# Organohalogen Residues of Fishes from Different Trophic Levels

A thesis submitted to the University of Dhaka, Bangladesh in the fulfillment of requirements for the degree of Doctor of Philosophy

**Submitted by** 

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**Registration Number: 26/2012-2013 Session: 2012-2013** 



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**June 2017** 

# **Dedicated**

To

My beloved parents Late A. K. M. Golam Mustafa and Tahura Begum and

My husband Engr. Md. Anamul Haque Bhuiyan

## **CERTIFICATION**

Trophic Levels" submitted to the Department of Zoology, Faculty of Biological Sciences, University of Dhaka, Bangladesh in partial fulfillment of the requirements for the degree of Doctor of Philosophy. We certified that the candidate, Tonima Mustafa (Registration No. 26/2012-2013) has been completed her research under our supervision and suggestions. We have read this dissertation and that, in our opinion, it is fully adequate in scope and quality as a dissertation for the degree of Doctor of Philosophy. Experimental work described in the thesis has been carried out by the author at the Department of Chemistry, University of Dhaka. The work has not been and will not be presented for any other degree. It is further certified that to the best of our knowledge the thesis contains original research.

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**DECLARATION** 

I do hereby declare that the research work entitled "Organohalogen Residues of

Fishes from Different Trophic Levels" submitted to the Department of Zoology,

Faculty of Biological Sciences, University of Dhaka, Dhaka, Bangladesh, for the

degree of Doctor of Philosophy is the result of my own analysis. The thesis or part of

it has not been presented before for any other degree.

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### **ACKNOWLEDGEMENTS**

Alhamdulillah, I am expressing my gratitude to Allah, as with the blessing of the Almighty I have been able to complete this thesis for the degree of Ph.D.

Completion of this doctoral dissertation was possible with the valuable support of several people. I would like to express my sincere gratitude to all of them. First and foremost I would like to express my special appreciation and thanks to all of my respected supervisors **Professor Dr. M. Niamul Naser**, Ph. D. (Dalhousie, Canada), Department of Zoology, University of Dhaka; **Professor Dr. Gulshan Ara Latifa**, Ph. D. (Manchester, UK), Department of Zoology, University of Dhaka; **Professor Dr. Nilufar Nahar**, Ph.D. (Uppsala, Sweden), Department of Chemistry, University of Dhaka, for their proper and effective guidance, cheerful enthusiasm, scholastic supervision, and constructive criticisms during the entire period of this research work.

Special mention goes to my supervisor, Professor Dr. Nilufar Nahar who has been a truly dedicated mentor. This research work was possible only because of the unconditional research support provided by Madam. A person with amicable and positive disposition, she has always made herself available to carry out the research work in her lab despite her busy schedules and I consider it as a great opportunity to do my doctoral program under her guidance and to learn from her research expertise. I thank her whloeheartly, for her tremendous support from the beginning to end of the research work.

It is my pleasure to express my sincere gratitude, cordial thanks, deepest sense of respect and appreciation to Professor Dr. Md. Anwarul Islam, Chairman, Department of Zoology, University of Dhaka, for his kind co-operation. I also wish to express my appreciation to all the teachers of the Department of Zoology, University of Dhaka, for their affection, sincere suggestions and guidance throughout the course of this work.

Cordial thanks and sincere gratitude to Professor Dr. Md. Iqbal Rouf Mamun, Department of Chemistry, University of Dhaka, Professor Dr. Mohammad Shoeb, Professor, Department of Chemistry, University of Dhaka, Mrs. Abida Sultana, Assistant Professor, Department of Chemistry, University of Dhaka, for their help, valuable suggestions and co-operation during this research work.

I am very grateful to the authority of Jagannath University, Dhaka, Bangladesh for giving me full time study leave. I am also grateful to the authority of department of Zoology, Jagannath University for helping me to get study leave and also do research work uninterruptedly.

I gratefully acknowledge the funding source that made my Ph. D. work possible. I am grateful to the Ministry of Science and Technology, Bangladesh for giving me Bangabandhu fellowship (July 2013-June 2017). I am also thankful to the International Science Programme (ISP), Upsalla University, Sweden for providing financial support to carry out of my research work.

I would like to thank research group members Abida Sultana (Assistant, Department of Chemistry, University of Dhaka), Md. Kamrul Hassan (Lecturer, Department of Chemistry, University of Dhaka) Dr. Md. Nashir Uddin Al Mahmud, Farzana Khalil, Anower Hossain, Zerin Sultana, Md. Akram Hossain (Technical officer, Dept. of Chemistry, DU) and other research students of Department of Chemistry, University of Dhaka, for their co-operation and encouragements. Special mention goes to Dr. Md. Nashir Uddin Al Mahmud, Farzana Khalil and Md. Akram Hossain for their help, cordial co-operation and good suggestions during my research work.

I also want to thank Md. Abul Fazol, Late Md. Mohsin Munshi, Md. Sohel Rana, Md. Mizanur Rahman, Md. Abdul Malek, Md. Emdadul Islam, Mohammad Abdulla, Md. Atikur Rahman, Md. Ishtiaq Ahmed, Md. Mizanur Rahman (2) and Narayan Chandra Das for their kind assistance during research.

Lastly, I would like to thank my family for all their love and encouragements. I owe a lot to my parents, who encouraged and helped me at every stage of my personal and academic life. I deeply miss my departed father A. K. M. Golam Mustafa who had a dream about my doctoral dissertation and longed to see this achievement come true. I lost my father at the beginning of my Ph. D. research. Although he is not now with us to share this joy but I believe his love and encouragement are always with me that inspire me to proceed forward.

