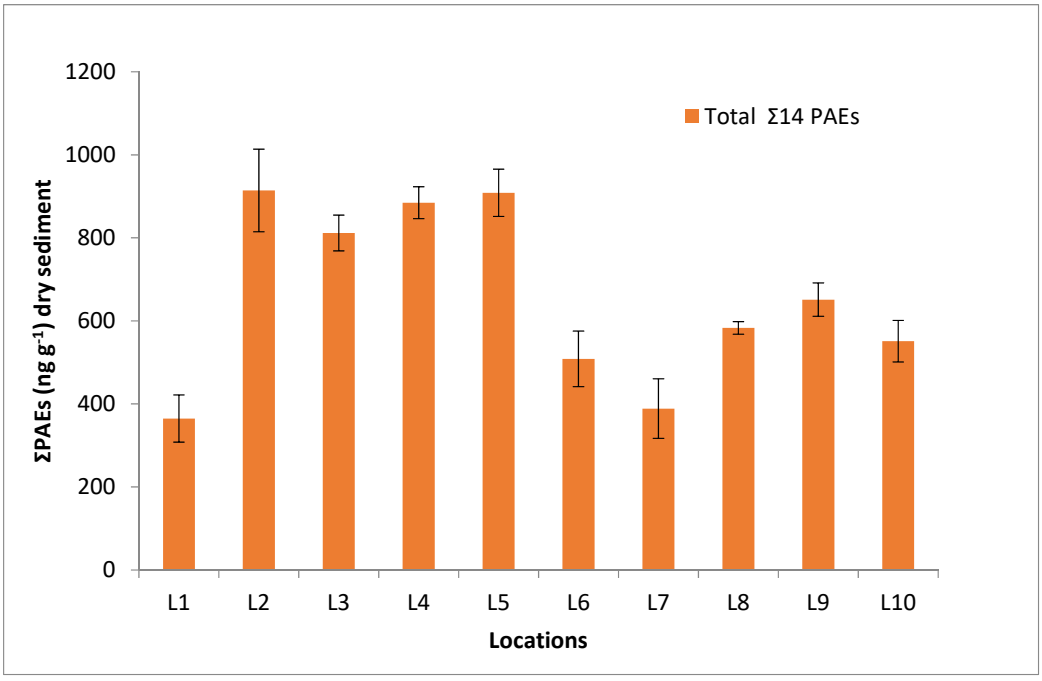


Fig. 4.2.1 Concentrations of total phthalates Σ_{14} PAEs (ng g^{-1}) in surface sediment (n=5, ±1σ) at ten different locations across Thane creek.

Average concentration and deviation of individual phthalates in surface sediments collected across Thane creek are shown in **Fig 4.2.2**. The order of abundance of phthalate esters (PAEs) in surface sediments was as DBP > DIBP > DMPP > DNOP > DEP > DBEP > DNP > DNPP > DMP > DEHP > DEEP > DMEP > DNHP ≈ BBP. DBP had the highest concentration of all fourteen phthalates and was detectable in all sediment samples. DBP level in sediment was on an average two order of magnitude higher than those of DEHP. Although, DEHP was in highest concentrations PAE reported in literature [**134**, **143**]. DNHP, BBP and DEEP are the least abundant with average concentration less than 1 ng g^{-1}. Factors, such as content in technical mixture of PAE products used in area, environmental conditions, photo oxidation and biotransformation in sediment environment may cause the abundance of PAEs in this study can be possible explanation from earlier reported values. DEHP level in eastern German sediment were reported as 0.21–8.44 mg kg^{-1} dw (median: 0.7 mg kg^{-1} dw), which is three order of magnitude higher than in this study (0.2 to 4.2 ng g^{-1} dw) [**133]**.

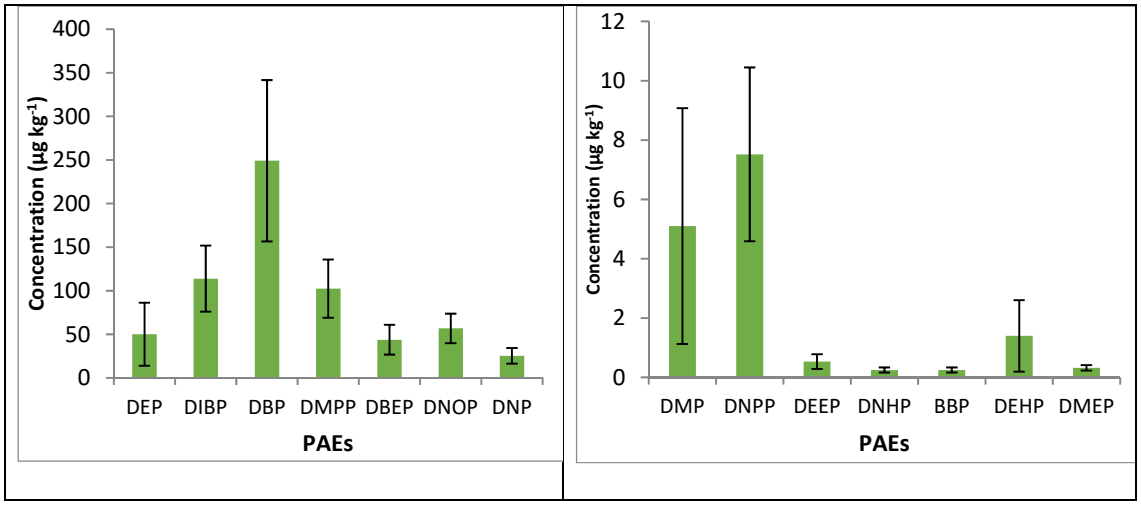


Fig 4.2.2 Average concentration of individual PAEs in surface sediment and variation in them.

The DBP levels of sediment in eastern Germany were reported as 0.2–1.7 mg kg^{-1} dw (median: 0.5 mg kg^{-1} dw), which are comparable with present study (0.13 – 0.4 mg kg^{-1}). Therefore, these results indicated the high environmental burden of DBP in sediments across Thane creek,

as well as that other factors may affect the distribution of phthalate. Spatial distribution of ∑PAEs in sediments across Thane creek, Mumbai is shown in **Fig 4.2.3**.

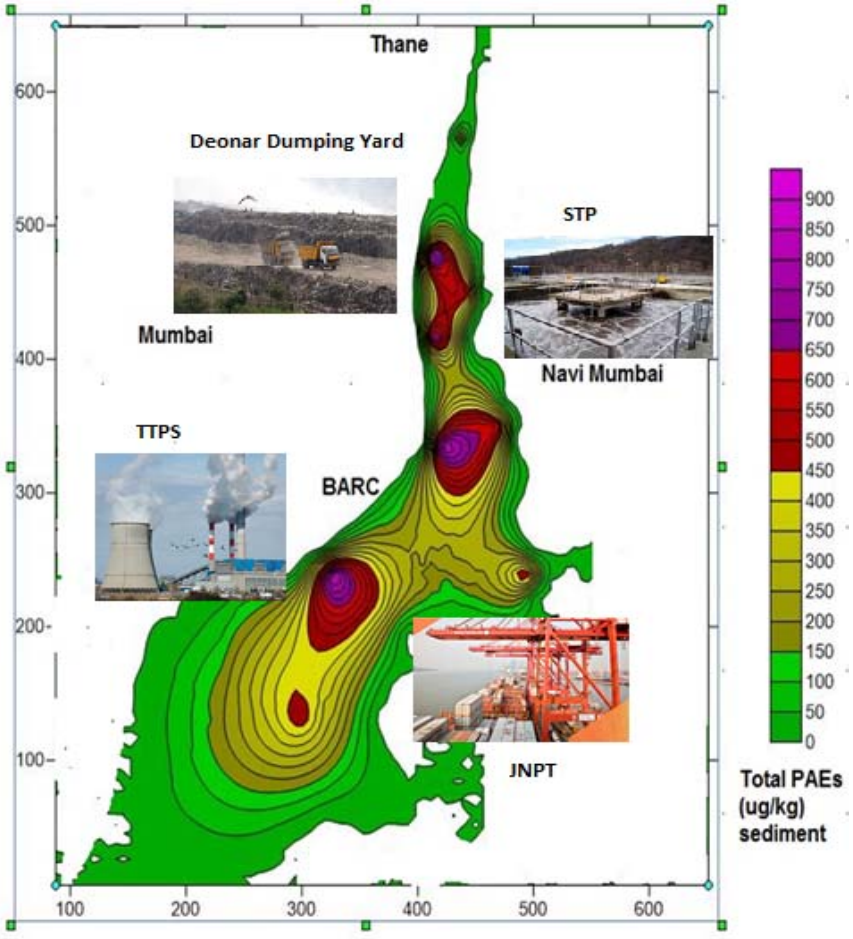


Fig 4.2.3 Spatial distribution of $\sum_{14}$PAEs in sediments across Thane creek Mumbai.

4.2.2 Correlation of organic carbon and PAEs in sediment

Total organic carbon content in sediment were analyzed using TOC analyzer (Shimadzu) and values were found in range of 2.33 to 4.2 % of total mass (dry weigh). Other physicochemical parameters were also measured for sediment sampled and briefly mentioned as following. pH values of sediment samples were measured using pH meter (Eutect PC 510) after dissolving the dry sediment in de-ionized water in 1:5 by volume. pH values of samples were recorded in the range of 7.9 to 8.3 with mean value of 8.2. Particle size characterization of collected grab

sediment samples were also carried out using electromagnetic sieve shaker (Electrolab EMS-8). Contribution of coarse sand (2000-250 µm), fine sand (250-53 µm) and silt and clay (<53 µm) in sediment samples were found to be 15- 49% (35.5%), (44- 67 %) 54.5% and 4-17% (10%) of the total mass respectively. To assess the accumulation pattern and distribution of PAEs in the estuarine surface sediment, total organic carbon content of individual samples was plotted against total PAEs concentration for each location. It was found that the spatial distribution of PAEs generally followed the distribution pattern of TOC. This fact can be well elucidated by correlating TOC (%) values and total PAEs contents of sediment samples.

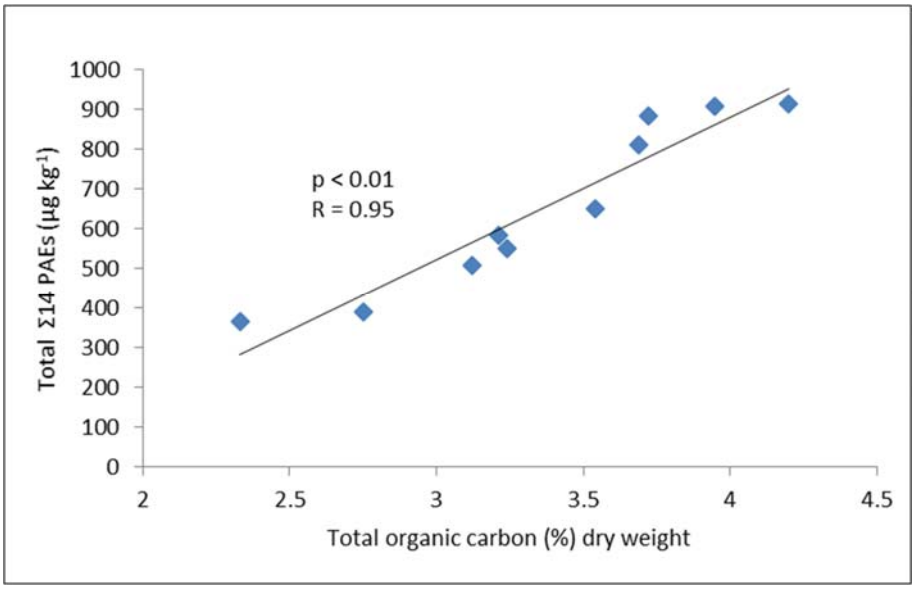


Fig. 4.2.4 Relationship between total organic carbon content and $\sum_{14}$PAEs (ng g^{-1}) in sediment.

A significant correlation (r = 0.95, p < 0.01) was observed between TOC (%) values of sediment samples and total PAEs (**Fig. 4.2.4**). On the basis of these statistical observations, it can be stated that the distribution of PAEs linearly depends upon the increasing %TOC in sediments. Similar observations were reported with river sediment samples in literature [**138]**.

4.2.3 Distribution of Phthalates in Sweater

Table 4.2.1 shows the concentration of PAEs compounds identified in seawater, fish and crab samples across Thane creek. Out of fifteen monitored PAEs except DCP (dicyclohexyl phthalate) were found in tested samples. DCP was not identified in any sample. Total phthalate

esters were found in seawaters at levels from 41.5 to 138.7 μg L^{-1}, with a mean concentration of 104.8 μg L^{-1} and standard deviation of 37.8 μg L^{-1}. DBP was found as most abundant phthalate compound with mean concentration of 25.1 ± 6.8 μg L^{-1} followed by DEHP (19.8 ± 7.1 μg L^{-1}), while DMEP was least abundant (ND - 1.1 μg L^{-1}) among tested phthalic acid esters on seawater. The order of PAEs abundance in seawater was found as DBP > DEHP > BBP > DEP > DIBP > DBEP > DNHP > DMPP > DNP > DNOP > DNPP > DMP ≈ DEEP >DMEP.

Table 4.2.1 Concentration of PAEs (mean ± SD (range)) in seawater (μg L^{-1}), fish (μg g^{-1}, dw) and crab (μg g^{-1}, dw) samples collected across Thane Creek, Mumbai India.

PAEs	Seawater (μg L^{-1})	Fish (μg g^{-1},dw)	Crab (μg g^{-1},dw)
	1.2 ± 0.2	2.6 ± 0.3	5.1 ± 1.3
DMP	(0.1 - 3.1)	(1.2 - 3.5)	(ND - 7.2)
	11.4 ± 4.7	1.2 ± 0.4	10.4 ± 2.1
DEP	(1.9 - 17.1)	(ND - 3.1)	(4.2 - 21.3)
	8.2 ± 1.5	4.6 ± 0.9	4.5 ± 0.8
DIBP	(0.5 - 13.4)	(1.1 - 7.4)	(7.4 - 30.3)
	25.1 ± 6.8	35.1 ± 8.5	54.6 ± 12.3
DBP	(20.1 - 28.4)	(19.1 - 40.2)	(40.2 - 73.2)
	0.5 ± 0.03	1.1 ± 0.2	1.1 ± 0.3
DMEP	(ND - 1.1)	(ND - 2.1)	(ND - 2.1)
	3.2 ± 1.4	2.1 ± 0.4	11.2 ± 0.9
DMPP	(1.4 - 5.9)	(0.5 - 3.1)	(10.2 - 14.2)
	1.2 ± 4.2	1.6 ± 0.5	1.1 ± 0.4
DEEP	(ND - 1.8)	(ND - 2.4)	(ND - 1.6)
	1.7 ± 0.9	1.7 ± 0.9	4.5 ± 1.5
DNPP	(ND - 3.1)	(0.3 - 2.9)	(ND - 7.1)
	3.5 ± 1.2	4.6 ± 1.1	1.1 ± 0.2
DNHP	(ND - 4.2)	(0.9 - 7.2)	(ND - 2.1)
	17.2 ± 5.6	16.7 ± 4.3	7.4 ± 2.5
BBP	(2.5 - 20.5)	(10.2 - 21.6)	(4.2 - 10.4)
	7.9 ± 2.3	7.65 ± 1.4	6.5 ± 2.1
DBEP	(1.5 - 9.5)	(1.2 - 10.7)	(1.2 - 10.8)
	19.8 ± 7.1	31.6 ± 6.7	8.9 ± 3.1
DEHP	(12.3 - 24.2)	(26.4 - 55.5)	(4.5 - 15.2)
	1.8 ± 0.8	2.1 ± 0.7	21.3 ± 6.1
DNOP	(ND - 2.6)	(ND - 4.2)	(4.5 - 40.2)
	2.1 ± 1.1	0.4 ± 0.1	17.2 ± 4.6
DNP	(1.2 - 3.8)	(ND - 0.9)	(7.5 - 21.8)

The total PAEs concertation in seawater across Thane creek was found below than the municipality wastewater influent (153 μg L^{-1}) reported from Catalonia, Spain [**275]**. Measured concentrations of DEP, DBP and BBP in seawater samples were found within range of earlier reported seawater from coastal area of Al-Khobar, Saudi Arabia [**276]**. Levels of DEHP, DEP, and DBP in seawater founds in this study were higher compare to lagoons water from Nigeria [**277]**. Study from North Indian wastewater treatment plants reports, in untreated wastewater with DEHP being present in the highest mean concentration of 28.4 ± 5.3 μg L^{-1}. The average concentrations for DBP and DEHP were 10.57 μg L^{-1} and 28.4 μg L^{-1} respectively in untreated wastewater and 1.3 μg L^{-1} (DBP) and 2.6 μg L^{-1} (DEHP) in treated wastewater [351]. Comparing these values with present study it is observed that DEHP has less concentration compare to untreated waste water effluent, while reverse is observed in case DBP.

