

**OCCURRENCE AND ACCUMULATION OF HCHs
AND DDTs IN WATER, SEDIMENTS AND EDIBLE
FISH SPECIES OF KABUL RIVER, PAKISTAN**



BY

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**DEPARTMENT OF ENVIRONMENTAL SCIENCES
UNIVERSITY OF PESHAWAR**

2015

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AND DDTs IN WATER, SEDIMENTS AND EDIBLE
FISH SPECIES OF KABUL RIVER, PAKISTAN



*A Thesis submitted to Department of Environmental Sciences,
University of Peshawar in fulfillment of the requirements for the
degree of*

DOCTOR OF PHILOSOPHY

IN

ENVIRONMENTAL SCIENCES

BY

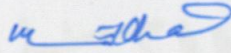
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2015

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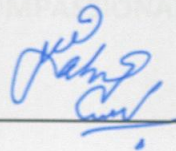


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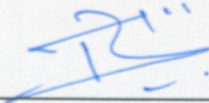


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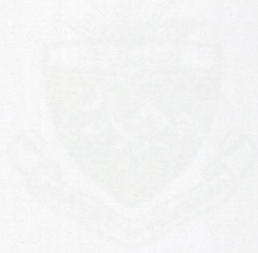
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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

IN THE NAME OF ALLAH, THD MOST COMPASSIONATE, EVER
BROTHERS, SISTERS, COUSIN MY AUNT
MERCIFUL

HUSSAIN WHO SUPPORTED ME AT EACH
STEP OF THE WAY



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"In the name of Allah the most merciful and beneficent"

All praises for Almighty Allah the most merciful and beneficent, without whose consent and permission nothing would ever be imaginable. Praise be to Holy Prophet for His life and work.

DEDICATION

First of all, I am highly indebted to my supervisor Dr. Sadiq Khan, Associate Professor, Department of Environmental Sciences, University of Peshawar, Pakistan for his dynamic supervision and able guidance without whom I may have not been able to complete this research thesis.

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Furthermore, it is the inspiration that I derived from the unconditional love, care and prayers of my parents, brothers, sister, all my other family members and friends that have propelled me to do as I have is grateful.

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MUHAMMAD AAMIR

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Contents Description

This dissertation comprises of 6 chapters is organized sequentially to achieve the aforementioned objectives. Chapter 1 deals with general introduction of HCHs and DDTs and it contains aims and objectives of this study. Chapter 2 discusses literature on the occurrences of HCHs and DDTs in sediments, water and their bioaccumulation in fish species.

Chapter 3 focuses on occurrence, enantiomeric signatures of HCHs isomers and DDTs metabolites in sediments from Kabul River and to assess their ecotoxicological risk to aquatic biota. This chapter is under review in *Chemosphere*.

Chapter 4 describes Contamination profile of HCH and DDT congeners in fish tissues from Kabul River, and also deals with carcinogenic and non-carcinogenic health risk assessment associated with the consumption of HCHs and DDTs contaminated fish. This chapter is under review in *Ecotoxicology and Environmental Safety*.

Chapter 5 provides detailed information on congener-specific evaluation of biota-sediment accumulation factor model for HCHs and DDTs to test the applicability and suitability of the model under small scale *in situ* riverine condition to know about the bioaccumulation potential of sediment-associated hydrophobic organic compounds. Chapter 6 provides conclusions and recommendations for future studies, based on this study.

Abstract

Globally, hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs) are of great concern because of their environmental persistent, long-range transport, bioaccumulative and potentially toxic nature and have been listed as possibly carcinogenic to humans.

The purpose of this work was to investigate the occurrence of HCHs and DDTs residues in three different compartments of aquatic ecosystem (sediments, water and fish) and to assess their eco-toxicological and human health risk associated with the consumption of contaminated fish, and to further evaluate the bioaccumulation potential of sediments-associated HCHs and DDTs into the investigated fish.

HCH isomers and DDT metabolites were analyzed in water, sediments (three different depths 0-10, 10-20 and 20-30 cm) and edible fish species (*Orienes plagiostomus*, *Tor putitora*, *Glyptothorax punjabensis* and *Cyprinus carpio*) collected from Kabul River, Pakistan in February 2014. In three different depths of sediments the occurrence levels, enantiomer fractions and potential ecological risk of these organochlorine pesticides (OCPs) were evaluated. The total concentrations of Σ HCHs and Σ DDTs in surface sediments ranged from 4.9-23.9 and 6.4-18.8 ng g⁻¹ (dry weight basis), respectively. The vertical contamination profile of DDTs was found in order of 20-30 cm > 10-20 cm > 0-10 cm, indicated that the residue levels of DDTs gradually decreased after it was banned. The ratios of β -HCH/HCHs ranged from 0.04 to 0.73 (69% of samples below 0.5) suggested the fresh input of HCHs, while isomeric ratios of α -HCH/ γ -HCH (ranged from 0.02–7.94), with 76% of samples less than 3, indicated the cocktail use of technical grade HCH and lindane in the study area. The ratio of (DDE+DDD)/DDTs (ranged from 0.42–0.90) indicated long-term biodegradation of parent DDT. The enantiomer of α -HCH was generally racemic or close to racemic for most of the samples, with EF value < 0.5 for some of the samples indicated the preferential biodegradation of (+)- α -HCH enantiomer, while for *o,p'*-DDT the EF value were > 0.5 indicated the depletion of (-)-*o,p'*-DDT enantiomer in most of the samples. According to sediment quality guidelines

(SQGs), HCHs contamination is the main concern for ecotoxicological risk in Kabul River.

The levels of HCH and DDT congeners were found significantly different in four different fish species. The average $\sum(\text{HCHs} + \text{DDTs})$ concentration in *G. punjabensis* (214 ng g⁻¹) (carnivores) was found higher than *T. putitora* (herbivores) (155 ng g⁻¹). The distribution of $\sum(\text{HCHs} + \text{DDTs})$ in all fish tissues was found in order of liver > muscle > stomach > gills. The congeners profile indicated that all fish species were contaminated with recent usage of HCH ($\beta\text{-HCH}/\sum\text{HCH}$ from 0.29-0.47), while DDT ((DDE+DDD)/ $\sum\text{DDT}$ from 0.61-0.78) contamination originated from long-time degradation mechanism of DDT. The average estimated daily dietary intake of $\sum\text{HCHs}$ (15.0 ng kg⁻¹ day⁻¹) was found higher than $\sum\text{DDTs}$ (12.5 ng kg⁻¹ day⁻¹) by the local consumers via fish consumption. On the basis of both 50th and 95th percentile exposure levels the carcinogenic hazard ratios for DDT and its congeners were exceeded one (safe limit) for all fish species, indicating a great potential cancer risk for local consumers with life time consumption of contaminated fish.

For evaluation of bioaccumulation potential of sediment-associated HCHs and DDTs into the investigated fish, the congener-specific biota-sediment accumulation factors (BSAFs) for HCHs and DDTs were determined in investigated fish species in *in situ* riverine conditions of Kabul River. BSAF model was used as predictive tool for bioaccumulation of sediment-associated hydrophobic organic compounds (HOCs) for small scale field data. The results from field data validated the model for predicting the bioaccumulation of sediment associated HCH and DDT congeners in the investigated fish species, and also suit the model for benthic fish (*G. punjabensis*) on the basis of 25th and 75th percentiles of congener-specific BSAF values. The BSAFs for *t*-HCH and *t*-DDT were ranged from 0.6-1.5 and from 0.2-1.6, respectively. The model was significantly influenced by both fish ($p = 0.004$) and chemical types ($p = 0.002$) at 95% confidence level. The sediment organic carbon fraction (f_{soc}) and fish lipid fraction (f_{lipid}) had not-to-weak effects on BSAF values for all fish species. The BSAF values were linear fitted with logarithmic octanol-water partition coefficients ($\log K_{\text{ows}}$) of HCH and DDT congeners, despite of the different chemical structures and different compound classes significant negative

correlations between log K_{ows} and BSAF values were found, indicating that hydrophobicity is one of the key factors influencing the bioaccumulation potential of the investigated compounds.

Keywords: Organochlorine pesticides; Hexachlorocyclohexane; Dichlorodiphenyltrichloroethane; Enantiomer signatures; Tissue distribution; Biota-sediment accumulation factor; *In situ* riverine condition; Ecotoxicological risk; Carcinogenic and non-carcinogenic risk; Water; Sediments; fish; Kabul River

Chapter-1

GENERAL INTRODUCTION

1.1. Background

Chemical pollution in the environment is a great concern of people around the world. Among a large number of synthetic chemicals, greater attention has been focused on the persistent organochlorine pesticides (OCPs) because of their high persistent and toxic nature (Iwata et al., 1994). The OCPs incorporate a large group of compounds having diverse structure and properties, and used for the control of human's vectors and agriculture pests. These toxic OCPs are very poisonous for human health and pose a great risk to global environment because of their long-range transportability, non-biodegradability and bioaccumulation in food chains and webs (Laws, 2000; Wang et al., 2015).

Organochlorine pesticides were introduced in early twentieth century with the development of the chlor-alkali industry. With the mass production of synthetic chlorinated organic compounds, the desire for biocide within chemical technologies and agriculture were increased and the use of synthetic pesticides were begun. An early hexachlorocyclohexane (HCH) insecticide was first introduced in 1933—historically known as benzene hexachloride (BHC). In 1939 with the outbreak of World War II (WWII), and the need to prevent typhus and malaria amongst civilians and troops led to the invention and development of persistent insecticide dichlorodiphenyltrichloroethane (DDT) (Turnbull, 1996).

Pesticides were introduced in Pakistan for the first time in 1954 with 254 metric tons of formulated products, when imported and distributed among the farmers free of cost. With this humble beginning in late 1960s and early 1970s thousand tons of pesticides were imported from Europe and USA for malaria eradication, handling crop pest infestation and locust control, during the green revolution in the country. Moreover, HCH and DDT were produced locally in 1980s when the business of pesticide imports and distribution was transferred from public to private sector and the uses of these pesticides were increased from 665 metric tons to 78133 metric tons annually (Azizullah et al., 2011).

Since WWII, OCPs have been extensively used in agriculture practices to control crop diseases. Among OCPs, DDT and HCH have been most frequently used worldwide (Hong-Yan et al., 2007; Ael et al., 2012). The OCPs were the first synthetic pesticides and were banned in 1970s in most of the developed countries—regulation and prohibition developed on the toxic chemicals (Zhao et al., 2009). The HCH and DDT were among the 12 persistent organic pollutants restricted globally in the Stockholm Convention (2001) on POPs, because of their non-biodegradability, long-range transportability, lipophilic nature and adverse eco-toxicological impacts on humans, and global environment (UNEP/UNDP, 2001). The convention was signed by 151 countries and ratified by 122 countries. In December 6, 2001 Pakistan signed this convention (SDPI 2006).

Among OCPs, HCH and DDT had been widely used as pesticides in agriculture and as insecticides in sanitation programs for vector control. These pesticides, their isomers and metabolites such as HCH: α -HCH, β -HCH, γ -HCH, δ -HCH and ϵ -HCH and DDT: *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD are linked with different health and environmental risk (Mishra et al., 2012).

1. 2. HCHs

HCH have total eight isomers and each isomer differs from one another at the chlorine atom equatorial and axial position. Isomer α -HCH is the one of isomers which exists in two enantiomeric forms (Wu et al., 2013).

In comparison with other organochlorine pesticides, HCH and their isomers have relatively high vapor pressure and high water solubility. Therefore, HCH and their isomers found in different compartments of the environment, in different state, usually present as gas in atmosphere or dissolved in water, and only small portion adsorbed onto particles or accumulated in organisms. The physicochemical characteristics of isomers of HCHs differ from one another. For instant, γ -HCH has somewhat greater vapor pressure than α -HCH. Therefore, γ -HCH exhibit to be slightly less lipophilic than α -HCH. In comparison α -HCH is relatively more likely to partition to air because α -HCH has Henry's law constant twice of that of γ -HCH. Among HCH isomers, β -HCH is the most persistent and stable isomer, it also shows more lipophilic characteristics than the others. These unique properties of β -HCH isomer are because of its smaller molecular volume. The β -

HCH molecule is small enough and denser, and easily stored in organism's lipids. Therefore, β -HCH accumulates in organisms predominantly (Walker et al., 1999; Wu et al., 2013).

The isomers α , β and γ -HCH are found most commonly in the environment, and as a result of metabolism in soil or sediment can be transformed into α - or β -HCH and during its long range transport may be transformed into α -HCH by sunlight (Walker et al., 1999; Li et al., 2015).

Hexachlorocyclohexane is found in two formulations, lindane (γ -HCH) and the technical grade HCH. The technical mixture of HCH composed of all eight isomers, out of which α -, β -, γ -, δ - and ϵ -HCH are generally identified and stable isomers (Fig. 1.1), while lindane made up entirely of γ -HCH. Commercial technical HCH consists of: (60-70%) α -HCH, (5-12%) β -HCH, (10-15%) γ -HCH, (6-10%) δ -HCH and (3-4%) ϵ -HCH (Li and Macdonald, 2005). Both formulations lindane and commercial HCH have been used as insecticide in agriculture practices and for vector control in sanitation programmes (Walker et al., 1999; Mishra et al., 2013).

In 1948 to 1997, approximately 10 Mt of commercial HCH has been received by the environment, predominantly HCH consumed by China, former Soviet Union and India were 4464 kt, 1960 kt and 1057 kt, respectively. Around half of the global usage of technical grade of HCH has been consumed by China. In western countries estimated pesticide used by France, Germany and Spain were about 520 kt, 142 kt and 133 kt, respectively (Li and Macdonald, 2005).

DDT and with minor part of the remaining metabolites. The DDT and its metabolites persist in the environment for long time; half-life of DDT is reported to be 75 years. In the environment DDT degradation occurs in two different conditions, aerobic and anaerobic. In aerobic condition, DDT effectively transformed to DDE and under anaerobic condition it transformed to DDD by reductive dechlorination and dehydrochlorination processes (Mishra et al., 2013).

The first environmental book "Silent Spring" was written by Rachel Carson in 1962. In her book she highlighted the environmental adverse impacts of the improper use of DDT in the USA and raised an issue on the release of these

Chemical name: 1, 2, 3, 4, 5, 6, -Hexachlorocyclohexane

Chemical formula: $C_6H_6Cl_6$

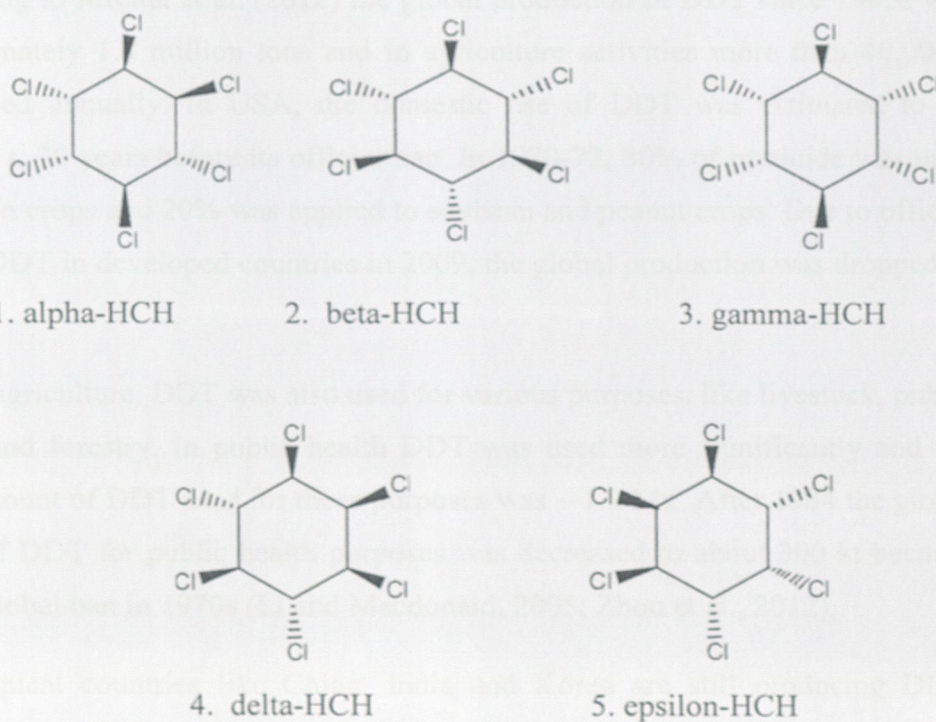


Fig. 1.1. Chemical formulas and structures of HCH.

1.3. DDTs

There are total of six metabolites of DDT found in the environment, these are *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE and *o,p'*-DDE (Fig. 1.2). The commercial DDT consist of 75% of *p,p'*-DDT, 15% of *o,p'*-DDT, 5% of *p,p'*-DDE and with minor percent of the remaining metabolites. The DDT and its metabolites persist in the environment for long time; half-life of DDT is reported upto 75 years. In the environment DDT degradation occurs in two different conditions, aerobic and anaerobic. In aerobic condition, DDT effectively transformed to DDE and under anaerobic condition it transformed to DDD by reductive dechlorination and dehydrochlorination processes (Mishra et al., 2012).

The first environmental book “Silent Spring” was written by Rachel Carson in 1962. In her book she highlighted the environmental adverse impacts of the improper use of DDT in the USA and raised an issue on the release of these

pesticides in large amount without have knowledge about the adverse effects on the human health and environment (Li and Macdonald, 2005).

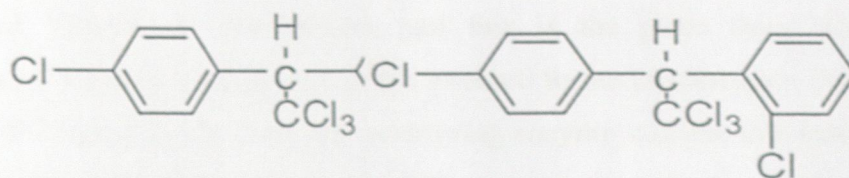
According to Mrema et al. (2012) the global production of DDT since 1940s was approximately 1.8 million tons and in agriculture activities more than 40,000 t were used annually. In USA, the domestic use of DDT was estimated to be 600,000 t, 30 years before its official ban. In 1970-72, 80% of pesticide was used on cotton crops and 20% was applied to soybean and peanut crops. Due to official ban of DDT in developed countries in 2009, the global production was dropped to 3,314 t.

Beside agriculture, DDT was also used for various purposes, like livestock, public health and forestry. In public health DDT was used more significantly and the total amount of DDT used for these purposes was ~ 1.9 Mt. After 1984 the global usage of DDT for public health purposes was decreased to about 300 kt because of the global ban in 1970s (Li and Macdonald, 2005; Zhou et al., 2012).

The tropical countries like China, India and Korea are still producing DDT, mainly for public health purposes. In 2000-2004, in China the annual production of DDT was 4,500 Mt and about 90% was consumed in the acaricide and Dicofol production. In India, about 1000 Mt DDT per year is used for the prevention of visceral leishmaniasis. In India a large amount of DDT was consumed annually, but this ratio was decreased in 2007 to one-fourth of the level of 2005 (van den Berg, 2009).

Chemical name: 1,1,1-trichloro-2,2-bis-(4-chlorophenyl) ethane

Chemical formula: $C_{14}H_9Cl_5$



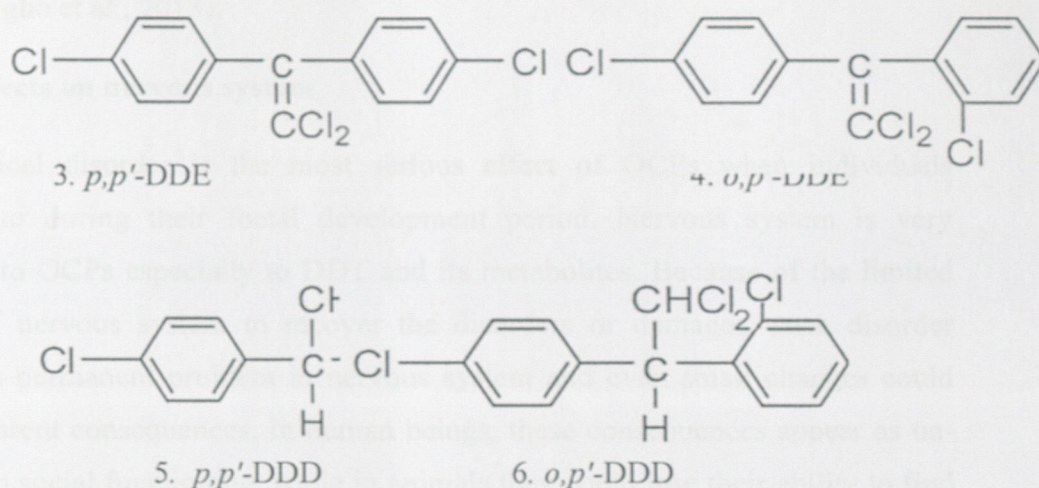


Fig. 1.2. Chemical formulas and structures of DDT

1.4. Toxicological Effects of OCPs

Organochlorine pesticides and their derivatives are harmful for humans and animals because of their toxicological effects. Health problems associated with short-term exposure include: dizziness, headaches, skin irritation, vomiting, fever, and difficulties in breathing. Chronic exposure to pesticides has been shown to cause breast, lungs, liver and kidney cancer, brain damage, and disruption of immune and nervous systems etc. Eventually exposure to high dose could cause death (Fig. 1.3) (Chopra et al., 2010). The most significant adverse effects of OCPs are described as follow.

1.4.1. Effects on function of liver and metabolism of Vitamin A

Vitamin A has great importance in all life sustaining processes, it plays important role in immune system and in the development and metabolism of normal foetal and their reproduction. Liver acts as accumulating body of Vitamin A where the surplus of Vitamin A accumulates and this is the place from where this accumulated Vitamin in regulated doses released to the bloodstream. Prior to the storage of Vitamin in the liver the esterifying enzyme convert this substance to the ester. The OCPs like DDTs and HCHs prevent the storage of Vitamin A in liver by inhibiting the function of this esterifying enzyme and thus the intake of

very small concentration of these pesticides disturb the function of liver (Dekebo, 2007; Berghe et al., 2013).

1.4.2. Effects on nervous system

Neurological disorder is the most serious effect of OCPs when individuals exposed to during their foetal development period. Nervous system is very sensitive to OCPs especially to DDT and its metabolites. Because of the limited ability of nervous system to recover the disorders or damages, such disorder become a permanent problem in nervous system and even small changes could have apparent consequences. In human beings, these consequences appear as unbalance in social functioning, while in animals they could lose their ability to find food and unable to reproduce. DDT and its metabolites enhance and make stronger the impulses of nerve system and which exist for long time than normal (Medehouenou et al., 2014).

1.4.3. Disruption of hormones function

The DDTs and HCHs have resemblances with sex hormones and disturb the hormonal natural balance, either in their parental state or derivatives. These pesticides have similarities with sex hormones act as oestrogens and play important role in the activation of oestrogen receptors. On the other hand, these pesticides are also capable of to lock oestrogen receptor, the substance with locked the oestrogen receptor is also called anti-oestrogenic, while the substance which reduces the function of male sex hormones are called anti-androgenic. Among organochlorine pesticides DDT and its metabolite DDE have the estrogenic effects, whereas DDE also exhibit anti-androgenic effect. Meanwhile HCH and its isomer Lindane (γ -HCH) have anti-estrogenic effect. The sensitivity of OCPs at the foetus development stage is very high and the disruption of the sex hormones can result in birth defects and developmental disorder. The exposure of male foetues to anti-androgenic substances will result in the sexual organs feminization and malformation, which appear as impaired fertility, reduce sperm production and modified mating behavior at the adulthood (Freirea et al., 2014; Rivero et al., 2015).

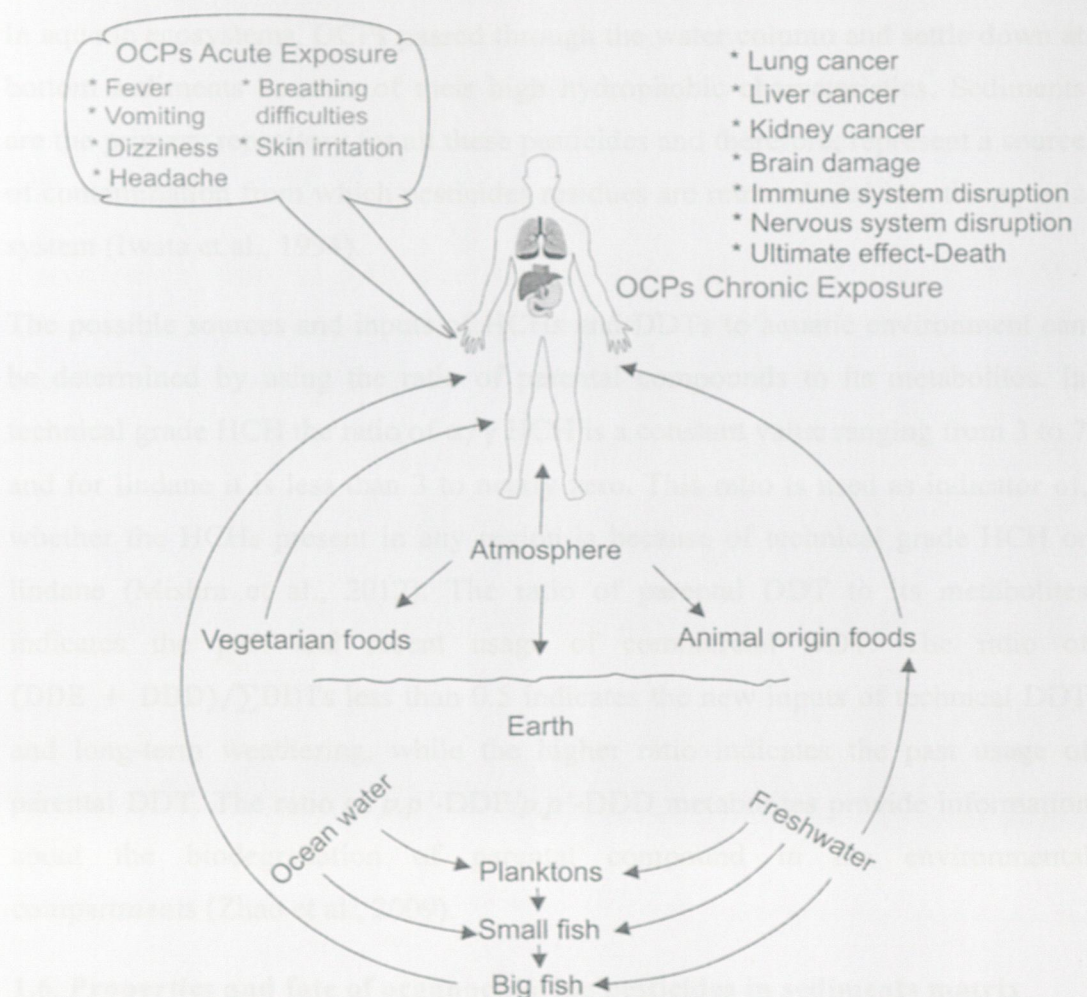


Fig. 1.3. Toxicological effects to human beings from exposure to OCPs contaminated food chains.

1.5. Sources and inputs of OCPs to aquatic environment

In the past, research studies on the occurrence of OCP residues in various environmental matrices are reported and also detected in aquatic environment: such as fish, sediment and water (Tanabe et al., 1994). OCPs applied on the land enter into aquatic environment by atmospheric wet and dry depositions, runoff from nonpoint sources, discharge of industrial wastewater and domestic sewage and direct dumping of waste (Doong et al., 2002). Therefore, pesticides ultimately bioaccumulate into food and ingest by human. The other sources/pathways are contaminated drinking water and consumption of contaminated fish and aquatic food stuffs (Zhou et al., 2012).

In aquatic ecosystems, OCPs passed through the water column and settle down at bottom sediments because of their high hydrophobic characteristics. Sediments are the primary repository for all these pesticides and therefore, represent a source of contamination from which pesticides residues are reintroduced into the aquatic system (Iwata et al., 1994).

The possible sources and inputs of HCHs and DDTs to aquatic environment can be determined by using the ratio of parental compounds to its metabolites. In technical grade HCH the ratio of α/γ HCH is a constant value ranging from 3 to 7 and for lindane it is less than 3 to nearly zero. This ratio is used as indicator of, whether the HCHs present in any region is because of technical grade HCH or lindane (Mishra et al., 2012). The ratio of parental DDT to its metabolites indicates the past and recent usage of commercial DDT. The ratio of $(DDE + DDD)/\sum DDTs$ less than 0.5 indicates the new inputs of technical DDT and long-term weathering, while the higher ratio indicates the past usage of parental DDT. The ratio of p,p' -DDE/ p,p' -DDD metabolites provide information about the biodegradation of parental compound in the environmental compartments (Zhao et al., 2009).