Word cannot express how grateful I am to my husband Engr. Md Anamul Haque Bhuiyan. Really he is a nice person, I want to express my appreciation to him for his endless support, sacrifices, patience, prayers, love, care, and encouragement in all my professional endeavors. I would like to thank to my two kids Md Addin- Jubayer and Md. Tawsin Juabyer for being such good boys and self-dependent that helping me to do my research work with deep attention. I also want to thank to my departed father in law and mother in law for their prayers, inspiration and encouragements. My siblings, friends, my relatives and my colleagues are hereby acknowledged for their constructive assessment, endless love, support and prayer.

Above all, I owe it all to Almighty Allah for Granting me the wisdom, health and strength to undertake this research task and enabling me to its completion.

Author

### **ABSTRACT**

Global contexts on human health hazards through pesticide residues become a serious focus and environmental issues today. Fishes are used extensively for environmental monitoring because they uptake contaminants directly from water and food. Generally the ability of the fish to metabolize organohalogen is moderate, thus contaminants load in fish are well reflective of the state of pollution in surrounding environments. The present study was conducted to assess the concentrations and patterns of organohalogen pesticide residues i.e. DDT and its metabolites (4,4'-DDT, 2,4'-DDT, DDD and DDE) in fishes and prawn species of different trophic levels of four seasons from Sonargaon Upazila of the Meghna River. The samples were collected between the periods of 2015-2016. The number of species available in the seasons varied from twenty-two to twenty-four i.e. rainy season (twenty-four), autumn (twenty-two), winter (twenty-three) and summer (twenty-two). Analysis of the samples for DDTs residues were carried out using Gas Chromatograph with Electron Capture Detector (GC-ECD). The samples were extracted by Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method and the sample extracts were cleaned-up by using  $H_2SO_4$  Linearities ( $r^2$ ) were > 0.9950 for calibrations. The recoveries were 88.67% -104.89% (20 ng g<sup>-1</sup>), 70.10% - 101.32% (10 ng g<sup>-1</sup>) and 71.64% - 113.83% (5 ng g<sup>-1</sup>). The limit of detection was found 0.0625 ng g<sup>-1</sup> in fish samples. The concentrations of total DDTs residue in fish and prawn tissues of rainy-season, autumn, winter and summer varied between  $2.64 \pm 0.35$  ng g<sup>-1</sup> to  $191.14 \pm 31.18$  ng g<sup>-1</sup>,  $16.42 \pm 1.90$  ng g<sup>-1</sup>  $^{1}$  to 271.50  $\pm$  6.17 ng g  $^{-1}$  , 3.88  $\pm$  0.60 ng g  $^{-1}$  to 141.57  $\pm$  10.24 ng g  $^{-1}$  , 157.58  $\pm$  1.15 ng  $g^{-1}$  to 1660.89 ± 157.9 ng  $g^{-1}$  wet weight (ww) respectively. The year round highest concentrations were observed in Bacha ( Eutropiichthys vacha). However the lowest levels of total DDTs were observed in Kachki (Corica soborna) in rainy-season and Khalisha (Trychogaster fasciata) in autumn and Goldachingri (Macrobrachium rosenbergii) in winter. Considering the average concentrations of total DDTs residue of four seasons, the twenty fish and prawn species that analysed in all seasons showed the chronology of Kachki (Corica soborna) < Chanda (Parambassis ranga) < Shing (Heteropnuestes fossilis) < Ghainna (Labeo gonius) < Rui (Labeo rohita) < Systomus sarana (Sharpunti) < Bata (Cirrhinus reba) < Jatpunti (Puntius sophore) < Goldachingri (Macrobrachium rosenbergii) < Foli (Notopterus notopterus) < Boal (Wallago attu) < Gulsha (Mystus cavasius) < Bele (Glossogobius giuris) < Tengra (Mystus vittatus) < Bajari-tengra (Mystus tengra) < Chewa (Pseudapocrypter elongates) < Meni (Nandus nandus) < Borobaim (Mastacembelus armatus) < Poa (Otolithoides pama) < Bacha (Eutropiichthys vacha). The other fishes that analysed in one or two seasons showed the chronology, Kaikka (Xenentodon cancila) < Khalisha (Trychogaster fasciata) <Gutum (Lepidocephalus guntea) < Magur (Clarius batrachus) < Tarabaim (Macrognatus aculiatus) < Shole (Channa striata) < Gojar (Channa marulius). In each season, the variation of DDT contents varied with fish species, when the concentrations in a fish varied between seasons. This might be attributed to the combine influence of the trophic position, feeding habits, lipid contents, physiological activities (metabolism rate, excretion rate and maturation stage etc.) of fishes and meteorological parameters i.e. temperature, humidity and rainfall to the accumulation of DDTs in fish tissues. Overall, the rank orders of average DDTs of different fish and prawn species of different seasons were carnivore > omnivore > herbivore and summer > autumn > rainy-season > winter. From the present study, it could be said that lower residues may be found in herbivores, lean and plant based omnivores and lean and zooplankton based carnivores; medium or higher residues may be found in fatty and animal based omnivores, lean and lower carnivores while much higher residues may be found in the fishes with bottom feeder carnivores, predators and fatty top carnivores. Analysis of total DDTs residues by one way ANOVA with LSD and Tukey HSD tests showed that significant differences in total DDTs between herbivore, omnivore and carnivore (p < 0.05). Highly significant differences between seasons (p < 0.001) were also observed. Pearson correlations analysis showed the positive relationships between DDE and DDD with lipid contents (p  $\leq$  0.05). Besides, highly significant positive relationships between total DDTs with temperature with  $(p \le 0.001)$  and negative relationship between humidity with total DDTs (p < 0.01) were observed. DDD (55.54%) was the major contributor to fish and prawn samples in rainy-season and followed by 4,4'-DDT (16.10%), DDE (14.35%) and 2,4'-DDT(14.00%). In autumn season, the major contributor was DDE (53.11%) and followed by the DDD (31.79%), 2,4'-DDT(8.52%) and 4,4-DDT(6.58%). In winter the major contributor was DDE (68.80%) and followed by DDD (18.23%), 2,4-DDT (7.63%) and 4,4-DDT (5.33%). In summer, the major contributor was 4,4-DDT(40.23%) and followed by 2,4-DDT(27.59%), DDE (18.60%) and DDD (13.58%). Compositional distribution of DDTs and the ratios of (DDE+DDD)/DDTs contributing to the values indicating both recent and past use of DDT in the region. The concentrations of total DDTs in all the samples were within the permissible Maximum Residue Level (MRL) i.e. for human consumption recommended by FAO-WHO. But 20.83% species of rainy-season, 68.18% of autumn, 13.04% in winter and 100% in summer exceeded the maximum admissible limit recommended by European Union. However, 4.55% species of autumn and 36.36% of summer were above concentrations associated with reproductive toxicity in several species of fish. To assess human health risks, Health risk Indexes (HI) of fish and prawn samples were calculated. HIs < 1 in all fish and prawn samples indicating that the fishes are safe to consume but the daily consumption of the fishes together with other contamination in food may cause human health hazard. As DDT is a long persistent and bioaccumulative substance in the environment, intake of significant amount of these poisonous elements with our diet is a matter of great health concern.