4.2.4 Distribution of phthalates in fish

The concentrations of the phallic acid esters identified in fish (*Trachinocephalus myops*) samples are shown in **Table. 4.2.1**. Total concentration of PAEs in fish samples was found between 60 and 164 μg g^{-1} dw with a mean value of 113 ± 36.4 (1SD) μg g^{-1}. DPB was most abundant phthalate compound in fish with concentration of 35.5 ± 8.5 μg g^{-1}, followed by Bis (2-ethylhexyle) with mean concentration of 31.6 μg g^{-1}. DNP was the least abundant among tested phthalate compounds with a concentration ranging from ND to 0.9 μg g^{-1}, and mean of 0.4 μg g^{-1}. The order of PAEs abundance in fish samples was observed as DBP > DEHP > BBP > DBEP > DNHP ≈ DIBP > DMP > DMPP > ≈ DNOP > DNPP > DEEP > DEP > DMEP > DNP. A study from Taiwan reports as DEHP was the predominant compound in fish samples, followed by BBzP and DBP. The highest mean DEHP level was 61.8 (1.7–253.9) μg g^{-1} dw for *L. subviridis*, followed by 33.6 (1.4–129.5) μg g^{-1} dw for *O. miloticus niloticus*, in individual fish samples [**143]**. The levels of phthalates(Σ7) in three fish species from the Orge river, of Ile-de-France was 2.250–5.125 μg g^{-1} dw, which was quite less from this study [**273]**. DEHP

values in fish samples Thane creek were found below the reported values in Huang et al. 2008, but high levels DPB compare to DEHP may be of concern.

4.2.5 Distribution of Phthalates in Crab

The concentration of PAEs in crab (*Brachyura*) samples from Thane creek are shown in **Table 4.2.1**. Phthalates concentration in crabs was found to be vary between 84 and 258 μg g^{-1}, with a mean value of 155 μg g^{-1}. DBP was found to be most abundant PAEs among tested compounds with average concentration of 46.6 ± 12.3 μg g^{-1}. The high concentration of DBP in crab samples from Thane creek can be due to its high levels in sediment from this area as discussed earlier. The order of abundance of PAEs in crab was found as DBP > DNOP > DNP > DMPP > DEP > DEHP > BBP > DBEP > DMP > DIBP ≈ DNPP > DMEP ≈ DEEP ≈ DNHP. DEHP concentration in crabs were found in similar order of magnitude (i.e. 1-10 μg g^{-1}) as reported from Urdaibai estuary (Bizkaia, Basque Country, Spain) by Chalera et al. (2004) **[237]**.

4.2.6 Inter-compartmental behaviour of PAEs in marine environment

Several lab scale studies have investigated the bio-concentration of phthalate esters in molluscs, crustacean, fish species, algae, polychaetes, aquatic insects, macrophytes, and in many other organisms. The data reported in these studies have been compiled and reviewed in Staples et al. (1997) **[4]**. In situ bio-concentration factors (BCFs) were calculated for all fourteen analyzed phthalates in both fish and crab samples with respect to their seawater concentration. BCFs values of individual phthalates are shown in **Fig 4.2.5** for fish and crab. The BCFs value for fish vary between 105 (DEP) with 2, 200 (DMEP) with average value of 1116. For crab samples BCFs values for most of the phthalates were found higher compare to fish in the range of 314 to 11, 833 with a mean value of 2,799. BCFs for DNOP and DNP in crab was found highest compare to other PAEs, this may due to their higher log K_{ow} values which are 8.1 and 9.52 respectively.

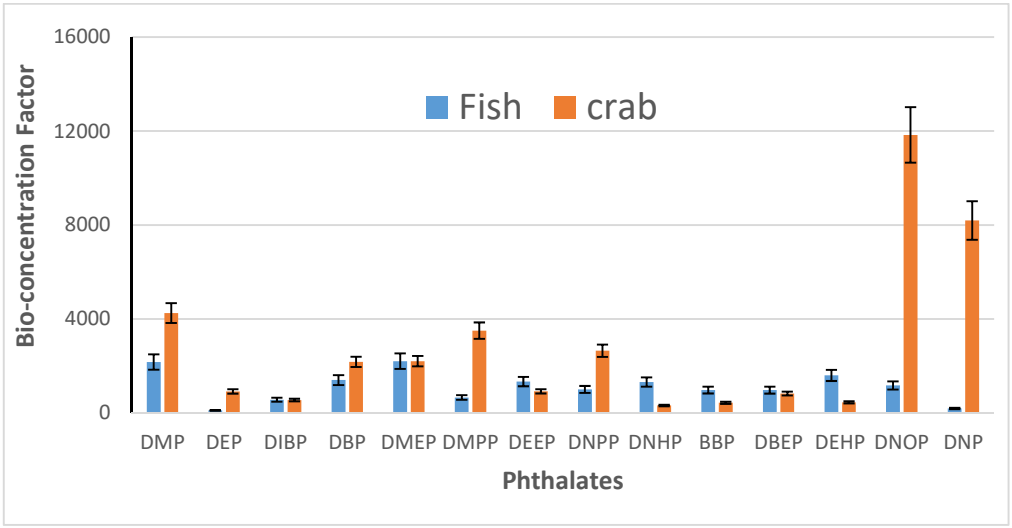
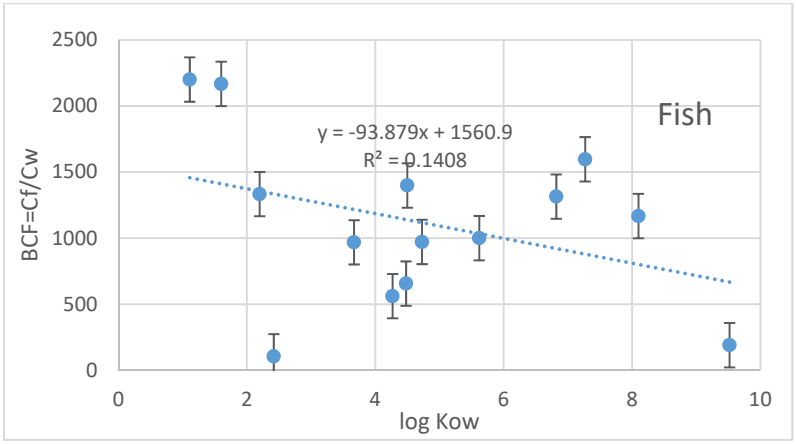
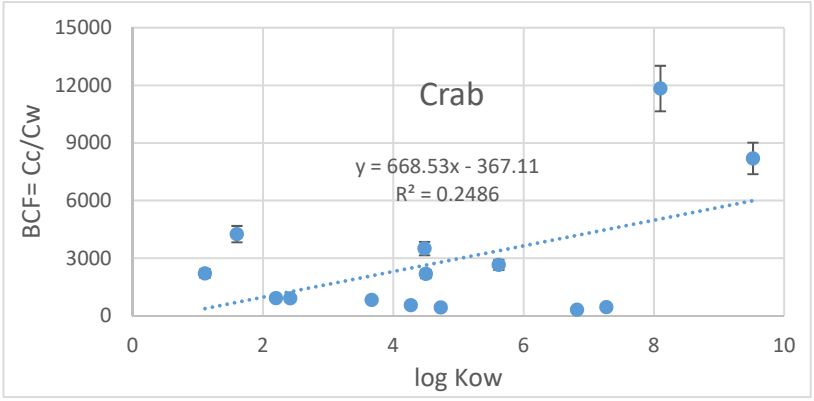


Fig. 4.2.5 Bio-concentration factor for analyzed phthalates in fish and crab samples from Thane creek.



(a)



(b)

Fig 4.2.6 Plots showing the dependency of bio concentration factor a) fish, b) crab to the log Kow of selected phthalates.

In situ fish and crabs BCFs are plotted as a function of log K_{ow} of phthalates and shown in **Fig 4.2.6.** The observed values of BCFs do not correlated to K_{ow} i.e. liner correlation coefficients ($p < 0.01$) were found 0.14 and 0.24 for fish and crab samples respectively. For fish samples BCFs values were found higher to the phthalates having low less K_{ow} values. In literature such results are attributed to metabolism and diffusion of phthalates in marine environment [**278, 279]**. BCFs for the water soluble phthalates (lower value of log k_{ow}) were underestimated by about an order of magnitude due to the high elimination from organisms. Data for aquatic organisms also indicate BCFs were lower compare to octanol-water partitioning and do not correlate with phthalate ester hydrophobicity [**4]**.

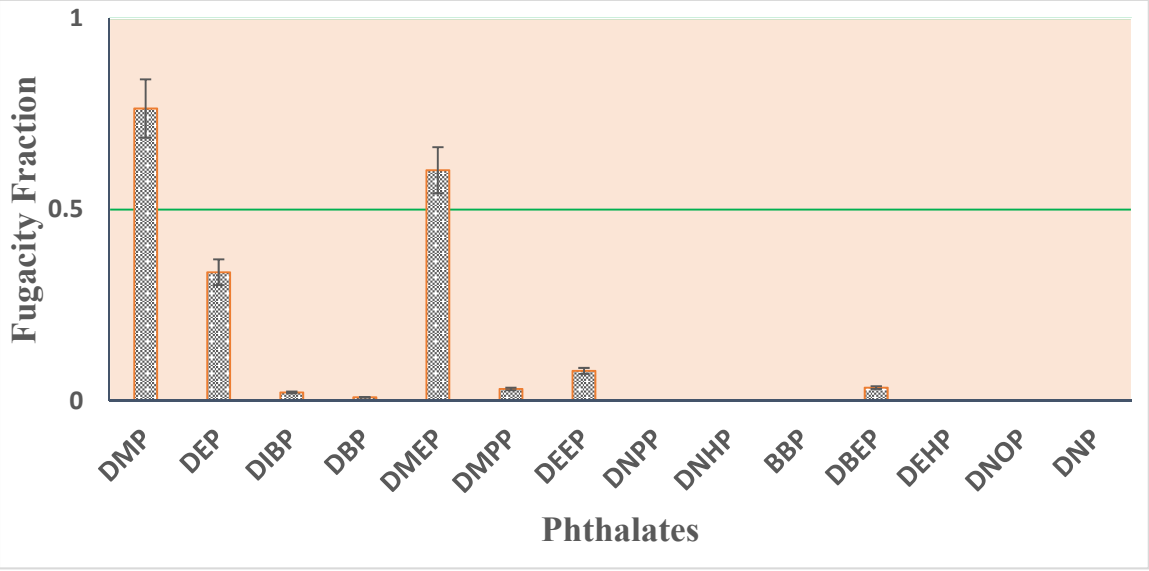


Fig 4.2.7 Fugacity fraction of phthalates for assessing their seawater-sediment exchange in Thane creek area.

Phthalates have high log K_{ow}, which makes them to attach with the organic matter present in the aquatic environment. To evaluate the sediment – seawater exchange of phthalate compounds their fugacity fractions were calculated and presented in **Fig 4.2.7**. It was observed that DMP and DMEP escape from sediment and tends to dissolve in seawater (ff > 0.5). Fugacity fraction values of all other phthalates indicates their accumulation (net flux) in sediment from seawater as their ff <0.5.

4.2.7 Eco-toxicological concern of Phthalates across Thane creek

The seawater concentrations of DBP and DEHP were compared with their predicted no effect concentration (PNEC) values recommended by European Commission (1996), which are 10000 ng L^{-1} and 1300 ng L^{-1} respectively [**280**]. Seawater concentrations of DBP and DEHP were found to be much higher compare to their PNEC values for Thane creek. The RQ is used to checked the ecological risk of the phthalates in creek environment, and RQ relates to measurable environmental concentration (MEC) and predicted no effect concentration (PNEC), as RQ = (MEC/PNEC). The calculation of RQ is an essential step for risk assessment of phthalate exposure. As MEC was larger than the PNEC resulting in RQ larger than 1, indicates chemical was risky to human being or the environment [**281]**. RQ values for DBP and DEHP in seawater were calculated and found as 2.5 and 15.2 respectively which indicated that these compounds had high ecological risk.

The estrogenic activity of seawater in terms of DEHP and DBP was calculated. The estrogenic potential of phthalates can be represented by the estradiol equivalent (EEQ) concentration, which can be obtained by the estradiol equivalency factor (EEF) and the MEC as EEQ = EEF × MEC. EEF values for DBP and DEHP were taken as 95th percentile of their distribution and were 2.5 × 10^{-6} and 1.3 × 10^{-5} respectively. EEF values were based on relative estrogenic potential of compound to estradiol (E2) for which EEF is unity [**11]**. Total EEQ i.e. due to phthalates (DBP and DEHP) is calculated as equation (5).

$$EEQ_{total} = EEQ_{DBP} + EEQ_{DEHP} \quad (5)$$

Calculated mean value of EEQ for DBP and DEHP were found as 0.06 and 0.25 ng L^{-1}, total value of EEQ due to both compound was calculated as 0.32 ± 0.1 ng L^{-1}. The EEQ_{Total} for sweater was less than 1 ng-E2/L, indicating that on average, the phthalates alone could not probably cause endocrine disruption in marine organism in Thane creek. As EEF values are

low for DBP and DEHP which results in low EEQ values for them, major attributors to estrogenic potential of seawater may be steroidal estrogens contains estrone (E1), E2, estriol (E3) and EE2; the second category phenolic compounds contain nonylphenol (NP) and bisphenol A (BPA).

Other types of endocrine disruption effects, which are not reflected through the EEQ are describe hereafter. The term endocrine disruptor can be used synonymously with hormone disruptor. The concept that endocrine disruptors encompass more than just environmental estrogen and include any agent that adversely affects any aspect of the entire endocrine system. Endocrine disruptors can mimic, enhance (an agonist), or inhibit (an antagonist) the action of hormones. They may act as hypertrophic (stimulatory) agents and tumor promoters in different organisms. Dose, body burden, timing, and duration of exposure at critical periods of life are important parameter to consider for assessing detrimental effects of an endocrine disruptor. Effects may be reversible or irreversible, immediate or latent. The endocrine system includes a number of central nervous system (CNS)-pituitary-target organ feedback pathways involved in regulating a multitude of bodily functions and maintaining homeostasis. Furthermore, due to complexity of the cellular processes involved in hormonal communication, any of these loci could be indulged mechanistically in a toxicant's endocrine-related effect.

To quantify dietary intake and associated risk of phthalates through marine food consumption daily intakes values were calculated. Calculation of daily intakes (DI, µg/kg-bw/day) of phthalate esters through fish and crab is carried out using equation (6).

$$DI = \frac{(C_{food} \times IR \times EF \times ED)}{(BW \times AT)} \quad (6)$$

C_{food}, PAE concentration in food (mg kg^{-1}); IR, ingestion rate of marine organism (kg day^{-1}) 0.014 kg (wet wt) day^{-1}; EF, exposure frequency (365-day $year^{-1}$); ED, exposure duration (62

years); BW is the average body weight of people (70 kg for adults); AT is the average lifespan (25,500 days) [**282, 331, 332]**. Parameters and values used were observed data such as PAE contents, published literature and reports on population exposure [**283]**. Daily intake (µg/kg-bw/day) values for tested phthalates were shown in **Fig. 4.2.8** for both fish and crab samples.

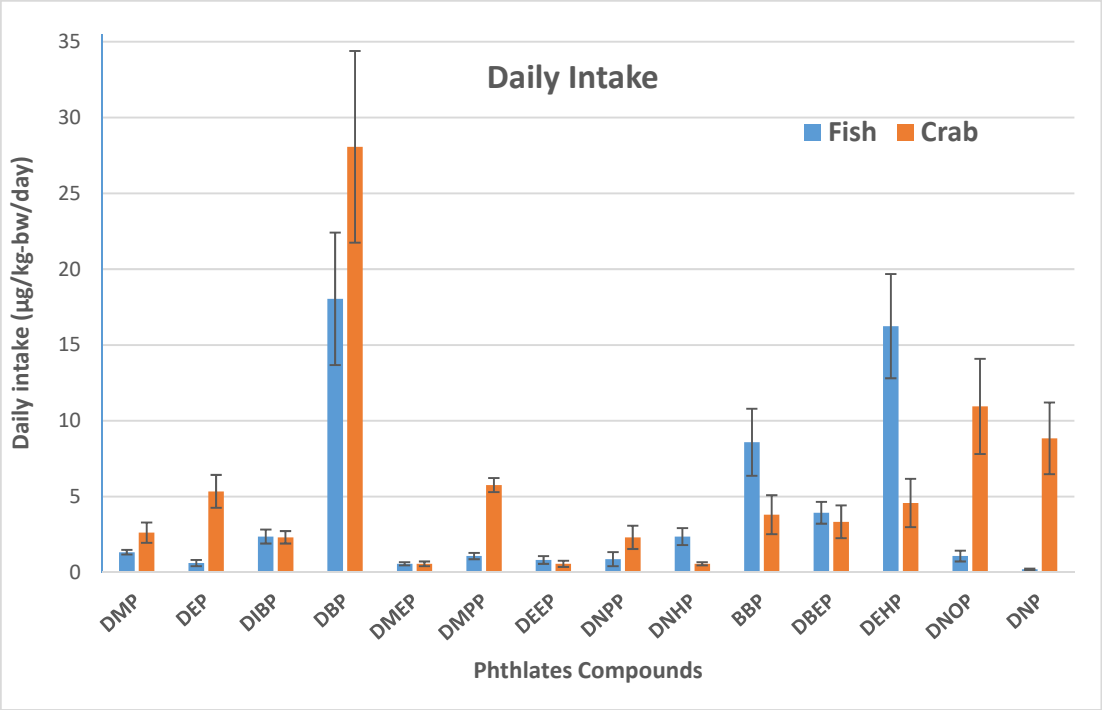


Fig 4.2.8 Daily intake values of PAEs on consumption of fish and crab from Thane creek.