1.6. Properties and fate of organochlorine pesticides in sediments matrix

The accumulation of OCPs in sediments is a sophisticated process depends on physicochemical properties of the surrounding environment, like organic matter content of sediments and partition coefficient of individual compound (Borgå et al., 2001). Many studies documented on the distribution of OCPs and physicochemical properties of sediments, such as sediments grain size, organic matter content and sediment water contents which play significant role in fate and distribution of OCPs in aquatic environment (Fig. 1.4). Moreover, these toxicants can be bioaccumulate in aquatic organisms and then further biomagnified through food chain, which pose threat to aquatic ecosystem and food safety and eventually causing health hazards (Zhao et al., 2009; Kuranchie-Mensah et al., 2012; Li et al., 2015).

Fate of OCPs in the sediments system can be describe as the retention or persistence, the degradation and mobility of pesticides in sediments which depends on the properties of compounds like solubility, partition coefficient and

vapor pressure Henry's law constant and the sediments properties such as pH, sediment moisture, sediments type and soil organic carbon content etc. (Fig. 1.4). These properties are the main contributing factors in the adsorption of OCPs to sediment such as organic matter, minerals and clay (Pereira et al., 2010).

The lower pH value of sediments enhances the accumulation of pesticides and reduces its degradation in sediments by reducing the microbial activities. According to presumption of Skrbic and Mladenovic (2007) the levels of DDT increased when value of pH dropped.

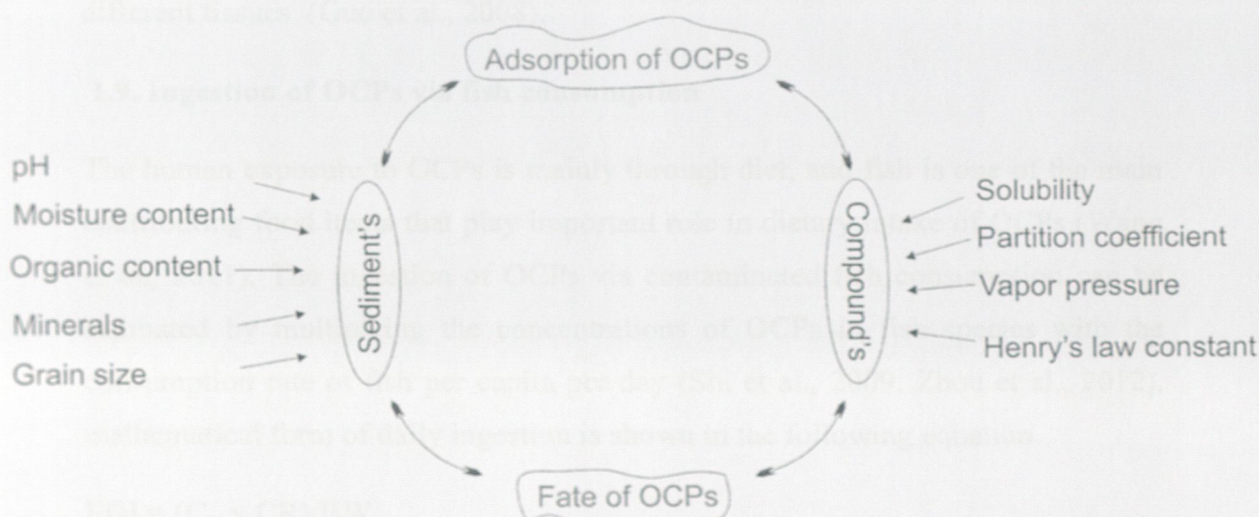


Fig.1.4. Physicochemical properties playing vital roles in adsorption and fate of OCPsin sediment matrix.

1.7. Bioindicator of OCPs in aquatic environment

Fish is a good bioindicator for pollution in aquatic ecosystems, because it possesses a low metabolism activity for OCPs and thus allows higher bioaccumulation of OCPs and should represent the contamination status of contaminants in the aquatic ecosystems (Muir et al., 1990). Moreover, fish can bioaccumulate OCPs from their surrounding environment (abiotic compartment), thus enabling us to measure pollutants concentration in their environment, and biomagnification through their food chain. Therefore, information on the occurrence and bioaccumulation of OCPs in edible fish are important from both human health and ecological perspectives (Davodi et al., 2010).

1.8. Bioaccumulation of OCPs in different fish tissues

As discussed in preceding section, fish can be used extensively for environmental monitoring of OCPs contamination because they uptake contaminants (i.e. OCPs) directly from water and diet. The loading in fish is well reflective of the pollution state of surrounding environment. In addition, this bioaccumulation of OCPs in fish and their distribution in different tissues i.e. liver, skin, gills, stomach and muscle would provide more clues about the path-ways along which OCPs load into fish and about their bioaccumulation and biotransformation pattern in different tissues (Guo et al., 2008).

1.9. Ingestion of OCPs via fish consumption

The human exposure to OCPs is mainly through diet, and fish is one of the main contributing food items that play important role in dietary intake of OCPs (Wang et al., 2011). The ingestion of OCPs via contaminated fish consumption can be estimated by multiplying the concentrations of OCPs in fish species with the consumption rate of fish per capita per day (Shi et al., 2009; Zhou et al., 2012), mathematical form of daily ingestion is shown in the following equation.

$$EDI = (C_m \times CR)/BW$$

Where EDI is estimated daily intake ($\text{ng}^{-1} \text{ kg}^{-1} \text{ d}^{-1}$); C_m is the concentration of OCPs in fish (ng g^{-1}); CR is the consumption rate of fish (g d^{-1}); BW is the average body weight (kg).

1.10. Assessment of health risk associated with OCPs exposure

Organochlorine pesticides are poisonous to human health and even exposure to very low dose can cause toxic effects to human being (discussed in section 1.4). Human health risk associated with consumption of OCPs contaminated fish can be assessed according to USEPA (2001) recommended guidelines. Non-carcinogenic risk from OCPs can be estimated by comparing their estimated daily intake (EDI) from contaminated fish consumption with the recommended reference doses (RfDs) set forth for OCPs, while for carcinogenic risk estimation, the hazard ratios (HRs) for OCPs were calculated using the following equation (Wang et al., 2011; Akoto et al., 2015).

$$\text{Hazard Ratios (HRs)} = \frac{\text{EDI}}{\text{CBC}}$$

Where CBC represent cancer benchmark concentration ($\text{ng kg}^{-1} \text{d}^{-1}$).

Despite a global ban on usage and production of persistent HCHs and DDTs these pesticides are still using in some developing countries of tropical and subtropical belt for vector control programs and agricultural activities because of their easily availability, versatility in pests and insects control and low cost (Iwata et al., 1994; Tanabe et al., 1994). Consequently, in Asian developing countries the environmental and health problems associated with these toxicants are of great concern of people. It is documented in annual report of WHO/UNEP working group, that approximately 200,000 people die and about three million get poisoned each year in developing countries from poisoning of pesticide (WHO, 1990; FAO, 2000).

In Pakistan, like other developing countries, OCPs have been banned by government of Pakistan since 2001 (Ejaz et al., 2004). Different studies conducted in different regions of the country and had determined OCPs in different environmental compartment like water, soil, food and air (Tariq et al., 2007) but there is still scarce of literature on OCPs residue and their isomers and metabolites in different environmental matrices around Pakistan. Prior to this study there is no study conducted on the assessment of contamination levels of HCH and DDT congeners in water, sediments and sediment-associated bioaccumulation in fish species of Kabul River.

Study aim and objectives

Aim

The aim of this work is to assess the potential hazards and health risks of HCHs and DDTs to aquatic environment and ultimately to human being. The approach of present work is to provide a source of scientific information on the determination and contamination levels of DDTs and HCHs residues in water, sediments and edible fish species and their relationship with physicochemical parameters of sediments. The present study is also focusing on, to identify the

possible input pathways of HCHs and DDTs and their bioaccumulation potential into fish species.

LITERATURE REVIEW

Specific Objectives

The objectives of this study are:

- To determine levels of HCH and DDT and their byproducts in water, sediments and fish species from Kabul River.
- To estimate the recent and past usage, and input sources of HCHs and DDTs residues.
- To evaluate bioaccumulation potential of sediment-associated HCHs and DDTs into tissues of edible fish species.
- To estimate the potential eco-toxicological impacts of OCPs to aquatic environment and the habitants inhabiting into the sediments.

Chapter 2

LITERATURE REVIEW

In the developed and some developing countries like Pakistan the use of OCPs have been banned from the last 30 years but due to their high persistence nature these OCPs are still present globally in different environmental matrices, like sediments, soil, water, atmosphere, suspended particulate matters (SPM), plants and animals or other biological organisms. Whereas, these OCPs can further transform and transport to spread among different compartment of environment and result in cross contamination (Yang et al., 2013).

2.1. Residues of HCHs and DDTs in freshwater

The water columns from Poyang Lake was studied to evaluate the fate of OCPs. The total concentration of OCPs was ranged from 19.10-111.78 ng L⁻¹. Among OCPs, HCHs and DDTs were found predominantly (Table 2.1). The results from this study suggested that technical DDTs and dicofol were the main sources of DDTs, whereas long-distance transmission and industrial products with separate lindane in use were considered as the main source of HCHs. In addition, the levels of HCHs and DDTs in Poyang Lake water were below the recommended threshold values (Zhi et al., 2015).

Yu et al. (2014) analyzed the possible influence of OCP on Miyun Reservoir and for that water samples were collected from Chao River, which is the main upstream source of the reservoir. Among the total of 24 types of OCPs measured (ranged 0.8145 to 16.8524 ng g⁻¹) HCHs and DDTs residues were found dominant (Table 2.1). The ratios of HCH and DDT parental compounds to their congeners suggested the recent (lindane) and past usage of HCH and DDT in the study area, respectively.

Hellar-Kihampa et al. (2013) monitor the Pangani River, Tanzania to determine OCPs in surface water, the sample were collected from twelve stations along the river in three distinct seasons: the dry season from September to October 2009, the rainy season from March to April 2010 and mid-season from January to February 2011. The seven compounds were determined, among DDTs and

HCHs concentrations were detected maximum, DDTs were dominated by *p,p'*-DDT and *p,p'*-DDE with concentration of 320-1300 pg L^{-1} and 160 -1460 pg L^{-1} , respectively. Whereas the total DDT was found in the range from 590-2030 pg L^{-1} , while α -HCH was detected at concentration of 4460 pg L^{-1} (Table 2.1). The concentration was detected low in the rainy season and high in dry season. The result showed that the concentrations were below the WHO guideline 2 $\mu\text{g L}^{-1}$ for drinking water.

In china intense survey and experiment was conducted to determine the levels of OCPs in Yangtze River to assess and evaluate the potential ecological risk and pollution levels. Total of twenty four OCPs were investigated, among residues of DDTs and HCHs were dominated with average concentration levels of HCHs: (0.970 ng L^{-1}) α -HCH, (0.657 ng L^{-1}) β -HCH, (0.261 ng L^{-1}) γ -HCH, (0.631 ng L^{-1}) δ -HCH and (2.52 ng L^{-1}) Σ HCH, while concentration of DDT and its metabolites were (0.997 ng L^{-1}) *p, p'*-DDT, (0.112 ng L^{-1}) *o, p'*-DDT, (0.008 ng L^{-1}) *p, p'*-DDD, (0.045 ng L^{-1}) *o, p'*-DDD, (0.004 ng L^{-1}) *p, p'*-DDE, (0.010 ng L^{-1}) *o, p'*-DDE and (1.17 ng L^{-1}) Σ DDT (Table 2.1). The results were compared with surface water quality guideline recommended by USEPA (2002) and environmental quality standards of China (GB3838-2002), all the samples were within the range of guidelines and contamination levels of OCPs were within the safe limits. In the study area metabolites of DDT (DDM, DBP and DDMU) were also found in trace amount for the first time (Tang et al., 2013).

The surface water of Lake Chao hu in China was analyzed for the residues of HCHs and DDTs to assess the potential health and eco-toxicological risk of these compounds during 2009. Total of fifteen water samples were collected and analyzed for the residues of HCHs and DDTs. The data showed that the residues of HCHs and DDTs were in the range of 1.58-31.66 and 1.52-21.79 ng L^{-1} , respectively. The results and margin of safety 10 (MOS10) values indicated that the residues of DDTs and γ -HCH associated with lake water pose potential eco-toxicological risk to the Lake and its biota. The human health risk (both carcinogenic and non-carcinogenic) associated with HCHs and DDTs were relatively lower (He et al., 2012).

Kuranchie-Mensah et al. (2012) conducted a study in 2012 and were determined the OCPs residues in water from Densu River, Ghana. The water samples were collected from Nsawam and Weija along Densu River. The result showed that the average levels of δ -HCH were higher than that of γ -HCH (Lindane). In the HCH isomers, the concentration of δ -HCH was found maximum at Nsawam with concentration of $0.130 \mu\text{g L}^{-1}$. Meanwhile p,p' -DDT and p,p' -DDE were recorded at concentrations of $0.025 \mu\text{g L}^{-1}$ and $0.015 \mu\text{g L}^{-1}$, respectively (Table 2.1). The concentrations were compared with the Australian guideline; the concentrations of γ -HCH (Lindane) and δ -HCH were found higher than the guideline values.

In Pakistan a study was conducted in 2010, to assess the contamination levels of pesticides in three provinces (NWFP, Punjab and Sindh), a total of 33 water samples were collected. Table 2.2 indicated that among OCPs Σ DDT, 2,4-DDT, 4,4-DDT, 2,4-DDD and 4,4-DDE were detected with concentration levels ranging from 0.009 to $0.06 \mu\text{g L}^{-1}$, 0.009 to $0.11 \mu\text{g L}^{-1}$, 0.063 to $0.03 \mu\text{g L}^{-1}$ and 0.019 to $0.04 \mu\text{g L}^{-1}$, respectively. However, β -HCH and γ -HCH were detected in study area with concentrations ranging from 0.018 to $0.09 \mu\text{g L}^{-1}$ and 0.014 to $0.16 \mu\text{g L}^{-1}$, respectively. The result showed that the concentrations of DDT and lindane exceeded the European Union Maximum Allowable Concentration (EU MAC) of $0.1 \mu\text{g L}^{-1}$ in 12% and 25% water samples, respectively (Ahad et al., 2010).

In another research in China the residues of DDTs and HCHs were determined in water from Daliao River estuary with concentration ranges of 0.02 – 5.2 and 3.4 – 23.8 ng L^{-1} , respectively (Table 2.1). The result of this study indicated that the concentration of DDTs and HCHs were below the quality standard established by the State Environment Protection Agency, China (SEPA) (Σ DDT $< 2000 \text{ ng L}^{-1}$ and for Lindane $< 1000 \text{ ng L}^{-1}$). According to USEPA guideline (2002), for surface water the γ -HCH criteria maximum concentration should be 950 ng L^{-1} . The concentrations of Lindane in all samples were recorded within the range of guideline set by UESPA and SEPA. Whereas the concentration of HCHs in some sample were higher than the environmental quality standard (2000/60/EC) established by European Union for HCHs (20 ng L^{-1}) in water, while DDTs

concentration were within the range of guideline (25 ng L⁻¹ for DDTs). The result showed that the study area has an ecological risk from HCHs (Tan et al., 2009).

Jan et al. (2009) worked out a study to examine the DDT residue around a DDT manufacturing factory in Amman Gharh, Nowshera, Pakistan during 2009. The water samples were taken from the main drain of factory leading to Kabul River and surrounding villages. The result showed that despite the demolishment of DDT factory many years ago the DDT residue were still found in most of the water samples, ranging from 0.20 ± 0.23 to 0.31 ± 0.03 ug ml⁻¹ (Table 2.2) and threaten the human life and aquatic ecosystem of Kabul River.

Darko et al. (2008) investigate the extent of contamination of selected OCPs in surface water of Lake Bosomtwi, Ghana during 2008. Total of 50 samples were collected randomly and analyzed for OCPs using Gas chromatograph with electron capture detector. The result showed that the Lindane was determined in 64% of all samples, while DDT was detected in 78% of all samples with average levels of 0.012 ng g⁻¹.

As shown in Table 2.2, Asi et al. (2008) conducted a study to determine pesticides residues in water samples from different regions of the country. The DDT and its metabolites were detected ranging from 84 to 91% in all samples, with the detection limit ranging from 0.02 to 0.10 ng ml⁻¹. In addition, many studies were conducted to examine the HCHs and DDTs residue levels from different areas of Pakistan as shown in Table 2.2.

Table 2. 1. Residues of HCHs and DDTs detected in freshwater from different countries.

Area	Type of Water (surface/ground)	Year	Detected residues	Concentration	Technique	References
Pangani River, Tanzania	Surface water	2013	<i>p,p'</i> -DDE	160-1460 pg L ⁻¹	GC-ECD	Hellar-Kihampa et al. (2013)
			<i>p,p'</i> -DDT	320-1300 pg L ⁻¹		
			α -HCH	4460 pg L ⁻¹		
			<i>p, p'</i> -DDT	0.997 ng L ⁻¹		
			<i>o, p'</i> -DDT	0.112 ng L ⁻¹		
Yangtze River, China	Surface water	2013	<i>p, p'</i> -DDD	0.008 ng L ⁻¹	GC-ECD	Tang et al. (2013)
			<i>o, p'</i> -DDD	0.004 ng L ⁻¹		
			<i>p, p'</i> -DDE	0.045 ng L ⁻¹		
			<i>o, p'</i> -DDE	0.010 ng L ⁻¹		
			Σ DDT	1.170 ng L ⁻¹		
			α -HCH	0.970 ng L ⁻¹		
			β -HCH	0.657 ng L ⁻¹		
			γ -HCH	0.261 ng L ⁻¹		
			δ -HCH	0.631 ng L ⁻¹		
			Σ HCH	2.520 ng L ⁻¹		
			<i>p,p'</i> -DDT	0.025 μ g L ⁻¹		
			<i>p,p'</i> -DDE	0.015 μ g L ⁻¹		
Densu River, Ghana	Surface water	2012	δ -HCH	0.130 μ g L ⁻¹	GC-ECD	Kuranchie-Mensah et al. (2012)
			DDTs	0.02-5.2 ng L ⁻¹		
			HCHs	3.4-23.8 ng L ⁻¹		
Daliao River estuary, China	Surface water	2008			GC-ECD	Tan et al. (2009)
Chao River, Thailand	Surface water	2014	Σ OCPs: including DDTs and HCHs	0.8145-16.8524 ng g ⁻¹	GC-ECD	Yu et al. (2014)
Poyagn Lake	Surface water	2015	Σ OCPs: including DDTs and HCHs	19.10-111.78 ng L ⁻¹	GC-ECD	Zhi et al. (2015)

Table 2. 2 Residues of HCHs and DDTs detected in freshwater from different areas of Pakistan ($\mu\text{g L}^{-1}$)

Area	Type of Water (surface/ground)	Year	Detected residues	Concentration ($\mu\text{g L}^{-1}$)	Technique	References
Cattle Colony, Karachi	Surface water	1984	HCH isomers <i>p,p'</i> -DDE <i>p,p'</i> -DDT	Traces-16.7 Traces Traces	GC-ECD	Parveen et al. (1988)
Samples collected from cotton growing area of the Multan	Groundwater	2000	Lindane	0.0-0.1	GC (FID,ECD and NPD)	Ahad et al. (2001)
SCARP (II-V) area of the Punjab	Surface water	2000	Lindane	0.0-0.001	GC (ECD, NPD)	PPSGDP, (2002)
Nine districts of Punjab	Both surface and groundwater	2008	DDT and its metabolites	0.02-0.10	HPLC	Asi et al. (2008)
Amman Gharh, Nowshera, NWFP	Both surface and groundwater	2009	DDT	0.20 - 0.31	GC-ECD	Jan et al. (2009)
Samples collected from Simly Lake and Rawal Lake, Islamabad	Surface water	2009	2,4-DDT 4,4-DDD 4,4-DDE Alpha-HCH	0.96-2.8 0.80-2.39 Traces 0.84	GC-ECD	Iram et al. (2009)
Obsolete pesticide stores in NWFP, Punjab and Sindh	Both surface and groundwater	2010	Lindane 2,4-DDT 4,4-DDT 2,4-DDD 4,4-DDE β -HCH γ -HCH	Traces 0.009-0.06 0.009-0.11 0.063-0.03 0.019-0.04 0.018-0.09 0.014-0.16	GC-ECD	Ahad et al. (2010)

2. 2. Occurrence of HCHs and DDTs in sediments

Wu et al. (2013) investigate the residues of HCHs and DDTs in sediments from Hangzhou, China in September 2009. Total of 15 sediment sampling sites were selected, where samples were collected from three different depth levels 0-5 cm, 5-10 cm, and 10-15 cm. The results showed that the determined residues in the study area were mainly originated from the past usage of OCPs, with concentration levels of DDTs and HCHs were 0.76–17 ng g⁻¹ and 0.74–5.8 ng g⁻¹, respectively (Table 2.2). The study suggested that the occurrence of HCHs and DDTs species in the study area have more eco-toxicological concern.

Yang et al. (2013) conducted study to explain the sources and fate of OCPs in the sediments from Quanzhou Bay, China. During the month of August 2011 sixteen sediment samples were collected from coastal areas of Quanzhou Bay. HCHs and DDTs were determined with average concentrations of 0.927 and 1.357 ng g⁻¹ in surface (0-20 cm) and subsurface (20-40 cm), while 4.737 and 3.207 ng g⁻¹ in surface and subsurface, respectively. The results were compared with the two sediment quality guidelines of threshold effects level and probable effects level issued by Canadian Council of Ministers of the Environment (2002) and effect range- low value and effects range-median value guideline (US-EPA). The result showed that the concentrations of γ -HCH and *p,p'*-DDD were higher than guideline values, while concentration of *p,p'*-DDE was lower than the threshold effect level (TEL). The higher concentrations of *p,p'*-DDT and *o,p'*-DDT than threshold effect level, probable effect level and effect range-low values suggesting that the DDTs pose a adverse ecological risk to the aquatic environment. According to sediment quality guideline values DDTs have potential to cause ecotoxicological risk to Quanzhou Bay.

Kuranchie-Mensah et al. (2012) investigated OCPs in two stations of Densu river basin, Ghana. The surface sediment samples were collected from 0-2 cm in 2011 from Weija and Nsawam stations with the help of stainless-steel grab sampler. The residues of *p,p'*-DDT, *p,p'*-DDE, γ -HCH and δ -HCH were determined using Gas Chromatograph mounted with electron capture detector with concentration ranged from 0.05-0.45 $\mu\text{g kg}^{-1}$, 0.14 - 1.67 $\mu\text{g kg}^{-1}$, 0.280-1.04 $\mu\text{g kg}^{-1}$ and 0.04-0.23 $\mu\text{g kg}^{-1}$, respectively (Table 2.2).

Hu et al. (2009) analyzed sediments of Bohai Sea, China for OCPs, to identify the residues, their sources and possible eco-toxicological risk. Total of fifty five (55) sediment samples were collected with the help of stainless steel grab sampler. DDTs and HCHs were detected with concentrations ranged from 0.24 - 5.67 ng g⁻¹ and 0.16 -3.17 ng g⁻¹, respectively. The ratio (DDE + DDD)/DDT indicated the residues identified were the result of past usage of DDT, while the recent usage was attributed to mainly agricultural activities, antifouling paint and industrial wastewater. The result showed DDTs pose more ecotoxicological risk to the Bohai Sea.

Poolpak et al. (2008) analyzed residues of OCPs in sediments from Mae Klong River, Thailand. The surface sediment samples were collected from 10-20 cm depth. The samples were analyzed with gas chromatograph, total OCPs concentration were determined with range from 4.12 to 214.91 µg g⁻¹ dw (first year, 2003) and 3.26–215.09 µg g⁻¹ dw (second year, 2005). The result showed the occurrence of DDTs species of OCPs was dominant in most of the samples.

In India from sunderban wetland sediments cores samples were taken from 0-30 cm depth in winter during the months of November-December, 2005. The samples were analyzed for OCPs. The results showed that residues of HCH, DDT and its isomers and metabolites were determined with concentration levels of 0.05–12 ng g⁻¹ (ΣHCHs) and 0.05–11.5 ng g⁻¹ (ΣDDTs) (Table 2.2). According to the sediments quality guideline the peak concentrations of ΣHCHs and ΣDDTs showed that results have the potential to cause ecotoxicological effects to aquatic environment (Sarkar et al., 2008).

In September 2004, surface sediment samples from two rivers Tajan and Neka in Iran were collected with the help of stainless steel grab sampler at a depth of 0-2cm from four sampling sites of each river. The sediments of two rivers were assessed using gas chromatograph (GC) to determine the levels of contamination of OCPs. The residue of HCHs were found the highest in comparison of global record, with concentration of up to 30 ng g⁻¹ dw., while DDTs were determined with concentration rang of 73-91 ng g⁻¹ dw (Table 2.2) (Kalantari et al., 2006).

Pazou et al. (2006) measured residues of OCPs in catchment area of Ouémé River, Republic of Bénin. Along the river total of 37 sediment samples were

collected with the help of grab sampler up to a depth of 10 cm. OCPs such as Σ DDT and Σ HCH were identified with a percentage of samples above detection limit of 86% and 62%, respectively.

Sapozhnikova et al. (2004) investigated the Salton Sea, California, USA to determine the residues of OCPs in the sediments and to assess the ecotoxicological risk of these pesticides to aquatic life. During 2000-2001 sediment samples were collected from six sites along the Salton Sea. Lindane (γ -HCH) and Σ DDT were identified in most of the samples; the observed concentration range of the Σ DDT was 10 to 304 ng g⁻¹ ww (Table 2.2), whereas the ratio of the DDT to its metabolites indicated the recent usage of DDT. The concentration of lindane and DDE detected in sediment samples were higher than probable effect levels and effect range-median values established by sediments quality guideline (Canadian Council of Ministers of the Environment and US-EPA, 2002). The results showed eco-toxicological risk is the concern of Salton Sea.

Barakat et al. (2002) investigated OCPs in surface sediments from Alexandria Harbor, Egypt. Total of twenty three surface sediment samples were collected from study site using Ekman grab sampler and analyzed with the help of gas chromatograph mounted with electron capture detector. The results showed the occurrence of DDT and its metabolites in most of the sediment samples with concentration range of < 0.25 ng g⁻¹ to 885 ng g⁻¹, while HCHs were found in trace amount in most of the samples. The ratio of DDT to its metabolite was 0.86, indicated the recent usage of DDT.

In Pakistan several research studies conducted to detect residues of OCPs in sediments/soil, samples were collected from different areas of Pakistan and were analyzed using gas chromatograph mounted with electron capture detector (GC-ECD). The result is compiled in Table 2.2 indicated that residues of DDTs and HCHs were detected in different areas of Pakistan (Tariq et al., 2007).

Table 2. 3 Residues of HCHs and DDTs detected in soil/sediments from different areas of Pakistan and other countries

Area	Country	Year	Detected residues	Concentration ($\mu\text{g g}^{-1}$)	Technique	References
Quanzhou Bay	China	2013	HCHs DDTs	0.00093-0.0047 0.00136-0.003217	GC-ECD	Yang et al. (2013)
Hangzhou	China	2013	HCHs DDTs	0.00076-0.017 0.00074-0.0058	GC-ECD	Wu et al. (2013)
Sediments samples from Degh Nullah , Lahore	Pakistan	1994	<i>o,p'</i> -DDT <i>p,p'</i> -DDT <i>o,p'</i> -DDD <i>p,p'</i> -DDD <i>o,p'</i> -DDE <i>p,p'</i> -DDE HCH	1.07-0.17 0.0062-2.41 0.181-1.98 0.614-2.32 0.0021-0.324 0.0008-0.825 Traces	GC-ECD	Tehseen et al. (1994)
Sunder ban wetland	India	2008	Σ HCHs Σ DDTs	0.00005-0.012 0.00005-0.0115	GC-ECD	Sarkar et al. (2008)
Cotton, rice and tobacco growing areas soils, Punjab and NWFP	Pakistan	1990	<i>p,p'</i> -DDT <i>o,p'</i> -DDD <i>p,p'</i> -DDE	Traces-0.0002 Traces-0.002 Traces-0.002	GC-ECD	Ali and Jabbar. (1992); Jabbar et al. (1993)
Densu river basin	Ghana	2012	<i>p,p'</i> -DDT <i>p,p'</i> -DDE γ -HCH δ -HCH	0.05-0.45 0.14 - 1.67 0.280-1.04 0.04-0.23	GC-ECD	Kuranchie-Mensah et al. (2012)
Coastal areas sediments, Karachi	Pakistan	1990	DDT DDE α -HCH	2.7-9.2 0.06-0.16 1.1-3.5	GC-ECD	Bano and Siddique. (1991)

Haleji Lake Sediments, Thatta(Sindh)	Pakistan	2002	HCH DDT	Traces 0.0-6.5	GC-ECD	Sanpera et al. (2002)
Tajan and Neka River	Iran	2004	HCHs DDTs	up-0.03 0.073-0.091	GC-ECD	Kalantari et al. (2006)
Salton Sea	USA	2004	ΣDDTs	0.01-0.304	GC-ECD	Sapozhnikova et al. (2004)
Ravi River	Pakistan	2014	HCHs DDTs	nd-0.016 0.0015-0.0585	GC-ECD	Syed et al. (2014)

2.3. Bioaccumulation of organochlorine pesticides in fish

Thomas et al. (2012) worked on bioaccumulation of OCPs in fish muscles. In 2008 during the production cycles of fish, samples were collected from five sites from fish ponds near Moselle River, France. OCPs were detected at low level in fish muscles, the concentration of *p,p'*-DDE was found the highest among OCPs with averaged concentrations of $0.57 \pm 0.44 \text{ ng g}^{-1} \text{ ww}$ (carp) and $0.58 \pm 0.29 \text{ ng g}^{-1} \text{ ww}$ (perch) (Table 2.3). Whereas among HCH isomers, the concentration of β -HCH were found highest with average concentration of $0.64 \pm 0.15 \text{ ng g}^{-1} \text{ ww}$.