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### **ABBREVIATIONS**

ADI Acceptable Daily Intake

ANOVA Analysis of Varience

ATSDR Agency for Toxic Substances and Disease Registry

BSTI Bangladesh Standards and Testing Institute

BDL Below detection limit

BW Body weight

C Celsius

DAE Department of Agriculture Extension

DDD 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane

DDE 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane

DDT 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane

DDTs DDT and its metabolites

DoE Department of Environment

DR Daily consumption rate

dw Dry weight

ECD Electron capture detector

EDI Estimated Daily Intake

EPA Environmental Protection Agency

e.g. Exempli gratia = For example

et al. Et alia = And others

etc. et. cetera = And the others = And so on

EU European Union

f.w. Fresh weight

FAO Food and Agriculture Organization

g Gram

G Granular

GC Gas chromatography

GC-ECD Gas chromatography- Electron capture detector

GDP Gross domestic product

HI Health risk Index

HSD Honestly Significant Difference

IARC International Agency for Research on Cancer

i.e. id est = That is

IUPAC International Union of Pure and Applied Chemistry

Kg Kilogram

LOD Limit of Detection

LOQ Limit of Quantification

LSD Least Significant Difference

lw Lipid weight

mg Milligram mL Milliliter

MRL Maximum Residual Limit

NA Not Analyzed

nd Non detectable

ND Not detected

ng g<sup>-1</sup> Nanogram / gram

NPD Nitrogen phosphorous detector

OCPs Organochlorine pesticides

OHCs Organohalogen compounds

PAHs Polycyclic aromatic hydrocarbons

PCA Priciple Component Analysis

PCB Polychlorinated biphenyls

PCDD Polychlorinated dibenzo-p-dioxins

PCDF Polychlorinated dibenzofurans

POPs Persistent Organic Pollutants

PRA Pesticide residue analysis

QuEChERS Quick, Easy, Cheap, Effective, Rugged, and Safe

*R*<sup>2</sup> Regression Coefficient

Rpm Revolutions per minute

RSD Relative Standard Deviation

RT Retention time

### Dhaka University Institutional Repository

S/N Signal to noise ratio

SD Standard Deviation

SPSS Statistical Package for the Social Sciences

UNEP United Nations Environment Programme

USA United States of America

USEPA United.States Environmental Protection Agency

WHO World Health Organization

ww Wet weight

4,4´-DDD 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane

4,4'-DDE 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane

2,4´-DDT 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane

4,4´-DDT 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane

% Percent

### 1. INTRODUCTION

#### 1.1. Background

Organohalogen compounds such as organochlorine pesticides (OCPs) represents a key group of persistent organic pollutants (POPs). One of the most hazardous and ubiquitous OCP is Dichlorodiphenyltrichloroethane (DDT). DDT and its metabolites are of great concern to the environmental scientist for several decades, due to their long-range transport, persistence, bioaccumulation, biomagnification, toxicity and adverse effects on environment and human health including reproduction and birth defects (Edwards 1987), endocrine disruptions, immune system dysfunction and cancer (Adeyemi *et al.* 2008). DDT is listed on Stockholm convention as a Persistent Organic Pollutant (POP) among 12 POPs where proposed the actions for elimination and restriction their fresh release in environments (UNEP 2001). Considering the adverse effects, many countries have restricted or banned their use (Wania and Mackay 1996, Sabljic 2001). In Bangladesh, DDT and its metabolites have been used extensively for agriculture as well as for mosquito control programs for long time. DDT was completely banned in the country in 1993 when their applications were officially phased out but it's use remains illegally (Matin *et al.* 1998).