The order of daily intake values was same as their abundance in organisms (fish and crab). The average values of total PAEs daily intake were calculated as 58.1 ± 13.6 and 79.6 ± 19.6 (µg/kg-bw/day) for fish and crab respectively to an adult population. DEHP daily intakes for fish and crabs were estimated as 16.24 ± 3.4 and 4.57 ± 1.6 (µg/kg-bw/day) respectively, were below the EPA's reference dose of 20 µg/kg-day for risk of increased liver weight and the EFSA's total daily intake of 50 µg/kg-day for developmental risk of testicular toxicity for women of reproductive age and adolescents [**284, 285]**. Although, in case of fish consumption exceeded the ADI for reproductive malformations in females (11.5 µg/kg-day) [**286]**.

4.2.8 Distribution of BPA in sediments

BPA is a widely used chemical in the manufacturing of Polycarbonate (PC) plastics and epoxy resins. Leaching of BPA from PC plastics into water has been reported earlier [**287]**. Kitada et al. (2006) suggest that sediments might offer an advantage in evaluating contamination by BPA because BPA concentrations in sediments are higher than in water [**288**]. Location wise BPA concentrations in surface sediment samples collected across Thane estuarine creek were represented in **Fig. 4.2.9**.

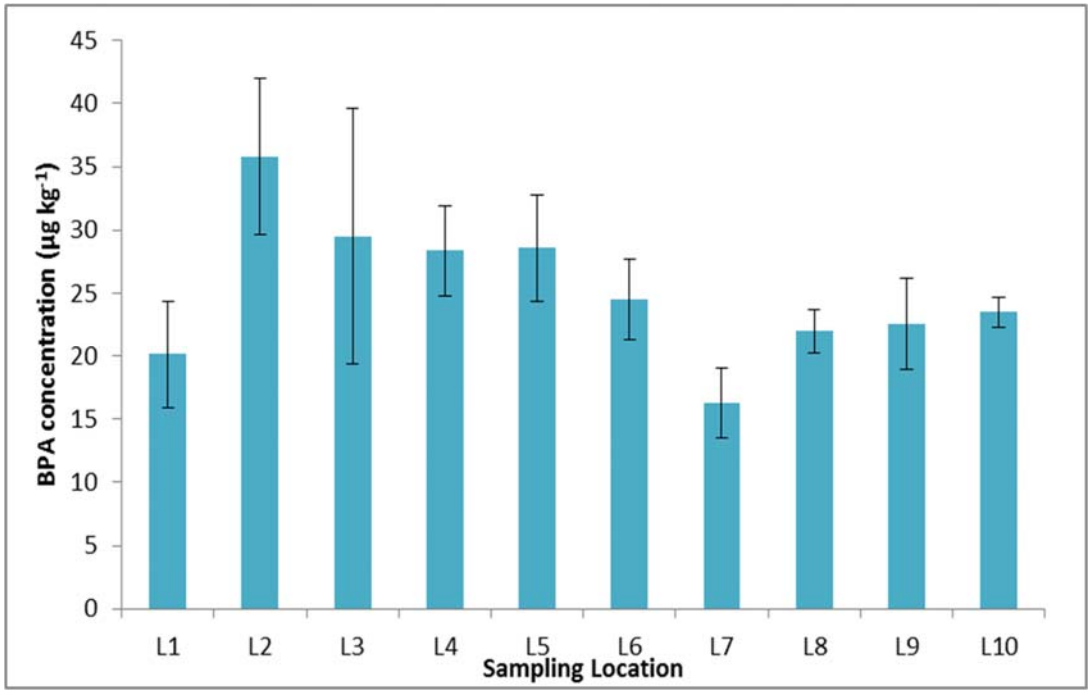


Fig 4.2.9 Spatial variation of BPA concentrations (n=5, ±1σ) in sediment samples at 10 selected sites across Thane creek.

BPA was detected in all samples; average BPA concentration varies from 16.3 to 35.79 ng g^{-1} with mean value 25.15 ng g^{-1} dry weight of sediment. Concentrations of BPA in sediment are found within range of values reported in earlier literature [**157]**. Studied the distribution of BPA in sediments collected in the Jiaozhou Bay (China) and surrounding rivers; BPA was detected in all samples; concentrations between 0.7 and 20.3 ng g^{-1} d.w. in Bay samples and at

concentrations between 2.4 and 27.3 ng g^{-1} in river sediments [**289]**. Another study investigated the occurrence of BPA in sediment samples collected at four stations in the Venice Lagoon close to municipal wastewater and industrial discharges; whereas BPA was detected values up to 118 µg/kg d.w. in the sampling station nearest to the plant discharge [**166]**. Liao et al. (2012) reports highest concentration of BPA was found in sediment from Korea (mean: 567, median: 6.02 ng g^{-1} dw), followed by Japan (8.17 and 8.30 ng g^{-1} dw), and the U.S. (5.14 and 1.49 ng g^{-1} dw) [**290**]. The spatial distribution of BPA in sediment found in very similar as for PAEs discussed earlier. The prime sources of BPA in Thane creek sediment are may be major heavy chemical industrial activity and domestic wastewater; because PC plastics are used for domestic applications such as food packaging and plastic bottles for water, and epoxies are also used as a coating for polyvinyl chloride water drainpipe walls.

4.2.10 Distribution of other EDCs in sediments

Apart from BPA and PAEs in surface sediment samples were also monitored for the levels of other EDCs viz. Estrone, 17β- estradiol, 17α- ethinylestradiol, Octylphenol, and Nonylphenol. To the best of our knowledge, these are the first data about contamination from EDCs in the surface sediment collected across Thane creek area. Order of average occurrence of EDCs in sediment samples observed in this study is Nonylphenol > Octylphenol > 17α- ethinylestradiol > Estrone > 17β- estradiol. The average concentrations of above mentioned EDCs from sampling locations are reported in **Table 4.2.2**. Spatial distribution of EDCs in sediment for Thane creek is represented in **Fig 4.2.10**. Nonylphenol was found at the highest concentration in surface sediment samples ranges from 234.6 to 537.8 ng g^{-1}, average 356.5 ng g^{-1}; as NP is lipophilic (K_{ow}: 5.76), which cause a dominant accumulation in sediment.

Table 4.2.2 Concentration (ng g^{-1}) of select EDCs in surface sediments samples ($\mu \pm 1\sigma$, n=3) from ten different stations across Thane creek.

Stations	Estron	17α Eth.estradiol	17β estradiol	4-ter Octylphenol	4-Nonylphenol
L1	13.4 ± 0.78	20.4 ± 2.02	6.8 ± 0.57	180.21 ± 7.21	353.42 ± 42.3
L2	9.4 ± 0.4	16.43 ± 1.45	5.47 ± 0.57	190.32 ± 12.2	413 ± 25.7
L3	12.4 ± 1.23	14.47 ± 1.34	4.82 ± 0.36	209.46 ± 15.9	418.93 ± 26.7
L4	12.2 ± 0.95	18.62 ±0.96	6.20 ± 0.54	217.4 ± 20.3	433.51 ± 25.4
L5	6.5 ± 0.57	20.42 ± 1.95	6.80 ± 0.75	187.92 ± 27.35	375.84 ± 15.2
L6	14.5 ± 1.23	29.17 ± 2.15	9.72 ± 1.24	107.35 ± 11.7	234.56 ± 25.34
L7	10.1 ± 1.21	23.12 ± 2.1	7.70 ± 0.85	127.5 ± 10.54	257.65 ± 30.2
L8	9.6 ± 0.75	26.74 ± 1.95	8.91 ± 0.75	268.89 ± 23.15	537.78 ± 60.75
L9	13.4 ± 1.32	19.14 ± 1.65	6.38 ± 0.77	135.86 ± 10.35	271.72 ± 21.75
L10	16.3 ± 1.57	22.29 ± 2.65	7.43 ± 0.57	134.14 ± 17.4	268.29 ± 26.35

Peng et al. (2006) reports NP were detected in the range from 204.2 to 664.5 ng g^{-1} in sediment samples from Pearl river estuary, south China sea [**291**]. However, the reported concentrations were generally similar or higher than those previously recorded in other environments [**166, 292, 293]**. Average Octylphenol concentration in the surface sediments of the Thane creek amounted to 176 ng g^{-1} dw (**Table 4.2.2**) and was significantly higher than in other regions of the world. Reported OP levels in sediments from the Thermaic Gulf in Greece were 10.3 ng g^{-1} dw [**294]**, and in sediments from the Mediterranean coast of Spain, OP levels were 61 ng g^{-1} dw [**147**]. Another study by Khim et al. (1999) reports bottom sediments from Masan Bay in South Korea were characterized by mean OP concentrations of 91.5 ng g^{-1} dw [**295**], and equally high concentrations were discovered in bottom sediments on the coast of Taiwan [**296]**. In coast Gulf of Gdansk (Baltic Sea), the highest NP (1.46 ng g^{-1} dw) and OP (6.56 ng g^{-1} dw) amounts were observed in autumn. As was the case with NP, the concentrations of OP were much higher in the sediments of South-East Asia than in the Gulf of Gdansk; while in present study NP were found significantly higher as compare to OP [**297]**.

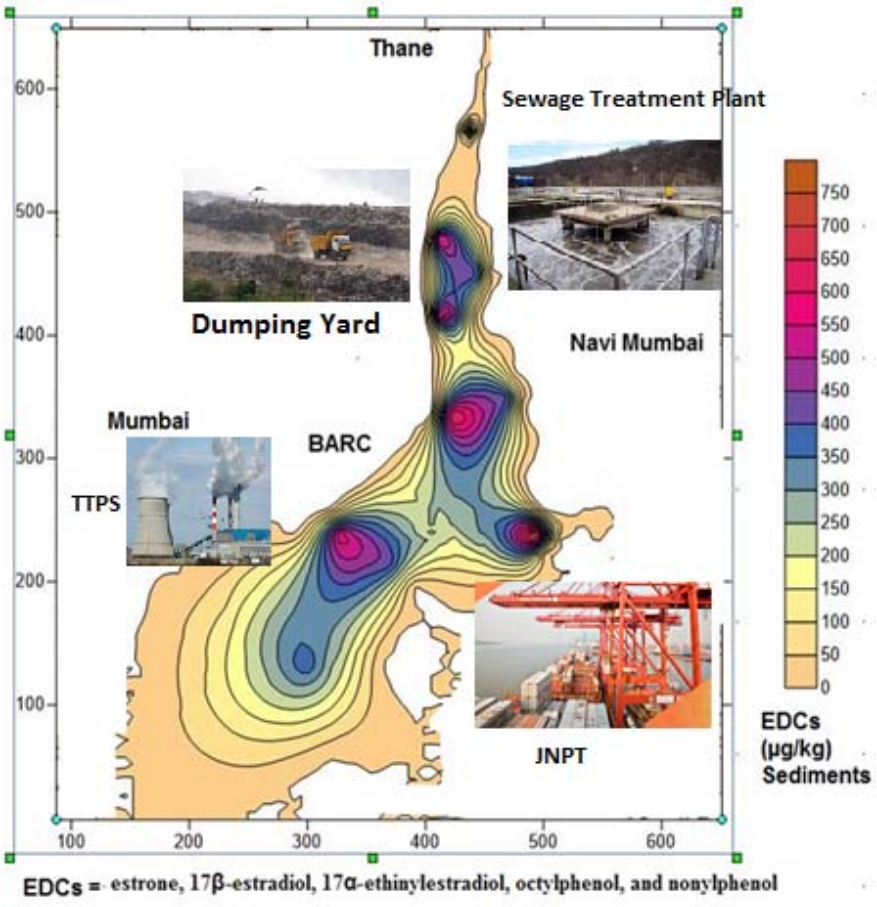


Fig 4.2.10 Map showing spatial distribution of EDCs in sediments across Thane creek.

Estrone was detected in all surface sediment samples; and its concentration varies from 6.5 to 16.3 ng g^{-1} with average value of 11.8 ng g^{-1}. 17α- ethinylestradiol was found to be in range of 14.5 – 29.5 17β- estradiol with average value 21.1 ng g^{-1}, while 17β- estradiol mean concentration was observed 7 ng g^{-1} across Thane creek (**Table 4.2.2**). It was observed that present data of estrogens in sediment samples were higher compare to earlier study; few estrogens were not found in sediment also reported [**166, 291, 298]**). German rivers sediments contained the natural estrogens estrone and 17β-estradiol were detected up to 2 ng g^{-1}; the 17α-ethinylestradiol was extracted with a maximum of 0.9 ng g^{-1} [**298]**. The most contaminated sediment was found at station 8 with the EDCs discussed in this section relative to other sampling station followed by station number 4, 3 and 2. Those concentrations were higher in

the part of the creek, which is directly receiving huge quantities of pollutants from adjacent rivers/ outflow containing a large amount of municipal and industrial (pharmaceutical) wastewater across thane creek. High concentrations of estrogen in sediments samples also indicate the input sewage sludge across Thane creek [**298, 299**].

4.2.11 Estimation of estrogenicity of sediments

The estrogenic activity can be described as an interference caused by the environmental EDCs which interfere or damage the organism's endocrine system in the ecosystem and the correlative system. The estrogenic activity interference encompasses interference effects on reproduction and growth of the organisms, disorders on endocrine system and nervous system, and abnormality on immune function [**239, 240]**. For environmental and ecological systems, EDCs is mainly studied based on the biological individual cells, sub-organ or organs and tissue to reveal the influence mechanism for biological population, biotic community and system level in the ecological system [**11]**. The estrogenicity of the sediment samples were calculated in terms of the estradiol equivalent concentrations (EEQs). The EEQs for analyzed compounds were calculated by using estradiol equivalent factor (EEF), defined as the quotient of $EC_{50E2}/EC_{50compound}$ [**241]**; shown in equation (7).

$$EEQ = EEF \times MEC \tag{7}$$

Each individual average EEF value was multiplied by the measurable environmental concentration (MEC) of the corresponding EDC to obtain an EEQ value for each analyzed chemical. To calculate the level of estrogenic potential in sediment, the total EEQ based on the EEQ of single estrogenic EDC were calculated by equation (8). EEQ of each compound was calculated by the 95th percentile of EEF reported elsewhere [**11]**.

$$EEQ_{total} = \sum EEQ_i = EEQ_{E1} + EEQ_{E2} + EEQ_{EE2} + EEQ_{BPA} + EEQ_{NP} + EEQ_{DBP} + EEQ_{DEHP} \quad (8)$$

Whereas; E1 (Estrone), E2 (17-β-Estradiol), EE2 (17-α-Ethynylestradiol), NP (4-para-nonylphenol), BPA (Bisphenol A), DBP (Di-n-butylphthalate), DEHP (Bis(2-ethylhexyl) phthalate). The concentrations of individual EDCs in sediments were determined using chromatography technique, while the EEFs were obtained from scrutiny examination of most recent values reported in the literature [**11]**. The EEF values were 0.61, 1, 5.11, 1.00×10^{-3}, 4.90×10^{-4}, 2.50×10^{-6} and 1.3×10^{-5} for E1, E2, EE2, BPA, NP, DBP and DEHP respectively, the EDCs contributing significantly (< 0.1%) to the total EEQs are E1, E2, EE2. The resulting EEQ values for surface sediment are shown in **Fig. 6** for all monitoring locations across Thane creek. The EEQs ranged between 87.1 to 169 ng g^{-1} with a mean value 123.1 ng g^{-1}. Station 6 exhibit the highest EEQs level, while station 3 was less contaminated with respect total estrogenic potential. Earlier study on the effects of EDCs contaminated sediments on the mud snail (*Potamopyrgus antipodarum*) reported increase in the number of sheltered embryos already at 1 μg kg^{-1} (d.w.) EEQ level [**300]**. Taking this into consideration that all of the analyzed sediment samples exhibited EEQ much more than 1 μg kg^{-1}, the Thane creek sediment is strongly expected to be, specifically for the sediment feeder biota.

It is worthwhile estimating EEQs from chemical analysis is to identify the compounds most contributing to the total estrogenic potential. Percentage contributions of compounds to the total EEQs of sediments were analyzed. 17-α-Ethynylestradiol (EE2), estrone (E1) and 17-β-Estradiol (E2) were the main contributors to the overall EEQs in sediment, their average percentage contributions are 87.53, 6.6 and 5.71% respectively. And the combined contribution from NP, BPA, DBP and DEHP is less than 1% to the total estrogenic potential; values of EEF were low for these compounds although have significant concentration in sediments as for E1, E2 and EE2. In this study Estriol (E3) and Diethylstibestrol (DES) were not considered for

total EEQs as they were not analysed in the sample; so EEQs value may higher than those reported in present study.