Zhang et al. (2012) measured the levels of OCPs in edible part of shellfish from Xiamen, China. During 2010 (August-September) fish samples were collected from different food markets and analyzed using GC (Agilent Technologies). The results showed the concentrations of Σ OCPs were ranged from 1.686 to 73.61 $\text{ng g}^{-1} \text{ ww}$ whereas the concentration of DDTs and HCHs were ranged from 0.86 to 48.65 $\text{ng g}^{-1} \text{ ww}$ and 0.03 to 3.18 $\text{ng g}^{-1} \text{ ww}$, respectively (Table 2.3). DDTs species were found predominant among OCPs, however according to US-EPA (Environmental Protection Agency) Carcinogenic risk assessment guideline (2005); carcinogenic risk of *p,p'*-DDT, *p,p'*-DDD, α -HCH and β -HCH were identified in certain shellfish species.

Wang et al. (2012) investigated the levels and biomagnifications of OCPs in Taihu Lake fish species from China. During September 2009, total of 193 fish samples were collected by fisher men and analyzed with gas chromatograph (Agilent 6890). OCPs concentration ranged from 121 to 904 $\text{ng g}^{-1} \text{ lw}$ revealed that fish species of taihu Lake is moderately contaminated by OCPs, it is detected that biomagnification factor of OCPs is higher than one, indicated that most of the organochlorine pesticides is biodiluted by aquatic food chain. However, *p,p'*-DDD and *p,p'*-DDE were significantly biomagnified. The ratio of Σ DDT to its metabolites was ranged from 0.77 to 0.95 indicated DDTs residues bioaccumulate in fish species is because of past usage of DDTs, whereas β -HCH was measured predominant among Σ HCH with a percent ranged from 77.7 - 96.0% (of Σ HCH).

Davodi et al. (2011) investigated the bioaccumulation of OCPs in fish species (used in local diet) and also evaluated the potential health risk associated with OCPs. In 2007 (October-November) total of 95 fish samples were collected from

Shadegan Marshes wetland, Khuzestan, Iran. Σ DDT and Σ HCH were detected with average concentrations of $330 \pm 335 \text{ ng g}^{-1} \text{ lw}$ and $170 \pm 200 \text{ ng g}^{-1} \text{ lw}$, respectively (Table 2.3). Among DDT metabolites *p,p'*-DDE was present in higher concentration in all fish samples with average value of $230 \pm 270 \text{ ng g}^{-1} \text{ lw}$, while α -HCH was found dominant isomer (of HCH) in all fish samples. However, the results suggested that low concentration of lindane (γ -HCH) in fish samples indicated that residue of HCHs in the study area is because of the recent use of technical HCH. The results showed that concentrations of Σ DDT in all samples were below the food safety guideline (tolerance limit of $1000 \text{ ng g}^{-1} \text{ lw}$.) recommended by US Food and Drug Administration and European Union.

Ondarza et al. (2011) worked out a study to analyze residues of DDTs in fish tissues. Brown trout fish species were caught from Quemquentreu River, Andean Patagonia, Argentina. DDTs were detected in different part of fish species including gills, muscle, gonads, stomach and liver with concentration levels of 958.5 ± 135.0 , 131.0 ± 82.3 , 143.9 ± 62.0 , 231.2 ± 44.6 , and $139.0 \pm 40.9 \text{ ng g}^{-1} \text{ lw}$, respectively (Table 2.3).

Christoforidis et al. (2008) quantified the bioaccumulation of DDTs and HCHs in fish species collected from River Nestos, Greece. Muscle and liver of the fish samples were analyzed for OCPs with the help of gas chromatograph (GC-MSD 5973 Agilent). OCPs were detected in all fish samples with average concentration of $30.71 \text{ ng g}^{-1} \text{ ww}$. Among OCPs, HCHs were determined with average concentration ranged from 0.10 to $0.91 \text{ ng g}^{-1} \text{ ww}$. and 2.70 to $9.87 \text{ ng g}^{-1} \text{ ww}$, in muscle and liver of fish species, respectively. The results showed that higher concentrations of DDTs were detected in fish liver with average concentration of $52.81 \text{ ng g}^{-1} \text{ ww}$ (Table 2.3). However, the results indicated that the concentrations of Σ DDT and Σ HCH were lower than the food safety guideline tolerance limits (Σ DDT = $1000 \text{ ng g}^{-1} \text{ lw}$; Σ HCH = $1300 \text{ ng g}^{-1} \text{ lw}$) recommended by European Union. The concentrations of Σ DDT were also lower than threshold concentrations ($140 \text{ ng g}^{-1} \text{ ww}$) of Canadian Environmental Quality Guideline, established by Canadian Council of Ministers of the Environment (CCME, 2001).

Coelhan et al. (2006) studied to assess the bioaccumulation of OCPs in fish species. Total of 12 fish species were caught (December, 2003) from Marmara

Sea, Turkey and analyzed for OCPs. The concentrations of OCPs were detected ranged from 329.41 to 1453.87 ng g⁻¹ lw. DDTs species were found the dominant among OCPs with concentration ranged from 211.58 to 810 ng g⁻¹ lw (Σ DDT), while average concentrations of Σ HCH were ranged from 17.25 to 224.70 ng g⁻¹ lw (Table 2.3). The ratio of Σ DDT to its metabolites was below than 0.6 in all samples indicated that the residues in the study area were because of fresh input of DDTs.

In Pakistan a study was conducted to investigate OCPs (DDTs and HCHs) in edible fish species and also to assess their health risk. During 2007 to 2009 a total of eleven edible fish species were collected from twenty five sampling stations along Chenab River. OCPs were detected with a total concentration ranged from 13 to 365ng g⁻¹ ww, whereas Σ DDT were detected in greater than 90% of all samples with concentration ranged from 4.6 to 88.5 ng g⁻¹ ww, while Σ HCH were detected in all samples with concentration ranged from 2.5 to 8.6 ng g⁻¹ ww (Table 2.3). The result showed that the hazard ratio of OCPs for the 50th and 95th percentiles in fish were exceeded than the value of 1, which indicated cancer risk from daily dietary exposure to OCPs (Eqani et al., 2013).

Saqib et al. (2005) conducted a study to determine the level of OCPs in edible fish species. In 2005 total of 45 fish samples were collected from Haleji and Kalri lakes, sindh, Pakistan. The results from this study indicated that DDTs and DDE were determined in 10 and 12 samples, respectively.

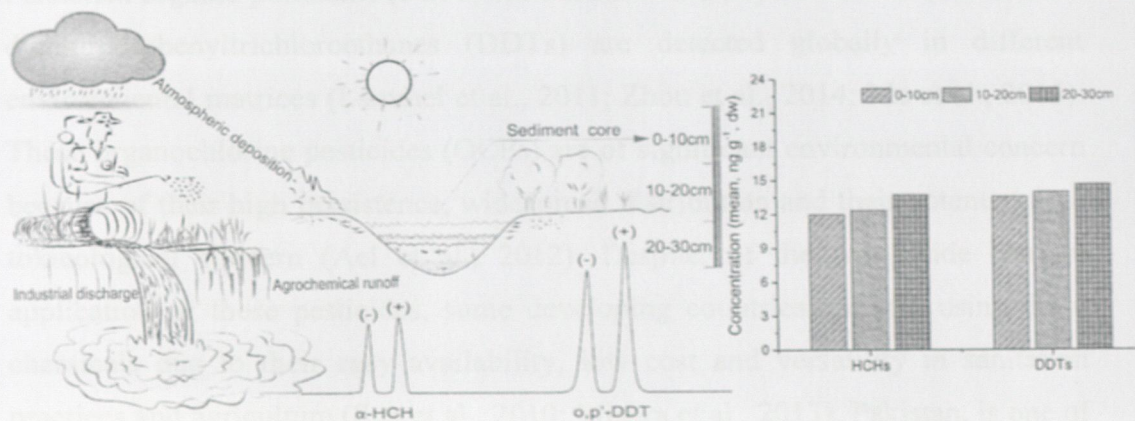
Table 2.4 Residues of HCHs and DDTs detected in fish from Pakistan and other countries.

Area	Country	Year	Detected residues	Concentrations (ng g ⁻¹ , ww)	Technique	References
Moselle River	France	2012	<i>p,p'</i> -DDE β-HCH	0.57±0.44-0.58±0.29 0.64±0.15	GC-ECD	Thomas et al. (2012)
Xiamen	China	2012	DDTs HCHs	0.86-48.65 0.03-3.18	GC-ECD	Zhang et al. (2012)
River Chinab	Pakistan	2013	DDT HCH	4.6-88.5 2.5-8.6	GC-ECD	Eqani et al. (2013).
Shadegan Marshes wetland	Iran	2011	ΣDDT ΣHCH	330±335 170±200	GC-ECD	Davodi et al. (2011)
Quemquentreu River	Argentina	2011	DDTs HCHs	131-958.5 ---	GC-ECD	Ondarza et al. (2011)
River Nestos	Greece		DDTs	52.81	GC-ECD	Christoforidis et al. (2008)
			HCHs	0.10-9.87		
Marmara Sea	Turkey		ΣDDT ΣHCH	211.58-810 17.25-224.70		Coelhan et al. (2006)

Chapter-3

OCCURRENCE, ENANTIOMERIC SIGNATURES AND ECOTOXICOLOGICAL RISK ASSESSMENT OF HCH ISOMERS AND DDT METABOLITES IN SEDIMENTS FROM KABUL RIVER, PAKISTAN

3.1. Graphical abstract



3.2 Abstract

Hexachlorocyclohexane (HCH) isomers and dichlorodiphenyltrichloroethane (DDT) byproducts were analyzed in sediments of three different depths (0-10, 10-20 and 20-30 cm) collected from Kabul River, Pakistan in February 2014. The occurrence levels, enantiomer fractions and potential ecological risk of these organochlorine pesticides (OCPs) were evaluated. The total concentration of HCHs and DDTs in surface sediments ranged from 4.9 to 23.9 and 6.4 to 18.9 ng g⁻¹ (dry weight basis), respectively. The vertical profiles of HCH and DDT contaminations were in order of 20–30 cm > 10–20 cm > 0–10 cm, indicating that the residue levels of these compounds gradually decreased after they were banned. The ratios of β -HCH/HCHs ranged from 0.04 to 0.73 (69 % of samples below 0.5) suggested the fresh input of HCHs, while isomeric ratios of α -HCH/ γ -HCH (ranged from 0.02–7.94), with 76% of samples less than 3, indicated the cocktail use of technical grade HCH and lindane in the study area. The ratio of (DDE+DDD)/DDTs (ranged from 0.42–0.9) indicated long-term biodegradation of parent DDT. The enantiomer of α -HCH was generally racemic or close to

racemic for most of the samples, with EF value < 0.5 for some of the samples indicated the preferential biodegradation of (+)- α -HCH enantiomer, while for o,p'-DDT the EF values > 0.5 indicated the depletion of (-)-o,p'-DDT enantiomer in most of the samples. According to sediment quality guidelines (SQGs), HCH contamination is the main concerned for ecotoxicological risk in Kabul River.

3.3. Introduction

Persistent organic pollutants (POPs) such as hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs) are detected globally in different environmental matrices (Lammel et al., 2011; Zhou et al., 2014; Ma et al., 2015). These organochlorine pesticides (OCPs) are of significant environmental concern because of their high persistence, widespread distribution and their potential ecotoxicological concern (Ael et al., 2012). Despite, of the worldwide ban on application of these pesticides, some developing countries are still using these chemicals due to their easy availability, low cost and versatility in sanitation practices and agriculture (Sun et al., 2010; Mishra et al., 2013). Pakistan, is one of the signatory countries of the Stockholm Convention (2001) on POPs, is being committed to Convention implementation to phase out POPs usage (Eqani et al., 2013). However, in Pakistan these banned compounds are still in use, moreover the world's largest stockpiles of obsolete toxic pesticides exist in Pakistan, along with DDT demolished factory located in Kabul River catchment area (Eqani et al., 2012). The inadequate handling and storage result in leakage of obsolete pesticides to the ambient environment (Tariq et al., 2007; Jan et al., 2009).

Organochlorine pesticides enter aquatic environment through direct and indirect sources such as effluents release, agriculture runoff and atmospheric deposition (Barakat et al., 2013). In aquatic environment OCPs tend to adsorb on bottom sediments due to their hydrophobic nature and strong particulate matter affinity and as a result sediment serve as a sink or primary repository for OCPs (Wu et al., 2013). Surface sediments provide information about recent deposition of contaminants, whereas the sediment core reflects historical contamination over decades (Wang et al., 2013). The sediments also act as a source of secondary contamination, once sediments are disturbed reintroducing the contaminants to the

aquatic environment and constitute threat to sediment associated biota and even aquatic food web (Kuranchie-Mensah et al., 2012).

In aquatic environment, sediments comprise a suitable compartment for monitoring and eco-toxicological risk assessment of OCPs (Barakat et al., 2013). The low level of OCPs in the sediments might still pose adverse ecological effects on aquatic ecosystem; even through aquatic food consumption, OCPs constitute health threats for human beings (Yang et al., 2013). Residual HCH isomers (α -, β -, γ - and δ -HCH) and DDT metabolites (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD and *o,p'*-DDD) are considered to cause variety of adverse effects including carcinogenic, teratogenic, immunologic, neurological and reproductive problems in living organisms. These compounds have been also reported as endocrine disrupters and biomagnified in food chain (Rajendran et al., 2005).

Among the congeners of HCHs and DDTs, α -HCH and *o,p'*-DDT are exist as chiral molecules in the environment and these enantiomers may be determined separately in environmental samples (Corsolini et al., 2006). Chiral analysis of OCPs enantiomers has recently received increased attention, in order to understand transport pathways, biological activity, and historical and recent input sources in different environmental compartments (Yang et al., 2010; Garrison et al., 2014).

In Pakistan, although reports on HCH isomers and DDT metabolites in sediments from Degh Nala, Haleji Lake, Chenab River and Ravi River (Tehseen et al., 1994; Sanpera et al., 2002; Eqani et al., 2011., Syed et al., 2014) in Sindh and Punjab provinces are available, but data is lacking on contamination profile of HCHs and DDTs in sediments from Khyber Pakhtunkhwa Province. Kabul River is one of the largest rivers of Khyber Pakhtunkhwa Province; it is associated with intense industrial activities and confined by traditional agriculture and highly populated areas. Kabul River is the ultimate effluents disposal site for most of the industries present in Khyber Pakhtunkhwa Province (MOE-PAK, 2005a).

This study aimed to investigate the occurrence of residual HCHs and DDTs, and their enantiomeric signatures in sediments of Kabul River. Additionally, the input sources and historical trends of contamination were also investigated for the purpose to assess the potential eco-toxicological risk of these compounds. To our knowledge, this is the first time that HCH isomers, DDT metabolites, and their enantiomers are measured in sediments collected from Kabul River.

3.4 Materials and Methods

3.4.1. Study area and sampling

Kabul River originates in Afghanistan and at a place of Shalman in the Khyber Agency enters Pakistan (Yousafzai et al., 2008). The section of Kabul River (Fig.3.1) under study is just downstream of warsak dam to its confluence at the place of Kund with Indus River. This section is about approximately 90 km long covering three administrative districts (Peshawar, Charsadda and Mardan).

Sediments samples were collected in February 2014. Total of 11 sampling stations along the Kabul River were selected as shown in Fig. 3.1. Surface sediment samples were collected with the help of long hand stainless steel scoop, while sediments core of 30 cm length were collected using stainless steel corer (8 cm diameter; 40 cm length). Sediment columns were sliced into three layers (0-10, 10-20 and 20-30 cm) and stored in pre-cleaned glass jars. All the samples were transported to the laboratory in icebox and stored at -20 °C until analysis.

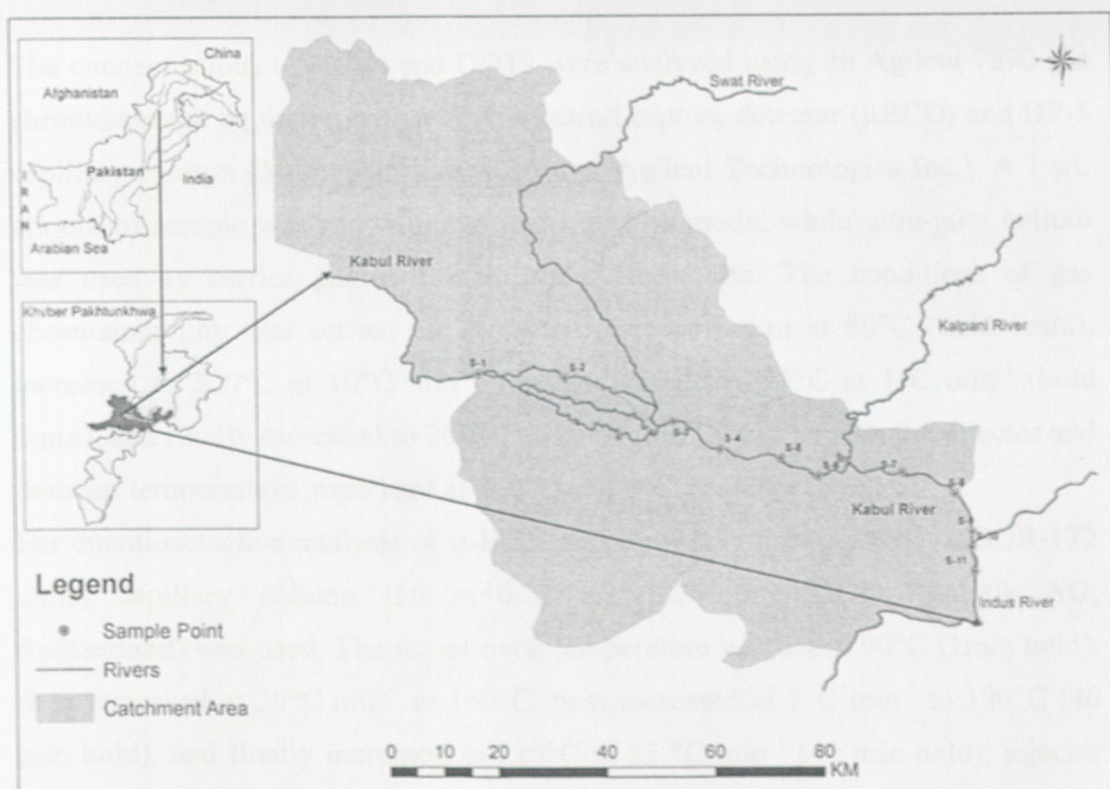


Fig.3.1. Map showing the sampling sites along Kabul River, Khyber Pakhtunkhwa-Pakistan

3.4.2. Extraction and cleanup

Dried sediments were homogenized and divided into two subsamples for physico-chemical analysis and extraction of HCHs and DDTs. Briefly, 20 g of sample was spiked with surrogate standard PCB-209 and Tetrachloro-m-xylene (TCmX) and Soxhlet extracted with 150 ml mixture of n-hexane: acetone (4:1 v/v) for 24 h at 50°C. The extract was concentrated and solvent-exchanged to hexane using rotary evaporator. The sample extract was purified with silica column, packed with 1 g anhydrous sodium sulfate (activated at 450 °C), 4 g silica gel (activated at 180 °C), 2 g alumina (activated at 250 °C) from bottom to top. The column was washed with 20 ml n-hexane, and then eluted with 70 ml mixture of n-hexane: dichloromethane (7:3 v/v) to recover the target analytes. Final elute was reduced to 5 ml using rotary evaporator and was concentrated to 0.5 ml with a gentle stream of pure nitrogen gas.

3.4.3. GC Analysis

The concentrations of HCHs and DDTs were analyzed using an Agilent 7890 gas chromatograph equipped with a 63Ni electron capture detector (μ ECD) and HP-5 capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m, Agilent Technologies Inc.). A 1 μ L aliquot of sample was run with splitless injection mode, while ultra-pure helium was used as carrier gas at 1.5 mL min⁻¹ flow rate. The conditions of gas chromatography was set as: the oven temperature began at 80°C (hold 1min), increased to 200°C at 10°C min⁻¹, next increased to 225°C at 1°C min⁻¹ (hold 1min), and finally increased to 260°C at 15 °C min⁻¹ (hold 5 min); the injector and detector temperatures were kept at 250°C and 300 °C, respectively.

For enantioselective analysis of α -HCH and *o,p'*-DDT enantiomers, a BGB-172 chiral capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m; BGB Analytik AG, Switzerland) was used. The initial oven temperature was set at 90°C (1min hold), then increased at 20°C min⁻¹ to 160°C, next increased at 1°C min⁻¹ to 190°C (40 min hold), and finally increased to 225°C at 25 °C min⁻¹ (40 min hold); injector and detector temperatures were set at 250°C and 280°C, respectively. Helium was used as carrier gas at the flow rate of 1mL min⁻¹.

3.4.4. Quality assurance and control

The levels of HCH isomers and DDT metabolites were determined under a strict quality control and assurance protocols. The correlation coefficients (r) of five-point calibration curves were all greater than 0.998. The procedural blanks, spiked blanks and duplicate samples were analyzed to assure the quality. The average surrogate recoveries for TCmX and PCB-209 were $84\pm 9\%$ and $91\pm 12\%$, respectively. The method detection limits (MDLs) of HCHs and DDTs were ranged from 0.05 to 0.08 ng g^{-1} , dw. For enantiomer analysis, the racemic α -HCH and o,p' -DDT standard solution was injected to ensure the reproducibility of chiral analysis. The mean value of enantiomeric fraction was 0.501 ± 0.001 . These parameters confirmed the practicability of the analytical protocol used herein.

3.4.5. Statistical Analysis

Principal component analysis with varimax rotation was performed to identify the concentrations variability and potential input sources of HCHs and DDTs in sediments. The two-tailed, Pearson correlation coefficient was used to examine the correlation between HCHs, DDTs and total organic carbon (TOC), whereas one way ANOVA was performed for significant differences among the whole dataset. All analyses and calculations were performed using statistical software, SPSS 17 (SPSS Inc., Chicago, Illinois, USA) and Microsoft Excel (2013).

3.5. Results and discussions

3.5.1. Occurrence of HCHs and DDTs residues in sediments

The residue levels of HCHs and DDTs, and TOC are given in Table 3.1. The detection frequencies for the targeted compounds in our samples ($n = 33$) were as high as 100% (Table 3.2), indicating a wide occurrence of HCHs and DDTs in sediments of Kabul River. The residue levels of Σ HCHs and Σ DDTs in surface sediments (0-10 cm) ranged from 4.9-23.9 ng g^{-1} , dw (mean 12 ng g^{-1} , dw) and from 6.4-18.9 ng g^{-1} , dw (mean 13.6 ng g^{-1} , dw), respectively. The concentrations of Σ HCHs and Σ DDTs in surface sediments of Kabul River, Pakistan were compared with other countries/region from Asia (Table 3.3). The levels of Σ DDTs in surface sediments of Kabul River were much lower than those of riverine

sediments from India, Thailand and Vietnam, whereas were found higher than those from China and Korea, while levels of Σ HCHs were found lower than those of India and Thailand, and higher than Vietnam, China and Korea as shown in Table 3.3. Beside the one way ANOVA results of non-significant differences ($F = 0.28$, $P > 0.05$) in the contamination levels of HCHs and DDTs among the three sediment core layers, the vertical contamination profile of total HCHs and DDTs were found in order of 20-30cm (mean = 28.4 ng g^{-1}) $>$ 10-20 cm (mean = 26.3 ng g^{-1}) $>$ 0-10 cm (mean = 25.5 ng g^{-1}), which indicated that the contamination levels of HCHs and DDTs were gradually decreased in Kabul River sediments which could be linked with the ban on the usage of these pesticides in the Stockholm Convention (2001) on POPs (UNEP/UNDP, 2001). Among the total concentrations of HCHs and DDTs, DDTs were found more abundant than HCHs (Table 3.1), which is consistent with previous studies of HCHs and DDTs contaminations in riverine sediments in China (Zhou et al., 2014). This might be due to the fact, that DDTs have a low water solubility, lower vapour pressure, low biodegradability, high particle affinity and high lipophilicity than HCHs (Niu et al., 2013). Thus HCHs more easily biodegraded in the sediments as compared to DDTs (Wu et al., 2013). The highest concentrations of total HCHs and DDTs in surface sediments were found at Station 5 and Station 6 (Fig. 3.2 and Table 3.1) which are surrounded by DDT demolished factory, chemical, papermaking, or pharmaceutical factories, corresponding to relatively the highest content of TOC; 0.74% and 0.62%, respectively. It was found, that the concentrations of total HCHs and DDTs, and TOC contents in the sediments were significantly positive correlated ($r = 0.77$; $p < 0.01$) in this study (Table 3.4), suggesting a significant influence of TOC on the HCHs and DDTs distribution in the sediments. Furthermore, masking the outlier and extreme stations (e.g. station 4, station 5 and station 6) with abnormal higher concentration of total HCHs and DDT, the linear fitting analysis showed that the total residual concentrations of HCHs and DDTs in surface sediments were increased from upstream to downstream along the river as shown in Fig.3.2. This was explained by the hypothesis that the sediment deposition rate would be higher at downstream zones when compared to upstream zones.

Table 3.1 Concentrations (ng g⁻¹, dw) of HCHs, DDTs and TOC contents in sediments from Kabul River.