Bangladesh is an agro-based riverine country enriched with vast fisheries resources. Fish is an essential and mostly preferred food, other than rice in the country. It accounts for the largest share of per capita food expenditures after rice (Minten *et al.* 2010) and is by far the most frequently consumed animal-source food, providing approximately 60% of animal protein in the diet as well as other essential nutrients (Belton *et al.* 2011, Roos *et al.* 2007). Fisheries sector plays an important role in the national economy and nutrition as it contributes about 2.73% of the total export earning, 3.69% to GDP and 22.60% to agricultural sector (BES 2014). In case of inland fisheries production, Bangladesh is ranked fourth largest in the world (NFW 2015). Although rapid population growth and one of the world's highest population densities (Talukder 2005), the country has attained close self-sufficiency in rice and vegetables. As a result, pesticide use in general is increasing for higher food

production to meet the demand of growing population. According to statistical data of the Government of Bangladesh, pesticides consumption increased from 7350 metric tons in 1992 (Rahman 2005, Rahman and Thapa 1999) to 16,200 metric tons in 2001, more than doubling in the past decade. (Rasul and Thapa 2003, Hossain 1988). The agricultural land lead to the contamination of aquatic environments by Persistent organic pollutants (POPs) mainly through the rain water runs off. Specially, fishes uptake pollutants through gills, skin and food intake which then transfer the contaminants to humans through consumption of these organisms (Zhou *et al.* 2008). As fishes of the country contribute great role in national nutrition and also exported in many countries of the world, detail research studies are needed to assess the occurrence and toxic effects of POPs of fishes while very few research have been conducted to such extent which give the true picture of contamination levels in the country as well as the region.

The Meghna River is one of the most important rivers in Bangladesh, one of the three that forms the Ganges Delta, the largest on earth fanning out to the Bay of Bengal. In the Sonargaon Upazila of Narayanganj District, the Meghna River plays a vital role in the economy of the district as they facilitate fisheries resources, irrigation, drainage, and water supply of the area. On the bank of the Meghna River at Sonargaon Upazila different types of rice, jute, vegetables, banana, betel leaf, sugar cane etc. are cultivated throughout the year (NDS 2013). Pesticides are used improperly due to lack of appropriate knowledge about their applications and untoward effects. Moreover several mills, factories and industries are present on the bank of the river in the upazila. It is obvious that the wash out of pesticide residues by rain water and industrial effluents discharge contaminate the aquatic environments of the rivers and lakes. These contaminants can be potentially bioaccumulated in the fatty tissues of fish and biomagnified from lower trophic level to the higher trophic levels through food chain.

This research aims to determine amount of organochlorine pesticides (OCPs) such as DDT and its metabolites residues in fishes of different trophic levels of the Meghna River during different seasons.

#### 1.2. Persistent Organic Pollutants (POPs)

Persistent organic pollutants are organic chemical substances, possess some physical and chemical properties in a particular combination therefore once released into the environment, they may

- remain intact for exceptionally long periods of time.
- transport over large distance and become widely distributed throughout the environment involving air, soil and water.
- accumulate in the fatty tissue of living organisms including human, biomagnified through the food chain that found at higher concentrations at higher trophic levels.
- pose a risk of causing adverse effects to the environment and human health.

POPs include pesticides such as DDT, industrial chemicals such as polychlorinated biphenyls (PCB) and unintentionally generated chemicals such as polychlorinated dibenzofurans (PCDF) and polychlorinated dibenzo-p-dioxins (PCDD). Over the past several decades, POPs are released to the environment through human activities. Due to the nature of semi-volatile, long half lives and long range transport, POPs are now extensively distributed throughout the world including some of the most remote areas (Daly et al. 2007, Fernandez and Grimalt 2003, Lohmann et al. 200, Ondarza et al. 2011) and the areas where POPs have never been used (eg. Polar region). POPs are accumulated in the fatty tissue of living animals and human beings therefore the concentrations of POPs residues in fatty tissue can become magnified by up to much higher (About 70 000 times ) than the initial levels. Because of it's biomagnifications property through the food chain, concentrations of POPs tend to increase so that animals at the top of the food chain such as fish, predatory birds, mammals, and humans tend to have the greatest concentrations of these chemicals, and therefore are also at the highest risk from acute and chronic toxic effects (Fernandez and Grimalt 2003).

POPs are highly toxic which enter in to environment through human activities but mainly unintentionally (Kennish 1997, Breivik *et al.* 2004). They negatively affect humans, plant and animal species and natural ecosystems both in close proximity and

at significant distances away from the original source of discharge. Exposure to POPs in humans, either acute or chronic, can be associated with a wide range of adverse health effects, including illness and even death. The adverse effects of POPs can include hypersensitivity, allergies, disruption of the immune system and endocrine, damage to the central and peripheral nervous systems, reproductive disorders and carcinogenicity (El Nemr *et al.* 2011, Amodio *et al.* 2012, El Nemr 2013). POPs cause endocrine disruption by altering the hormonal system and damage the reproductive and immune systems of exposed individuals as well as their offspring. Moreover the higher levels of POPs residues in human blood serum can be associated to diabetes (Lee *et al.* 2006).