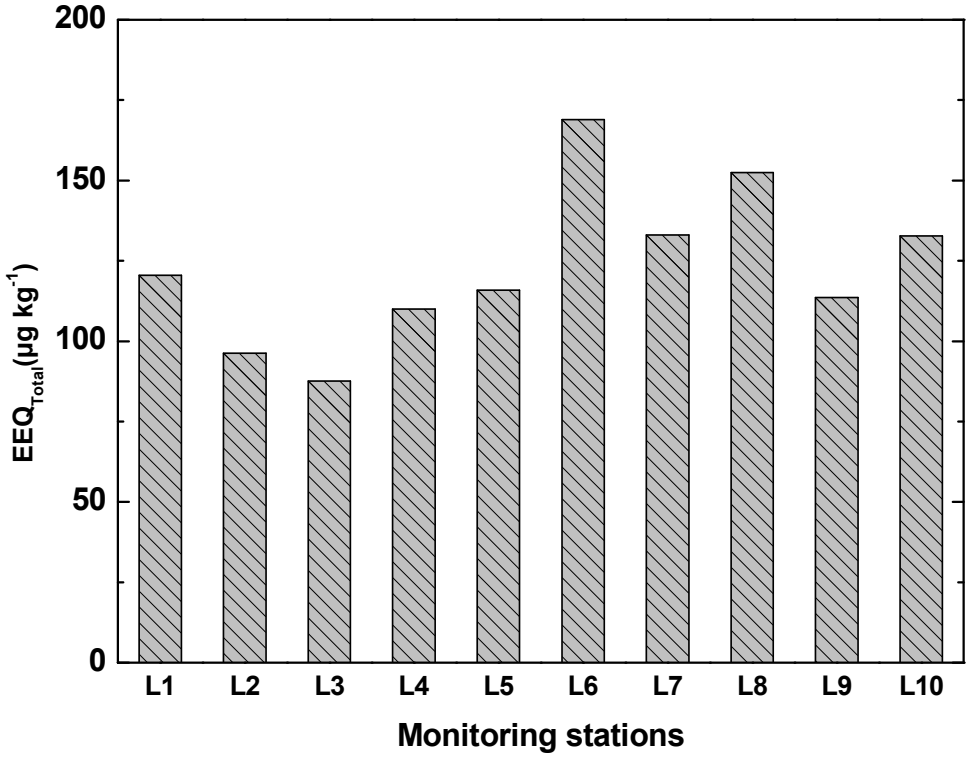


Fig. 4.2.11 EEQ_{Total} (estradiol equivalent concentrations) of estrogenic EDCs in surface sediment samples collected across Thane creek.

4.3 Polycyclic aromatic hydrocarbons (PAHs) in Thane creek, inter-compartmental behavior and their ecotoxicological concerns.

Concentrations of sixteen US EPA priority PAH in sediments, seawater and biota samples for two season (winter and summer) are presented in this section. Distribution data of PAHs are further used for inter-compartmental behavior to assess their fate in creek environment. PAHs level in sediments are compared with sediment quality guideline to assess their ecotoxicological. Finally, human health risk assessment was carried out for the exposure of PAHs via marine consumables from study area.

4.3.1 PAHs in sediment

Total concentrations of all measured sixteen USEPA priorities PAHs (Σ_{16}PAHs) at each sampling location of the Thane creek in different seasons (winter and summer) in a year are shown in **Fig. 4.3.1,** while their spatial distributions in terms of BaP_{eq} are shown in **Fig 4.3.2.**

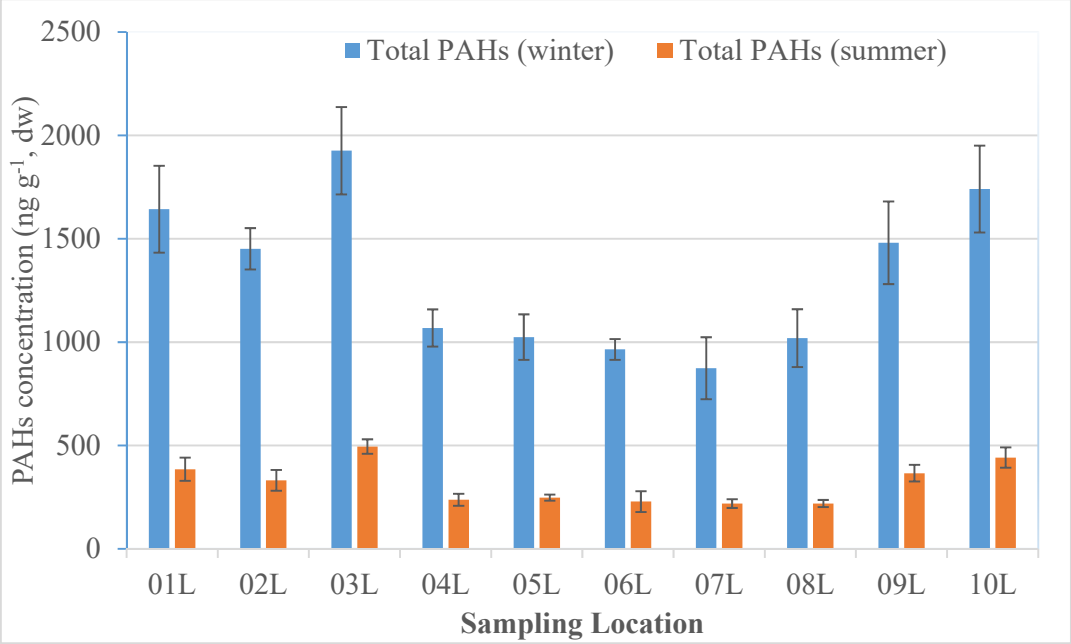


Fig 4.3.1 $\sum_{16}$PAHs concentration (ng g^{-1}, dry weight) in grab sediment sample collected in difference location across Thane creek for winter and summer season (mean ± SD, n = 3).

PAHs concentrations in sediments were two to three times higher in winter than summer. Total PAHs concentrations in sediments varies from 874 to 1925 ng g^{-1} with mean value of 1391 ng g^{-1} in winter, during summer these values varies between 219 and 495 ng g^{-1} with a mean value of 317 ng g^{-1}. The concentration of total PAHs have been widely reported in sediments from different coastal regions around world, are in range (ng g^{-1}) with the results of present study. Such as, the concentrations (ng g^{-1}, dw) of ΣPAHs were in range of 294 –1381 in East China Sea, China [**302]**, Miki et al. (2014) reports PAHs in sediment from Osaka Bay, Japan in range of 6.4 – 7800 ng g^{-1} [**303**], a Malaysian study reports these values in range of 12.3 to 1450 ng g^{-1} [**304]**, for Dalian, northeast China 31.5 to 4520 ng g^{-1} in grab sediment [**86]**.

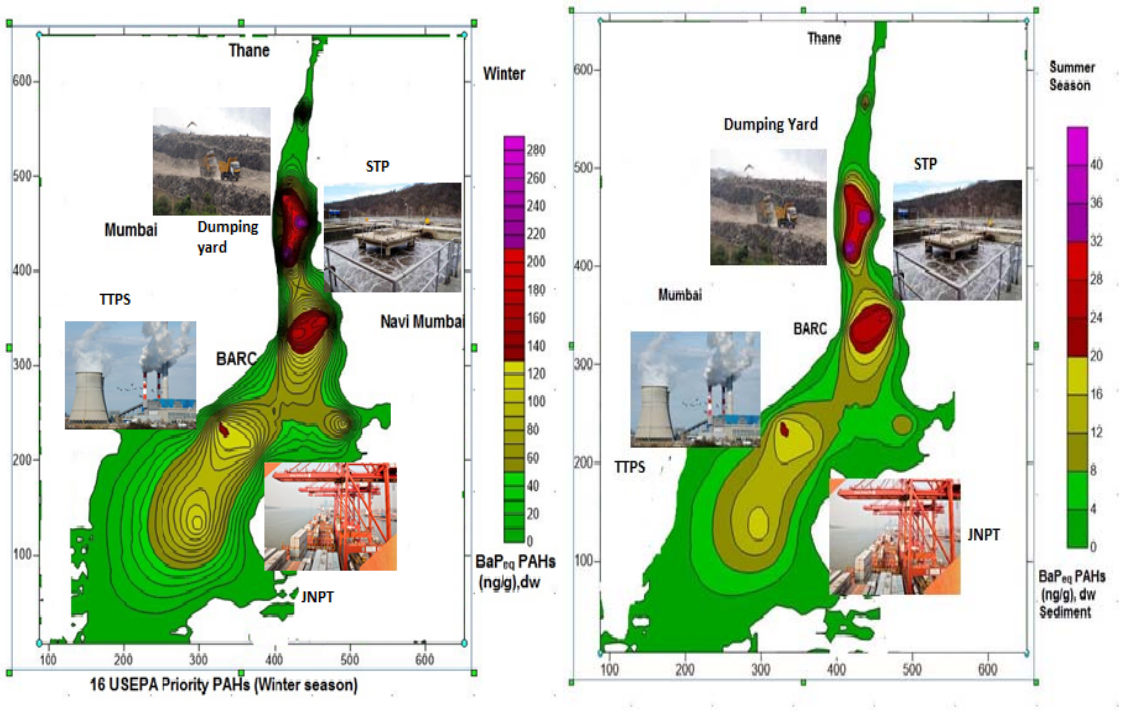


Fig 4.3.2 Spatial distribution of BaPeq PAHs across Thane creek, during winter and summer.

The difference in PAHs concentrations during summer (pre-monsoon) and winter (post-monsoon) may be explained as, during monsoon season lot of rain fall carry those PAHs in runoff from area surrounding Thane creek. Therefore, high concentration was observed during winter, afterword those PAHs may undergo various environmental degradation processes like

photochemical oxidation, biodegradation, alkylation etc. and concentrations were reducing in summer. Wang et al. (2008) and Hong et al. (2016) found that the concentrations of PAHs in soil and sediment from Dalian, China were much higher in winter than those in summer, and the main reasons were the low temperature and residential heating (using coal and biomass) in winter [**86, 305**]. **Table 4.3.1** shows PAHs comparisons contamination levels in seawater and sediment at Thane creek and other recently studied area.

Table 4.3.1 Comparison of $\sum_{16}$PAHs contamination levels in seawater and sediment at Thane creek and global sites.

Sample and Location	PAHs Concentration	Reference
Sediment, East China Sea, China	294 –1381ng g^{-1}	Deng et al., 2013 [**302**]
Sediment, Osaka Bay, Japan	6.4 – 7800 ng g^{-1}	Miki et al., 2014 [**303**]
Sediment, Malaysia	12.3 – 1450 ng g^{-1}	Retnam et al., 2013 [**304**]
Sediment, Dalian, Northeast China	31.5 – 4520 ng g^{-1}	Hong et al., 2016 [**86**]
Sediment, Thane creek, India	157 – 1926 ng g^{-1}	This study
Seawater, East and South China Seas	30.4 – 120.29 ng L^{-1}	Ren et al., 2010 [**306**]
Seawater, western Mediterranean	272 – 1392 pg L^{-1}	Marrucci et al., 2013 [**307**]
Seawater, Western Strait, China	12.3 – 58.0 ng L^{-1}	Wu et al., 2011 [**308**]
Seawater, Thane creek, India	180 – 1090 ng L^{-1}	This study

The concentration range (i.e. maximum and minimum PAHs concentration), and mean of PAHs in the surface sediment from the Thane creek during winter and summer are shown in **Table 4.3.2**. Average BaP equivalent concentration were 194.7 and 27.9 ng g^{-1} in winter and summer respectively. All USEPA priority PAHs were detected in the all sediment samples for both period of sampling. Abundance of PAHs in sediment was observed as BbF > BaA > BkF > CHR > FLUO > PHEN > PYR > BaP > BghiP > DBA > IND > NAP > FLU > ANT > ACE > ACY during winter, likewise for summer was as BaA > FLUO > PHEN > PYR > CHR > BkF > BbF > NAP > BaP > FLU > DBA > BghiP > ANT > IND > ACY > ACE. Based on the number of rings in the PAHs, the 16 PAHs can be divided into three groups: (2 + 3)-ring (NAP, ACE, ACY, FLU, PHEN, and ANT), 4-ring (PYR, FLUO, CHR, and BaA) and (5 + 6)-ring

(BaP, BbF, BkF, IND, DBA and BghiP) components, representing low-, medium- and high-molecular weight PAHs, respectively [**309, 310]**. Pie charts of PAHs abundance in grab sediments categorized by the number of rings for winter and summer shown in **Fig. 4.3.3.** The abundance of (5+6)-ring PAHs in total PAHs was highest and followed by 4-ring, and (2+3)-ring PAHs during winter. Such observations indicate during winter (5+6)-ring PAHs mainly by pyrogenic sources (high-temperature combustion) [**311]**. All though, pyrogenic sources of PAHs are constant during winter and summer in Mumbai, slight difference in temperature can change the PAHs profile in atmosphere so in sediment.

Table 4.3.2 Concentration of PAHs (ng g^{-1}, dw) and total BaP equivalent in grab sediments during winter and summer; across Thane creek.

PAHs	Winter			Summer		
	Max	**Min**	**Average**	**Max**	**Min**	**Average**
NAP	47.2	10.2	26.6	33.2	7.2	18.4
ACY	7.5	1.1	3.2	4.9	1.2	2.7
ACE	8.1	1.1	3.6	5.1	1.1	2.4
FLU	39.5	12.3	23.9	13.1	3.9	8.1
PHEN	177.3	69.0	115.1	69.0	12.5	41.6
ANT	39.0	9.6	22.7	7.2	1.4	4.0
FLUO	149.0	84.0	118.5	57.0	29.8	42.6
PYR	142.0	69.0	107.3	55.2	24.3	41.3
BaA	310.0	112.0	183.9	97.0	36.0	60.5
CHR	157.0	82.6	120.2	42.7	19.5	29.8
BbF	300.5	142.2	219.6	34.5	3.6	18.9
BkF	197.5	124.5	153.5	31.2	7.2	21.4
BaP	149.5	52.3	99.2	23.6	3.6	13.4
DBA	57.2	4.7	37.7	9.2	2.4	5.6
BghiP	81.2	9.5	50.5	8.4	1.2	4.1
IND	63.2	12.2	33.7	4.8	1.9	3.3
Total BaP_{eq}	**302.6**	**104.1**	**194.7**	**46.4**	**10.7**	**27.9**

The abundance of ring number wise PAHs is totally different in summer compared to winter, in summer 4-ring PAHs contribution was observed maximum to total PAHs followed by (2+3)-ring PAHs, while (5+6)-ring PAHs were least contributing to the total PAHs. Urban runoff

discharge to creek may dominantly affect the PAHs accumulation in sediment. As this increase the proportion of 4-6 rings PAHs in the sediment particularly in winter [**310]**.

Location wise BaP_{eq} concentrations were calculated for PAHs in sediment samples using toxicity equivalency factors (TEFs). TEFs for cancer induction relative to B[a]P were used to convert PAHs concentration in same scale of toxicity. PEFs have been derived only for PAHs with demonstrated carcinogenicity in bioassays. A much larger number of PAHs and PAH derivatives are considered mutagenic or genotoxic and may have limited evidence for carcinogenicity. However, until that time the TEFs proposed for use in risk assessment were estimated only for PAHs currently classified as carcinogens. The calculated TEF is 0.001 for NAP, ACY, ACE, FLU, PHEN, FLUO and PYR, 0.01 for ANT, CHR and BghiP, 0.1 for BaA, BbF, BkF and IND, 1 for BaP and DBA [**312- 314]**.

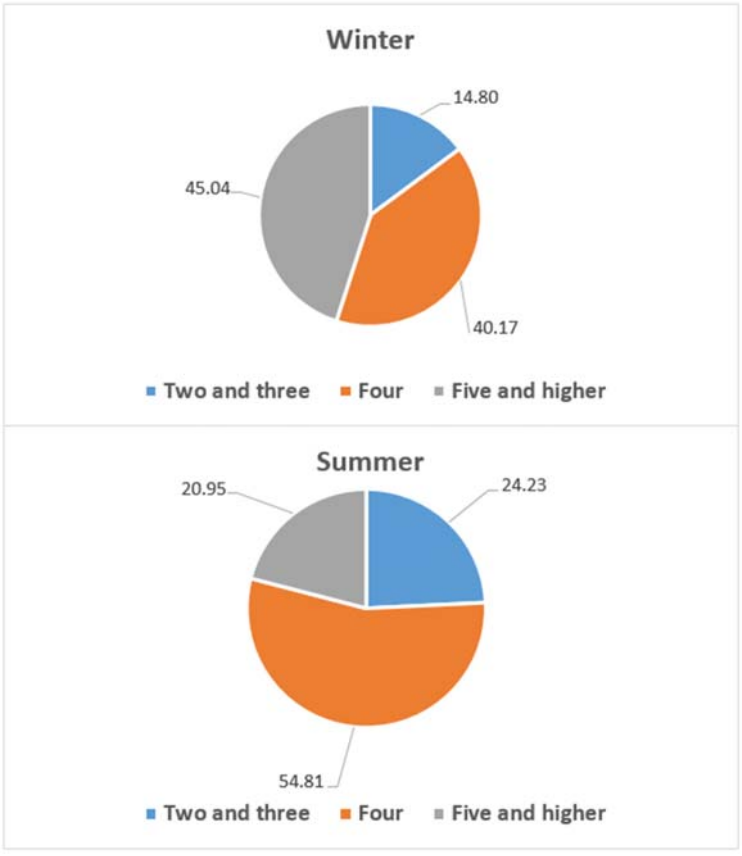


Fig. 4.3.3 Pi-chart showing the ring number wise PAHs distribution in sediments sample during winter and summer.

Location wise B(a)P_{eq} PAHs concentration in sediment across Thane creek were also calculated. BaP_{eq} concentrations of PAHs in surface sediments vary from 122 to 302 ng g^{-1} (dw) during winter, while these values were 13 to 45.5 ng g^{-1} for summer. Monitoring station 03L was found most contaminated with respect to BaP_{eq} PAHs abundance followed by 01L, 10L and 02L stations. The probable reason for this observation is likely the urban and industrial run off discharge to near to these locations. Secondly these monitoring stations were very close to shore. Station 05L, 06L, 07L and 08L were less contaminated with PAHs compare to other sampling stations in both season i.e. winter and summer. Relatively low concentration of BaP_{eq} PAHs in these station may be due to distance from shore so they may get diluted with tidal current and other physiochemical processes.