Stations	TOC (%)	α-HCH	β-HCH	γ-HCH	δ-HCH	ΣHCHs ^a	o,p'-DDE	p,p'-DDE	o,p'-DDD	p,p'-DDD	o,p'-DDT	p,p'-DDT	ΣDDTs ^b	Σ(HCHs + DDTs)
0-10cm														
S1	0.28	1.43 ± 0.01	2.92 ± 0.19	0.18 ± 0.02	0.39 ± 0.01	4.90	0.29 ± 0.02	1.77 ± 0.02	0.35 ± 0.01	1.75 ± 0.02	0.75 ± 0.09	3.59 ± 0.02	8.50	13.42
S2	0.32	2.38 ± 0.02	3.07 ± 0.02	2.46 ± 0.01	1.14 ± 0.03	9.00	0.70 ± 0.01	1.09 ± 0.02	0.48 ± 0.01	0.67 ± 0.10	1.31 ± 0.02	2.22 ± 0.11	6.40	15.52
S3	0.47	1.05 ± 0.02	3.99 ± 0.02	0.19 ± 0.01	1.39 ± 0.02	6.60	0.64 ± 0.03	0.98 ± 0.01	1.98 ± 0.02	3.17 ± 0.02	0.83 ± 0.10	4.33 ± 0.02	11.9	18.55
S4	0.50	3.16 ± 0.02	4.93 ± 0.03	3.22 ± 0.02	3.50 ± 0.02	14.8	2.16 ± 0.01	3.47 ± 0.03	2.99 ± 0.02	3.20 ± 0.03	2.64 ± 0.04	3.10 ± 0.04	17.6	32.37
S5	0.74	8.04 ± 0.05	9.06 ± 0.05	3.90 ± 0.04	2.86 ± 0.02	23.9	2.79 ± 0.04	4.41 ± 0.02	2.54 ± 0.01	2.57 ± 0.01	2.92 ± 0.02	3.72 ± 0.04	18.9	42.81
S6	0.62	5.66 ± 0.32	8.22 ± 0.41	2.04 ± 0.03	3.95 ± 0.03	19.9	4.24 ± 0.02	3.58 ± 0.03	0.54 ± 0.01	3.92 ± 0.03	0.59 ± 0.02	2.79 ± 0.02	15.6	35.53
S7	0.44	1.61 ± 0.01	2.29 ± 0.01	2.46 ± 0.24	2.81 ± 0.01	9.20	1.98 ± 0.07	2.17 ± 0.01	3.59 ± 0.04	2.36 ± 0.14	1.98 ± 0.02	2.11 ± 0.01	14.2	23.36
S8	0.21	3.30 ± 0.02	3.35 ± 0.03	1.24 ± 0.01	2.23 ± 0.09	10.1	2.14 ± 0.04	3.43 ± 0.03	0.16 ± 0.01	2.95 ± 0.10	2.13 ± 0.02	1.96 ± 0.11	12.8	22.89
S9	0.32	2.34 ± 0.18	3.07 ± 0.03	0.31 ± 0.02	1.96 ± 0.01	7.70	1.96 ± 0.03	2.41 ± 0.30	1.10 ± 0.03	1.48 ± 0.02	3.13 ± 0.11	4.68 ± 0.01	14.7	22.44
S10	0.35	4.23 ± 0.03	4.28 ± 0.03	2.60 ± 0.01	3.21 ± 0.02	14.3	0.47 ± 0.01	2.37 ± 0.03	0.21 ± 0.01	1.10 ± 0.03	2.95 ± 0.03	2.75 ± 0.02	9.80	24.17
S11	0.29	1.53 ± 0.01	5.87 ± 0.02	1.29 ± 0.01	2.53 ± 0.03	11.2	2.61 ± 0.21	3.74 ± 0.05	2.46 ± 0.02	3.29 ± 0.20	3.06 ± 0.03	3.60 ± 0.01	18.8	29.98
Mean *						12.0 ± 5.78							13.6 ± 4.14	25.5 ± 8.85
10-														
20cm														
S1	0.31	0.91 ± 0.02	5.50 ± 0.03	0.28 ± 0.10	0.81 ± 0.09	7.50	1.00 ± 0.01	2.12 ± 0.02	0.64 ± 0.02	1.86 ± 0.02	2.95 ± 0.02	3.39 ± 0.10	12.0	19.46
S2	0.39	2.05 ± 0.01	4.05 ± 0.03	0.26 ± 0.09	0.26 ± 0.10	6.60	0.54 ± 0.02	0.86 ± 0.01	1.90 ± 0.03	2.43 ± 0.03	0.52 ± 0.02	1.98 ± 0.03	8.20	14.85
S3	0.40	3.15 ± 0.03	3.75 ± 0.02	3.16 ± 0.02	2.06 ± 0.01	12.1	1.97 ± 0.02	2.77 ± 0.03	2.99 ± 0.02	1.38 ± 0.03	1.69 ± 0.02	2.44 ± 0.02	13.2	25.36
S4	0.57	1.16 ± 0.01	6.93 ± 0.04	2.25 ± 0.02	2.50 ± 0.02	12.8	2.20 ± 0.01	3.48 ± 0.03	0.09 ± 0.02	3.21 ± 0.02	2.51 ± 0.03	3.17 ± 0.03	14.7	27.50
S5	1.00	10.2 ± 0.05	9.27 ± 0.03	3.71 ± 0.02	4.12 ± 0.20	27.3	2.01 ± 0.04	4.78 ± 0.21	3.97 ± 0.04	0.60 ± 0.04	1.25 ± 0.21	2.76 ± 0.02	15.4	42.70
S6	0.65	2.02 ± 0.02	7.14 ± 0.06	2.66 ± 0.10	2.01 ± 0.10	13.8	2.93 ± 0.03	1.33 ± 0.01	2.60 ± 0.02	2.11 ± 0.03	0.63 ± 0.11	3.33 ± 0.03	12.9	26.76
S7	0.41	3.31 ± 0.03	3.13 ± 0.02	1.01 ± 0.02	3.98 ± 0.02	11.4	3.21 ± 0.03	2.74 ± 0.03	1.70 ± 0.03	3.72 ± 0.20	2.90 ± 0.02	1.99 ± 0.02	16.3	27.69
S8	0.44	1.10 ± 0.06	4.74 ± 0.04	1.24 ± 0.01	2.23 ± 0.20	9.30	2.98 ± 0.02	3.44 ± 0.03	2.89 ± 0.02	2.97 ± 0.02	0.92 ± 0.02	2.86 ± 0.21	16.1	25.37

S9	0.28	2.03 ± 0.10	0.16 ± 0.01	1.95 ± 0.11	0.08 ± 0.02	4.20	3.28 ± 0.02	3.61 ± 0.03	3.60 ± 0.02	2.84 ± 0.02	2.75 ± 0.03	2.99 ± 0.03	19.1	23.29
S10	0.39	2.10 ± 0.02	3.44 ± 0.03	2.60 ± 0.01	5.31 ± 0.04	13.4	0.11 ± 0.09	2.87 ± 0.02	1.35 ± 0.02	1.05 ± 1.02	2.87 ± 0.01	3.02 ± 0.03	11.3	24.72
S11	0.56	2.09 ± 0.02	6.72 ± 0.03	3.90 ± 0.02	4.61 ± 0.03	17.3	1.76 ± 0.02	3.88 ± 0.01	0.47 ± 0.03	0.93 ± 0.03	3.04 ± 0.03	4.61 ± 0.01	14.7	32.01
Mean*						12.4 ±							14.0 ±	26.3 ±
						6.22							2.90	7.05
20-30cm														
S1	0.28	0.60 ± 0.05	1.95 ± 0.08	1.45 ± 0.03	0.44 ± 0.01	4.40	1.91 ± 0.11	2.15 ± 0.01	1.85 ± 0.02	1.89 ± 0.02	1.97 ± 0.01	1.36 ± 0.01	11.1	15.57
S2	0.35	2.36 ± 0.03	2.05 ± 0.03	4.60 ± 0.06	1.16 ± 0.01	10.2	1.44 ± 0.02	1.89 ± 0.02	0.96 ± 0.10	1.63 ± 0.02	2.60 ± 0.01	3.96 ± 0.01	12.5	22.65
S3	0.34	3.57 ± 0.03	2.97 ± 0.04	1.77 ± 0.03	3.29 ± 0.03	11.6	0.74 ± 0.02	2.96 ± 0.02	3.00 ± 0.01	2.47 ± 0.01	2.70 ± 0.02	2.80 ± 0.02	14.7	26.27
S4	0.53	4.16 ± 0.51	3.93 ± 0.06	2.90 ± 0.04	2.41 ± 0.03	13.4	2.91 ± 0.10	3.56 ± 0.02	0.59 ± 0.02	2.75 ± 0.02	0.16 ± 0.02	1.21 ± 0.02	11.2	24.58
S5	0.72	17.07 ± 1.2	11.2 ± 0.06	3.23 ± 0.02	6.83 ± 0.09	38.4	1.08 ± 0.03	3.40 ± 0.03	2.99 ± 0.20	4.17 ± 0.21	1.30 ± 0.02	3.78 ± 0.02	16.7	55.07
S6	0.66	0.12 ± 0.03	9.90 ± 0.08	2.76 ± 0.03	4.16 ± 0.02	16.9	0.89 ± 0.02	4.32 ± 0.02	3.72 ± 0.03	3.18 ± 0.02	4.68 ± 0.01	5.37 ± 0.02	22.2	39.10
S7	0.42	6.90 ± 0.09	4.07 ± 0.05	1.32 ± 0.03	5.16 ± 0.03	17.5	3.27 ± 0.05	3.80 ± 0.11	0.73 ± 0.09	3.79 ± 0.02	2.99 ± 0.01	2.01 ± 0.02	16.6	34.04
S8	0.21	0.06 ± 0.02	2.48 ± 0.04	3.34 ± 0.21	1.23 ± 0.02	7.10	2.00 ± 0.02	1.50 ± 0.02	2.99 ± 0.01	1.02 ± 0.02	1.80 ± 0.02	2.88 ± 0.02	12.2	19.30
S9	0.32	1.20 ± 0.11	4.47 ± 0.02	1.08 ± 0.02	1.30 ± 0.02	8.00	0.34 ± 0.02	3.67 ± 0.08	0.62 ± 0.03	3.07 ± 0.03	3.78 ± 0.01	4.75 ± 0.01	16.2	24.28
S10	0.41	2.50 ± 0.04	3.27 ± 0.03	1.48 ± 0.02	5.27 ± 0.07	12.5	3.38 ± 0.01	2.91 ± 0.01	1.40 ± 0.02	2.44 ± 0.02	0.93 ± 0.10	3.27 ± 0.01	14.3	26.85
S11	0.32	1.90 ± 0.06	3.30 ± 0.01	2.02 ± 0.04	3.84 ± 0.03	11.1	2.80 ± 0.02	3.94 ± 0.02	2.51 ± 0.02	1.47 ± 0.02	0.12 ± 0.01	2.65 ± 0.02	13.5	24.55
Mean*						13.7 ±							14.7 ±	28.4 ±
						9.04							3.22	10.9

*Mean = Mean concentrations.

^aΣHCHs = α-HCH + β-HCH + γ-HCH + δ-HCH.

^bΣDDTs = o,p'-DDE + p,p'-DDE + o,p'-DDD + p,p'-DDD + o,p'-DDT + p,p'-DDT.

Table 3.2 Compounds with the percentage of detection

S. No	Compounds	Detection frequency (%)
1	α -HCH	100
2	β -HCH	100
3	γ -HCH	100
4	δ -HCH	100
5	<i>o,p'</i> -DDE	100
6	<i>p,p'</i> -DDE	100
7	<i>o,p'</i> -DDD	100
8	<i>p,p'</i> -DDD	100
9	<i>o,p'</i> -DDT	100
10	<i>p,p'</i> -DDT	100

Table 3.3 Comparison of HCH and DDT residue concentrations in surface sediments of Kabul River and other Asian countries (ng g⁻¹ dw).

Location	Year	Σ HCHs	Σ DDTs	References
Kabul River, Pakistan	2014	4.9-23.9 (12.0)	6.4-18.9 (13.6)	This Study
Brahmaputra River, India	2009-2011	39.2-728 (279)	69.1-852 (291)	Mishra et al. (2013)
Mae Klong River, Thailand	2003-2005	640-6920 (NA)	80-1830 (NA)	Poolpak et al. (2008)
Ravi River, Pakistan	2011	nd-16.0 (4.1)	1.5-58.5 (22)	Syed et al. (2014)
Hanoi Rivers, Vietnam	2006	< 0.2-36 (3.8)	4.4-1100 (135)	Hoai et al. (2010)
Huaihe River, China	2007	1.95-11.05 (4.5)	4.07-23.9 (11.1)	Sun et al. (2010)
Han River, Korea	2005	NA	1.05-8.94 (3.9)	Kim et al. (2009)

nd= Not detected

NA= Not available

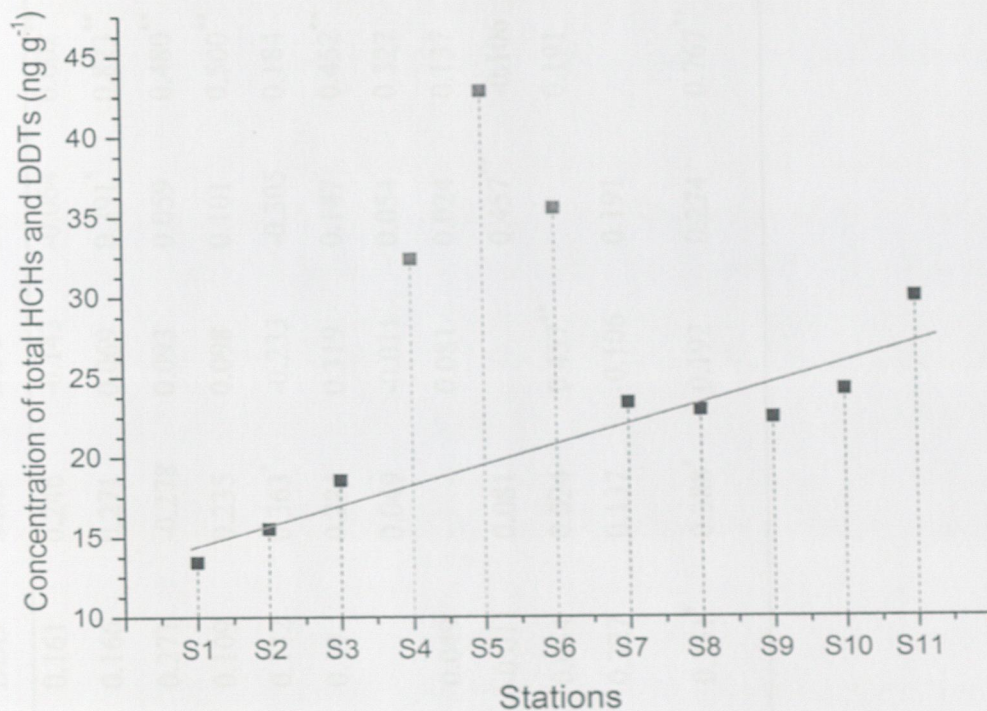


Fig. 3.2 Linear fitting analysis and total concentrations of HCHs and DDTs (ng g⁻¹ dw) in surface sediments. The line represents the gradual increase from upstream to downstream along Kabul River. The higher points represent the masking outliers and extreme.

Table 3.4 Correlation matrix for HCHs, DDTs and TOC

	α -HCH	β -HCH	γ -HCH	δ -HCH	<i>o,p'</i> -DDE	<i>p,p'</i> -DDE	<i>o,p'</i> -DDD	<i>p,p'</i> -DDD	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	TOC	Total OCPs
α -HCH		0.575**	0.345*	0.582**	0.112	0.378*	0.161	0.246	-0.143	-0.064	0.602**	0.780**
β -HCH	0.575**		0.329	0.515**	0.044	0.469**	0.166	0.271	0.069	0.391*	0.823**	0.812**
γ -HCH	0.345*	0.329		0.339	0.111	0.324	0.271	-0.278	0.093	0.059	0.480**	0.499**
δ -HCH	0.582**	0.515**	0.339		0.196	0.541**	0.109	0.235	0.098	0.101	0.500**	0.754**
<i>o,p'</i> -DDE	0.112	0.044	0.111	0.196		0.396*	0.132	0.363*	-0.233	-0.305	0.184	0.272
<i>p,p'</i> -DDE	0.378*	0.469**	0.324	0.541**	0.396*		0.19	0.284	0.319	0.147	0.452**	0.704**
<i>o,p'</i> -DDD	0.161	0.166	0.271	0.109	0.132	0.19		0.049	-0.011	0.054	0.327	0.351*
<i>p,p'</i> -DDD	0.246	0.271	-0.278	0.235	0.363*	0.284	0.049		0.081	0.024	0.137	0.388*
<i>o,p'</i> -DDT	-0.143	0.069	0.093	0.098	-0.233	0.319	-0.011	0.081		0.457**	-0.106	0.192
<i>p,p'</i> -DDT	-0.064	0.391*	0.059	0.101	-0.305	0.147	0.054	0.024	0.457**		0.191	0.274
TOC	0.602**	0.823**	0.480**	0.500**	0.184	0.452**	0.327	0.137	-0.106	0.191		0.767**
Total	0.780**	0.812**	0.499**	0.754**	0.272	0.704**	0.351*	0.388*	0.192	0.274	0.767**	
OCPs												

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

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

3.5.2. Compositions of HCHs and DDTs

The composition diversities of HCH isomers and DDT metabolites could provide a better understanding of these compounds origin, transport, and their historical and recent usage in the environment (Iwata et al., 1994; Li et al., 2015). HCHs were introduced into environment, mainly from two sources, technical grade HCH mixture (60–70% of α -HCH, 5–12% of β -HCH, 10–12% of γ -HCH, 6–10% of δ -HCH and 3–4% of ϵ -HCH) and lindane (contained 99% of γ -HCH) (Willett et al., 1998; Mishra et al., 2013). Among HCH isomers, β -HCH is the most persistent and dominant isomer found in different environmental compartments (soil, sediment, animal tissue and water), while α -HCH is more likely to transport over long distances (Wu et al., 2013). Moreover, α -HCH and γ -HCH can be photochemically transformed and biodegraded to β -HCH in the environment (Willett et al., 1998). In the present study, the ratio of β -HCH/ Σ HCHs varied from 0.04 to 0.73, with 69% of samples below 0.5, suggesting that the recent HCH input occurred in the study area. The ratio of α/γ -HCH typically ranged from 3 to 7 for technical HCH, whereas less than 3 for the use of lindane (Zhou et al., 2014). In addition, the ratio of α -HCH/ γ -HCH were ranged from 0.02 to 7.94, with 76% of samples less than 3 (Table 3.5), indicating the usage of both historical technical grade HCH and lindane; while 39% of samples were less than 1, which further supported the recent input of lindane (Wang et al., 2013). It may be considered, that HCHs contamination originated mainly from fresh input, as well as from past usage of technical grade HCH in some sites, but the recent usage of lindane had superimposed on the background of historical technical grade HCH.

A technical grade DDT mixture composed of 75% *p,p'*-DDT, 15% *o,p'*-DDT, 5% *p,p'*-DDE and rest of metabolites (Yang et al., 2010). In the environment, DDT can be biodegraded into DDD under anaerobic conditions and into DDE under aerobic conditions through dechlorination and dehydrochlorination processes, respectively.

The ratio of DDE/DDD could indicate the aerobic/anaerobic conditions of DDT biodegradation (Hu et al., 2009). A ratio DDE/DDD value greater than 1 denoted the occurrence of aerobic degradation of parent DDT, while a ratio less than 1 implied that the DDT loss was predominant by anaerobic degradation. As shown

in Table 3.5, the DDE/DDD ratios were ranged from 0.31 to 4.03, with 73% of the ratio values were greater than 1, demonstrating that aerobic degradation was the main pathway of parent DDT loss in the study area; similar to sediments from Ravi River, Pakistan (Syed et al., 2014), High Park in Toronto, Canada (Wong et al., 2009) and Hangzhou, China (Wu et al., 2013). The ratio $(DDE+DDD)/\sum DDTs$ is used to assess the possible historical and recent pollution sources of DDT. The ratio $(DDE+DDD)/\sum DDTs$ value less than 0.5 would indicate fresh application of DDT, while a value greater than 0.5 would indicate historical usage of DDT (Hitch and Day, 1992). In this study, the $(DDE+DDD)/\sum DDTs$ ratios were found in the range of 0.42-0.9, with 76% ratio values > 0.5 , suggesting that the DDT contamination mainly caused by long-term degradation of historical DDT. Furthermore, the ratio o,p' -DDT/ p,p' -DDT could be used to estimate whether DDT came from technical grade DDT or from dicofol- type DDT. Thus, for technical grade DDT the ratio o,p' -DDT/ p,p' -DDT ranged from 0.2–0.3, whereas it ranged from 1.3–9.3 or higher for dicofol (Qiu et al., 2005). In the present study, ratio o,p' -DDT/ p,p' -DDT ranged from 0.05-1.49, with a mean value of 0.69, suggested that the application of both technical and dicofol-type DDT may be occurred in the study area.

type DDT may be occurred in the study area.			0.69	0.19	
3.27	0.27	1.10	0.70	1.46	
0.39	0.51	1.19	0.76	0.32	
1.04	0.04	1.07	0.78	0.02	
0.81	0.36	1.24	0.48	0.93	
0.54	0.39	4.03	0.48	0.66	
0.41	0.44	1.05	0.70	1.45	
0.51	0.28	1.29	0.47	0.60	
2.01	0.36	0.68	0.62	0.96	
1.43	0.39	1.94	0.88	0.13	
5.29	0.29	0.63	0.78	0.39	
0.04	0.34	0.76	0.53	0.87	
3.21	0.23	1.35	0.70	1.49	
0.02	0.55	0.87	0.62	0.63	
1.11	0.56	1.09	0.47	0.30	
1.69	0.26	1.64	0.71	0.28	
0.94	0.30	1.70	0.79	0.05	
2.28	0.39	1.36	0.63	0.69	
Range	0.02-7.34	0.04-0.73	0.31-4.03	0.43-0.83	0.05-1.49

Table 3.5 Diagnostic ratios of parental OCPs with their isomers and metabolites

Samples	α/γ -HCH	β -HCH/ Σ HCHs	DDE/DDD	(DDE + DD)/ Σ DDTs	<i>o,p'</i> -DDT/ <i>p,p'</i> -DDT
0-10cm					
S1	7.94	0.59	0.98	0.49	0.21
S2	0.97	0.34	1.56	0.45	0.59
S3	5.53	0.60	0.31	0.57	0.19
S4	0.98	0.33	0.91	0.67	0.85
S5	2.06	0.38	1.41	0.65	0.79
S6	2.77	0.41	1.75	0.78	0.21
S7	0.65	0.25	0.70	0.71	0.94
S8	2.66	0.33	1.79	0.68	1.09
S9	7.55	0.40	1.69	0.47	0.67
S10	1.63	0.30	2.17	0.42	1.07
S11	1.19	0.52	1.10	0.64	0.85
10-20cm					
S1	3.20	0.73	1.25	0.47	0.87
S2	7.90	0.61	0.32	0.70	0.26
S3	1.00	0.31	1.08	0.69	0.69
S4	0.52	0.54	1.72	0.61	0.79
S5	2.76	0.34	1.49	0.74	0.45
S6	0.76	0.52	0.90	0.69	0.19
S7	3.27	0.27	1.10	0.70	1.46
S8	0.89	0.51	1.10	0.76	0.32
S9	1.04	0.04	1.07	0.70	0.92
S10	0.81	0.26	1.24	0.48	0.95
S11	0.54	0.39	4.03	0.48	0.66
20-30cm					
S1	0.41	0.44	1.09	0.70	1.45
S2	0.51	0.20	1.29	0.47	0.66
S3	2.01	0.26	0.68	0.62	0.96
S4	1.43	0.29	1.94	0.88	0.13
S5	5.29	0.29	0.63	0.70	0.34
S6	0.04	0.58	0.76	0.55	0.87
S7	5.21	0.23	1.56	0.70	1.49
S8	0.02	0.35	0.87	0.62	0.63
S9	1.11	0.56	1.09	0.47	0.80
S10	1.69	0.26	1.64	0.71	0.28
S11	0.94	0.30	1.70	0.79	0.05
Mean	2.28	0.39	1.30	0.63	0.69
Range	0.02-7.94	0.04-0.73	0.31-4.03	0.42-0.88	0.05-1.49

3.5.3. Principal component analysis

A multivariate analytical tool, principal component analysis (PCA) was performed on the datasets containing these individual HCH isomers, DDT metabolites and TOC, the scree plot of Eigenvalues depicted that 73.1% of the data variance could be illustrated by four principal components (Fig. 3.3 and Table 3.6), while the remaining 26.9% of variance could not be explained clearly, possibly due to large diversity among original datasets variables. The scree plot was also become flat indicating a less contribution of any additional components to the total data variance. In this study, we considered the first three components with 63.2% of the total variance.

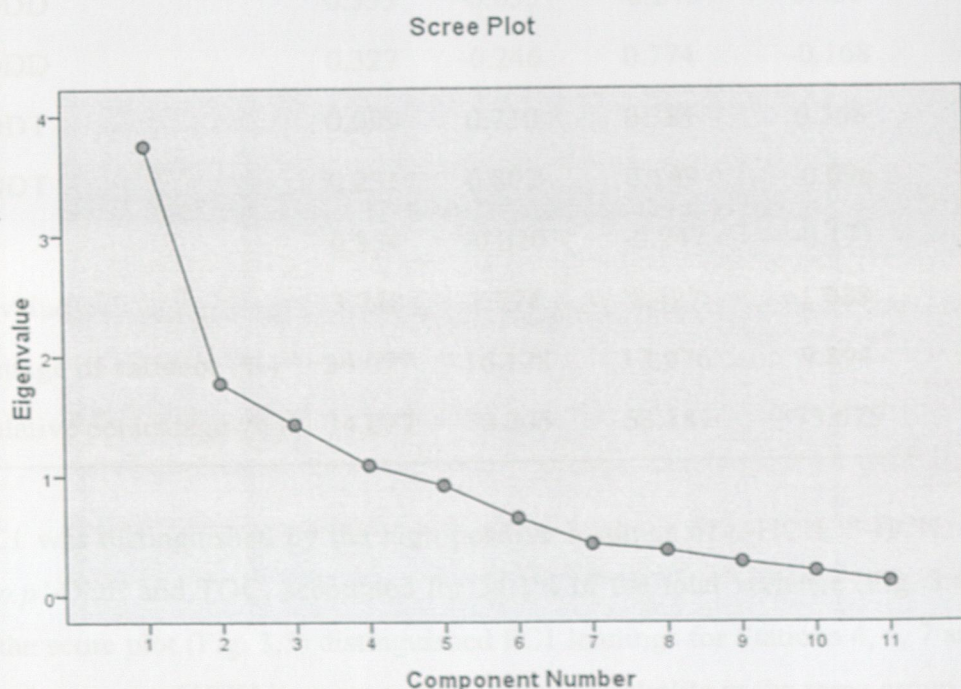


Fig. 3.3 The scree plot of Eigenvalue for total of HCHs and DDTs

Table 3.6 The first four principal components (PCs) matrix and variance explained

Variable	PC1	PC2	PC3	PC4
α -HCH	0.741	-0.216	-0.137	-0.334
β -HCH	0.834	0.197	-0.014	-0.337
γ -HCH	0.400	0.443	-0.563	0.185
δ -HCH	0.753	-0.029	0.090	-0.063
<i>o,p'</i> -DDE	0.307	-0.660	0.410	0.310
<i>p,p'</i> -DDE	0.723	0.035	0.316	0.372
<i>o,p'</i> -DDD	0.353	-0.055	-0.246	0.450
<i>p,p'</i> -DDD	0.327	-0.246	0.774	-0.168
<i>o,p'</i> -DDT	0.099	0.730	0.381	0.368
<i>p,p'</i> -DDT	0.231	0.802	0.149	-0.096
TOC	0.854	-0.020	-0.247	-0.171
Eigenvalue	3.748	1.774	1.427	1.088
Percentage of variance (%)	34.077	16.128	12.976	9.894
Cumulative percentage (%)	34.077	50.205	63.181	73.075

The PC1 was distinguished by the high positive loadings of α -HCH, β -HCH, δ -HCH, *p,p'*-DDE and TOC, accounted for 34.1% of the total variance (Fig. 3.4), where the score plot (Fig. 3.5) distinguished PC1 loadings for Stations 4, 6, 7 and 8. The occurrence of HCH isomers and *p,p'*-DDE metabolite in the same group of PC1 could suggest the same origin of these compounds. β -HCH and *p,p'*-DDE were relatively more persistent than other isomers and metabolites. The isomer α -HCH can be photochemically transformed into β -HCH more easily in the environment (Willett et al., 1998). Moreover, TOC gave a high positive loading on the PC1 as shown in Fig.3.4, and there were significant positive correlations between TOC and HCH isomer and DDT metabolites (e.g. *p, p'*-DDE; $r = 0.5$; $p < 0.01$) (Table. 3.4). This indicated that beside input sources of these compounds the distribution of historical accumulated HCHs and DDTs residues could also be influenced by organic matter in sediments due to post-depositional sorption of

these compounds to sediments organic matter (Hung et al., 2007). According to Fig. 3.4 and Fig. 3.5, PC2 was mainly related with *o,p'*-DDT, *p,p'*-DDT and γ -HCH, and stations 1, 9 and 11, accounted for 16.1% of the total data variance. The presence of *p,p'*-DDT and *o,p'*-DDT in the same group of PC2 could suggest that they had a common source of contamination. The PC3 accounting for 13% of total variability was related with *p,p'*-DDD and *o,p'*-DDE, which was distinguished by score plot (Fig. 3.5) for stations 3, 5 and 10 and had a distinct loading in PCA compared with *p,p'*-DDT, indicating a distinguish source of contamination.