According to the U.S Environmental Protection Agency (EPA) there are links between POPs exposure and the increased frequency of diseases and abnormalities in wildlife species, including certain kinds of fish, birds, and mammals. The negative effects of pesticides in the marine and coastal environments include changes in reef community structure, such as decreases in live coral cover and increases in algae and sponges and damage to sea grass beds and other aquatic vegetation from herbicides (EPA 2012).

Considering the adverse effects of POPs, great concern has arisen about the occurrence of the POPs in environment for the last decade. Scientists and policymakers around the world became concerned about these harmful compounds and decided to make a convention in a meeting in Sweden on May 23, 2001 known as the "Stockholm Convention". In May 2001, the United Nations Environment Programme (UNEP) through the Stockholm convention investigated POPs, initially the Convention recognized only twelve POPs i.e. aldrin, chlordane, dieldrin, DDT, endrin, heptachlor, mirex, toxaphene, hexachlorobenzene, PCBs, PCDDs and PCDFs as the primary problem compounds, and are commonly known as the 'Dirty dozen'. At present, the number of POPs has increased to 19. The aim of the convention is to protect human health and the environment by phasing out of these hazardous pollutants from the earth.

#### 1.2.1. Classification of persistent organic pollutants

POPs are classified into two important subgroups including both the polycyclic aromatic hydrocarbons and some halogenated hydrocarbons. The halogenated group includes several organochlorines which are generally the long term persistent of all the halogenated hydrocarbons.

#### 1.2.1.1. Organohalogen compounds (OHCs)

Organic halogen compounds are a large class of natural and synthetic chemicals that contain one or more halogens (fluorine, chlorine, bromine, or iodine) combined with carbon and other elements. Organohalogens are widely used in industry and society. Most of the organohalogen compounds in use recently are synthetic in origin. However, huge amount of synthetic halogen compounds are used as pesticides, aerosol propellants, cleaning solvents, anaesthetics, polymers, refrigerants and so on. The wisdom of this massive use of materials that are foreign to our natural environment gradually is being reevaluated as the long-term detrimental effects of many of these chemicals become known. For example, many of the chlorinated hydrocarbons such as DDT, Chlordane, and Lindane, which have been used very widely as insecticides, now are at least partially banned because of concern for their long-term effects on non-target species, including man.

#### 1.2.1.2. Organochlorine pesticide residues (OCPs)

The chemical compounds used to control pests and diseases of plants, to eradicate weeds, to kill pests and microorganisms that spoil agricultural products, materials and articles, and to control parasites and vectors of dangerous diseases of human and animals are called pesticides (Grugdyev *et al.* 1983). The term pesticide includes insecticide, herbicide, acaricide, ovicide, larvidice, molluscicide, nematicide, rodenticide, fungicide, bactericide, etc. Pesticides can be classified according to chemical class such as organochlorine, carbamate, organophosphorus, chlorophenoxy compounds.

Organochlorine pesticides are chemically produced insecticides composed primarily of carbon, hydrogen and chlorine which include DDT, dieldrin, heptachlor, chlordane,

endosulfan and dicofol. Their carbon-chlorine bond is very stable towards hydrolysis and, the greater the number of chlorine substitutions, the greater the resistance to biological and photolytic degradation. By virtue of their high degree of halogenations, they have very low water solubility and high lipid solubility, leading to their propensity to pass readily through the phospholipid structure of biological membranes and accumulate in fat deposits. Chlorinated pesticides, with molecular weights 28 greater than 236 g/mol, have the ability to accumulate in biological tissues and to concentrate in organisms that occupy positions in the upper trophic levels. The general characteristics of OCPs; high chemical stability, low volatility, high lipid solubility, slow biotransformation and degradation, make them persistent, bioconcentrate and biomagnify through food chain (UNEP 2001). Finally these persistent compounds cause several environmental and human health hazards. Considering these adverse effects, the worldwide environmental scientists and general public pay their attention to these pollutants for the last decade (Law *et al.* 2003, Jiang *et al.* 2009).

OCPs, their bioaccumulation tendency and global contamination resulted in their ban and restriction in most countries. Banned OCPs include DDT, aldrin, dieldrin, toxaphene, chlordane and heptachlor. Although the production and use of OCPs have been banned in most of the country but they are still used illegally in some developing countries where there are evidence of their continued usage and presence in ecosystems (Darko and Acquaah, 2007). In some countries like Africa, Malaysia, Chine and India, they are still used in many sectors including agricultures, industry and public health (FAO 1999, Karlsson *et al.* 1997). However, the extensive use of OCPs for the past several decades and recent illegal use in agriculture and public health sectors has resulted an accumulation of these toxic residues in various environmental compartments such as soil, sediment, water, fish, birds, wildlife, vegetables and human body (Nishina *et al.* 2010, Sharma *et al.* 2013, Wang *et al.* 2013, Osafo and Frempong 1998, Ntow 2001).