4.3.2 PAHs in seawater

Concentrations of all tested sixteen unsubstituted US-EPA priority PAHs in seawater sampled from Thane creek are shown in **Table 4.3.3**, in winter and summer. The mean value of Σ_{16} PAHs concentration during winter was recorded 706 ng L^{-1}, with a variation of 193 ng L^{-1}; for summer those values were recorded as 337 and 79 ng L^{-1} respectively. Concentration of PAHs are significantly higher ($p < 0.01$) in winter compare to summer. PAHs values in seawater are on higher side of reported values from other part of globe, such as PAHs reported from the east and south China sea as ranged between 30.4 and 120.29 ng L^{-1} of seawater **[306]**, study from western Mediterranean reports total concentrations of the PAHs ranged from 272 to 1392 pg L^{-1}, with a mean value of 623 pg L^{-1} **[307]**. The results from Western Taiwan Strait, China showed that the total concentrations of PAHs in the dissolved phase and particulate phase were ranged from 12.3 to 58.0 ng L^{-1}, and 10.3–45.5 ng L^{-1} **[308]**. Higher concentration of total PAHs in seawater during winter and summer were observed in this study compare to reported

from Dalian, Northeast China (375 ± 128 ng L^{-1} in winter and 297 ± 265 ng L^{-1} in summer) and from Mexico (76.6-384 ng L^{-1} in winter and 30.1-746 ng L^{-1} in summer) [**86, 315]**.

Table 4.3.3: Concentration of PAHs (ng L^{-1}, x ± 1s, n=30) in seawater during winter and summer.

PAHs	Winter	Summer
NAP	170 ± 70	34 ± 21
ACY	12.4 ± 7.1	4.1 ± 2.3
ACE	5.6 ± 1.7	2.5 ± 1.9
FLU	50 ± 11.4	21.3 ± 5.3
PHEN	250 ± 60.3	100.1 ± 15.3
ANT	40.2 ± 7.9	20.1 ± 8.4
FLUO	21 ± 4.7	18.2 ± 4.2
PYR	107.2 ± 17.4	90.2 ± 11.6
BaA	16.5 ± 3.6	10.2 ± 2.1
CHR	10.5 ± 4.2	9.6 ± 2.1
BbF	1.4 ± 0.4	4.2 ± 0.7
BkF	10.2 ± 2.1	10.8 ± 1.7
BaP	5.1 ± 1.2	6.2 ± 1.3
DBA	1.9 ± 0.1	1.2 ± 0.14
BghiP	2.1 ± 0.15	3.1 ± 0.4
IND	1.8 ± 0.1	1.7 ± 0.21

Phenanthrene (PHEN) was found to most abundant PAHs in seawater during both monitoring period, its concentration was 250 ± 60.3 ng L^{-1} in winter, while 100.1 ± 15.3 ng L^{-1} in summer. High molecular weight PAHs such as BaP, DBA, BghiP and IND were found in less concentration among other tested PAHs.

Percent contribution of **(**2+3)-ring, 4-ring, and (5+6)-ring PAHs to total PAHs in seawater were also calculated and shown in **Fig 4.3.4** for winter and summer. **(**2+3)-ring PAHs were found most contributing to total PAHs i.e. more than 50% in both monitoring periods. Higher molecular weight PAHs (5 and 6 ring PAHs) were least contributing to total PAHs concentration in seawater with mean value of 8 and 3 % during summer and winter respectively. This observation was opposite to concentration of PAHs in sediment as discussed earlier. A recent study from Colombian Cauca River also reports Benzo[b]fluoranthene,

Benzo[k]fluoranthene, and Pyrene in sediments were most detected; and Fluorene, Acenaphtylene, and Anthracene in water **[316]**. The lower concentration of high molecular weight PAHs in seawater probably due to their high hydrophobic nature unlike low molecular weight PAHs. The contributions of 4-ring PAHs were found 22 and 38 % to total PAHs in winter and summer.

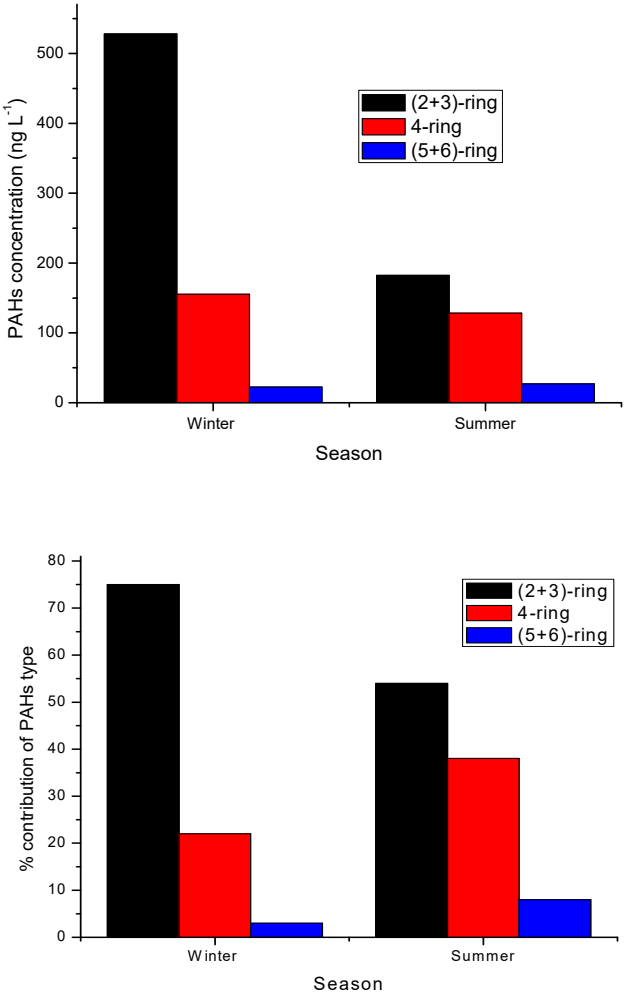


Fig 4.3.4 Distributions of PAHs according to ring numbers (% contribution to total and concentration (ng L^{-1}) wise) in seawater during summer and winter.

4.3.3 PAHs in marine organisms

PAHs were monitored in lizard fish, Bombay duck and crab samples, results are shown in **Fig. 4.3.5** for both sampling periods i.e. winter and summer. Total concentrations of PAHs in consumable portion of lizard fish were found 156.8 ± 18 and 122 ± 24.5 ng g^{-1} (wet weight) during winter and summer respectively. Σ_{16}PAHs in bombay duck samples were determined and founds as 117.4 ± 17.65 in winter, and 95.8 ±16.2 in summer. The Student's t-test was applied for comparison of mean PAHs contamination levels in summer and winter season for all tested environmental matrices. The statistic t was calculated using equations (9) and (10).

$$t_{exp} = \frac{\bar{X}_s - \bar{X}_w}{S_{sw}\sqrt{\frac{1}{n_s}+\frac{1}{n_w}}} \quad (9)$$

Where $$S_{sw} = \sqrt{\frac{(n_s-1){S_s}^2+(n_w-1){S_w}^2}{n_s + n_w - 2}} \quad (10)$$

$\overline{X}_s$ and $\overline{X}_w$ **a**re mean value of PAHs concentration in summer and winter respectively. And n_s and n_w are sample size (number of samples) analyzed in winter and summer, while S_s and S_w are standard deviation respectively. t_{exp} value is compared with the critical (theoretical) t_{th} value corresponding to given degree of freedom ($n_s + n_w - 2$) and the confidence level chosen. The experimental values of t were 3.69 and 2.89 for lizard fish and Bombay duck respectively, which are higher compared with a critical value of t(2.87) for 18 degrees of freedom and a significance level of 0.01 indicating that there was significant difference between PAHs concentration in winter and summer. Naphthalene and phenanthrene were most abundant PAHs in both fish samples, while high molecular weight PAHs were least abundant. Earlier study from natural reserve of Camargue also report the high abundant PAHs are phenanthrene, naphthalene and fluorene (about 10 ± 100 ng g^{-1}) then fuoranthene, pyrene and chrysene (5 ± 30 ng g^{-1}) [**317]**.

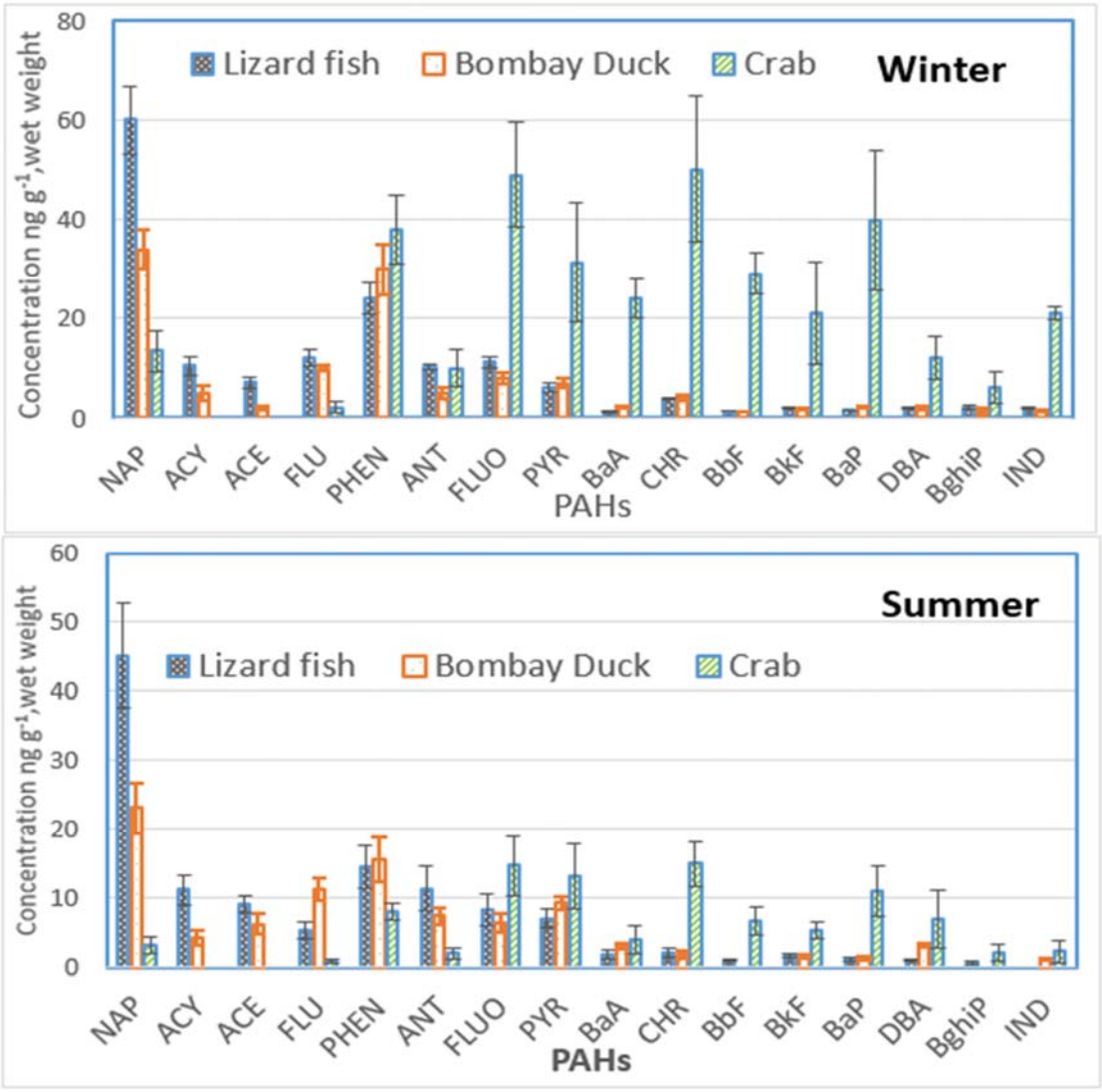


Fig 4.3.5 PAHs concentration (ng g^{-1}, wet weight) in lizard fish, bombay duck and crab samples from Thane creek during winter and summer.

A recent study from Ghana also reports the dominance of low molecular weight PAHs (naphthalene and phenanthrene) in fish samples [**318]**. Abundance of PAHs was higher in winter compare to summer in fish samples, which may be a due to higher concentration of those pollutants in seawater as discussed earlier, and contamination in fishes are mostly affected by their levels in seawater. Total PAHs content in crab samples was 348 ± 94.5 ng g^{-1} (wet weight) in winter, and 95.62 ± 31.9 ng g^{-1} in summer. The probable reason for this

observation was high concentration of these PAHs in sediment of Thane creek. A previous study from Bay of Biscay also report high concentration of PAHs during winter and low in summer ranging from 9.4 to 221.6 ng g^{-1} in crab samples with predominance of 4- and 5-rings PAHs [**319]**. PAHs content in crab were dominated by high molecular weight PAHs opposite to fish samples discussed above may be due high feeding from sediment. Ternary plots were drawn to find the contribution of three categories of PAHs to total PAHs for fish and crab samples, shown in **Fig 4.3.6**.

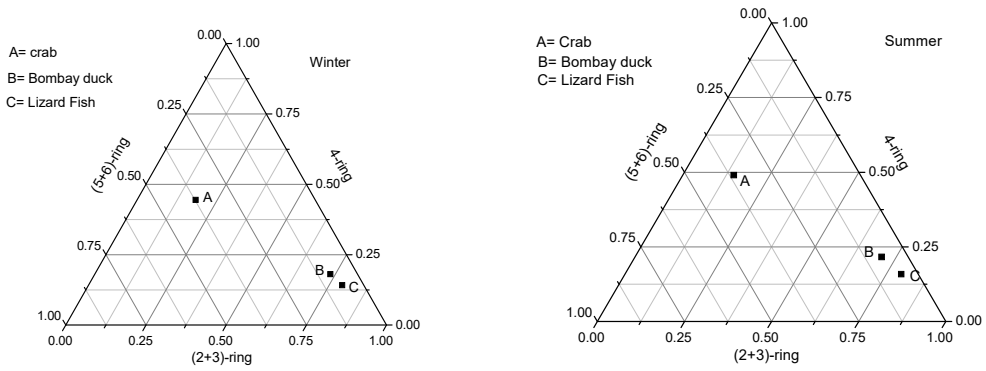


Fig 4.3.6 Ternary plot showing proportion of (2+3)-ring, 4-ring and (5+6)-ring PAHs in crab, Bombay duck and lizard fish, collected in winter and summer.

Ternary plots show very similar results of proportion of ring number wise PAHs for monitored marine species in winter and summer. Fish samples (lizard fish and bombay duck) were categorized as once with dominance of two and three ring PAHs. Similar PAH congeners abundance in fish with prevalence of 2–3 ring PAHs are in accordance with previous studies [**320- 323**]. PAH distribution according to molecular weight could be related to metabolic processes; as fish have a high metabolic capacity to bio-transform the higher molecular weight PAHs with a greater efficiency [**324]**. In crab samples 4-ring and (5+6)-ring PAHs were found to contributing majorly and almost similar proportion while (2+3)-ring PAHs were least contributing. The abundance pattern of PAHs in crab samples was very similar to previous studies i.e. predominance of 4- and 5-rings PAHs [**319, 325]**.

4.3.4 Inter compartmental behavior of PAHs

The bioconcentration factor (BCF) for each PAHs were calculated using their concentration in biota and seawater. BCF values for Bombay duck fish species found to be vary between 21 and 443 with mean value of 136, while for lizard fish species mean BCF was 561 (range, 21-443). The BCF of PAHs for crab was found one order of magnitude higher than fish, ranging from 58 to 12057 with mean values of 3719. To find the correlation between K_{ow} and BCF of PAHs in biota, a liner fitting was tested (**Fig 4.3.7**). It was found that BCF were poorly correlated with K_{ow} values of PAHs, in other words the variation in BCFs of individual PAHs was not explanatory using only their octanol-water partitioning behaviour.

In situ organic carbon–water partition coefficient (K'oc) were also calculated for PAHs using the following equation (11).

$$K'oc = \frac{C_s}{C_w \varphi_{oc}} \quad (11)$$

Where, C_S is chemical concentration in sediment, in the unit of ng g^{-1} dw, C_W is chemical concentration in water, in the unit of ng mL^{-1}, Organic carbon fraction (φ_{OC}) for each sediment sample can be calculated by assuming ($\varphi_{OC} = \varphi_{OM}$ x 0.55).

The K'oc values of PAHs in Thane creek were founds between 4.1×10^3 and 4.1×10^6 with mean values of 4.7×10^5 during winter, while between 6.9×10^3 and 2.6×10^5 with mean values of 6.8×10^4 during summer. The one order of magnitude difference during summer and winter can be explain by high concentration of PAHs during winter in sediments. K'_{OC} values were plotted against Kow of PAHs in **Fig 4.3.8** to evaluate their interdependency. It was found that K'oc moderately correlated ($r^2 = 0.5$-0.8) with Kow of PAHs.