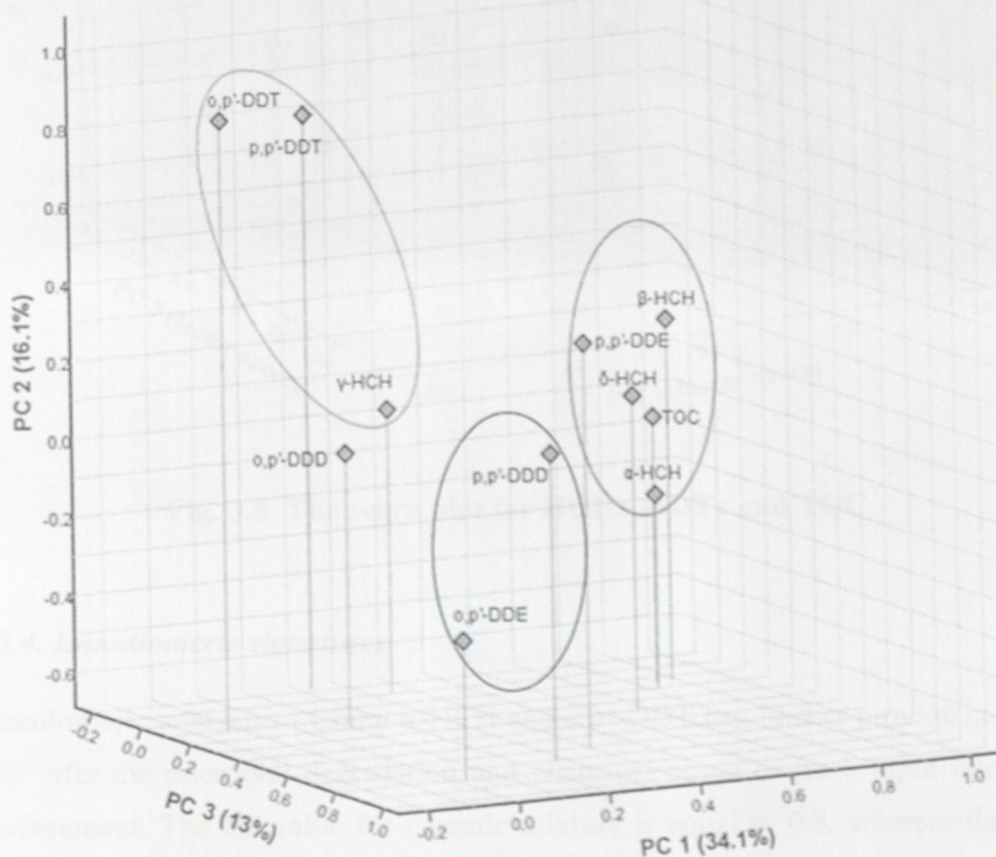


Fig.3.4. Principal component analysis for HCHs, DDTs and TOC

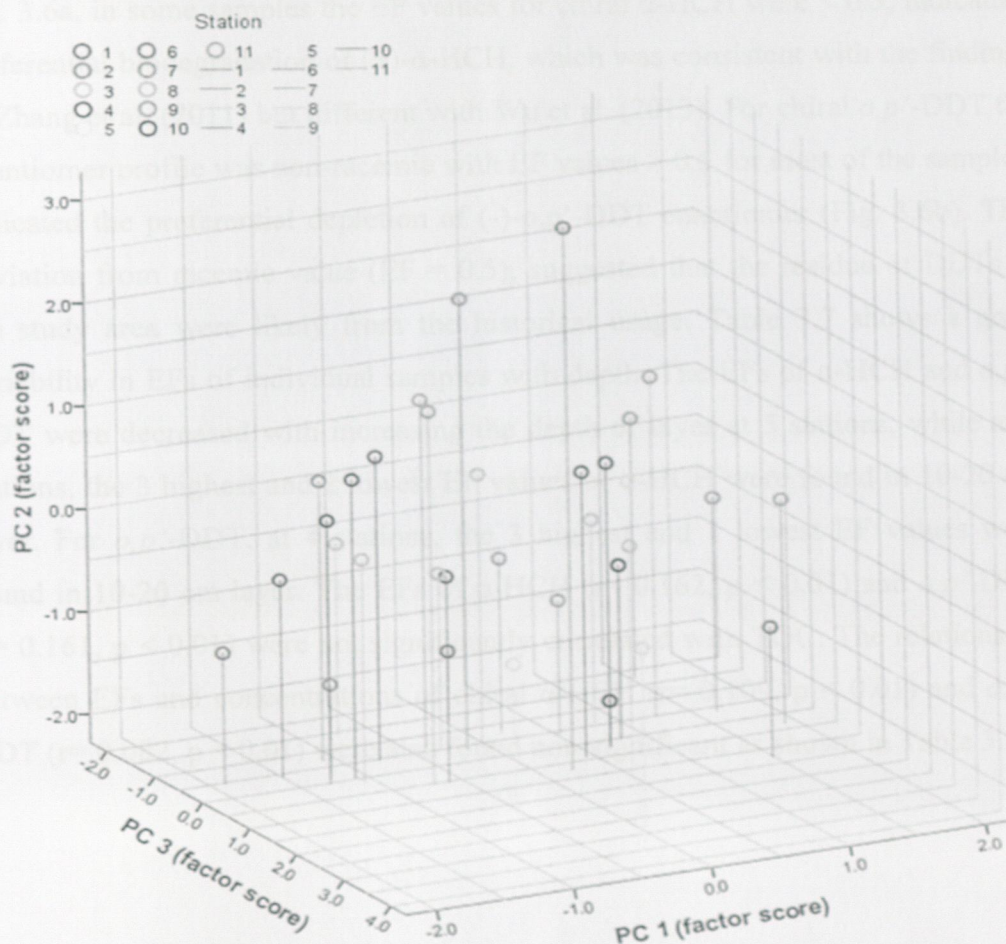


Fig. 3.5 The score plot for HCHs, DDTs and TOC

3.5.4. Enantiomeric signatures

Enantiomeric analysis of Chiral α -HCH and *o,p'*-DDT can further provide insight into infer the microbial degradation and historical usage or fresh input into the environment. The EF value for racemic mixture is equal to 0.5, whereas the EF value > 0.5 and < 0.5 indicate preferential degradation of (–) and (+)-enantiomer, respectively (Niu et al., 2013). In the present study, the EF values for the chiral compounds in sediments varied from 0.25 to 0.8, with a mean values of 0.49 and 0.53 for α -HCH and *o,p'*-DDT, respectively (Table 3.7). The enantiomer signatures of α -HCH were generally racemic or close to racemic for most of the samples (Fig. 3.6a), suggesting the possible fresh input of HCHs. The similar trend was also observed by Li et al. (2006) and Covaci et al. (2010). As shown in

Fig. 3.6a, in some samples the EF values for chiral α -HCH were < 0.5 , indicating preferential biodegradation of (+)- α -HCH, which was consistent with the findings of Zhang et al. (2011) but different with Wu et al. (2013). For chiral o,p' -DDT the enantiomer profile was non-racemic with EF values > 0.5 for most of the samples, indicated the preferential depletion of (-)- o,p' -DDT enantiomer (Fig. 3.6b). This deviation from racemic value (EF = 0.5), suggested that the residue of DDTs in the study area were likely from the historical usage. Table 3.7 shows a great variability in EFs of individual samples with depth. The EFs of α -HCH and o,p' -DDT were decreased with increasing the depth of layer at 5 stations, while at 5 stations, the 3 highest and 2 lowest EF values of α -HCH were found in 10-20 cm layer. For o,p' -DDT, at 4 stations, the 3 highest and 1 lowest EF values were found in 10-20 cm layer. The EFs of α -HCH ($r = 0.162$, $p < 0.01$) and o,p' -DDT ($r = 0.161$, $p < 0.01$) were not significantly correlated with TOC. The relationship between EFs and concentrations of chiral α -HCH ($r = 0.109$, $p < 0.01$) and o,p' -DDT ($r = 0.082$, $p < 0.01$) were also found non-significant as shown in Table 3.8.

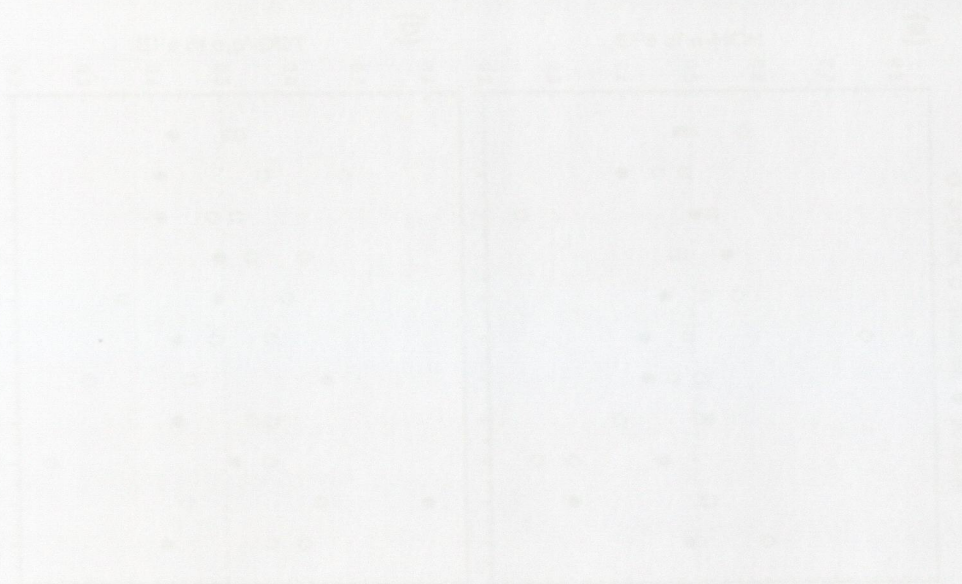


Fig. 3.6 Enantiomeric profile of chiral α -HCH and o,p' -DDT: (a) EFs of α -HCH; (b) EFs of o,p' -DDT. The dashed red line represents racemic value.

Table 3.7 Enantiomeric fractions of chiral α -HCH and *o,p'*-DDT

Stations	EFs of chiral α -HCH			EFs of chiral <i>o,p'</i> -DDT		
	0-10cm	10-20cm	20-30cm	0-10cm	10-20cm	20-30cm
S1	0.58	0.50	0.49	0.62	0.51	0.43
S2	0.48	0.51	0.40	0.78	0.56	0.52
S3	0.25	0.53	0.50	0.49	0.62	0.51
S4	0.49	0.48	0.55	0.75	0.54	0.50
S5	0.56	0.52	0.46	0.59	0.35	0.49
S6	0.75	0.51	0.43	0.49	0.57	0.43
S7	0.51	0.47	0.43	0.30	0.45	0.65
S8	0.39	0.51	0.52	0.67	0.54	0.43
S9	0.42	0.37	0.55	0.25	0.56	0.51
S10	0.52	0.52	0.32	0.45	0.64	0.79
S11	0.60	0.50	0.49	0.61	0.56	0.41
Mean		0.49			0.53	

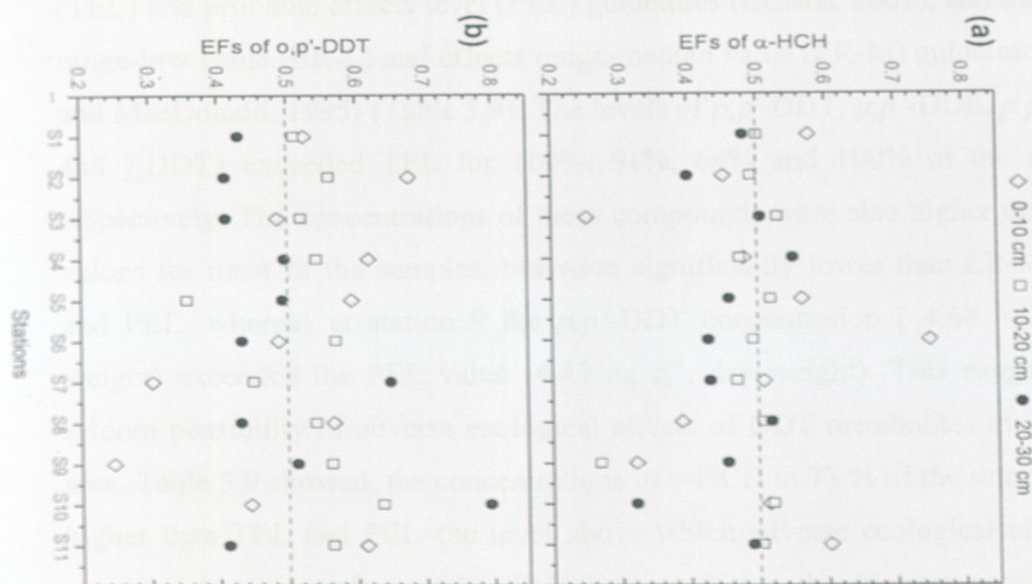


Fig. 3.6 Enantiomeric profile of chiral α -HCH and *o,p'*-DDT: (a) EFs of α -HCH; (b) EFs of *o,p'*-DDT. The dash-red line represents racemic value.

Table 3.8 Correlation between EFs and concentrations of α -HCH and *o,p'*-DDT, and TOC

	EFs of α -HCH	EFs of <i>o,p'</i> -DDT	α -HCH	<i>o,p'</i> -DDT	TOC
EFs of α -HCH		-0.094	0.109	-0.104	0.162
EFs of <i>o,p'</i> -DDT	-0.094		-0.037	-0.082	-0.161
α -HCH	0.109	-0.037		-0.143	0.602**
<i>o,p'</i> -DDT	-0.104	-0.082	-0.143		-0.106
TOC	0.162	-0.161	0.602**	-0.106	

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

3.5.5. Ecotoxicological concern of HCHs and DDTs

Contaminated sediments can pose potential ecological risks to aquatic life. In this study, a level-screening ecotoxicological risk assessment was conducted, using two international sediments quality guidelines (SQGs), the threshold effects level (TEL) and probable effects level (PEL) guidelines (CCME, 2002), and the effects range-low value (ER-L) and effects range-median value (ER-M) guidelines (Long and MacDonald, 1995) (Table 3.9). The levels of *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, and Σ DDTs exceeded TEL for 100%, 91%, 64% and 100% of the samples, respectively. The concentrations of these compounds were also higher than ER-L values for most of the samples, but were significantly lower than ER-M values and PEL, whereas at station 9 the *p,p'*-DDT concentration (4.68 ng g⁻¹, dry weight) exceeded the PEL value (4.47 ng g⁻¹, dry weight). This suggested the seldom possibility of adverse ecological effects of DDT metabolites in the study area. Table 3.9 showed, the concentrations of γ -HCH in 73 % of the samples were higher than TEL and PEL—the level above which adverse ecological effects are expected to appear frequently. This suggested that the highest possibility of ecotoxicological risk to sediments dwelling species are associated with exposure to γ -HCH in this area. There is lack of information about the potential ecotoxicological risk of chiral OCPs at enantiomeric level and assessment of ecological effects at isomeric/metabolites level is not sufficient. Further study must be needed to revise sediment quality guide lines for chiral OCPs at enantiomeric level.

Table 3.9 Assessment of potential ecotoxicological risk of HCHs and DDTs in surface sediments using two sediment quality guidelines.

Compound	Study area (ng g ⁻¹ , dw)	Above ER-L		Above ER-M		Above TEL		Above PEL (%) [*]
		ER-L ^a	(%) [*]	ER-M ^b	(%) [*]	TEL ^c	(%) [*]	
<i>p,p'</i> -DDE	0.98-4.41	2.2	64	27	0	1.22	91	7.81
<i>p,p'</i> -DDD	0.67-3.92	2	64	20	0	2.07	64	374
<i>p,p'</i> -DDT	1.92-4.68	1	100	7	0	1.19	100	4.47
ΣDDTs	6.45-18.95	1.58	100	46.1	0	3.89	100	51.7
γ-HCH	0.18-3.94	—	—	—	—	0.32	73	0.99
								73

^{*}Percent samples above corresponding limits

^aEffect range-low value

^bEffect range-median value

^cThreshold effects level

^dProbable effects level

3.6. Conclusions

The present study investigated the contamination profile of HCHs and DDTs at isomeric/metabolites and enantiomeric level in sediments of Kabul River, Pakistan. DDTs were found the most dominant contaminants of total of HCH and DDT congeners. The concentrations of Σ HCHs and Σ DDTs are comparable with other riverine sediments from Asian countries. The TOC contents are significantly correlated with total residual concentrations of HCHs and DDTs. Compositional and chiral analyses indicated that DDTs originate from historical application in most of the sampling sites, while HCHs contaminations were associated with fresh input in most of the samples, with evidence exhibiting the fresh input of lindane. PCA showed, in addition to input sources the distribution of historical accumulated HCHs and DDTs could also be influenced by organic matter in sediments due to post-depositional sorption of these compounds on sediments organic matter. In accordance, with sediment quality guidelines (SQGs), more ecotoxicological concern should be paid to HCHs in sediments of Kabul River, Pakistan.

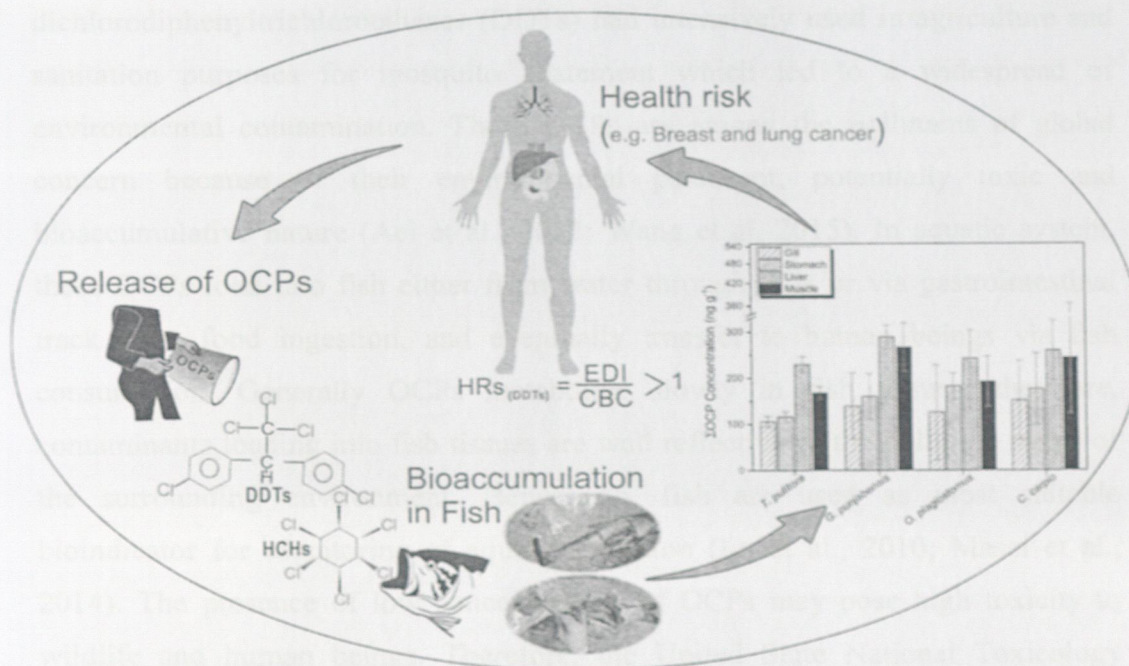
4.2. Abstract

Distribution of HCH and DDT congeners in sediments of Kabul River, Pakistan and their associated potential health risk to local consumer are presented. The average Σ (HCHs + DDTs) concentration in *Glyptotendipes pinnipes* (1.4 ng g^{-1}) (sediment) was found higher than for *Glyptotendipes pinnipes* (1.55 ng g^{-1}) (sediment). The distribution of Σ (HCHs + DDTs) in all 5 sites was in order of $1.4 > 1.3 > 1.2 > 1.1 > 1.0 \text{ ng g}^{-1}$. The congener profile indicated that all the species were contaminated with recent usage of hexachlorocyclopentadiene (HCH) (p-HCH/total HCH from 0.24-0.47%) while p,p'-DDE/p,p'-DDT (DDT/total DDT from 0.61-1.78%) contaminant originated from historical degradation mechanism. The average estimated daily dietary intake of HCHs ($0.10 \text{ ng kg}^{-1} \text{ day}^{-1}$) was found higher than DDTs ($0.23 \text{ ng kg}^{-1} \text{ day}^{-1}$) by the local consumer via fish consumption. On the basis of both 50^{th} and 95^{th} percentiles

Chapter-4

TISSUE DISTRIBUTION OF HCH AND DDT CONGENERS IN FISH COLLECTED FROM KABUL RIVER, PAKISTAN AND HUMAN HEALTH RISK ASSOCIATED WITH FISH CONSUMPTION

4.1. Graphical Abstract



4.2. Abstract

Distribution of HCH and DDT congeners in tissues of four different fish species and their associated potential health risk to local consumer are presented. The average $\sum(\text{HCHs} + \text{DDTs})$ concentration in *Glyptothorax punjabensis* (214 ng g⁻¹) (carnivores) was found higher than *Tor putitora* (herbivores) (155 ng g⁻¹). The distribution of $\sum(\text{HCHs} + \text{DDTs})$ in all fish tissues was found in order of liver > muscle > stomach > gills. The congeners profile indicated that all fish species were contaminated with recent usage of hexachlorocyclohexane (HCH) ($\beta\text{-HCH}/\sum\text{HCH}$ from 0.29-0.47), while dichlorodiphenyltrichloroethane (DDT) ((DDE+DDD)/ $\sum\text{DDT}$ from 0.61-0.78) contamination originated from long-time degradation mechanism. The average estimated daily dietary intake of $\sum\text{HCHs}$ (15.0 ng kg⁻¹ day⁻¹) was found higher than $\sum\text{DDTs}$ (12.5 ng kg⁻¹ day⁻¹) by the local consumer via fish consumption. On the basis of both 50th and 95th percentile

exposure levels the carcinogenic hazard ratios for DDT and its congeners were exceeded one (safety limit) for all fish species, indicating a great potential cancer risk for local consumer with life time consumption of contaminated fish.

4.3. Introduction

Organochlorine pesticides (OCPs) such as hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs) had intensively used in agriculture and sanitation purposes for mosquito abatement which led to a widespread of environmental contamination. These OCPs are among the pollutants of global concern because of their environmental persistent, potentially toxic and bioaccumulative nature (Ael et al., 2012; Wang et al, 2015). In aquatic system, these OCPs load into fish either from water through gills or via gastrointestinal track from food ingestion, and eventually transfer to human beings via fish consumption. Generally OCPs metabolize slowly in fish tissues; therefore, contaminants loading into fish tissues are well reflective of the pollution status of the surrounding environment. Henceforth, fish are used as most suitable bioindicator for monitoring of aquatic pollution (Lu et al., 2010; Masci et al., 2014). The presence of low concentrations of OCPs may pose high toxicity to wildlife and human beings. Therefore, the United State National Toxicology Program (USNTP) have been listed HCHs and DDTs as “reasonably anticipated to be a human carcinogen” (Wang et al., 2011).

The Kabul River contains more than 54 fish species, of which most of the species are used for human consumption (Yousafzai et al., 2008). Kabul River is the ultimate disposal site for industrial chemicals, sewage wastewater and agricultural runoff, which increased the possibility of pesticide emission to the river (MOE-PAK, 2005a). Consequently, pesticides distribute among river ecosystem, and bioaccumulate in biota. In addition, DDT demolished factory is also located in the river catchment area. These special circumstances surrounding the Kabul River provide an opportunity to investigate the contamination profile of HCH and DDT congeners in fish species and examine the state of pollution of their habitat. In Pakistan, numerous studies investigated the occurrence of OCPs in the blood serums and fat samples of the residents of Punjab, Sindh and Balochistan provinces, and the cancer incidence rate have also been correlated with OCPs

levels during the years 1994-2002 (Naqvi and Jahan, 1999; Parveen and Masud, 2001;), while the prior studies showed, consumption of OCPs contaminated fish are the major contribution to human carcinogenic risk (Wang et al., 2013; Yohannes et al., 2014).

The present study aimed to investigate the contamination profile of HCH and DDT congeners in different tissues of different edible fish species and to further examine their composition and input sources. The study is also focused on the assessment of carcinogenic and non-carcinogenic risks associated with the consumption of HCHs and DDTs contaminated fish.

4.4. Materials and methods

4.4.1. Study area and fish samples

Kabul River located in Khyber Pakhtunkhwa Province of Pakistan, stretches approximately 90 km and covering three administrative districts (Peshawar, Charsadda and Nowshera). The river provides water for irrigation and domestic uses and somehow for drinking purpose. The river has rich biodiversity, with a large diversity of fish. It is a main fishing source for local residents. In February 2014, four different edible fish species (n=16), namely *Cyprinus carpio*, *Orienes plagiostomus*, *Glyptothorax punjabensis* and *Tor putitora* were caught at 11 different sites along Kabul River (Fig. 4.1). The selected species are plentiful and most commonly consumed by the local population. The species were identified using taxonomic key of Muhammad et al. (2014) and Ullah et al. (2014) (Table 4.1). Each individual fish was carefully dissected to obtain muscle, gill, stomach and liver.

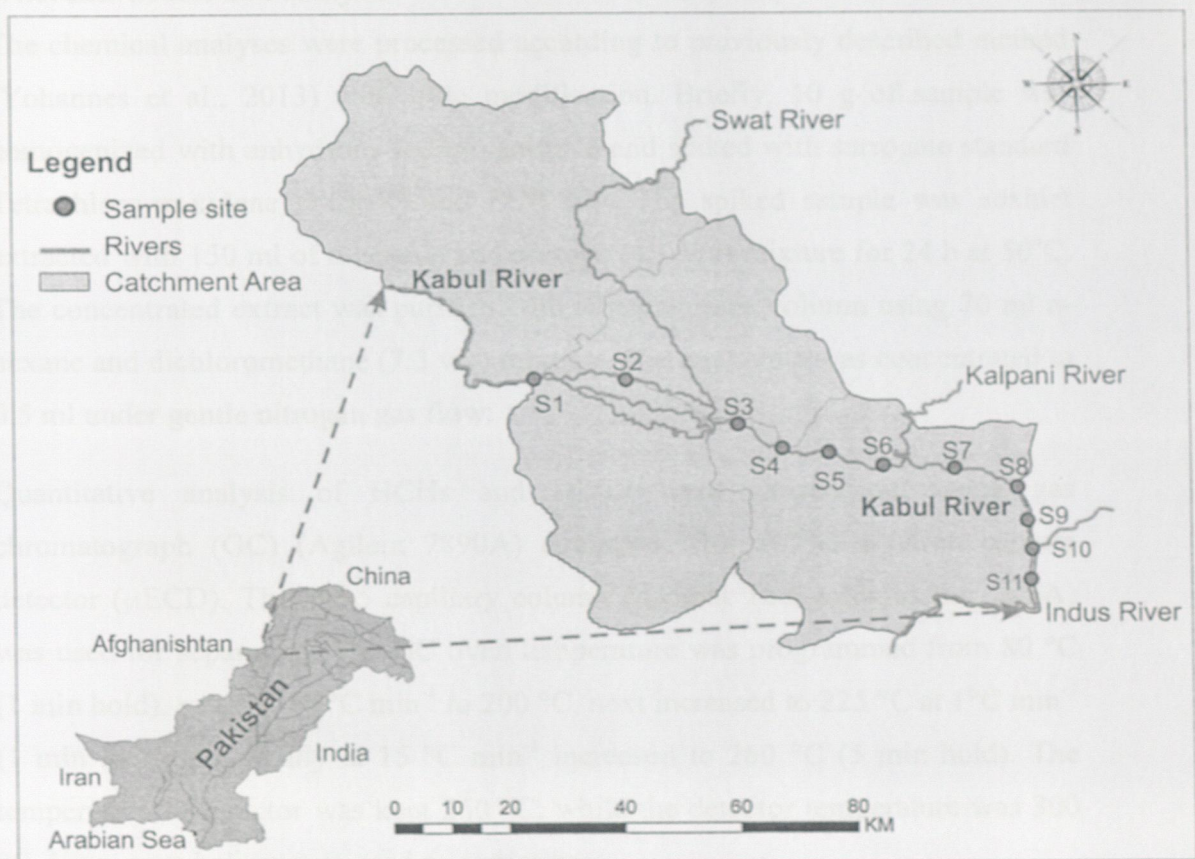


Fig. 4.1 Map showing the sampling sites along Kabul River, Khyber Pakhtunkhwa- Pakistan

Table 4.1 Fish identification taxonomic key

S. No	Order	Family	Genus and species	Local Name	References
1	Cypriniformes	Cyprinidae	<i>Tor putitora</i>	Mahseer	(IUCN., 1994)
			<i>Orienus</i>		(Muhammad et
2			<i>plagiostomus</i>	Swati machli	al., 2014)
					(Ullah et al.,
3			<i>Cyprinus carpio</i>	China kub	2014)
			<i>Glyptothorax</i>		(Muhammad et
4	Siluriformes	Sisoridae	<i>punjabensis</i>	Sulamanne	al., 2014)

4.4.2. Extraction and analysis

The chemical analyses were processed according to previously described method (Yohannes et al., 2013) with little modification. Briefly, 10 g of sample was homogenized with anhydrous sodium sulphate and spiked with surrogate standard Tetrachloro-m-xylene (TCmX) and PCB-209. The spiked sample was soxhlet extracted with 150 ml of n-hexane and acetone (4:1 v/v) mixture for 24 h at 50°C. The concentrated extract was purified with silica/alumina column using 70 ml n-hexane and dichloromethane (7:3 v/v) mixture. The final elute was concentrated to 0.5 ml under gentle nitrogen gas flow.

Quantitative analysis of HCHs and DDTs were carried out using gas chromatograph (GC) (Agilent 7890A) equipped with a ^{63}Ni electron capture detector (μECD). The HP-5 capillary column (Agilent Technologies Inc., USA) was used for separation. The GC oven temperature was programmed from 80 °C (1 min hold), ramp at 10 °C min^{-1} to 200 °C, next increased to 225 °C at 1 °C min^{-1} (1 min hold) and finally at 15 °C min^{-1} increased to 260 °C (5 min hold). The temperature of injector was kept 250 °C, while the detector temperature was 300 °C. Ultra-pure helium was used as carrier gas.

4.4.3. Quality control and assurance

The quantification of HCHs and DDTs were based on five point calibration curves with correlation coefficients ($R^2 > 0.998$ for all coefficients). For each batch of 10 samples, a procedural blank, a spike blank, and duplicate samples were processed to assure quality. The surrogate standards TCmX (84±9% recovery) and PCB-209 (91±12% recovery) were used to determine the recovery rates. The limits of detection (LOD) of both HCHs and DDTs were ranged from 0.14-0.98 ng g^{-1} , ww. These strict quality control and assurance protocols confirmed the accuracy and precession of the analytical procedures used in this research.

4.4.4. Risk assessment

Human health risk of HCHs and DDTs from consumption of contaminated fish was assessed according to USEPA recommended guidelines. Non-carcinogenic risk was estimated by comparing the measured estimated daily intake (EDI) (Eq. 1) with the recommended reference doses (RfD) listed in Table 4.2. For

carcinogenic risk, the hazard ratios (HRs) were calculated using Eq. 2 (Dougherty et al., 2000; Wang et al., 2011; Akoto et al., 2015).

$$EDI = \frac{C_m \times ACR}{BW} \quad (1)$$

Where EDI, C_m , ACR and BW represent the estimated daily intake ($\text{ng}^{-1} \text{kg}^{-1} \text{d}^{-1}$), the concentration of HCHs and DDTs in fish muscle (ng g^{-1} , ww), average consumption rate of fish (g d^{-1}) and the average body weight (kg), respectively.

$$\text{Hazard Ratios (HRs)} = \frac{EDI}{CBC} \quad (2)$$

The CBC is cancer benchmark concentration ($\text{ng kg}^{-1} \text{d}^{-1}$) of pesticides calculated by Eq. 3.

$$CBC = \frac{(MRL/OSF) \times BW}{CR} \quad (3)$$

Where MRL is the maximum acceptable risk level (1×10^{-6} , dimensionless), OSF is the cancer oral slope factor ($(\text{mg kg}^{-1} \text{d}^{-1})^{-1}$) and CR is the consumption rate (g d^{-1}). The cancer benchmark concentration for carcinogenic risk is derived by setting cancer risk to one in one million for lifetime exposure. The HR greater than 1 indicates a potential cancer risk to human health.