Generally pesticides are used in terrestrial ecosystems which then flushed away through rain water to rivers and end up in marine ecosystems. Moreover the deposition of organochlorine pesticides in the environment occurs through several input mechanisms of which include urban runoff, municipal, sewage, industrial waste, outflow from agricultural areas, chemical spills, and atmospheric deposition (Vallack *et al.* 1998). After enter into the environment, they pass through food chain and enter into organisms through their food. In general, the principal route of human exposure to OCPs is dietary intake, accountin for >90% as compared to dermal and inhalation exposure pathways (Mansour *et al.* 2009)

As mentioned before, despite their restrictions, these compounds are still detected in the environment and in tissue samples such as blood, adipose tissue and breast milk of humans. But recently, body burdens have declined since these organochlorines were banned, yet virtually the entire population still carries detectable levels of the toxic chemicals. Chronic exposure to low levels of OCPs can cause a wide range of serious harmful effects in animals and humans. However, a number of studies have demonstrated that pose serious health hazards to human (Adeyemi *et al.* 2008, Nakata *et al.* 2002, Sverdrup *et al.* 2002, Jayashree and Vasudevan 2007). The chlorinated organic pesticides can pass through the mother placenta to the unborn child (Nakata *et al.* 2002). They cause many harmful effects such as abnormal development of the immune system, birth defects and fetal death (Ayejuyo *et al.* 2008). Thus organochlorine pesticides are considered as one of the main environmental and human health problems in the world (Darko and Acquaah 2007, Doong *et al.* 2002).

#### 1.3. Dichlorodiphenyltrichloroethane (DDT)

DDT is an organochlorine insecticide. As an important group of POPs, DDT is mainly characterized by highly toxicity, long range transport, long life span and lipophilic properties. Because of it's lipophilic nature, it can be accumulated in the fat body of living organisms which then biomagnified to the top consumers than the lowers. Finally this hazardous chemical causes serious health effects in human and threat to environment.

#### 1.3.1. Physical properties

DDT is a white crystalline powder with minimal odor. It is nearly insoluble in water but soluble in fats, oils and most organic solvents. DDT does not occur naturally, but is produced by the reaction of chloral (CCl<sub>3</sub>CHO) with chlorobenzene (C<sub>6</sub>H<sub>5</sub>Cl) in the presence of sulfuric acid, which acts as a catalyst. The Chemical formula for DDT is  $C_{14}H_9C_{15}$ . It's melting point is 108.5 °C; boiling point is 185 at 0.05 mm Hg and degradation point is above 250 °C. Its structure is similar to the pesticides dicofol and methoxychlor.

#### 1.3.2. Isomers of DDT

Commercial DDT is a mixture of some related compounds. The components include the 4,4'-DDT isomer (77%), 2,4'-DDT (15%), dichlorodiphenylethane (DDE) and dichlorodiphenyldichloroethane (DDD). In the environment and in the body, dichlorodiphenyltrichloroethane (DDT) breaks down into dichlorodiphenylethane (DDE) and dichlorodiphenyldichloroethane (DDD) over time. DDE and DDD are the major metabolites of DDT. The total DDT in a sample refers to the sum of all DDT congeners (4,4'-DDT, 2,4'-DDT, DDE and DDD) (WHO, 1973 and Nahar *et al.* 2008). From this context, the term  $\Sigma$ DDTs ( $\Sigma$  is used to mean sum of) will be used. In some cases, the DDT will be used to refer to the collection of all forms of DDT, DDE and DDD.

Table 1.1. Physical properties of DDT and its Metabolites (Beyer 2000)

Name and Structure	Physical Properties
CI_CI	Melting point: 89°C
	Boling point:306°C
	Solubility (water):
CI	0.12 mg/L (25°C)
Dichlorodiphenyldichloroethylene(DDE)	Log Kow: 6.51
CI_CI	Melting point: 109-110°C
	Boiling point: : 350°C
	Solubility (water):
CI	0.090 mg/L (25°C)
Dichlorodiphenyldichloroethane(DDD)	Log Kow: 6.02
CI CI_ I_CI	Melting point: 74.2°C
5. 45.	Solubility (water):
	0.085 mg/L (25°C)
CI	Log Kow: 6.79
2,4'-Dichlorodiphenyltrichloroethane(DDT)	
CI CI、I、CI	Melting point: 109°C
01	Boiling point: : 260°C
	Solubility (water):
	0.025 mg/L (25°C)
CI CI	Log Kow: 6.91
4,4'-Dichlorodiphenyltrichloroethane(DDT)	

### 1.3.3. History of DDT

DDT is one of the well known synthetic pesticides. In 1874, it was first synthesized in the laboratory by a German chemist named Othmar Zeidler. Then Paul Muller discovered its insecticidal properties in 1939 (Mischke *et al.* 1985). DDT was first manufactured in 1943 (Pretty and Hine 2005). During Second World War, it was used by the military and civilians to control the spread of malaria and typhus by mosquitoes and lice respectively.

After the war, during 1945, DDT was used as agricultural pesticide and also to control insect-borne human diseases (EPA 2012). From that period, DDT was used extensively for around thirty years. Increasing environmental and human health hazards causes by this persistent and toxic chemical made the scientist concern about it (Carson 1962, Longnecker *et al.* 2005). Therefore, during 1959, the use of DDT as agricultural pesticides and also vector control insecticide was declining and since 1970s DDT was banned in most of the developed countries though it is still being used in some underdeveloped countries for disease vector control (Biscoe *et al.* 2004). Even so, in many developing countries still DDT is used illegally and also some countries produce DDT for agriculture purpose and malaria vector combat (Biscoe *et al.* 2004).

#### 1.4. DDT, DDE, and DDD in to the environment

#### 1.4.1. How DDT enters into the environment

DDT is manufactured chemically which is not known to occur naturally in the environment (WHO 1979). Historically, DDT was released to the environment during its extensive use as a pesticide in agriculture and vector control application. Because of its persistence and long half life, most DDT in the environment is a result of past use but still DDT enters into the environment due to its current use in different areas of the world. After sprayed on the crop as pesticide or use to vector control, this pollutants release in the environment and persist in the air, water, soil and organisms which then cyclically move through food chain, biomagnified and finally cause hazard to biota.