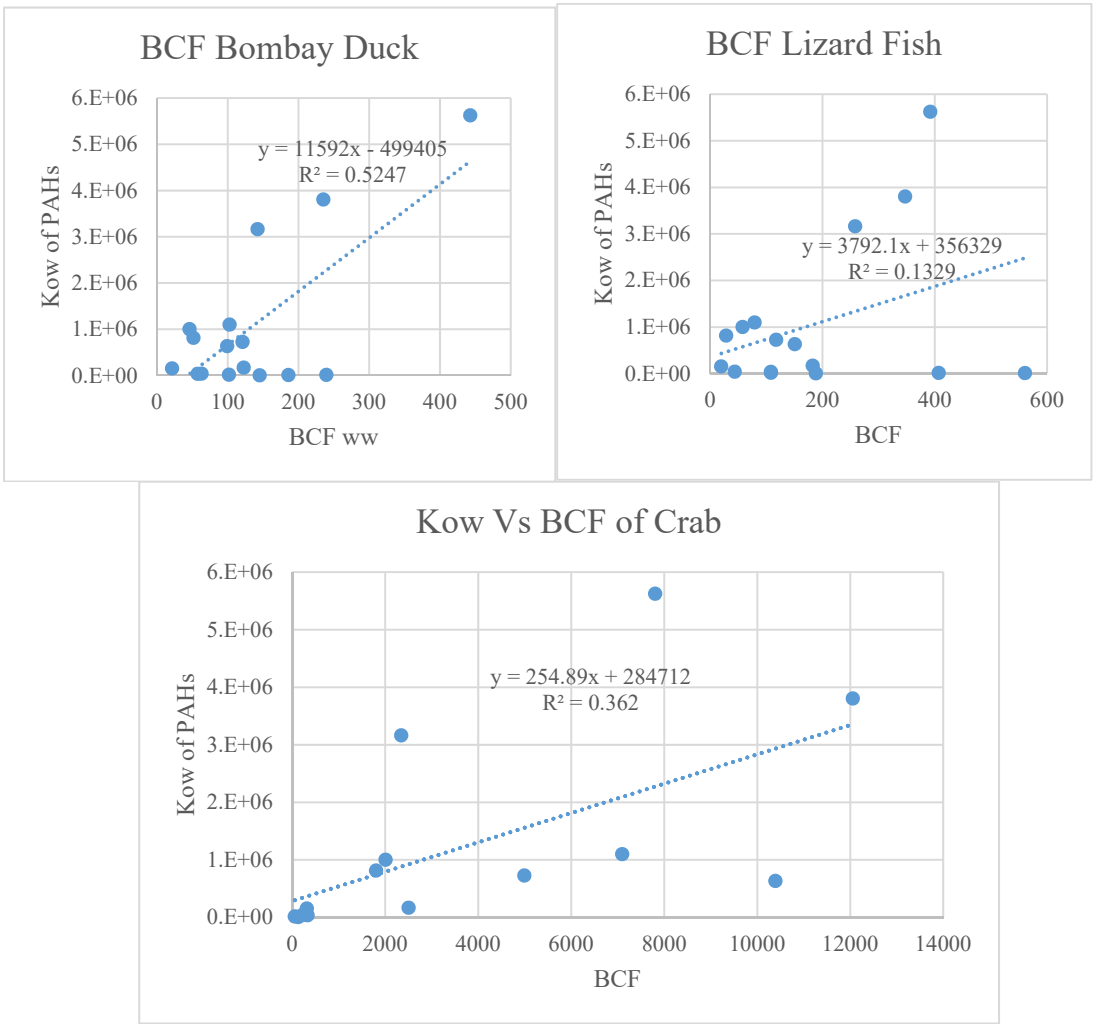


Fig 4.3.7 Scattered plot of BCF Vs Kow of PAHs for fish (Bombay duck, Lizard fish) and crab samples from Thane creek.

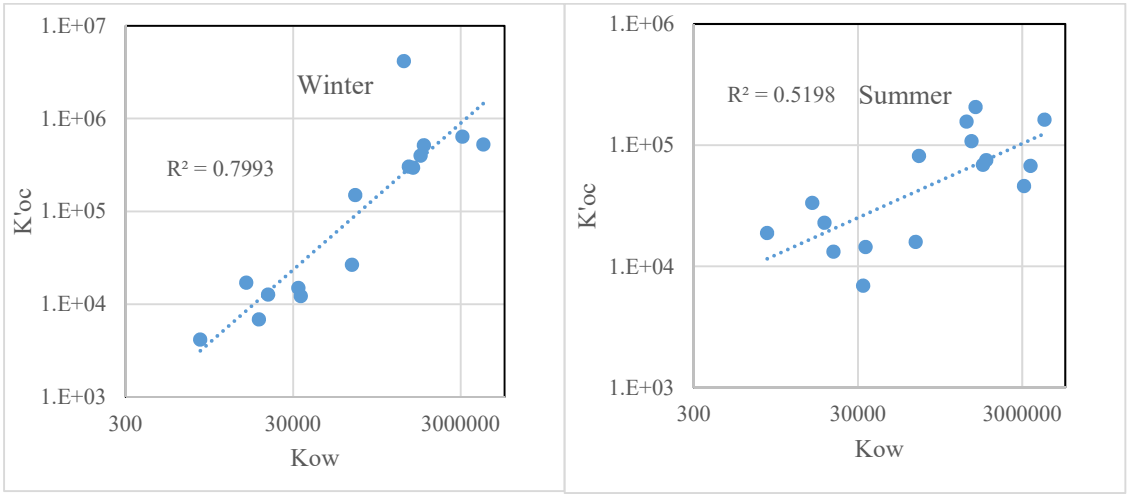


Fig 4.3.8 correlation between K_{ow} and K'_{OC} for PAHs during winter and summer.

The results of fugacity fraction (ff) values of PAHs are shown in **Fig. 4.3.9**. The values of ff < 0.5, indicating a net flux from seawater to sediment, ff ≈ 0.5, indicating water-sediment equilibrium and no net exchange, ff > 0.5, which indicated a net flux from sediment to seawater [**84**]. In general, distribution pattern of water-sediment exchange for LMW PAHs (NAP, ACY and ACE) showed a similar trend during the winter and summer. Mean ff values for more than LMW PAHs were larger than 0.5 (except for Acenapthylene in winter), indicating that sediment acted as the source to seawater for these chemicals. However, the ff values for remaining PAHs were lower than 0.5 (except for BbF in winter) indicating their net flux to sediment. In other words, in Thane creek environment HMW PAHs have tendency to accumulate in sediments. Hong et al. (2016) also reported similar observation for HMW PAHs as, mean ff values of benzo(k)fluoranthene, benzo(a)pyreneand, perylene were lower than 0.3 in summer, causing the net flux of these PAHs from seawater to sediment of Dalian, Northeast China [**86**].

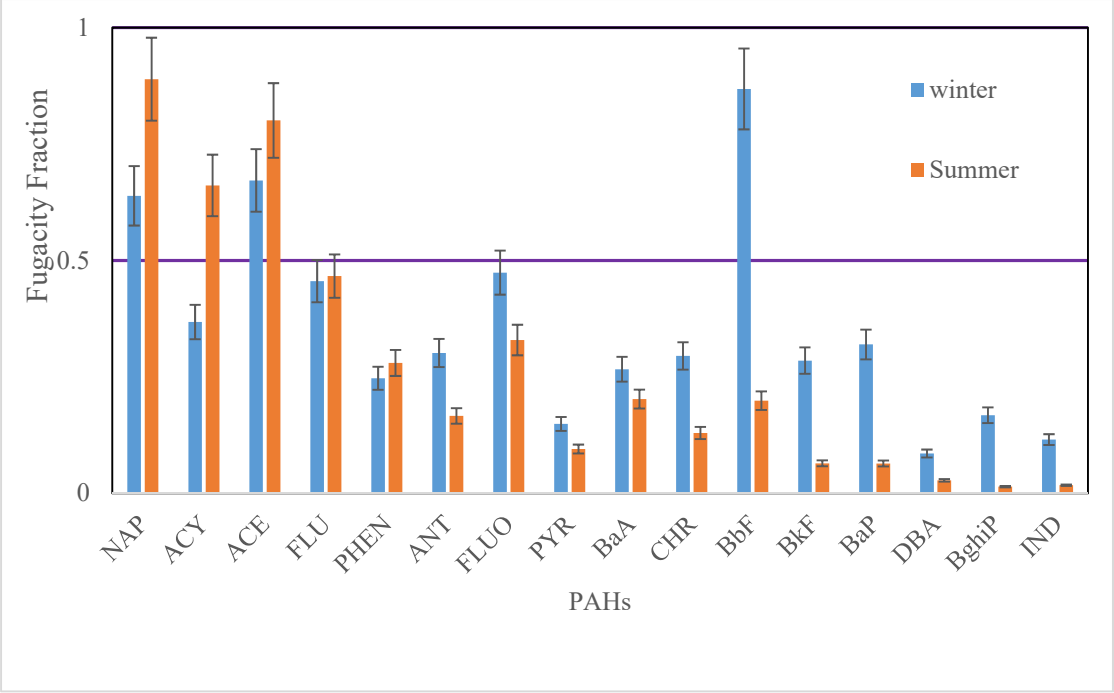


Fig 4.3.9 Fugacity fraction(ff) between seawater and sediments in Thane creek for PAHs.

4.3.5 Ecotoxicological risk of PAHs in sediment

Concentration of PAHs in sediment samples were compared with to sediment quality guidelines and presented in **Table 4.3.4**. Effect-range low (ERL) values, and effect range-median (ERM) values were taken from Long et al. (1995) [326], while threshold effect levels (TEL), and probable effect levels (PEL) were from Macdonald et al. (1996) **[327]**.

Table 4.3.4 Comparison of the sediment PAHs from Thane creek, Mumbai with sediment quality guidelines (ng g^{-1}, dw) for winter and summer time sampling.

PAHs	ERL*	ERM*	TEL#	PEL#	% of sample above ERL		% of sample above TEL	
					Winter	Summer	Winter	Summer
NAP	160	2100	34.6	391	None	None	40%	None
ACY	44	640	6.71	128	None	None	10%	None
ACE	16	500	5.87	88.9	None	None	20%	None
FLU	19	540	21.2	144	60%	None	50%	None
PHEN	240	1500	86.7	544	None	None	70%	None
ANT	85.3	1100	46.9	245	None	None	None	None
FLUO	600	5100	113	1494	None	None	60%	None
PYR	665	2500	153	1398	None	None	None	None
BaA	261	1600	74.8	693	20%	None	100%	30%
CHR	384	2800	108	846	None	None	50%	None
BbF	320	1880	-	-	None	None	-	-
BkF	280	1620	-	-	None	None	-	-
BaP	430	1600	88.8	763	None	None	60%	None
DBA	430	1600	6.22	135	None	None	90%	40%
BghiP	63.4	260	-	-	30%	None	-	-
IND	-	-	-	-	-	-	-	-

ERL -Effect-range low values, ERM -Effect range-median values (Long et al., 1995 **[326])*
*#TEL -Threshold effect levels, PEL -Probable effect levels (Macdonald et al., 1996 **[327]**)*

On comparing the ERL values to PAHs concentration in sediment from Thane creeks it was found that 60%, 20% and 30% of sampling sites of total were exceeding the guideline values of FLU, BaA, and BghiP respectively in winter. For all other PAHs in winter were below ERL values, and none of PAHs exceeded the ERL values. All the tested PAHs in sediments were found much below than ERM guideline values in both summer and winter. Concentration of most of the PAHs was found exceeding TEL values in winter, BAA was found above TEL in

all sampling sites during winter time sampling followed by DBA with 90% of sampling sites, indicates potential of causing toxic effects on benthic organisms. In summer, 30% and 40% of sampling sites were found above TEL for BaA and DBA abundance, for all other PAHs were found below TEL values. None of PAHs concentrations were observed above PEL values during monitoring.

4.3.6 ILCR calculation of PAHs

The carcinogenic risk of PAHs in food is often expressed by its BaP equivalent concentration (BaPeq) and is calculated from the concentrations of individual PAHs and their toxicity equivalency factors (TEFs) [**328, 329]**. Toxicity equivalency factors (TEFs) of PAHs were discussed in earlier section, BaP_{eq} concentrations were calculated of fish and crab samples. The calculated BaP_{eq} concentrations were found as 3.12 ± 1.1, 3.9 ± 0.47 and 43.3 ± 27.3 ng g^{-1} (wet weight) in lizard fish, bombay duck and crabs respectively averaged over monitoring period. Those values were much higher than the recommended BaP_{eq} of PAHs (0.67 ng g^{-1}, ww) suggested by USEPA (2000) for human fish consumption [**330**]. Additionally, the estimated daily dietary (EDI) and the incremental life time cancer risk (ILCR) of the dietary exposure to PAHs was also calculated using following equations (12, 13) [**323, 328]**.

$$EDI = \frac{CR \times C}{BW} \tag{12}$$

where EDI is the estimated daily dietary PAH exposure for human (ng kg^{-1} body weight (bw) day^{-1}); CR is the consumption rate of fish (14 g day^{-1}) [**331, 332]**, C is BaP_{eq} PAH concentration (ng g^{-1}) in consumable organisms; BW is the average body weight of people (70 kg for adults).

$$ILCR = \frac{ED \times EF \times EDI \times SF \times CF}{AT} \tag{13}$$

where ILCR is the incremental lifetime cancer risk of PAHs ingestion through marine consumable foods (dimensionless); ED is the exposure duration (62 year, Average life

expectancy); EF is the exposure frequency (365 day yr^{-1}); SF is the oral cancer slope factor of BaP (geometric mean of 7.3 mg kg^{-1} day^{-1}); CF is the conversion factor (1.0×10^{-6} mg ng^{-1}); AT is the average lifespan (25,500 days); EDI is the estimated daily dietary PAH exposure for human (ng kg^{-1} body weight (bw) day^{-1}). A public screening criteria for carcinogens which is set at a carcinogenic risk level of 1.0×10^{-6}, was used for assessment. One in a million chance of additional human cancer over a 70-year lifetime (ILCR = 1.0×10^{-6}) is the level of risk considered acceptable or inconsequential. In **Fig 4.3.10** a box plot was drawn to represent risk associated with consumption of fish and crab with respect to PAHs. Calculated risk values were found in range from 1.0×10^{-6} to 8.0×10^{-6} for fish samples, with mean values of 4.3×10^{-6} and 4.5×10^{-6} for lizard fish and bombay duck respectively. For crab samples ILCR values were found in between 1.5×10^{-5} and 1.2×10^{-4}, with average of 5.6×10^{-5}. So for all tested species the ILCR values were slightly higher for fishes and significantly high for crab consumption, compare to public screening criteria for carcinogens i.e. carcinogenic risk level of 1.0×10^{-6}.

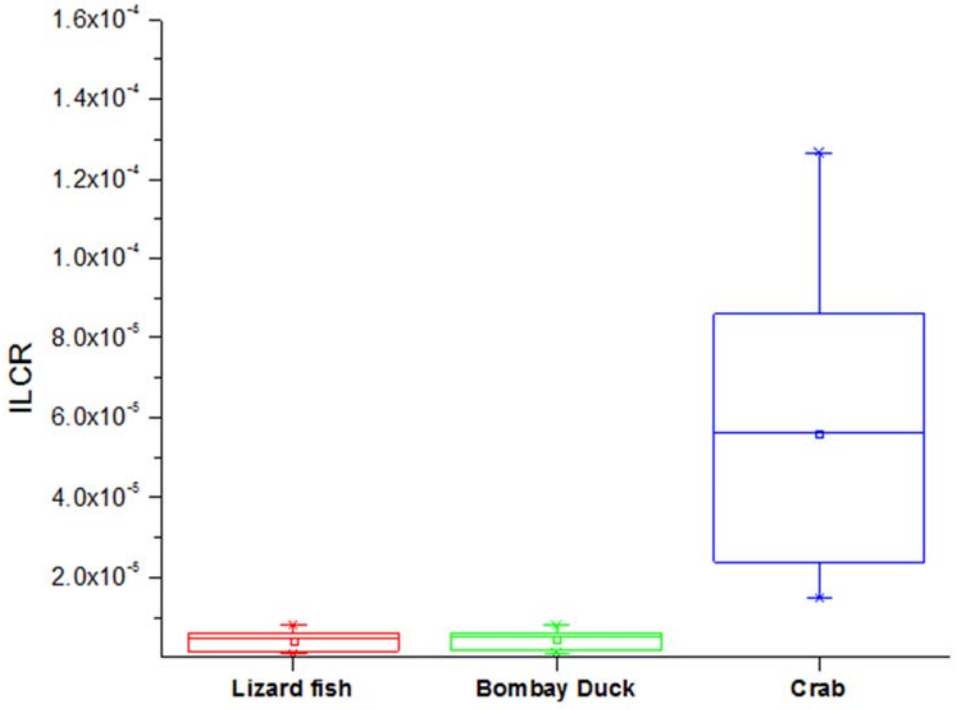


Fig 4.3.10 Box plot of calculated incremental lifetime cancer risk (ILCR) associated with consumption of tested marine consumables with respect of PAHs abundance in them.

4.4 Poly brominated diphenyl ethers (PBDEs) in marine sediment and their chronological assessment and source contribution.

The levels of 15 polybrominated diphenyl ether (PBDE) congeners in grab sediment and sediment cores from the Thane creek are reported, and spatial and temporal distribution is discussed. Sedimentation rate at the creek is also evaluated using Pb^{210} dating technique. Average percentage contribution of commercial penta-BDE (*f*P), octa-BDE (*f*O), and deca-BDE (*f*D) in sediments are determined using least square method. Levels of all measured PBDEs in sediment are also compared with sediment quality guideline.