4.4.5. Statistical analyses

In the present study, the significance differences were defined at $P \leq 0.05$ using ANOVA analyses. Principal component analysis was performed to investigate the loading pattern of HCHs and DDTs into fish and their biotransformation in different tissues. All datasets were examined for 50th and 95th percentile concentrations of investigated compounds before calculation of estimated daily dietary exposure to assess carcinogenic risk. All these analyses and calculations were performed using SPSS v 17 (SPSS Inc., Chicago, Illinois, USA) and Origin 9 (OriginLab Corporation, USA).

4.5. Results

4.5.1. Tissue distribution of HCH and DDT congeners in fish species

The occurrence frequencies of HCHs and DDTs in our samples ($n = 64$) were as high as 100%. The concentrations of total HCHs and DDTs were ranged from 51.9-493 ng g^{-1} , ww (mean 199 ng g^{-1} , ww), when all fish species were considered. The highest concentration of $\sum(\text{HCHs} + \text{DDTs})$ was found in *Glyptothorax punjabensis* (214 ng g^{-1}) followed by *Cyprinus carpio* (208 ng g^{-1}), *Orienus plagiostomus* (186 ng g^{-1}) and *Tor putitora* (155 ng g^{-1}) (Fig. 4.2). This may be attributed to their different feeding habits and habitats. *Tor putitora* individual is mainly feeding on aquatic vegetation, whereas *Glyptothorax punjabensis* is benthopelagic species and scavenged the bottom for benthos and zooplanktons (IUCN, 2014).

Among fish tissues significantly different ($p < 0.05$) concentrations of $\sum(\text{HCH} + \text{DDT})$ congeners were observed (Fig. 4.2). The highest concentrations of $\sum(\text{HCHs} + \text{DDTs})$ were found in liver of all fish species, varied from 80.7 to 289 ng g^{-1} , ww. The burden of $\sum(\text{HCHs} + \text{DDTs})$ levels in liver compare to other tissues (gill, stomach and muscle) was 1 to 2 times higher in all fish species. Guo et al. (2008) obtained $\sum(\text{HCHs} + \text{DDTs})$ levels in liver of mandarin fish that were 2 to 4 times higher than those in other tissues (skin, gill, gastrointestinal tract and muscle). The contamination pattern of $\sum(\text{HCHs} + \text{DDTs})$ levels in different tissues were found in order of liver > muscle > stomach > gills, for all fish species. Excluding liver, the concentrations of $\sum(\text{HCHs} + \text{DDTs})$ among gills, stomach and muscle were not significantly different in each species, e.g. *T. putitora* ($p > 0.25$), *G. punjabensis* ($p > 0.21$), *O. plagiostomus* ($p > 0.71$) and *C. carpio* ($p > 0.58$). This indicated that OCPs are able to equilibrate quickly in fish tissues

The concentrations of $\sum\text{HCH}$ and $\sum\text{DDT}$ in selected fish species were ranged from 10.7-329 ng g^{-1} and from 16.9-402 ng g^{-1} , ww, respectively. The $\sum\text{DDT}$ levels in different tissues were also found significantly different ($p < 0.02$), and their contamination pattern was nearly identical to that of $\sum(\text{HCHs} + \text{DDTs})$, while the concentrations of $\sum\text{HCH}$ were relatively similar among all fish species ($p > 0.79$) . (Fig. 4.2).

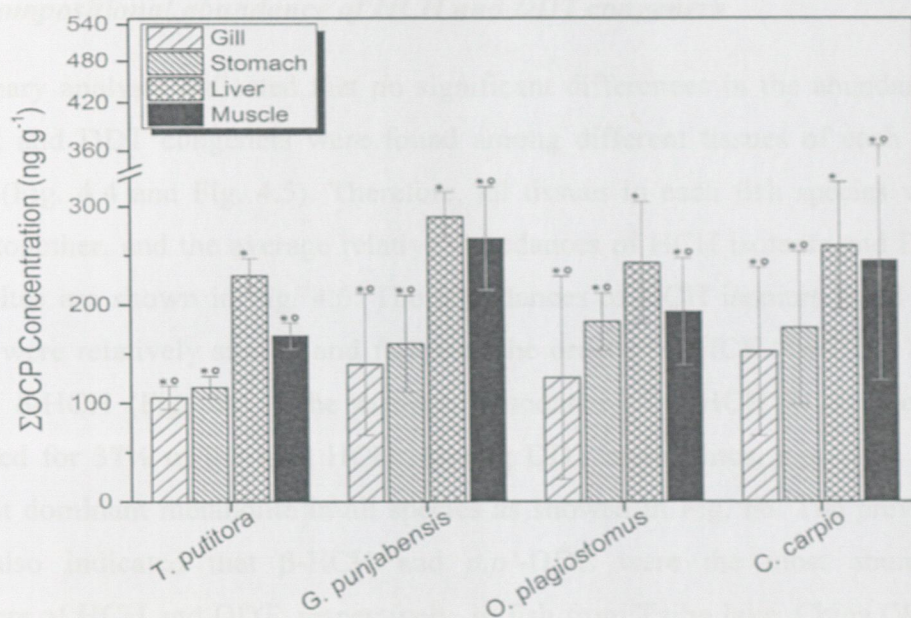


Fig. 4.2 Contamination profile of Σ OCP levels in tissues of different fish species. The error bars represent ± 1 standard deviation. (*) represent significant differences among all fish tissues, while excluding liver (o) represent non-significant differences among fish tissues.

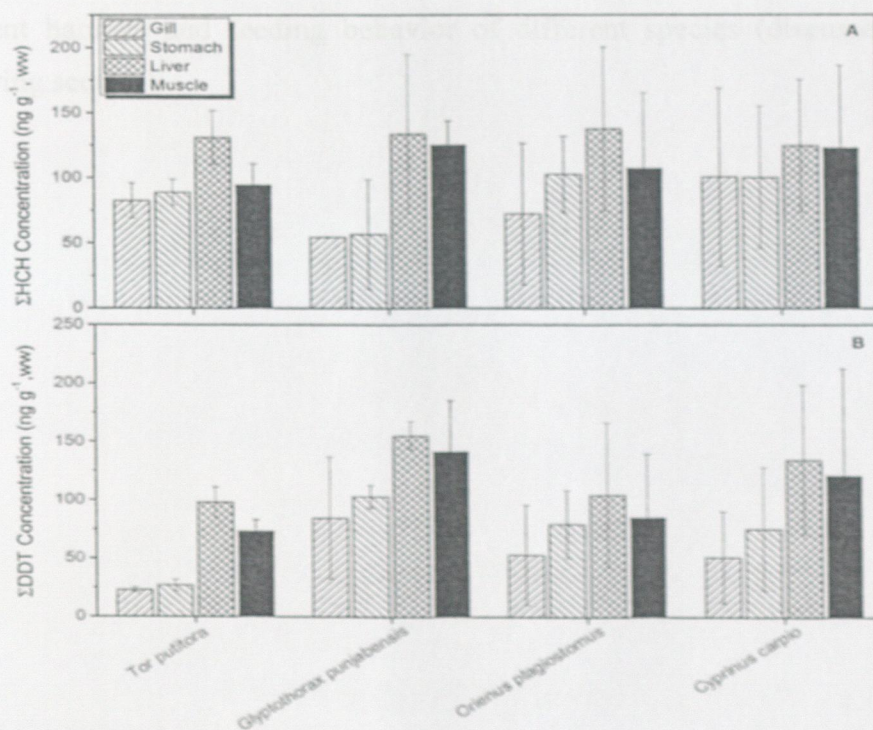


Fig. 4.3 Tissue distribution of Σ HCH (A) and Σ DDT (B) concentrations in different fish species

4.5.2. Compositional abundance of HCH and DDT congeners

Preliminary analysis indicated that no significant differences in the abundances of HCH and DDT congeners were found among different tissues of each fish species (Fig. 4.4 and Fig. 4.5). Therefore, all tissues in each fish species were pooled together, and the average relative abundances of HCH isomers and DDT metabolites are shown in Fig. 4.6. The abundances of HCH isomers in all fish species were relatively similar and followed the order of β -HCH > γ -HCH > α -HCH > δ -HCH (Fig. 6a). The average abundance of β -HCH in all species accounted for 37% of the total HCH. Among DDT metabolites, p,p' -DDE was the most dominant metabolite in all species as showed in Fig. 6b. The previous study also indicated that β -HCH and p,p' -DDE were the most abundant congeners of HCH and DDT, respectively, in fish from Taihu lake, China (Wang et al., 2013). In addition, the relative abundance of p,p' -DDT was found higher in *T. putitora* (27%), while lower in *O. plagiostomus* (22%) (Fig.3b). Whereas p,p' -DDD and o,p' -DDT were relatively similar in all species. These findings are probably attributed to different HCH and DDT congeners properties and/or different habitats and feeding behavior of different species (discussed in the following section).



Fig. 4.5 Abundance of DDT congeners in different fish tissues

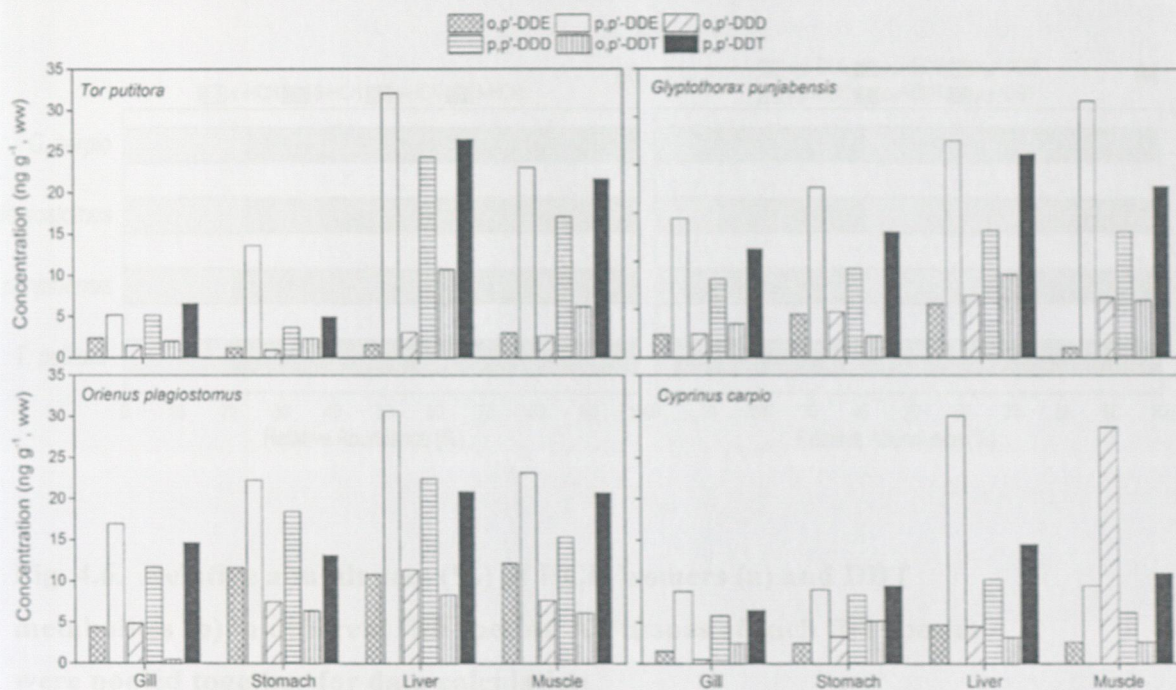


Fig. 4.4 Abundances of HCH congeners in different fish tissues

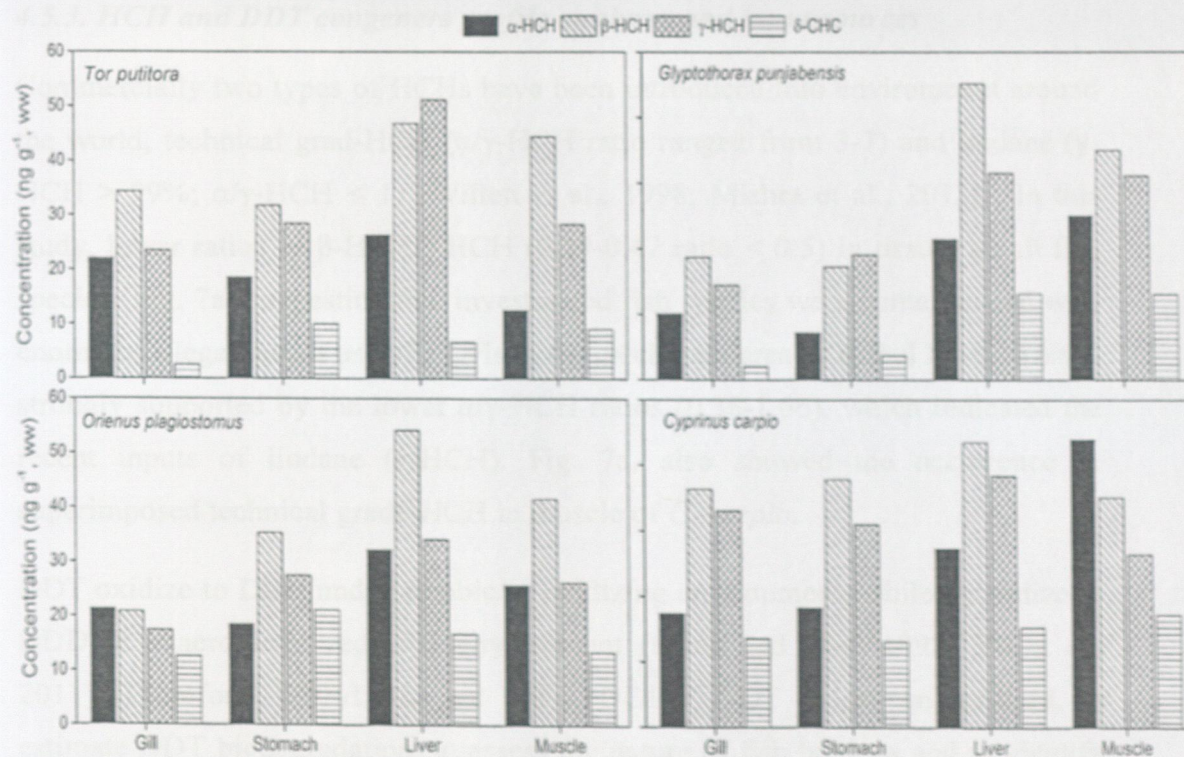


Fig. 4.5 Abundances of DDT congeners in different fish tissues

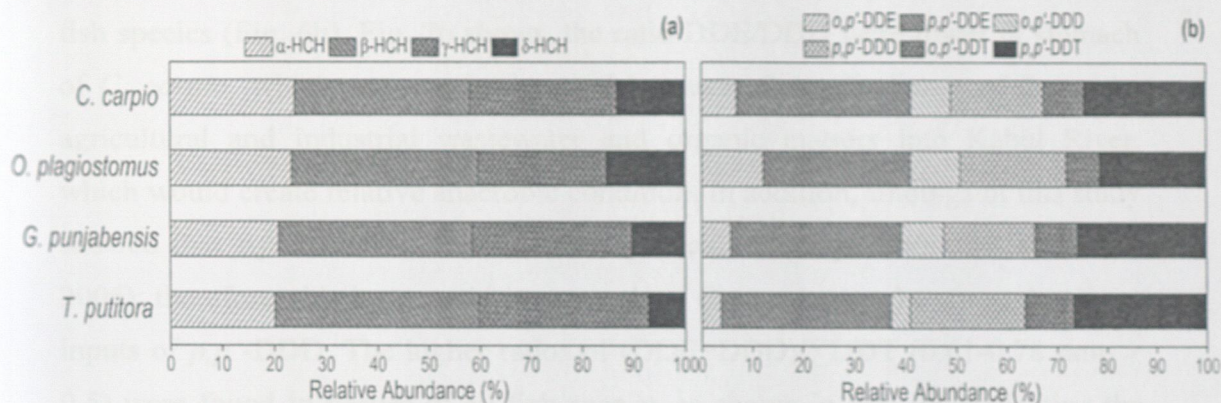


Fig. 4.6. Relative abundances (%) of HCH isomers (a) and DDT metabolites (b) in different fish species. All tissues of each fish species were pooled together for data calculation

4.5.3. HCH and DDT congeners profile analyses and input sources

Commercially two types of HCHs have been introduced into environment around the world; technical grad-HCH (α/γ -HCH ratio ranged from 3-7) and lindane (γ -HCH > 99%; α/γ -HCH < 1) (Willett et al., 1998; Mishra et al., 2013). In this study, lower ratios of β -HCH/ Σ HCH (0.29-0.47 ratio < 0.5) in tissues of all fish species (Fig. 7a) suggesting that investigated fish species were contaminated with enormous illegal recent use of HCHs in the catchment area of Kabul River. This is strongly supported by the lower α/γ -HCH ratios (0.38-1.66), which indicated the recent inputs of lindane (γ -HCH). Fig. 7a, also showed the occurrence of superimposed technical grade-HCH in muscle of *C. carpio*.

DDT oxidize to DDE under aerobic/or oxidizing environment, while deoxidize to DDD in anaerobic/or reducing environment (Hitch and Day, 1992; Yu et al., 2011). Therefore, DDE/DDD and (DDE+DDD)/ Σ DDT ratios can be used, to estimate DDT biodegradation, to assess the nature of fish habitats and to identify input sources (Wu et al., 2013). Excluding stomach of *C. carpio*, the present study showed that DDE/DDD ratios (1.11-3.19) in the tissues of all fish species were higher than unity (Fig. 7b), demonstrating that the dwelling environment of most of the species were aerobic. In addition, to higher persistency of *p,p'*-DDE

metabolite this may be also the reason for higher abundance of *p,p'*-DDE in all fish species (Fig. 6b). Fig. 7b shows, the ratio DDE/DDD were lower in stomach of *C. carpio*, which was not unexpected because of the discharge of domestic, agricultural and industrial wastewater and organic matters into Kabul River, which would create relative anaerobic condition. In addition, findings of this study showed that *p,p'*-DDD was also used as pesticide (Sethajintanin and Anderson, 2006); therefore, this lower ratio in stomach of *C. carpio* may be from the direct inputs of *p,p'*-DDD. The higher ratios of (DDE+DDD)/ Σ DDT (0.61-0.78 ratio > 0.5) were found in tissues of all fish species as shown in Fig. 7b, indicating the historical inputs of DDT into fish habitats. The higher ratios of *o,p'*/*p,p'*-DDT (1.3-9.3, or higher) characterize the usage of dicofol, while the lower ratio indicate the usage of technical DDT mixture (Qiu et al., 2005; Muñoz-Arnanz and Jiménez., 2011). In the present study, lower ratios of *o,p'*/*p,p'*-DDT (0.03-0.55) were occurred in tissues of all fish species, indicated that all fish species were contaminated with historically used technical grade-DDT mixture.

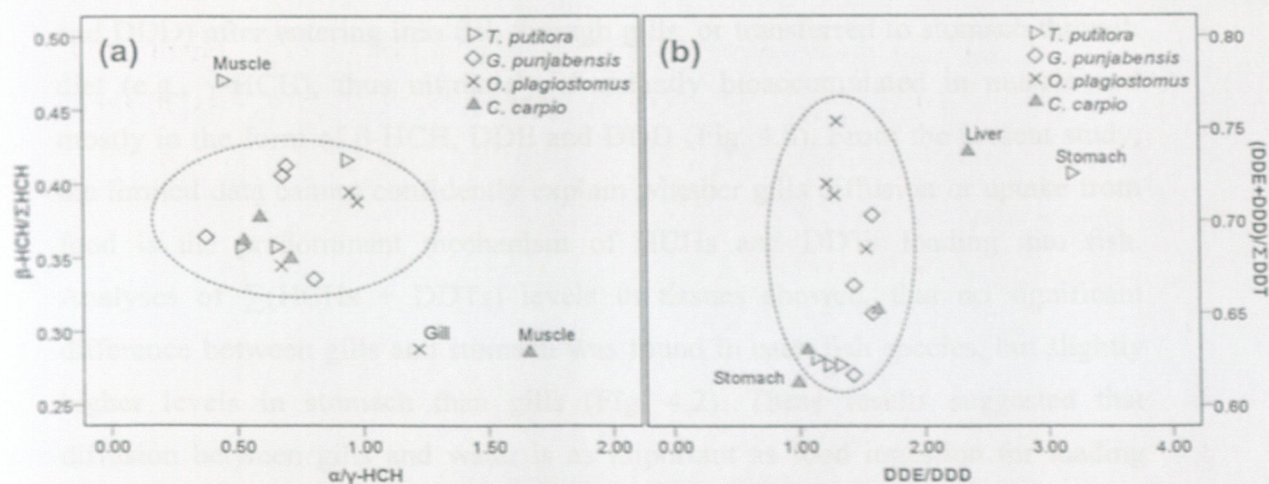


Fig. 4.7 Ratios of Σ HCH and Σ DDT to their isomers and metabolites, respectively, in different tissues of different fish species. (a) α/γ -HCH vs β -HCH/ Σ HCH ratio (b) (DDE/DDD) vs (DDE+DDD)/ Σ DDT ratio. Four points of the same legend represents the four different tissues.

4.5.4. Bioaccumulation and biotransformation of OCPs in fish tissues

Principal component analysis was performed to investigate HCHs and DDTs loading into fish and their transformation in different tissues (Fig. 4.8). PC1 shows positive loadings of DDE, DDD, and DDT, accounted for 54% variance, while PC2 was distinguished by high negative loading of γ -HCH and positive loadings of α -, β - and δ -HCH, explaining 31% variance (Fig. 4.8a). In the score plot (Fig. 4.8b); compared with other tissues, livers of all and muscles of two fish species (Fig. 4.8c) had positive scores of PC 1, indicating higher bioaccumulation of DDE, DDD and DDT in liver and muscle. Whereas, gills of all and stomach of two species had negative scores of PC 2, indicating higher bioaccumulation of γ -HCH in gill and stomach, which is further supporting the recent usage of HCH (Fig. 4.7a). In addition, muscles of *C. carpio* and *O. plagiostomus* showed relatively higher accumulation of β -HCH, α -HCH and δ -HCH, where α -HCH can be easily transformed to β -HCH (Willett et al., 1998; Mehboob et al., 2013).

In fact, the investigated compounds were metabolized in liver (e.g., DDT to DDE and DDD) after entering into fish through gills, or transferred to stomach through diet (e.g., γ -HCH), thus ultimately abundantly bioaccumulated in muscle and mostly in the form of β -HCH, DDE and DDD (Fig. 4.8). From the present study, the limited data cannot confidently explain whether gills diffusion or uptake from food is the predominant mechanism of HCHs and DDTs loading into fish. Analyses of Σ (HCHs + DDTs) levels in tissues showed, that no significant difference between gills and stomach was found in each fish species, but slightly higher levels in stomach than gills (Fig. 4.2). These results suggested that diffusion between gills and water is as important as food ingestion for loading HCHs and DDTs into fish. The previous study also demonstrated that the exchange of OCPs through gills is at least as important as gastrointestinal uptake from food (Guo et al., 2008).

Fig. 4.8 Principal component analysis of average relative abundance of OCPs in tissues of all species. (a) Loading plot, and (b) and (c) score plots.

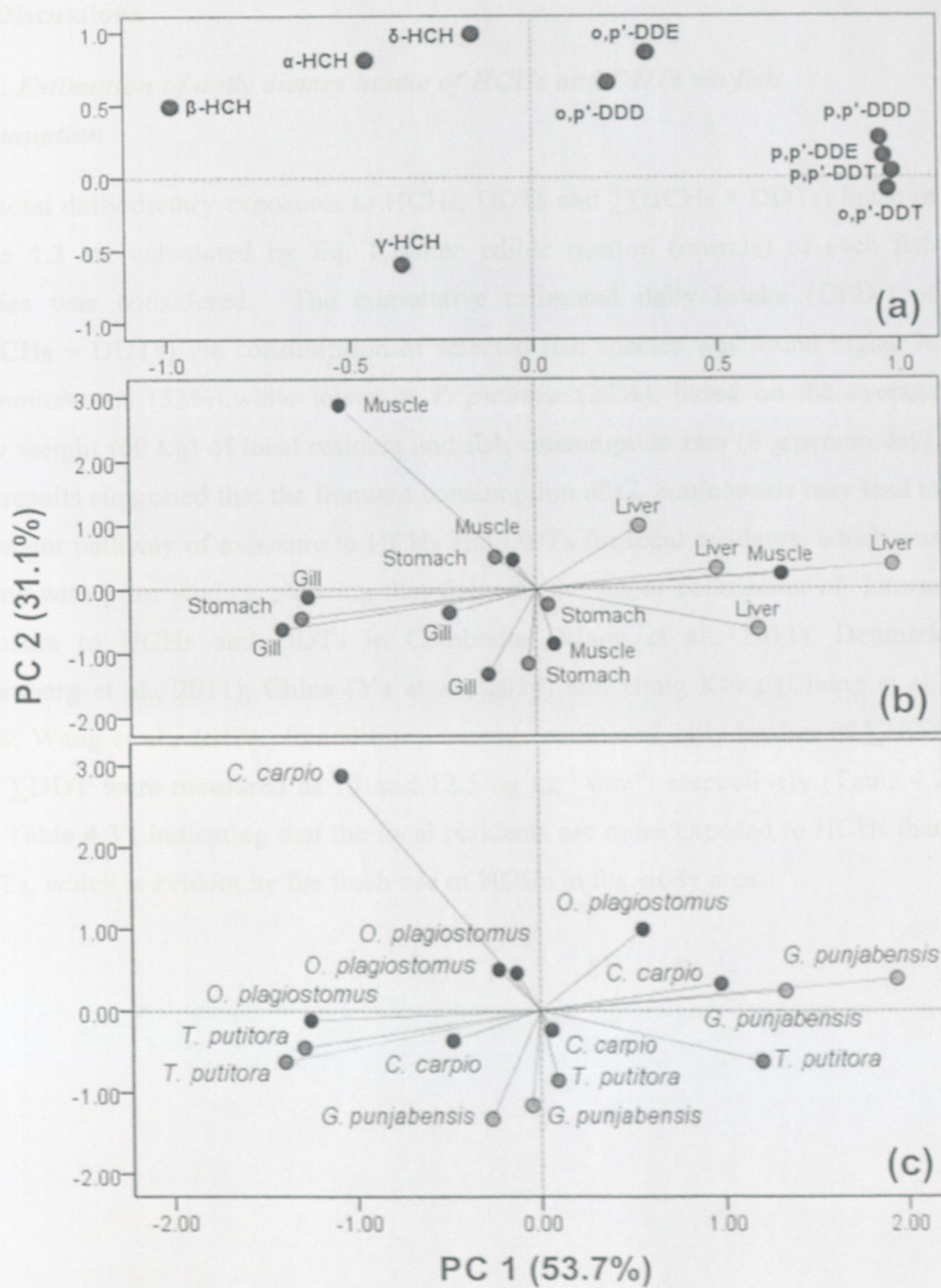


Fig. 4.8 Principal component analysis of average relative abundances of OCPs in tissues of all species. (a) Loading plot, and (b) and (c) score plots.

4.6. Discussions

4.5.1. Estimation of daily dietary intake of HCHs and DDTs via fish consumption

The total daily dietary exposures to HCHs, DDTs and $\Sigma(\text{HCHs} + \text{DDTs})$ listed in Table 4.2 are calculated by Eq. 1, when edible portion (muscle) of each fish species was considered. The cumulative estimated daily intake (CEDI) of $\Sigma(\text{HCHs} + \text{DDTs})$ via consumption of selected fish species was found higher in *G. punjabensis* (32%), while lower in *T. putitora* (20%), based on the average body weight (60 kg) of local resident and fish consumption rate (8 g/person/day). The results suggested that the frequent consumption of *G. punjabensis* may lead to the major pathway of exposure to HCHs and DDTs for local residents, which was in line with prior studies, showing that fish was the major contributor of human exposure to HCHs and DDTs in Cambodia (Wang et al., 2011), Denmark (Fromberg et al., 2011), China (Yu et al., 2014) and Hong Kong (Chung et al., 2008; Wang et al., 2013). In addition, average estimated daily intakes of ΣHCH and ΣDDT were measured as 15 and 12.5 ng kg⁻¹ day⁻¹, respectively (Table 4.2 and Table 4.3), indicating that the local residents are more exposed to HCHs than DDTs, which is evident by the fresh use of HCHs in the study area.