#### 1.4.1.1. Air pollution

DDT may be released in land or in atmosphere. In case of land region, from soil and water, some parts of released DDT evaporate to the air. When in the air or atmosphere, about 50% of DDT will be adsorbed to particulate matter and remaining will exist in vapor phase (Bidleman 1988). During the journey in soil, water and air, some amounts of DDT may be changed to DDD and DDE. However DDT, DDE, and DDD in the air will then be deposited on land or surface water again. This cycle of evaporation and deposition may be repeated many times which would help to

transport them to long distances in the atmosphere. Therefore, these chemicals have been found in bogs, snow, and animals in the Arctic and Antarctic regions, far from where they were ever used.

#### 1.4.1.2. Soil pollution

After application in terrestrial region, some DDT may have entered the soil from waste sites. Even though some parts of soil'DDT may be evaporated to air, most of them stick strongly to soil particles, and therefore generally remain in the surface layers of soil. Most DDT breaks down slowly into DDE and DDD, generally by the action of microorganisms.

DDT, DDE, and DDD last in the soil for a very long time, potentially for hundreds of years. The length of time that DDT will last in soil depends on many factors including temperature, type of soil, and whether the soil is wet. DDT disappears faster when the soil is flooded or wet than when it is dry. DDT disappears faster when it initially enters the soil. DDT lasts for a much shorter time in the tropics where the chemical evaporates faster and where microorganisms degrade it faster. In tropical areas, total DDTs ( $\Sigma$ DDTs) may disappear in much less than a year. In temperate areas, half of the  $\Sigma$ DDTs initially present usually disappear in about 5 years. However, in some cases, half of the  $\Sigma$ DDTs initially present will remain for 20, 30, or more years.

Some soil particles with attached DDT, DDE, or DDD may get into adjacent rivers and lakes in runoff. Only a very small amount, if any, will seep into the ground and get into groundwater. DDT in soil can also be absorbed by some plants and by the animals or people who eat those crops.

#### 1.4.1.3. Water pollution

DDT enters into the aquatic habitat through surface run off from contaminated lands and also deposition from air. After entering in the water body, it will bind to particles which then settle down and be deposited in the bottom sediment. From the water column, DDT is taken up by phytoplankton, zooplanktons, small organisms, fishes, molluscs and other aquatic organisms. It accumulates to high levels in fish and marine

mammals (such as seals and whales) through repeated feeding of other organisms. In those animals, level of DDTs residues may be reached many thousands of times higher than in water.

#### 1.4.1.4. Fish pollution

Aquatic ecosystems are the ultimate reservoirs of many contaminants including DDT, from where contaminants can easily be accumulated to aquatic organisms due to their (contaminants) persistent and lipophiclic properties (Ondarza *et al.* 2012). Particularly, fishes are able to uptake contaminants directly from water and or through their food chain. Therefore, fish can bioaccumulate DDT by two main routes in their natural aquatic habitat; from water via gill and body surfaces and from food intake (Fisk *et al.* 2001, Blocksom *et al.* 2010, Ondarza *et al.* 2011, Poma *et al.* 2014). However fishes with organochlorine residues can reflect the pollution status of the surrounding environments as the metabolisms rate of DDT in fish is moderate (Guo *et al.* 2008, b). Additionally, fish from the same site may occupy different trophic positions in their food chain, reflecting in different degrees of contaminant biomagnification (Hoekstra *et al.* 2003). After uptake contaminants, fishes eventually transfer them to piscivorous birds, wild animals and human through consumption of these organisms (Zhou *et al.* 2008).

#### 1.4.1.5. Human in the food chain

People may be exposed to DDT, DDE, and DDD through several ways. During the handling or application, DDT could be entered into the body through inhalation or absorption through the skin. While the common route of exposure is through the food intake containing small amounts of these compounds. Particularly fatty foods such as fish, meat, milk, poultry and dairy products contain DDTs residues. In case of vegetables, leafy vegetables contain more DDT than other vegetables, possibly because DDT in the air is deposited on the leaves.

After enter into the body, DDT can break down to DDE or DDD. Because of lipophilicity these contaminants are stored mostly in the fat body, especially DDE. Some of these stored amounts leave the body very slowly, mostly in urine or by breast

milk to the newborn. The amount of DDT in the body decreases with decrease exposure. But DDT levels may be increased with continuous exposure and may cause health hazard.

Mother can transfer DDT to her unborn baby through placenta or to the newborn through breast milk. However, in most cases, the benefits of breastfeeding outweigh any risks from exposure to DDT in mother's milk (Bouwman *et al.* 1990).

#### 1.5. Toxicology of DDT and its metabolites

DDT was first synthesized in 1874 but its insecticidal properties were not discovered until 1939 (Smith 1991), and large scale industrial production started in 1943.

DDT is given credit for having helped 1 billion people live free from malaria, thus saving millions of lives. In 1973, after 30 years of worldwide use of DDT, a World Health Organization (WHO) report concluded that the benefits derived from use of this pesticide were far greater than its possible risks (WHO 1973). After 25 additional years, the benefits of DDT can be confirmed, but its stability, ubiquitous presence, and persistence in the environment, its accumulation in adipose tissues, and its estrogenic properties raise concern about its possible long-term adverse effects. In addition to a possible carcinogenic effect, DDT has been reported to affect neurobehavioral functions and to be associated with premature births (VanWendel *et al.* 2001, Longnecker *et al.* 2001). No living organism may be considered DDT free. DDT is stored in all tissues, but the highest concentration occurs in fat. It has been calculated that it would take between 10 and 20 years for DDT to disappear from an individual if exposure would totally cease, but that DDE would possibly persist throughout the life span (Smith 1991).