4.4.1 Sedimentation rate

The allochthonous activity of ^{210}Po in grab sediments across Thane creek was observed between 21.5 and 60.3 Bq kg^{-1}. The total ^{210}Po activity in layers of the core sediments was found to be vary between 14.3 and 90.8 Bq kg^{-1}. The trend of allochthonous ^{210}Po activity was decreasing with depth in all cores sediment samples. Sedimentation rate was determined using the ^{210}Pb activity profile of the sediment core. For the determination of sedimentation rate Constant Initial Concentration (CIC) model was used. The CIC model based on the assumption that the sediment accumulation rate does not affect the ^{210}Pb activity concentration and so it will remain constant **[333]**. The average sedimentation rate was determined as 0.66 cm y^{-1}, this value agrees with that observed in earlier study from Thane creek, which was reported to be 0.67 cm yr^{-1} using the CIC model [**13]**.

4.4.2 PBDEs in surface sediment

PBDEs were detected in the surface sediment samples as well as in sediment cores collected from different locations across Thane creek. The levels as well as the pattern of vertical distribution of PBDE congeners at the five locations were considerably different from each

other. Mean concentrations of BDE congeners in grab sediment samples at the different locations were presented in **Table 4.4.1.** The order BDE-congeners abundance in grab sediments was found as BDE-209 > BDE-47 > BDE-28 > BDE-99 > BDE-100 > BDE-154 > BDE-190 > BDE-183 > BDE-66 > BDE-138 > BDE-77 > BDE-153 > BDE-85 > BDE-75 > BDE-71. The total PBDE (ΣPBDE) concentrations at the five locations were ranging from 15.98 ng g^{-1} at location 1 to 132.72 ng g^{-1} at location 5. PBDE sediment concentrations were generally comparable to the studies carried out in different parts of the world, and values summarized in **Table 4.4.2**.

Table 4.4.1 Mean concentrations of select PBDE congeners in Grab sediment samples

PBDE Congeners	**Mean Concentrations of PBDE Congeners (ng/g)**				
	Location 1	**Location 2**	**Location 3**	**Location 4**	**Location 5**
BDE-28	1.16	0.40	0.29	0.19	1.07
BDE-75	0.08	0.03	0.09	0.05	0.07
BDE-71	0.02	**BDL**	0.14	0.06	0.02
BDE-47	1.61	0.24	3.49	1.77	4.19
BDE-77	0.49	0.08	0.08	0.04	0.22
BDE-66	**BDL**	0.64	0.13	0.05	**BDL**
BDE-100	0.50	0.76	**BDL**	**BDL**	0.06
BDE-99	0.54	1.77	0.01	**BDL**	0.21
BDE-85	0.34	**BDL**	**BDL**	**BDL**	0.09
BDE-154	**BDL**	**BDL**	0.62	0.19	**BDL**
BDE-153	0.14	**BDL**	**BDL**	**BDL**	0.13
BDE-138	0.05	0.34	0.40	0.33	0.04
BDE-183	0.04	1.22	0.12	0.02	0.01
BDE-190	0.35	**BDL**	**BDL**	**BDL**	0.27
BDE-209	3.16	4.52	11.50	9.22	8.95

Spatial distribution reveals the order of PBDEs concentration in grab sediment as Location 3 > Location 5 > Location 4 > Location 2 > Location 1. PBDEs contamination in sediment from location 3 was found highest among monitoring sites, across Thane creek. Location 3 was very near to a dumping yard (Deonar) which may be leaching PBDEs to creek, a discharge location is also located near this location. The probable reason for this spatial distribution is likely the urban and industrial run-off discharge near those locations have high PBDEs concentration in sediment. A previous study on organic pollutants in surface sediments from the Clyde Estuary also showed higher levels of contamination associated with sediments close to dockyards as compared to the main channel [**334]**.

In coastal environments BDE-209 (Decabromodiphenyl) was the predominant congener in surface sediments with percentage contribution in the range of 19 to 35%. The contribution from 2,2′,4,4′-tetrabromodiphenyl (BDE-47) was in the range of 1.3 to 12.8%. Most of the studies indicted the predominance of BDE-209, BDE-47 and BDE-99 in the sediment [**335-337]**. The reason being explained is the predominant use of the commercially available pentabromodiphenyl mixture in the earlier days (which consists of 38-42% BDE-47 and 45-9% BDE-99). BDE-47 was found to be the compound with the largest bio accumulative index and these penta mixture was banned and phased out. The congener specific concentration of PBDEs in sediment can be accounted for as individual congeners possess different physical and chemical properties [**338]**. BDE-71 was least abundant congener among analysed BDE-congeners in sediments, while BDE-85, -153, -190, -85, -154, -77, -66 concentration was observed below detection limit in most of the analysed samples. Brominated compounds can enter the coastal ecosystem from polymer production, through leaching during usage, from waste streams such as incinerators, landfill sites and/or automotive scrap yards as well as from sewage sludge dumping. The buildup of PBDEs in sediments may be due to their

physiochemical properties such as low water solubility, high log K_{OW} values and resistance to biodegradation [**339]**.

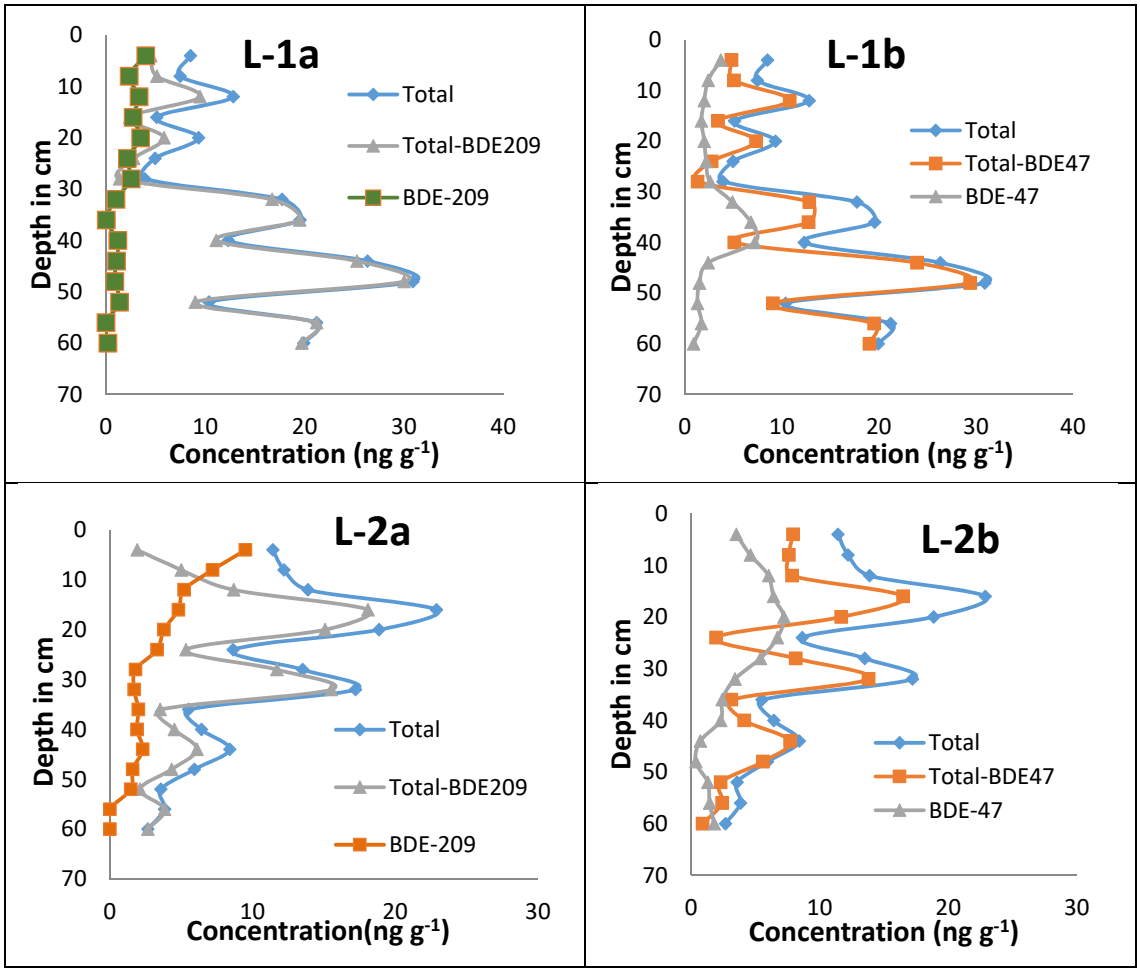
Table 4.4.2 PBDE concentrations in sediment in different parts of the world.

	Study	**ΣPBDE**	**Congeners analysed**	**Major Contributors**
1.	Russia (Labunska et al, 2010 [**340**])	0.1 – 3.4 ng/g	BDE17,28,47,66,85,99,100, 138,153,154,183,197,207 and 209	BDE 99 and 47
2.	United kingdom (Vane et al., 2010 [**336**])	1 – 2645 ng/g	BDE28, 47, 66, 100, 99, 85, 154,153, 138, 183, 197,203,196,208, 207 and 209	BDE 209
3.	China (Mai et al, 2005 [**112**])	0.04 - 7340 ng/g	BDE 28, 47, 66, 100, 99, 154,153, 138, 183 and 209	BDE 209
4.	Singapore (Wurl and Obbard, 2005 [**252**])	3.4– 13.8ng/g	BDE 47	BDE 47
5.	South Korea (Moon et al, 2006 [**341**]).	0.45– 494ng/g	BDE 3, 7, 15, 17,28, 47,49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 183, and 209	BDE47, 49, 99 and 209
6.	Spain (Eljarrat et al, 2005 [**342**])	2.7 to 134 ng/g	BDE 28, 33, 47, 66, 77, 100, 99, 118, 154, 153, 183 and 209.	BDE47 and 209
7.	West Bengal, India (Binelli et al., 2007 [**343**])	0.08 - 29.03 ng/g	BDE17, 28, 71, 47, 66, 100, 99, 85, 154, 153, 138 and 183	BDE47, 185,99 and 154
8.	China, Shanghai (Wu et al., 2016 [**213**])	0.11 to 13.07 ng/g	BDE-17, BDE-28, BDE-47, BDE-66, BDE-71, BDE-85, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-190 and BDE-209	BDE-209
9.	Mumbai, India (Present Study)	15.98 - 32.72 ng/g	BDE28, 47,66, 71, 75, 77, 85, 99, 100, 138, 153, 154, 183, 190 and 209	BDE47, 99 and209

4.4.3 PBDEs in core sediment

The concentration total PBDEs, BDE-209, BDE-47 and their differences in sediment cores from different location (L1 to L5) are presented in **Fig. 4.4.1**. Total PBDE show multimode concentration with depth in core sediments, among which 10 cm depth mode is predominant in all locations. Total PBDEs concentration profile at location 1 (L-1) shows multiple modes at depth of 30-60 cm, and values varies between 3.91 to 30.9 ng g^{-1}. The concentration of BDE-

209 in core sediment at L1 found from BDL to 4.0 ng g^{-1} with a mean of 1.74 ng g^{-1}. Contribution of BDE-209 to total BDE concentration was significantly small for below 30 cm depth. BDE-47 concentration was observed highest at a depth of 40 cm, and its abundance varies between 0.9 and 7.2 ng g^{-1} in sediment core from L1. At L2 location the trend of BDE-209 was observed to be decreasing with length of core, while total PBDEs concentration was showing a tri model distribution with modes at 15, 30 and 45 cm of depth. The BDE-47 concentration was found between 0.35 and 7.2 with mean value of 3.56 ng g^{-1} at that location.



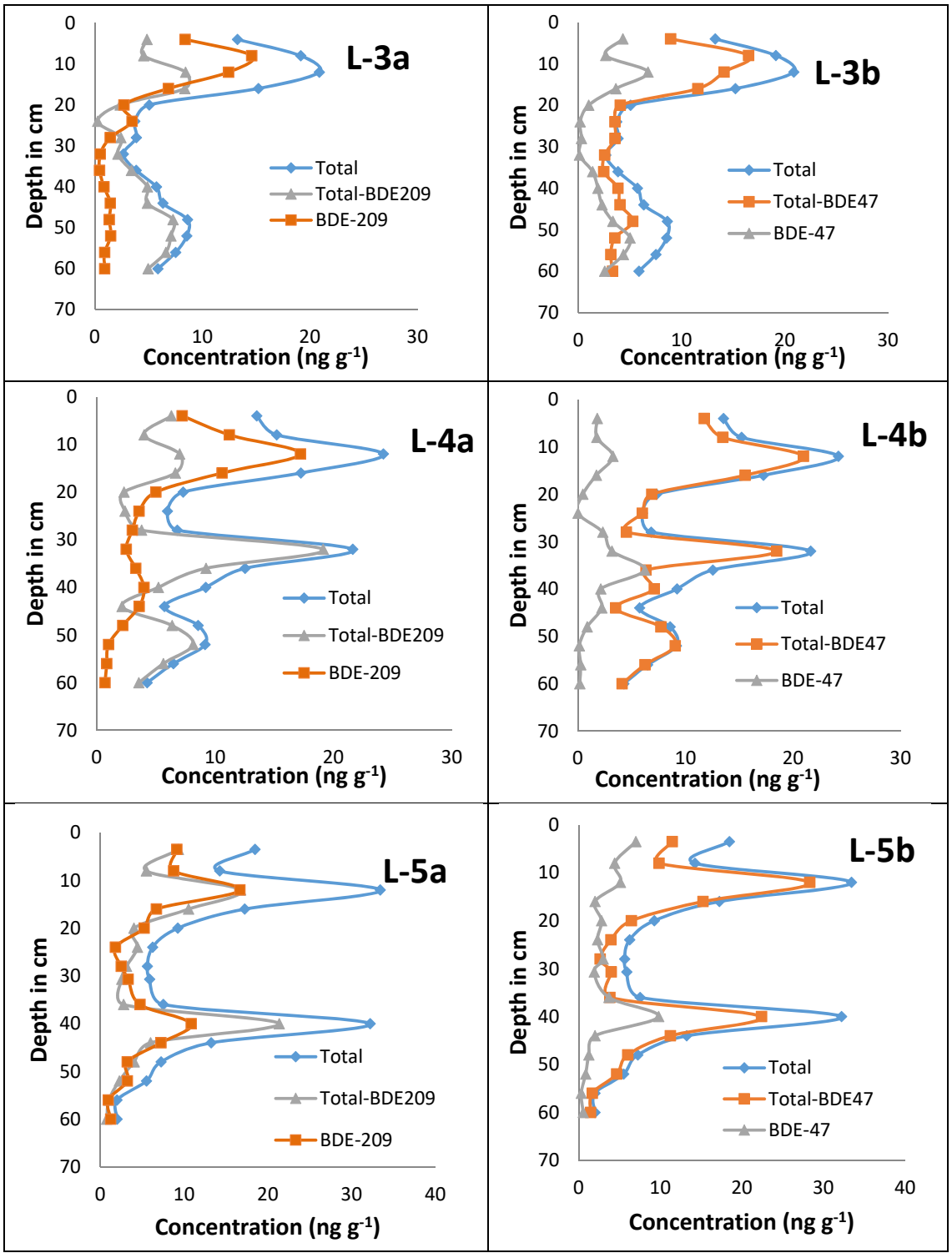


Fig 4.4.1 Depth profile of total BDE, BDE-209, BDE-47 and their difference in sediments collected from different location across Thane creek.

Although at location 3 (L3) total PBDEs concentration was highest in surface sediments compare to other monitoring sites, such finding was not consistent in case of core samples. Their concentration varies between 2.6 and 20.87 with mean values of 8.68 ng g^{-1} in core sediments. The concentration of BDE-209 varied from 0.4 to 14.5 ng g^{-1} and comprised on average 40-80% and 10-20% of all BDEs measured for top 0-30 cm and < 30 cm of core respectively. BDE-47 was found in the range of 0.1 to 6.75 with average concentration of 2.65 ng g^{-1} and contributes more in depth of core (< 30 cm) samples to total of PBDE, as reverse of BDE-209 congener.

The total PBDEs concertation in core sediments from location 4 (L4) was found in the range of 4.26 to 24.22 ng g^{-1} with average of 11.19 ng g^{-1}, and three peak were observed in the depth of 15, 35 and 35 cm. The mean concentration of BDE-209 and BDE-47 congeners were 5.07 (range 0.7-17.21 ng g^{-1}) and 1.75 ng g^{-1} (range BDL-6.21 ng g^{-1}) respectively. For sampling location 5 (L5) the total average PBDEs concentration was12.01 ng g^{-1} (range, 2-33.12 ng g^{-1}) in core samples, with a bimodal distribution peaked at 15 and 40 cm depth. The concentration BDE-209 and BDE-47 were in ranges of 0.95-16.67 (mean, 5.71) and 0.32-9.8 (mean, 3.13) ng g^{-1} respectively in core sediments. BDE-209 was found to be following similar trend as of total PBDEs, while BDE-47 was peaked at depth of 40 cm only.

Most of BDEs have peaked concentration at the depth of 10-15, 30-40 and 50 cm of depth, which are corresponding to years 2003-2008, 1988-1994 and 1980 respectively. This indicates PBDEs were enormously used in last two decades which gets accumulated in the bottom of sea. **Fig 4.4.2** showing the depth of core sediment and corresponding year of sediment, which calculated using Pb^{210} dating technique as discussed earlier.