Table 4.2 Estimated daily exposure to HCHs and DDTs via fish consumption and comparison with standard guidelines

Compound	<i>T. putitora</i> AC [*]	EDI ^e	<i>G. punjabensis</i> AC [*]	EDI ^e	<i>O. plagiostomus</i> AC [*]	EDI ^e	<i>C. carpio</i> AC [*]	EDI ^e	TDI/ADI ^a (ng kg ⁻¹ d ⁻¹)	RfD/MRL ^b (ng kg ⁻¹ d ⁻¹)	References
α-HCH	12.62	1.68	30.06	4.01	25.78	3.44	44.03	5.87	1000 (TDI)	8000 (MRL)	Sloof and Matthijsen (1988); ATSDR (2001)
β-HCH	44.52	5.94	42.07	5.61	41.58	5.54	35.12	4.68		600 (MRL) ¹	ATSDR (2001)
γ-HCH	28.37	3.78	37.40	4.99	26.37	3.52	26.45	3.53	8000 (ADI)	300 (RfD) 10 (MRL) ¹	US EPA (2001); ATSDR (2001)
δ-HCH	9.12	1.22	15.72	2.10	13.46	1.79	17.27	2.30			
HCHs	94.64	12.6	125.3	16.7	107.2	14.3	122.9	16.4			
<i>o,p'</i> -DDE	3.03	0.40	2.10	0.28	12.19	1.62	5.340	0.71			
<i>p,p'</i> -DDE	23.05	3.07	53.37	7.12	23.22	3.10	18.95	2.53			
<i>o,p'</i> -DDD	2.64	0.35	12.58	1.68	7.680	1.02	9.390	1.25			
<i>p,p'</i> -DDD	17.09	2.28	26.10	3.48	15.38	2.05	12.42	1.66			
<i>o,p'</i> -DDT	6.20	0.83	11.93	1.59	6.160	0.82	5.240	0.70			
<i>p,p'</i> -DDT	21.64	2.89	35.48	4.73	20.74	2.77	21.95	2.93			
DDTs	73.65	9.82	141.57	18.88	85.36	11.38	73.29	9.78	20,000 (ADI)	500 (RfD) 500 (MRL) ¹	US EPA (2001); ATSDR (2001)
ΣOCP	168.29	22.44	266.83	35.58	192.56	25.67	196.16	26.16			

^{*}AC: Average concentration (ng g⁻¹, ww)

^aTDI/ADI: Tolerable/Acceptable daily intake

^bRfD/MRL: Reference dose/Minimal risk level

^eEDI: Estimated daily intake (ng/kg/day), the average fish consumption rate and estimated body weight were 8 g/person/day and 60 kg, respectively, in Pakistan (Eqani et al., 2013).

¹Value is based on intermediate exposure durations (>14 ± 364 day).

Table 4.3 Estimated daily intake of Σ HCH and Σ DDT via fish consumption in different countries/regions

Country/Region	year	Σ HCH (EDI, ng/kg/day)	Σ DDT (EDI, ng/kg/day)	References
Pakistan	2014	14.99*	12.46*	Present study
China	2002	0.47	14.7	(Yang et al., 2006)
India	2004	18000	900	(Muralidharan et al., 2009)
Republic of Bénin	2003	-- ^a	30.8	(Pazou et al., 2006)
Pakistan	2007-2009	0.80	10.7	(Eqani et al., 2013)
South China	2004-2005	-- ^a	5.84	(Guo et al., 2010)
Korea	2010	-- ^a	5.73	(Lee et al., 2015)

* Average estimated daily intake

^aNot available

The present study revealed that the dietary exposure levels of HCHs and DDTs via daily intakes from fish consumption were much lower in comparison with the neighbor country India (Σ HCH: 18000 ng/kg/day and Σ DDT: 900 ng/kg/day) (Muralidharan et al., 2009), whereas were found higher than the surrounding countries such as Korea (Σ DDT: 5.73 ng kg⁻¹ day⁻¹) (Lee et al., 2015) and South China (Σ DDT: 5.84 ng kg⁻¹ day⁻¹) (Guo et al., 2010) (Table 4.3). These indicated that the local residents of Pakistan, especially in Khyber Pakhtunkhwa Province, may intakes higher levels of HCHs and DDTs via fish consumption than the people in Korea and China. In the present study, the food processing (e.g. freezing and cooking) dissipation of HCHs and DDTs residue were not taken into consideration (Kaushik et al., 2009).

4.6.2. Carcinogenic and non-carcinogenic health risk assessment

The non-carcinogenic risk of local residents exposed to HCHs and DDTs via contaminated fish consumption was assessed by comparing the EDI of HCHs and DDTs with their standard limits set forth by United States Environmental Protection Agency (USEPA, 2001) and Agency for Toxic Substances and Disease Registry (ATSDR, 2001) (Table 4.2). The body weight normalized CEDI of α -, β -, and γ -HCHs and DDTs by local residents were 15, 22, 16 and 50 $\text{ng kg}^{-1} \text{d}^{-1}$, respectively, when all fish species were considered. Compared to tolerable/or acceptable daily intake (TDI/ADI) and minimal risk levels (MRL) of HCHs and DDTs, the EDIs of these compounds were well below the recommended limits, but these lower EDI levels do not necessarily suggested that there are no potential health risks for the local residents associated with the consumption of HCHs and DDTs contaminated fish. For example, comparing the present results with maximum admissible concentrations of DDTs (14.4 ng g^{-1} , ww) and HCHs (10 ng g^{-1} , ww) for fish consumption established by USEPA (2000) and European Union, respectively (Eqani et al., 2013), showed that 100% of the samples exceeded these limits. Although the exposure levels of HCHs and DDTs via fish consumption were lower than the reference values, but these compounds could be bioaccumulated in fish tissues and pose adverse effects to human health. Moreover, this approach ignored synergistic effects when being exposed to OCPs synergistically.

A carcinogenic risk assessment was conducted using cancer benchmark concentrations (CBCs) –derived from cancer oral slope factors (USEPA) (Wang et al., 2011; Akoto et al., 2015) and estimated daily exposure levels of HCHs and DDTs. Two hazard Ratios (HRs) at 50th and 95th percentile exposure levels were used to estimate the potential carcinogenic effects. The hazard ratio greater than unity indicate that there is potential cancer risk to human health (Eq. 2 and 3). As shown in Table 4.4, DDT and its congeners have higher ratios than unity in all fish species, on the basis of both 50th and 95th hazard ratios, indicating that the local residents would have a chance to develop cancer from DDTs. In addition, on the basis of 95th hazard ratios *G. punjabensis* and *C. carpio* species showed relatively higher ratios (bold values), suggesting that consumption of these species would contribute more potential carcinogenic threats to human health than other

two species. In general, the results indicated that cumulative daily dietary intake of DDTs via fish consumption would yield life time cancer risk to human health; at considered risk one in one million for lifetime exposure.

The limitation in this study is associated with the daily dietary exposure to HCHs and DDTs which could lead to uncertainty in risk assessment. For example, skin tissue of fish is not taken into consideration in this study, which is commonly being eaten with muscle. A more refined estimation of daily dietary intake and subsequent health risk assessment are needed in the future.

Table 4.4 Cancer benchmark concentrations and hazard ratios for OCPs in different fish species from Kabul River, Pakistan.

Compound	OSF ^a ((mg kg ⁻¹ d ⁻¹) ⁻¹)	CBC ^b (ng kg ⁻¹ d ⁻¹)	50 th concentrations HRs ^c				95 th concentrations HRs ^c			
			<i>T. putitora</i>	<i>G. punjabensis</i>	<i>O. plagiostomus</i>	<i>C. carpio</i>	<i>T. putitora</i>	<i>G. punjabensis</i>	<i>O. plagiostomus</i>	<i>C. carpio</i>
α-HCH	6.3	47.25	0.04	0.08	0.04	0.04	0.04	0.09	0.13	0.44
β-HCH	1.8	13.5	0.43	0.42	0.43	0.23	0.44	0.42	0.61	0.79
γ-HCH	1.3	9.75	0.40	0.51	0.50	0.24	0.42	0.52	0.50	0.91
<i>p,p'</i> -DDE	0.34	2.55	1.28	2.79	1.46	0.95	1.35	3.56	1.67	1.79
<i>p,p'</i> -DDT	0.34	2.55	1.09	1.86	1.29	0.86	1.13	3.08	1.55	3.12
ΣDDTs	0.34	2.55	3.70	7.40	5.77	4.29	3.84	9.14	6.02	16.84

^a OSF; Oral slope factors (were obtained from USEPA's Integrated Risk Information System (IRIS), 2001).

^b CBC; Cancer benchmark concentration (ng/ kg body weight/day) (Fish consumption rate and average body weight were derived from Table 1).

^c HRs; Hazard Ratios.

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4.7. Conclusions

Tissues distribution of HCHs and DDTs were investigated in different edible fish species collected from Kabul River, Pakistan. The contamination levels of Σ (HCHs + DDTs) were found significantly different both among tissues and different fish species. β -HCH and *p,p'*-DDE congeners of HCH and DDT, respectively, were found predominant in all fish species. The congeners profile analyses indicated that HCHs contamination of fish species was mainly originated from the illegal recent usage of HCHs in the catchment area of Kabul River, while DDTs contamination originated from historical application. PCA analysis confirmed that diffusion between gills and water was as important as dietary intake via food ingestion, for bioaccumulation of HCHs and DDTs in fish. Risk characterizations of HCHs and DDTs on the basis of both 50th and 95th hazard ratios suggested that there were potential cancer risks for local residents due to DDTs daily intake via life time consumption of investigated fish species. Thus, the consumption of selected fish species should be limited to avoid the potential harmful chronic exposures to HCHs and DDTs, especially, *G. punjabensis* and *C. carpio* species.

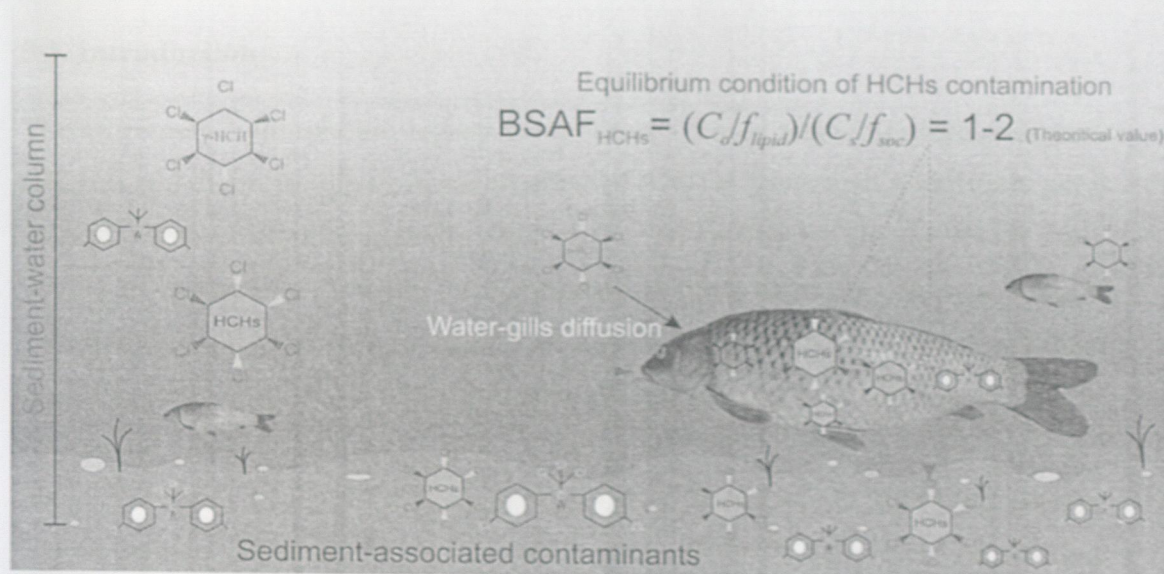
5.2. Abstract

The congeners specific bioaccumulation of hexachlorocyclohexanes (HCHs) and dichlorodiphenylchloroethanes (DDTs) were determined in four freshwater fish species (*Carassius auratus*, *Glyptocheilus punjabensis*, *Cyprinus carpio* and *Labeo rohita*) collected from the different catchments of Kabul River from Pakistan. BSAFs model was used as predictive tool for bioaccumulation of sediment-associated hydrophobic organic compounds (HOCs) for available field data. The results have shown that the model is useful for predicting the bioaccumulation of sediment-associated HCH and DDT congeners in investigated fish species and also indicated that the *G. punjabensis* on the basis of 25th and 75th percentile of sediment-associated BSAF values. The BSAFs for HCHs and DDTs were ranged from 0.1 to 1.0 and 0.1 to 1.0, respectively. The model was significantly influenced by fish species ($p < 0.001$) and chemical types ($p < 0.001$) at 95% confidence level. The sediment associated

Chapter-5

CONGENER-SPECIFIC EVALUATION OF BIOTA-SEDIMENT ACCUMULATION FACTOR MODEL FOR HCHS AND DDTs UNDER SMALL SCALE *IN SITU* RIVERINE CONDITION

5.1. Graphical abstract



5.2. Abstract

The congener-specific biota-sediment accumulation factors (BSAFs) for hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs) were determined in four freshwater fish species (*Orius plagiostomus*, *Tor putitora*, *Glyptothorax punjabensis* and *Cyprinus carpio*) in *in situ* riverine conditions of Kabul River from Pakistan. BSAF model was used as predictive tool for bioaccumulation of sediment-associated hydrophobic organic compounds (HOCs) for small scale field data. The results from field data validated the model for predicting the bioaccumulation of sediment associated HCH and DDT congeners in investigated fish species, and also suit the model for benthic fish (*G. punjabensis*) on the basis of 25th and 75th percentiles of congener-specific BSAF values. The BSAFs for *t*-HCH and *t*-DDT were ranged from 0.6-1.5 and from 0.2-1.6, respectively. The model was significantly influenced by both fish ($p = 0.004$) and chemical types ($p = 0.002$) at 95% confidence level. The sediment organic

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carbon fraction (f_{soc}) and fish lipid fraction (f_{lipid}) had not-weak effects on BSAF values for all fish species. The BSAF values were linear fitted with logarithmic octanol-water partition coefficients ($\log K_{\text{ows}}$) of HCH and DDT congeners, despite of the different chemical structures and different compound classes significant negative correlations between $\log K_{\text{ows}}$ and BSAF values were found, indicating that hydrophobicity is one of the key factors influencing the bioaccumulation potential of the investigated compounds.

5.3. Introduction

The extremely hydrophobic legacy compounds such as hexachlorocyclohexanes (HCHs) and dichlorodiphenyltrichloroethanes (DDTs) have been intensively used as pesticides/insecticides globally (Ael et al., 2012). Despite its worldwide long history of ban nearly 15 years, the occurrence of these highly persistent and toxic compounds were reported in recent studies in various environmental matrices including, air, soil, water and sediments (Ali et al., 2014; Khairy et al., 2014; Nasir et al., 2014; Tang et al., 2014; Wang et al., 2015), wildlife (Cervený et al., 2014; Yohannes et al., 2014; Jürgens et al., 2015; Torres et al., 2015) and even in human beings (Bu et al., 2015; Campagna et al., 2015; Gascon et al., 2015) from different parts of the world, indicating the pervasiveness of these compounds in the environment. In aquatic environment these lipophilic compounds are highly bioaccumulative to fish, where the low metabolism rate for these compounds declared fish as a most suitable reflective bioindicator for pollution status of the surrounding aquatic environment (Masci et al., 2014).

Sediments have high sorptive and storage capacity for hydrophobic organic compounds (HOCs). They can act as secondary source of contaminants to the water column, and aquatic biota that take contaminants from sediments (Wu et al., 2013). The determination of bioaccumulation of sediment-associated HOCs is crucial in assessment and management of contaminated sediments and their risk to ecosystem and ultimately human health. A simple approach to link sediment contamination to bioaccumulation of HOCs in benthic and pelagic organisms is based on the extrapolation of equilibrium partitioning (EqP) theory (Jahnke et al., 2014). The EqP approach described in details by Di Toro et al. (1991), stated that distribution of contaminants in sediments tends toward a thermodynamic

equilibrium among sediments organic carbon, corresponding water column and associated biota. The concept of EqP has often been extrapolated to estimate HOC bioaccumulation from sediment into biota/or food web using biota-sediment accumulation factor (BSAF).

The BSAF model based on EqP approach assumes that HOCs equally partition between the organic carbon content of sediments and lipids of biotic tissue, and its theoretical values (ranged from 1-2) are independent of species and sediment type, or physiochemical properties (e.g., hydrophobicity) of HOCs. This model also assumes that there is no chemical transformation, differential biotic uptake and depuration, or no mass transfer resistance (Wong et al., 2001; Burkhard et al., 2004; Burkhard, 2009). If the value of BSAF deviates from the expected theoretical values, then one or more of the assumptions behind BSAF model are not satisfied. To evaluate the utility of BSAF model as screening tool for predicting bioaccumulation potential of HOCs, numerous studies undertaken on large scale targeted a wide assortment of HOCs, e.g., polychlorinated biphenyls (PCBs), dechlorane plus (DP), polycyclic aromatic hydrocarbons (PAHs), DDTs and perfluorinated compounds (PFCs) and/or species type, such as fish, eel, bivalve and clam, (van der Oost et al., 1996; Yunker et al., 2011; Zhang et al., 2011; Mirsadeghi et al., 2013; Verhaert et al., 2013; Ma et al., 2014; Zhao et al., 2014; Casatta et al., 2015) but the results are inconsistent among chemicals, or species type and ecosystem. In general these studies somehow supported the use of BSAF model but caveated with the conditions that violate one or more of its assumptions (e.g., biotransformation, mass transfer limitation, chemical disequilibrium). Wong et al. (2001) evaluated BSAF model for HOCs from paired observations of fluvial sediments and large range of biotic species (fish and bivalves) across United States. The authors concluded from the results, the real-world worst case scenario of the model and invalidated the model over a large spatial scale for multiple species under *in situ* environmental conditions. The authors also assumed the suitability of the model for laboratory and small-scale field studies. However, the systematic evaluation of BSAF model under small scale *in situ* environmental condition, using limited number of biotic species has so far been lacking, we address this issue in the present study.

The aim of present study is to evaluate the BSAF model for total HCH and DDT and their congeners (their logarithm octanol water partitioning coefficients ($\log K_{ow}$) ranged from 3.7-4.1 and from 5.4-6.8, respectively) in river bed sediment and for limited number of species (benthic and pelagic) under small scale. The study uses paired observations (sediment-biota) of chlorinated HOCs, to test BSAF model in *in situ* environmental conditions, to analyze the influences of various environmental parameters such as chemical type, species type, sediment organic carbon, biota lipid content and hydrophobicity of chemicals, for a reason to further evaluate the applicability and suitability of this model in predicting bioaccumulation potential of HOCs under natural environmental conditions.

5.4. Materials and methods

5.4.1. Sampling

In February 2014, four different freshwater fish species, i.e., *Orieneis plagiostomus* (n = 4), *Tor putitora* (n = 3), *Glyptothorax punjabensis* (n = 3) and *Cyprinus carpio* (n = 4) were caught randomly at four spatially variable sites from Kabul River, Pakistan (Fig. 5.1). Multiple surficial sediment and water (collected from 20-30 cm below water surface) samples were concurrently collected with fish samples and composited to homogenize the spatial variability at each site within the river. The detailed information of the feeding behaviour, habitat, body weight and body length of these fish are summarized in Table 5.1.

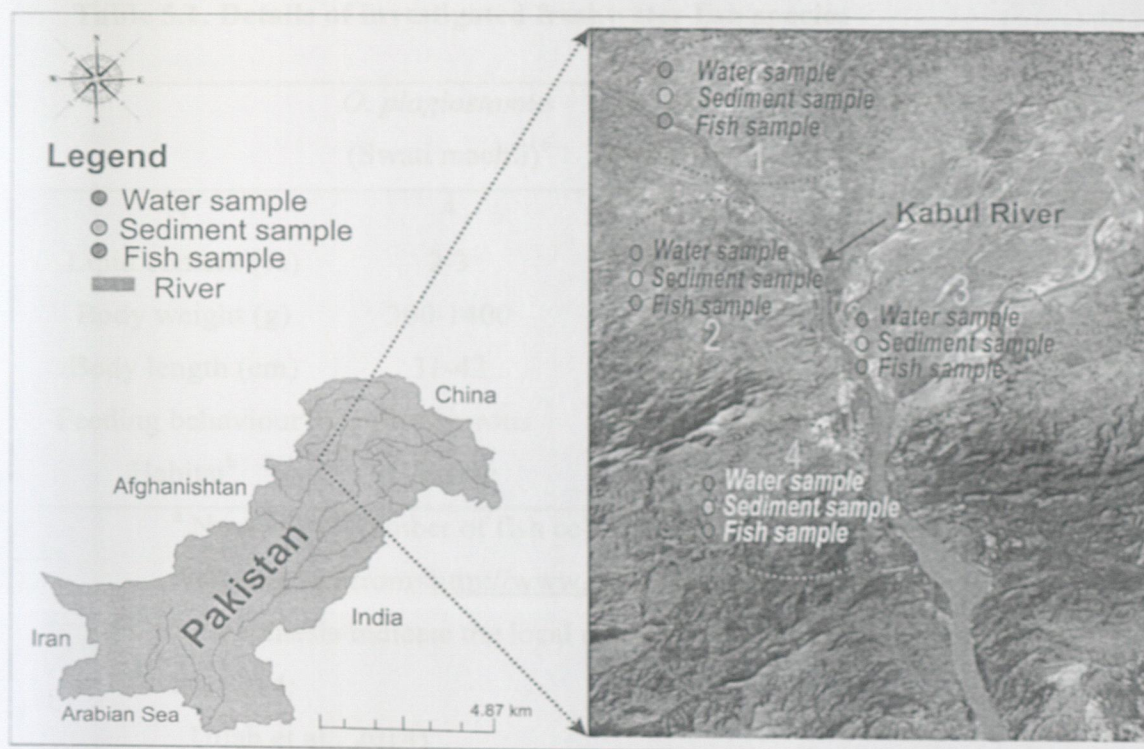


Fig. 5.1. Kabul River map showing sampling sites-Pakistan.

5.1.2 Chemical analysis

The chemical analyses for water, sediment and fish were performed according to previously methods described by Karamchahi-Monash et al. (2012) and Tufekci et al. (2014), respectively. Briefly, air dried sediment and whole fish samples were freeze-dried and spiked with appropriate sediment spikes and surrogate spiked *Leucisnon-maylene* (TCM) and PCB-27, respectively were added and then samples extracted. The water sample was extracted using liquid-liquid extraction technique. The concentrated extracts were purified with multiple silica-alumina column, and the final extracts extracted and concentrated in 0.5 ml under gentle flow of nitrogen gas. Sediment organic carbon and total lipid content were determined gravimetrically (Yehannes et al. 2014).

The congeners of HCH and DDT were analyzed using gas chromatograph equipped with a ^{63}Ni electron capture detector (GC-ECD). Agilent 7890A Technologies Inc. (USA). The HP-5 capillary column (30 m x 0.25 mm x 0.25 μm) was used for separation. The conditions of GC were set as: 1 min at 50°C, 1 min at 200°C at 10°C/min, and increased to

Table 5.1. Details of investigated freshwater fish species

	<i>O. plagiostomus</i> (Swati machli) ^c	<i>T. putitora</i> (Mahseer) ^c	<i>G. punjabensis</i> (Sulamanne) ^c	<i>C. carpio</i> (China kub) ^c
N ^a	4	3	3	4
Lipid content (%)	2-3	4-5	1.5-2	2-5
Body weight (g)	300-1400	320-500	250-500	500-3000
Body length (cm)	31-42	20-24	24-25	31-70
Feeding behaviour ^b	Omnivorous	Omnivorous	Benthivorous	Omnivorous
Habitat ^b	Pelagic	Pelagic	Benthic	Pelagic

^a Number of fish collected

^b Information from <<http://www.fishbase.org/search.php>>

^c Parenthesis indicate the local name of each species (Muhammad et al., 2014; Ullah et al., 2014)

5.4.2 Chemical analysis

The chemical analyses for water, sediment and fish were processed according to previously methods described by Kuranchie-Mensah et al. (2012) and Yohannes et al. (2014), respectively. Briefly, air dried sediment and whole fish samples were homogenized and spiked with anhydrous sodium sulfate and surrogate standard Tetrachloro-m-xylene (TCmX) and PCB-209, respectively, were added and then soxhlet extracted. The water sample was extracted using liquid-liquid extraction technique. The concentrated extracts were purified with multiple silica-alumina column, and the final hexane exchanged elute was concentrated to 0.5 ml under gentle flow of nitrogen gas. Sediment organic carbon and fish lipid content were determined gravimetrically (Yohannes et al., 2014).

The congeners of HCH and DDT were quantified using gas chromatograph equipped with a ⁶³Ni electron capture detector (GC-μECD, Agilent 7890A Technologies Inc., USA). The HP-5 capillary column (30 m×0.25 mm×0.25 μm) was used for separation. The conditions of GC were set as; the oven temperature began at 80°C with 1 min hold, ramp to 200°C at 10°C min⁻¹, next increased to

225°C at 1°C min⁻¹ with 1 min hold, and finally increased to 260°C at 15°C min⁻¹ with 5 min hold.

For quality assurance, the procedural blanks, spiked samples and standard reference materials were analysed along with field samples. The surrogate standards TCmX (with 84±9% recovery) and PCB-209 (with 91±12% recovery) were used to determine HCH and DDT congener's recovery rates. All experiments were performed in triplicates to reduce errors in data.

Calculation of BSAF

The BSAFs were calculated from Eq. 1, defined by Burkhard et al. (2004) as given below:

$$\text{BSAF} = (C_o / f_{\text{lipid}}) / (C_s / f_{\text{soc}}) \quad (1)$$

Where C_o is concentration (ng g⁻¹, wet weight) of desire chemical in the organism, f_{lipid} is the organism lipid fraction, C_s is the corresponding chemical concentration (ng g⁻¹, dry weight) in surface sediment, and f_{soc} is the sediment organic carbon fraction. HCH and DDT congener concentrations in water (ng L⁻¹), and lipid and organic carbon-normalized concentrations in different fish species (*O. plagiostomus*, *T. putitora*, *G. punjabensis* and *C. carpio*) (ng g⁻¹ lipid) and sediment (ng g⁻¹ organic carbon), respectively, are summarized in Table 5.2.

Table 5.2. Levels (mean $\pm$ SD^a) of HCH and DDT congeners in water (ng L⁻¹), sediments (organic carbon-normalized, ng g⁻¹ organic carbon) and different fish species (lipid-normalized, ng g⁻¹ lipid) from Kabul River, Pakistan.

Compound	Water n = 4	Sediments n = 4	<i>O. plagiostomus</i> n = 4	<i>T. putitora</i> n = 3	<i>G. punjabensis</i> n = 3	<i>C. carpio</i> n = 4
α -HCH	0.23 $\pm$ 0.03	8.22 $\pm$ 3.49	10.9 $\pm$ 7.70	3.89 $\pm$ 0.12	10.9 $\pm$ 7.70	10.0 $\pm$ 7.68
β -HCH	0.27 $\pm$ 0.03	14.0 $\pm$ 5.55	16.9 $\pm$ 11.6	8.21 $\pm$ 0.47	19.2 $\pm$ 11.6	17.7 $\pm$ 12.6
γ -HCH	1.16 $\pm$ 0.21	4.28 $\pm$ 3.23	11.9 $\pm$ 10.8	6.60 $\pm$ 0.03	16.2 $\pm$ 10.8	13.7 $\pm$ 8.60
δ -HCH	0.09 $\pm$ 0.04	8.01 $\pm$ 1.65	7.23 $\pm$ 3.93	1.79 $\pm$ 0.52	5.49 $\pm$ 3.93	7.97 $\pm$ 5.00
<i>t</i> -HCH ^b	1.74 $\pm$ 0.16	34.5 $\pm$ 9.12	46.9 $\pm$ 33.9	20.5 $\pm$ 0.90	51.9 $\pm$ 33.9	49.6 $\pm$ 33.7
<i>o,p'</i> -DDE	0.04 $\pm$ 0.02	5.49 $\pm$ 3.87	4.06 $\pm$ 2.53	0.42 $\pm$ 0.01	3.86 $\pm$ 2.54	3.80 $\pm$ 2.42
<i>p,p'</i> -DDE	0.67 $\pm$ 0.12	9.07 $\pm$ 3.34	10.0 $\pm$ 6.05	3.85 $\pm$ 0.21	24.7 $\pm$ 6.05	16.9 $\pm$ 11.7
<i>o,p'</i> -DDD	0.02 $\pm$ 0.01	4.17 $\pm$ 3.99	3.48 $\pm$ 2.72	0.61 $\pm$ 0.28	6.03 $\pm$ 2.72	2.53 $\pm$ 1.25
<i>p,p'</i> -DDD	0.25 $\pm$ 0.09	6.37 $\pm$ 4.37	7.28 $\pm$ 4.54	2.28 $\pm$ 0.33	12.6 $\pm$ 4.54	6.13 $\pm$ 3.03
<i>o,p'</i> -DDT	0.12 $\pm$ 0.05	9.59 $\pm$ 1.07	2.30 $\pm$ 1.54	1.13 $\pm$ 0.09	5.97 $\pm$ 1.54	2.99 $\pm$ 1.13
<i>p,p'</i> -DDT	0.61 $\pm$ 0.19	11.6 $\pm$ 3.45	7.70 $\pm$ 4.77	2.98 $\pm$ 0.01	19.0 $\pm$ 4.77	6.74 $\pm$ 3.41
<i>t</i> -DDT ^c	1.71 $\pm$ 0.27	46.3 $\pm$ 18.4	34.8 $\pm$ 21.8	11.3 $\pm$ 0.24	72.1 $\pm$ 21.6	39.0 $\pm$ 20.5

^aSD : represents $\pm$ 1 standard deviation

^b*t*-HCH: sum levels of α , β , γ and δ -HCH

^c*t*-DDT: sum levels of (*o,p'* + *p,p'*)-DDE, (*o,p'* + *p,p'*)-DDD and (*o,p'* + *p,p'*)-DDT

5.4.4. Data analyses

The BSAF for each congener (HCHs and DDTs) was calculated from paired observations of congener's measured concentrations in fish and sediment taken at same site. At each site sediment samples (composite of three samples) taken from the foraging range (Eq. 2) (Minns, 1995) of fish were paired with the corresponding fish samples. Statistical differences within and between the congeners and fish species-specific BSAF distributions were calculated by two-factor ANOVA with Tukey-honestly significant difference (HSD) multiple comparison test, differences at 95% confidence level ($\alpha = 0.05$) were considered significant. Bivariate correlations with Pearson's correlation coefficients at two-tailed test of significance were performed to determine the influence of f_{soc} and f_{lipid} on BSAF. Linear regression analysis was performed to investigate the relationship between BSAFs and logarithm of octanol-water partition coefficients ($\log K_{\text{ow}}$ s) of HCH and DDT congeners. For all statistical tests $\alpha = 0.05$ was the criterion for significance.

$$\ln H = -2.91 + 3.14 \text{ HAB} + 1.65 \ln L$$

Where H is the foraging range (home range) size (m^2), HAB = 0 for river, and = 1 for lake and L is fish body length (mm).

5.5. Results and discussions

5.5.1. BSAFs for HCH and DDT congeners in fish

The calculated values of BSAFs for HCH and DDT congeners in four different freshwater fish species (*O. plagiostomus*, *T. putitora*, *G. punjabensis* and *C. carpio*) are shown in Fig. 5.2. The BSAFs for *t*-HCH and *t*-DDT ranged from 0.6-1.5 (mean= 1.2) and from 0.2-1.6 (mean= 0.8), respectively, when all species were considered. According to BSAF model based on assumptions of equilibrium partitioning theory, when in condition of chemical equilibrium and no metabolism/or no chemical transformation, the HOCs partition equally between both the sediment and lipid-carbon pools. Under these conditions the theoretical BSAF values ranged from 1-2. Excluding *Tor putitora*, the BSAF values for HCH congeners (α , β and δ -HCH) and total-HCH (*t*-HCH) in three fish species fall between and on the lines corresponding to the theoretical BSAF values (1-2) (Fig.

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5.2), which explicate that HCH congeners were partition equally in both sediment and lipid carbon pools, indicated that levels of HCH contamination between sediment and fish were in equilibrium condition. This finding further supported the suitability of BSAF model as screening tool for predicting bioaccumulation of HOCs under small scale *in situ* riverine conditions. This also evident the assumption of BSAF model, that BSAF values for HCH is independent of species and sediment type. Whereas the less exposure to sediment-associated γ -HCH (12% of the total HCH) (Table 5.2) was not only the reason for relatively unusual high BSAFs for γ -HCH, in the present study higher concentration of γ -HCH (66% of the total HCH) than the other HCH congeners was found in water compartment (Table 5.2). Where it can be assume, that the higher BSAF values for lindane (γ -HCH) may be because of higher bioaccumulation of γ -HCH in fish species through the gills-water diffusion mechanism, which is as important as food ingestion in OCPs transfer to fish (Guo et al., 2008). Additionally, it is possible, that both fish and their food web were exposed to same levels of γ -HCH in water, which create the strong possibility of biomagnification of γ -HCH in the the food web of investigated fish.

As shown in Fig. 5.2, the low BSAF values for DDT congeners and total-DDT (*t*-DDT) were observed in present fish species, indicated the non-equilibrium condition of DDT contamination in the study area. These low BSAFs are reasonable for fish due to wide ranges of factors including chemical bioavailability/or bioaccessibility, sediment-water column chemical disequilibrium, differential biotic uptake efficiencies and biotic chemical metabolism, which could influence the levels of HOCs between fish and sediment (Burkhard et al., 2004; Zhang et al., 2011). The low BSAF corresponding to chemical metabolism within the fish species and/or their food web can be explained in next section. Therefore, it is important to recognise that in *in situ* riverine conditions the assumptions behind the theoretical value (1-2) of BSAF model are often violated due to non-equilibrium conditions (Verhaert et al., 2013). In general, these BSAF values for DDT and its congeners outside theoretical range (1-2) for fish do not violate the general definition of BSAF model nor invalidate the applicability/or utility of BSAF model in predicting bioaccumulation of DDT and its congeners in fish for assessing sediment-associated contamination of DDTs (Burkhard et al.,

2004; Burkhard., 2009). Additionally, these findings of equilibrium (HCHs) and non-equilibrium (DDTs) conditions are in a close agreement with the OECD (The Organisation for Economic Co-operation and Development) guidelines for testing of chemicals, that in aquatic environment the chemicals with a K_{ow} of 4 takes approximately five days to reach to effective steady state, while chemicals with K_{ow} of 6 takes approximately one year (OECD, 2012).

In summary, the measured BSAF values for HCH and DDT congeners (with log K_{ow} ranged from 3.7-6.8) validated the applicability/or usefulness of BSAF model for HCH and DDT congeners as screening tool for predicting bioaccumulation potential of sediment associated contaminants (HCH and DDT congeners) under small scale *in situ* reverine condition for the investigated fish species.

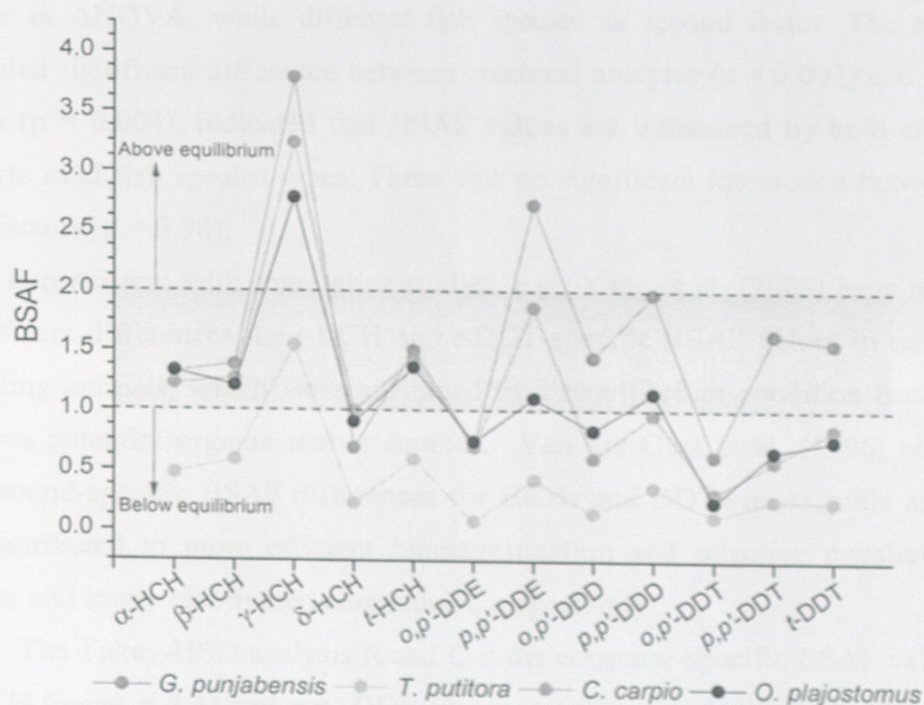


Fig. 5.2. Biota-sediment accumulation factors (BSAFs) (median values) for HCH and DDT congeners in four different freshwater fish species (*O. plagiostomus*, *T. putitora*, *G. punjabensis* and *C. carpio*) from Kabul River. Shaded area represents equilibrium condition (theoretical BSAFs ranged from 1-2)

5.5.2. Effect of chemical and species type on BSAF

The BSAF model assumes that chemical analyte is not transformed/or not metabolized in sediment or biota, and the value of BSAF is independent of chemical and species type, as it is based on the equilibrium partitioning between two carbon pools (sediment and lipid carbon) (Wong et al., 2001). To assess the validity of the model the effect of chemical and species type on BSAF needs to be examined. A two-factor ANOVA with subsequent Tukey-HSD multiple comparison test at 95% confidence level (significant at $\alpha = 0.05$) was performed on BSAF data pairs to investigate the effect of chemical analytes (HCH and DDT congeners) and fish species types (*O. plagiostomus*, *T. putitora*, *G. punjabensis* and *C. carpio*) on BSAF. HCH and DDT congeners were considered as first factor in ANOVA, while different fish species as second factor. The analysis revealed significant difference between chemical analytes ($p = 0.002$) and species types ($p = 0.004$), indicated that BSAF values are influenced by both chemical analyte and fish species types. There was no significant interaction between the two factors ($p = 0.98$).

This is consistent with some prior studies, e.g., Yang et al. (2006) have reported significant differences for *t*-HCH and *t*-DDT-specific BSAF values in sediment-dwelling animals, which were attributed to disequilibrium condition because of various potential organic matter sources. Van der Oost et al. (1996) observed compound-specific BSAF differences for HCHs and DDTs in *Anguilla anguilla*, and attributed to more efficient biomagnification and selective metabolism of higher and lower chlorinated compounds, respectively.

The Tukey-HSD analysis found that the congener-specific BSAF values for γ -HCH (mean = 3.4) and *p,p'*-DDE (mean = 1.7) were significantly higher than the BSAF values for other HCH and DDT congeners, while BSAF values for *o,p'*-DDT (median = 0.3) and *p,p'*-DDT (mean = 0.9) were found significantly lower than other congeners (both HCHs and DDTs) and DDT congeners, respectively (Fig. 5.2). This higher BSAF values for γ -HCH and *p,p'*-DDE make the overall distribution of BSAFs appear bimodal. These higher and lower BSAF values for *p,p'*-DDE and *p,p'*-DDT, respectively, indicated the preferential enrichment and depletion of *p,p'*-DDE and *p,p'*-DDT congeners, respectively, in fish species as compared to sediments. Manifestly, the assumption of BSAF

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model of no metabolism/or no transformation does not hold for DDT congeners. This is supported by previous field and laboratory studies, which revealed that DDT biodegrade to DDE under aerobic condition, while reductively transformed to DDD in sediment (Hu et al., 2009; Yu et al., 2011). Guo et al. (2008) assumed that DDT metabolized to DDE and DDD in the liver of freshwater fish species (e.g., bighead carp, northern snakehead and mandarin fish). Khan et al. (1977) conducted in vivo and in vitro study and observed that many fish species transform DDT to DDE and DDD. Whereas, high BSAF value for γ -HCH was attributed to higher bioaccumulation of γ -HCH in fish from water (discussed in preceding section), which is strongly supported by the lower ratios of α/γ -HCH ranged from 0.2-1.9 for overall dataset (including water, sediment and fish), indicating rich input of lindane (γ -HCH > 99%; α/γ -HCH < 3) (Willett et al., 1998; Mishra et al., 2013) in the study area.

As stated, BSAFs are significantly influenced by both chemical and fish species type, a multiple comparison Tukey-HSD analysis showed that the species-specific BSAF values for *G. punjabensis* (mean = 1.6) and *Tor putita* (mean = 0.4) were found higher and lower, respectively, than those for other species type (Fig. 5.3). These differences in BSAF values for different fish species are attributed to numerous factors, including different; species life histories, rout of exposures to HOCs, metabolism capacities, feeding behaviors and different habitats etc. Zhang et al. (2011) observed different BSAFs for HOCs in different fish species (crucian carp, mud carp and northern snakehead), which were attributed to different diet composition and different exposure time. Wu et al. (2010) and Tomy et al. (2007) assumed and interlinked the low BSAFs for HOCs with chemical metabolism capacities of different fishes in field investigations. Zhao et al. (2009) observed higher BSAFs for HCHs and DDTs in benthic fish (*C. fluminea*), and were attributed to higher uptake from the sediments and biomagnification, which is consistent with the higher BSAF values for benthic fish (*G. punjabensis*) (Fig. 5.2 and Fig. 5.3) in this study. Additionally, the 25th and 75th percentiles of both HCH and DDT congener-sepecific BSAF values for benthic species (*G. punjabensis*) fall between the theoretical values range of 1-2, which strongly supported the assumption of Wong et al (2001) that BSAF model may be suitable applicable to benthic fish. This finding further evident the

suitability of BSAF model to benthic organism –which are more directly expose to sediment associated contaminants.

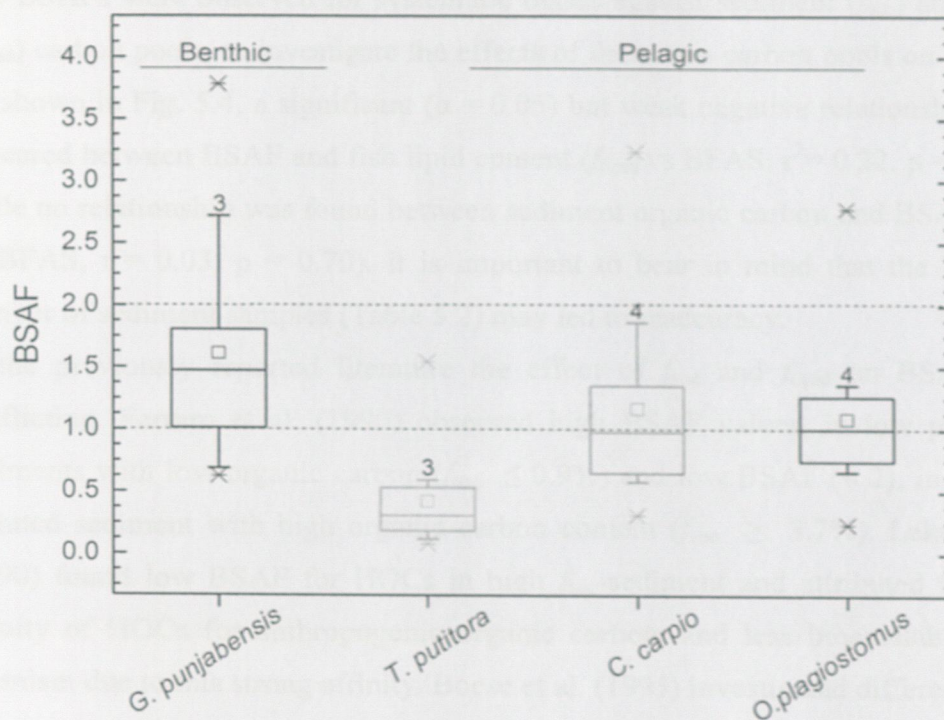


Fig. 5.3. Box plot of BSAFs (by both habitat and species type). Each box shows median values, 25th and 75th percentiles; whiskers show 10th and 90th percentiles, while cross symbols shows 1-99th percentiles. Small box represents mean value. Specie-specific BSAF values for benthic species (*G. punjabensis*) is relatively higher than those for pelagic species (two-factor ANOVA and Tukey-HSD analysis, $\alpha = 0.05$, $p = 0.004$). Number on top of each whisker represents number of data paired measured for each fish type.

5.5.3. Effect of f_{soc} and f_{lipid} on BSAF

The BSAFs were observed for systematic biases against sediment (f_{soc}) and lipid (f_{lipid}) carbon pools, to investigate the effects of these two carbon pools on BSAF. As shown in Fig. 5.4, a significant ($\alpha = 0.05$) but weak negative relationship was appeared between BSAF and fish lipid content (f_{lipid} vs BFAS, $r^2 = 0.22$, $p = 0.01$), while no relationship was found between sediment organic carbon and BSAF (f_{soc} vs BFAS, $r^2 = 0.03$, $p = 0.70$). It is important to bear in mind that the limited number of sediment samples (Table 5.2) may led to inaccuracy.

In the previously reported literature the effect of f_{soc} and f_{lipid} on BSAF are conflicting. Ferraro et al. (1990) observed high BSAF values, in low polluted sediments with low organic carbon ($f_{soc} \leq 0.9\%$) and low BSAF (< 2), in highly polluted sediment with high organic carbon content ($f_{soc} \geq 3.7\%$). Lake et al. (1990) found low BSAF for HOCs in high f_{soc} -sediment and attributed to high affinity of HOCs for anthropogenic organic carbon, and less bioavailability to organism due to this strong affinity. Boese et al. (1995) investigated differences in BSAF values and linked that with the use of different extraction solvent, which results in different degrees' extraction of lipids and HOCs. In laboratory exposure, Pruell et al. (1993) found higher BSAF values for HOCs in shrimps and clams than sandworms and attributed to different amount and type of lipid extracted. Therefore, it is uncertain that why the BSAFs have weak dependency on fish lipid content, and not for sediment organic content in this study.

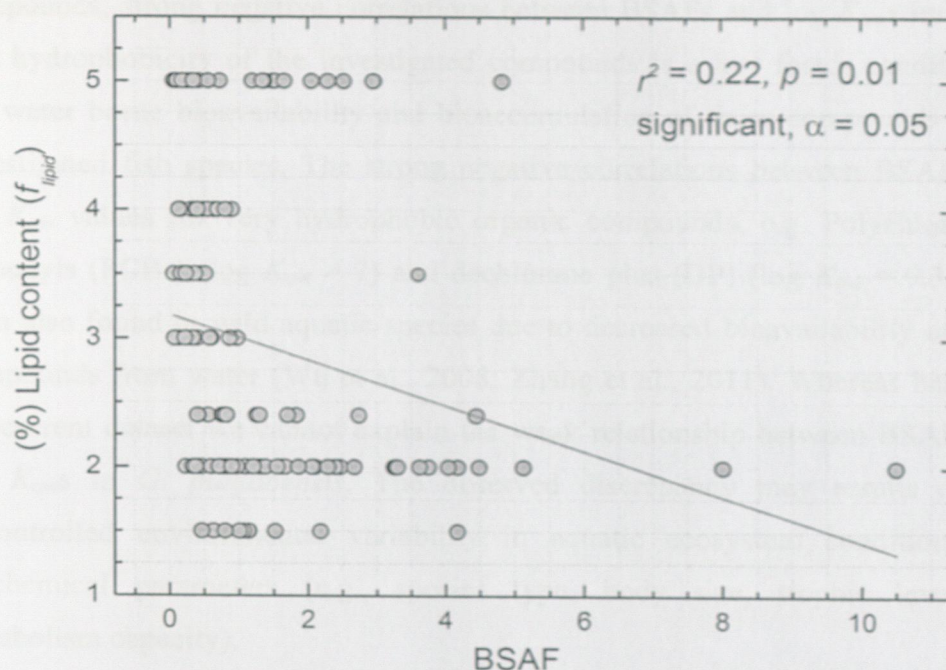


Fig. 5.4. Relationship between BSAF and fish lipid content (f_{lipid} vs BSAF)

5.5.4. Relationship between BSAFs and K_{ow} values of HCH and DDT congeners

The octanol-water partition coefficient (K_{ow}) is a parameter used to estimate the partitioning and bioaccumulation potential of HOCs (Linkov et al., 2005). To investigate the influence of K_{ow} of the HOCs on the BSAFs in the present fish, a linear regression analysis was performed to investigate the relationships between the log K_{ow} s and the BSAFs for the hydrophobic HCH and DDT congeners in the investigated freshwater fish species (Fig. 5). The log K_{ow} values for HCH (3.7-4.1) and DDT (5.4-6.8) congeners are from Paschke and Popp. (2003) and Han et al. (2011), respectively. In the present study, the relatively unusual high BSAFs for γ -HCH and p,p' -DDE (Fig. 5.2 and Fig. 5.5) were masked for the whole dataset before analysis. At a 95% confidence level for parameters a significant strong negative correlations between BSAFs and log-normal K_{ow} values of HCH and DDT congeners were found in *O. plagiostomus* ($r^2 = 0.82$, $p = 0.001$), *T. putitora* ($r^2 = 0.72$, $p = 0.011$) and *C. carpio* ($r^2 = 0.92$, $p < 0.001$), while a weak negative, non-significant correlation was found in *G. punjabensis* ($r^2 = 0.20$, $p > 0.05$). Despite of the different chemical structures of these different classes of

compounds, strong negative correlations between BSAFs and $\log K_{ow}$ s indicated that hydrophobicity of the investigated compounds is a key factor conditioning the water borne bioavailability and bioaccumulation of these compounds in the investigated fish species. The strong negative correlations between BSAFs and $\log K_{ow}$ values for very hydrophobic organic compounds, e.g. Polychlorinated biphenyls (PCBs) ($\log K_{ow} > 7$) and dechlorane plus (DP) ($\log K_{ow} = 9.3$) have been also found in wild aquatic species due to decreased bioavailability of these compounds from water (Wu et al., 2008; Zhang et al., 2011). Whereas based on the current dataset we cannot explain the weak relationship between BSAFs and $\log K_{ow}$ s in *G. punjabensis*. The observed discrepancy may results due to uncontrolled environmental variability in aquatic ecosystem conditions and biochemical parameters (e.g., species type, body size, trophic level and metabolism capacity).

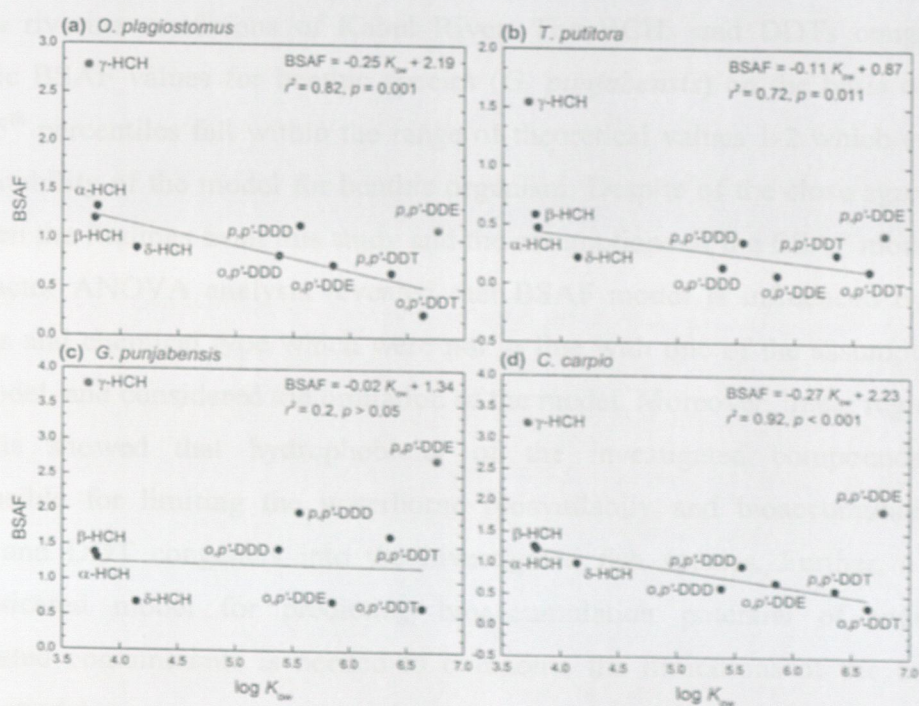


Fig. 5.5. Linear fitting between biota-sediment accumulation factors (BSAFs) and logarithm of octanol-water partition coefficients ($\log K_{ow}$ s) of hydrophobic HCH and DDT congeners in (a) *O. plagiostomus*, (b) *T. putitora*, (c) *G. punjabensis* and (d) *C. carpio*. The red points represent masked data points. The $\log K_{ow}$ values of HCH and DDT congeners are from Paschke and Popp.(2003) and Han et al.(2011), respectively.

5.6. Conclusions

The authors concluded from the results, that the current BSAFs for HCHs were in close agreement with the assumptions of BSAF model, that HCH and its congeners were equally partitioned between the two carbon pools of sediment and lipid, and its values were independent of investigated fish species type, but the BSAF values for DDT and its congeners deviated with the disequilibrium condition and biotransformation of DDT to its metabolites within the investigated fish species which indicated that the assumptions of BSAF model does not hold for DDT congeners. Whereas according to BSAF definition, such disequilibrium condition is reasonable for fish due to uncontrolled environmental variability. These findings from the present study validated the applicability/or utility of BSAF model as screening tool for predicting bioaccumulation of sediment-associated HCH and DDT congeners in investigated fish species under small scale *in situ* riverine conditions of Kabul River. The HCHs and DDTs congeners-specific BSAF values for benthic species (*G. punjabensis*) on the basis of 25th and 75th percentiles fall within the range of theoretical values 1-2 which evident the suitability of the model for benthic organism. Despite of the close agreement between the findings from this study and the assumptions of the BSAF model, the two factor ANOVA analysis revealed that BSAF model is influenced by both species and chemical type which were not in line with one of the assumption of the model, and considered the limitation of the model. Moreover, linear regression analysis showed that hydrophobicity of the investigated compounds was responsible for limiting the waterborne bioavailability and bioaccumulation of HCH and DDT congeners into the investigated fish species. Further, a more sophisticated model for predicting bioaccumulation potential of sediment-associated contaminants is needed to overcome the limitations of the existing BSAF model.

CONCLUSIONS AND RECOMMENDATIONS

The present study investigated the contamination profile of HCHs and DDTs at isomeric/metabolites and enantiomeric level in sediments of Kabul River, Pakistan. DDTs were found the most dominant contaminants of total of HCH and DDT congeners. The concentrations of Σ HCHs and Σ DDTs are comparable with other riverine sediments from Asian countries. The TOC contents are significantly correlated with total residual concentrations of HCHs and DDTs. Compositional and chiral analyses indicated that DDTs originate from historical application in most of the sampling sites, while HCHs contaminations were associated with fresh input in most of the samples, with evidence exhibiting the fresh input of lindane. PCA showed, in addition to input sources the distribution of historical accumulated HCHs and DDTs could also be influenced by organic matter in sediments due to post-depositional sorption of these compounds on sediments organic matter. In accordance, with sediment quality guidelines (SQGs), more ecotoxicological concern should be paid to HCHs in sediments of Kabul River, Pakistan.

Contamination profile of HCHs and DDTs in four different freshwater fish species were found significantly different both among tissues and different fish species. β -HCH and p,p' -DDE congeners of HCH and DDT, respectively, were found predominant in all fish species. The congeners profile analyses of HCHs and DDTs in fish species also indicated that HCH contaminations of fish were mainly originated from the illegal recent usage of HCH in the catchment area of Kabul River, while DDTs contamination originated from historical application. According to PCA water-gills diffusion of HCHs and DDTs were also responsible for bioaccumulation of HCHs and DDTs into investigated fish species. On the basis of both 50th and 95th hazard ratios, the investigated fish species were highly contaminated with DDTs, which indicated that there were potential cancer risks for local residents due to life time consumption of these DDTs contaminated fish species. To assure the better health of the local residents the consumption of investigated fish species should be limited to avoid the potential harmful chronic exposure to HCHs and DDTs.

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The BSAF model was evaluated for HCH and DDT congeners in the investigated fish species in *in situ* riverine condition. BSAFs for HCHs were in close agreement with the assumptions of BSAF model, while not for DDTs due to its disequilibrium and biotransformation within the fish species. According to definition of BSAF, these findings validated the applicability/or utility of BSAF model as screening tool for predicting bioaccumulation of sediment-associated HCH and DDT congeners in investigated fish species under small scale *in situ* riverine conditions of Kabul River. On the basis of 25th and 75th percentiles BSAF values for HCH and DDT congeners the model was best suit for benthic fish (*G. punjabensis*). The limitations of the model were, that its values were influenced by both species and chemical type revealed by two factor ANOVA analysis. According to linear regression analysis hydrophobicity of HCH and DDT congeners played important role in the conditioning of waterborne bioavailability and bioaccumulation of these hydrophobic compounds into the investigated fish species.

This study concluded that sediment, water and fish species from Kabul River were highly contaminated with HCHs and DDTs, which is a ecotoxicological threat for aquatic life and ultimately human health risk. The study showed that contaminated sediments was responsible for bioaccumulation of HCHs and DDTs into the investigated fish species. Therefore, it is recommended that the concerned authorities should conduct the crucial monitoring and proper management of the aquatic ecosystem of Kabul River. There should be appropriate regulations for the discharge of domestic sewage and industrial discharge to the Kabul River. The National Environmental Quality Standards (NEQSS) should be revised for sediment and implemented in accordance with anthropogenic activities responsible for effluent discharge. For the abatement of the illegal fresh usage of HCH in the study an appropriate regulation with penalty should be set to control the further usage of these toxic pesticides. The consumption of the investigated and other fish species from Kabul River should be avoided to reduce the chances of human health risk especially cancer risk. The concerned departments should enforce their authorities for the betterment of the aquatic life and ultimately human beings. A more comprehensive and large scale monitoring and assessment of the aquatic ecosystem of Kabul River is needed.

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