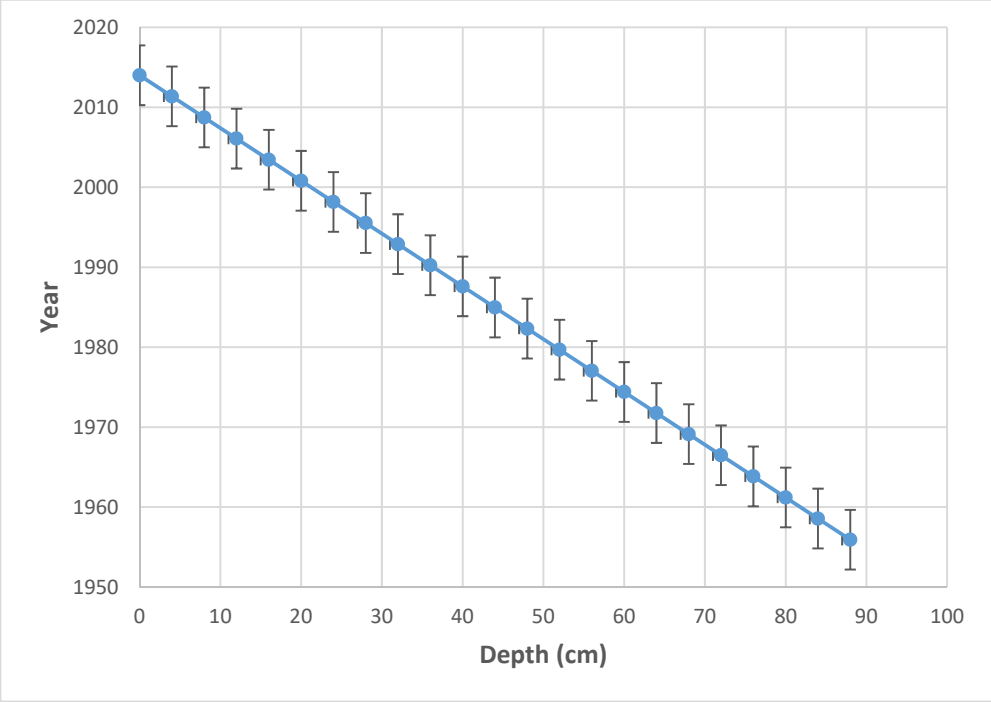


Fig. 4.4.2 Dating of sediment core using Pb^{210} techniques, with average liner sedimentation rate of 0.66 cm y^{-1}.

4.4.4 Profiles of PBDE homologues in sediments

To check the contribution of different homologues of PBDEs in sediments, analysed chemicals were classified in 6 groups base on their degree of bromination, from the most brominated homologue (DecaBDE) to the least (TriBDE). Percentage contributions of each PBDE homologues in sediments are shown in **Fig 4.4.3** for all monitoring sites across Thane creeks. In sediments from Thane creek, the average concentration of homologues profiles decreased in the following order: TetraBDE > DecaBDE > PentaBDE > HexaBDE > HeptaBDE > TriBDE. Homologues TetraBDE was recorded as most abundant PBDEs at monitoring sites L1 and L2, while DecaBDE was at remaining monitoring sites i.e. L3, L4 and L5. At all monitoring sites BDE-47 was highest contributing congeners of tetraBDEs (**Fig 4.4.3**). TriBDEs was least abundant homologue with a contribution of 1-2% to the total PBDEs. From toxicological and

bio-accumulation point of view, the tetra-, penta- and hexa-homologues of PBDEs are of more concern than the octa-, nona- and deca-brominated counterparts [**344]**

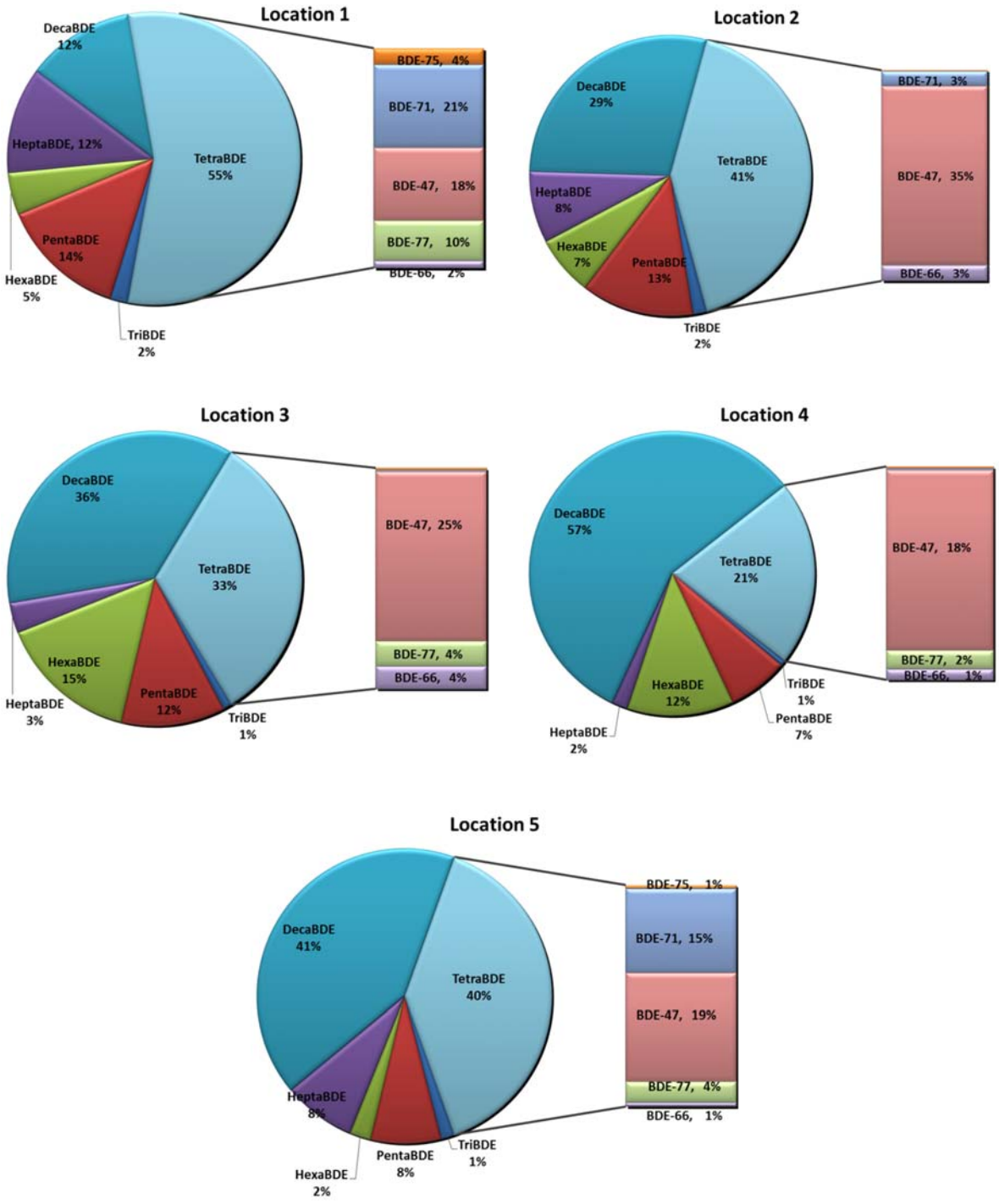


Fig. 4.4.3 Profiles of PBDE homologues in sediments from different locations across Thane creek, Mumbai.

4.4.5 Congener profile of PBDEs using least square method

To estimate the percentage contribution of the three popular commercial mixtures of PBDE to the sediments, it is possible to fit the observed congener profile to a linear combination of the profiles of the commercial products. The observed congener profiles were fitted to the profile of the three commercial mixtures by the least squares method. Solver feature of Excel was used for least squares procedure implemented, in which the following function (equation 15) was minimized [**345, 346]**.

$$\xi_c = \sum\left[\left(f_p C_{i,p} + f_o C_{i,o} + f_d C_{i,d}\right) - C_{i,obs}\right]^2 \quad (15)$$

where f_P is the fraction of the commercial penta-BDE in the sediment, $C_{i,P}$ is the percent of congener i in the penta BDE technical mixture, f_O is the fraction of the octa-BDE in the sedimet, $C_{i,O}$ is the percent of congener i in the octa-BDE product, f_D is the fraction of the deca-BDEs in the sediment, $C_{i,D}$ is the percent of congener i in the deca product, and $C_{i,obs}$ is the average observed percent of congener i in the sediment. Polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures was taken from literature [**347]**. Average percentage contribution of commercial penta-BDE (f_P), octa-BDE (f_O), and deca-BDE (f_D) to the profile found in sediments collected across Thane creek were 24 ± 5, 5 ± 1 and 69 ± 7 % ($p < 0.001$) respectively. These results suggest that fully brominated PBDEs i.e. BDE-209 (deca-BDE) accumulates in sediments, and its use was high compare to other two class of commercial mixtures as discussed above. The second dominant BDE commercial mixture in sediment was penta-BDE followed by octa-BDE. The source profile of PBDEs in sediments from Thane creek were in line with the use of commercial penta-BDE, octa-BDE and deca-BDE in 2001 globally, with estimated value of 7500, 3700 and 56000 t respectively [**348]**.

4.4.6 Comparison of PBDEs with sediment quality guideline

Levels of PBDEs in sediment from Thane were compare with the Federal Sediment Quality Guidelines (FSeQG) which are intended to protect sediment dwelling animals as well as pelagic animals which bioaccumulate PBDEs from sediments. Federal Sediment Quality Guidelines (FSeQG) provide benchmarks for the quality of sediments, and if measured concentration of contaminants met them so there is low likelihood of adverse effects on the protected use (e.g., aquatic life) [**349]**.

Table 4.4.3 Federal Sediment Environmental Quality Guidelines (FSeQG) for selected PBDEs and location wise (L1 to L5) average environmental concentration in sediment from Thane creek.

Homologue		FSeQG (ng/g dw)	L1 (ng/g dw)	L2 (ng/g dw)	L3 (ng/g dw)	L4 (ng/g dw)	L5 (ng/g dw)
triBDE	Total[1]	44	1.16	0.4	0.29	0.19	1.07
tetraBDE	Total[2]	39	2.2	0.99	3.93	1.97	4.5
pentaBDE	Total[3]	0.4	**1.38**	**2.53**	0.01	0	0.36
pentaBDE	BDE-99	0.4	**0.54**	**1.77**	0.01	0	0.21
pentaBDE	BDE-100	0.4	**0.5**	**0.76**	0	0	0.06
hexaBDE	Total[4]	440	0.19	0.34	1.02	0.52	0.17
decaBDE	BDE-209	19	3.16	4.52	11.5	9.22	8.95

1- triBDE only BDE-28; 2- tetraBDE includes BDE-47, BDE-66, BDE-71, BDE-75 and BDE-77
3-pentaBDE includes BDE-85, BDE-99, BDE-100; 4- hexaBDE includes BDE-138, BDE-153, BDE-154.

A comparison of FSeQG values of PBDEs and location wise (L1 to L5) average environmental concentration in sediment from Thane creek is shown in **Table 4.4.3**. Most of PBDEs met with those guideline values except the pentaBDE (total, BDE-99 and BDE-100) at location L1 and L2. These observations suggest that pentaBDE may cause adverse health effect on sediment dwelling animals across Thane creek.

Chapter 5

SUMMARY AND CONCLUSIONS

In present study monitoring of persistent organic pollutants (POPs) was carried out in aquatic environment of creek system. The study provides, levels of organic pollutants in variety of environmental matrices such as seawater, sediments and biota. Both spatial and temporal variation in contamination levels of the organic pollutants in sediment was also investigated in detail. Chromatographic techniques, one of the most convenient and effective tools for analytical chemist, was used for identification and quantification of POPs in different matrices. Levels of POPs in different matrices were compared with studies carried out in recent past from other parts of the globe. Levels of POPs were compare with various environmental quality guidelines to assess their potential threat to marine inhabitants. Various eco-toxicological indices such as $[BaP]_{eq}$, estradiol equivalent concentration (EEQ) were also calculated for addressing ecotoxicological concerns of particular group of contaminants e.g. polycyclic aromatic hydrocarbons, endocrine disrupting chemical. Incremental lifetime cancer risk (ILCR) and daily intake (DI) values were calculated for exposure of selected POPs through consumption of fish and crab from study area.

Data reported in this study will serve as a baseline over future trends of pollution and for subsequent adoption of appropriate remediation policies or techniques. In many parts of the world, the discovery of the persistent, bio accumulative toxic chemicals such as POPs in the environment led to formulation of regional, national and international policies for restriction or to ban the production and usage these chemicals. Present study definitely indicates a build-up of these organic contaminants in the creek eco-system which calls for more efficient and stringent effluent water treatment policies and facilities to prevent these contaminants from

getting released to the environment. Few major conclusions that can be drawn from the study are following.

- Spatial distribution of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) reveals relatively higher concentration at the wastewater receiving point of the creek compared to other sampling points, which are at a distance from the source. The Significant reduction in concentrations of OCPs and PCBs in surface sediments in current study compared with values from deeper sediments indicates the input of OCPs and PCBs in Thane creek area declined over the decades.
- Among OCPs, Lindane (γ-HCH) was the most abundant congener among all hexachlorocyclohexanes (HCHs). Concentration of γ -HCH was also found to be above TEL and PEL sediment quality guideline values. The fugacity fraction (FF) for DDT and its metabolites, indicates these chemicals have tendency to accumulate in sediment from seawater, while HCH congeners tend to follow the reverse pattern.
- PCB profile in grab sediments was also found to be lower from core samples, which again indicate that the sources of PCBs in the Thane creek have diffused and declined over time. Concentrations of PCBs in sediment samples were found to be within sediment quality guidelines given by USEPA and CCME.
- On extrapolation of concentration of POPs in sediment core shows their levels are declining exponentially and may fall below the detection levels in upcoming decades, provided there will be no new inputs of POPs in future to creek environment.

- Total phthalate ester concentrations in seawater, fish and crab samples were found to be slightly higher than what reported in literature in similar studies. The advent of huge quantities of pollutants from rivers/outflow containing a large amount of urban runoff and industrial discharge across the Thane creek must be contributing to this higher concentrations of Phthalates in sediments.
- DBP was found to be in the highest concentration among phthalate esters in sediment and found to be significantly higher than DEHP (PAE reported as most abundant in literature). Probable reasons for such observations may be lesser use of DEHP as compare to DBP or quick biotransformation of DEHP in sediment. Strong correlation was observed in organic carbon content and PAEs concentration for all sediment samples.
- Risk quotient (RQ) values for DBP and DEHP in seawater indicates that these compounds have high ecological risk. Total EEQs values of DBP and DEHP, which are less than 1 ng-E2/L, suggests that the current concentrations pose no threat of estrogenic activity to marine organism in seawater.
- Spatial distribution of Bisphenol A (BPA) in sediments was found very similar to phthalates. 17α-ethinylestradiol (EE2) was main contributor to EEQs in sediment in terms of estrogenic potential, followed by estrone (E1) and 17β-estradiol (E2); which indicates those compounds should be the priority EDCs concerns in the Thane Creek sediment.

- PAHs accumulation in sediment and marine organism was found higher in winter compared to summer. Low molecular weight PAHs (2+3-ring PAHs) were dominant in seawater and fishes compare to high molecular weight PAHs (5+6-ring PAHs), unlike in cases of sediment and crab samples.
- Water-sediment exchange for low molecular weight PAHs (NAP, ACY and ACE) in term of fugacity fraction indicates their net flux is from sediment to seawater. However, rest of the heavier PAHs indicated a net flux from seawater to sediment.
- It was found that, sediment concentrations of FLU, B(a)A, and B(g,h,i)P were exceeding Effect Range-Low (ER-L) values for PAHs at half of the monitoring sites.
- The levels of PAHs in the Fish and crab samples are also of concern based on their $[BaP]_{eq}$ concentration. ILCR values were slightly higher for fishes but significantly high for crab consumption, when compared to the public screening criteria for carcinogens.
- Total Polybrominated Diphenyl Ethers (PBDEs) concentration show multimode distribution with depth in sediment, with a predominant peak at the top layer of sediment. Results of sediment core also indicates PBDEs were heavily used in last two decades in surrounding area.
- Contribution of commercial penta-BDE (f_P), octa-BDE (f_O), and deca-BDE (f_D) to the profile found in sediments collected across Thane creek were in the proportion of their worldwide consumption. Levels of all measured PBDEs in sediment met with guideline values except for the penta-BDE (total, BDE-99 and BDE-100) at few locations.

Overall implications of present thesis can be concluded as following. Monitoring data of POPs in different matrices were helpful in understanding the environmental distribution, fate, and toxicity of these chemicals to human and environment. Data reported in this study can also serve as baseline and will be useful in future to estimate their trend over time. In response to the continuing discovery of the persistent, bio accumulative properties, and toxicity of POPs, regional, national and international policies ban the intentional production of these chemicals. However, the levels of some of these banned compounds in environment are hovering that could still be problematic rather than dwindling. Present study also indicates that effluent water treatment facilities surrounding study area are not efficient to remove these organic contaminants and there is build-up of these chemical in creek environment. Organic pollutants in marine consumables were found in significant concentration, and may cause adverse human health effect, and therefore people consuming marine product from study area should exercise caution.

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