#### 1.5.1. Human health hazard

#### 1.5.1.1. Short term health hazard

If anybody takes large amounts of DDT over a short time by eating food or inhalation then he would be experienced sweating, headache, nausea, vomiting, and dizziness. These could finally affect nervous system, reproductive system and also adrenal glands.

# 1.5.1.2. Long term health hazard

People exposed for a long time to small amounts of DDT (less than 20 mg per day), such as people who worked in factories where DDT was made, had some minor changes in the levels of liver enzymes in the blood. A study in humans showed that increasing concentrations of DDE in human breast milk were associated with reductions in the duration of lactation. An additional study in humans found that as the DDE levels in the blood of pregnant women increased, the chances of having a pre-term baby also increased.

Animal studies show that long-term exposure to moderate amounts of DDT (20-50 mg kg<sup>-1</sup> of body weight every day) may affect the liver. Studies in animals have shown that oral exposure to DDT can cause liver cancer. The International Agency for Research on Cancer (IARC), the Department of Health and Human Services and also the Environmental Protection Agency (EPA) together has determined that DDT, DDE, and DDD are possibly carcinogenic to humans.

### 1.5.1.3. Experimental evidence of carcinogenicity of DDT and its metabolites

The carcinogenicity of DDT and its metabolites has been studied in a number of laboratories in animals including non human primates. DDT induced tumors in mice after treatment for a limited period of time. When treatment was continued for 15 or 30 weeks and mice were killed at various intervals, similar proportions of mice bearing liver tumors were observed 65, 95, and 130 weeks after the beginning of the experiment. More mice had large liver tumors at 95 and 120 weeks than at 65 weeks. These results indicate that although no new tumors were induced after cessation of exposure to DDT, the persistence of DDT-induced hematomas did not depend on the continuous administration of DDT because the tumors that have already appeared continue to grow (Tomatis *et al.* 1974, Tomatis 1975). After treatment of mice with DDT for six generations at four doses, no increase in the incidence of liver tumors was observed from generation to generation as might have been expected in the presence of a genotoxic carcinogen (Turusov *et al.* 1973). DDT also increased incidences of lung tumors and lymphomas in mice, incidences of liver tumors in rats, and incidences of adrenal adenomas in hamsters (IARC 1991). Long-term oral

administration of DDT to nonhuman primates was reported to result in hepatic toxicity, and a few malignant and benign tumors at various sites were also found at an incidence that was of borderline statistical significance (Takayama *et al.* 1999). In the absence of tumors in the controls, the few tumors observed may be considered to be biologically significant, thus confirming the carcinogenic effect observed in rodents (Tomatis 2000).

A number of reports have indicated that organochlorine insecticides, including DDT and its metabolites may act as endocrine disruptors (Keith *et al.* 1997, Longnecker *et al.* 1997). Because a considerable proportion of all cancers in women are hormonally mediated, the possibility that xenoestrogenic substances, such as organochlorine insecticides, contribute to an increased cancer risk is particularly alarming (Wolf *et al.* 1996). Early reports showed higher concentrations of DDT and DDE in fat tissue of individuals with mammary cancer (Wasserman *et al.* 1974) and an association between DDE blood levels and mammary cancer (Wolf *et al.* 1993, Dewailly *et al.* 1994, Krieger *et al.* 1994).

#### 1.5.3. Effects on wildlife

Exposure to DDT through the environment and/or food chain, may alter the immune system of birds and mammals; induce thyroid dysfunction in shellfish and mammals; decrease fertility rates in some wild animals (e.g. mink) and may also cause disruptions in the sex characteristics of individual animals, thereby altering the sex ratio of the population (Colborn and Smolen 1996). DDT leads to great deal of negative effects in the wildlife such as on eggshell thinning of herring gull, peregrines, falcons and hawks; led to raised level of endocrine disrupting chemicals in the alligator's tissue and a decrease of 50% of juvenile alligator numbers; resulted to the disease syndrome in grey and ringed seals in Baltic which caused to decline of seal population; DDT is also a neuro-developmental toxicant with a lot of evidences in changes of behavior and neurochemistry into adulthood of mice which exposure to DDT in stage of pre-natal and neonatal nervous system development. The endocrine disrupting effects of DDT may be one of their major impacts on wildlife (Thuy 2015).

# 1.5.4. Effects on fish species

DDT is highly toxic to fish species. Schoenthal. in 1963 reported that the mortality rates of rainbow trouts were increased by feeding aquatic insects treated with DDT. DDT also induce thyroid dysfunctions in fish and aquatic mammals (Colborn and Smolen 1996). Atlantic salmon exposed to DDT as eggs experienced impaired balance and delayed appearance of normal behavior patterns. DDT also effects on temperature selection in fish. Moreover DDT might lead to toxic effects in fish, causing endocrine disruption and altering biochemical, physiological, histological and morphological parameters (Norena-Barroso *et al.* 2004, Da Cuna *et al.* 2011, 2013, Hued *et al.* 2013).

# 1.6. Occurrence of DDT in different country

DDT have penetrated almost all the ecosystems and are now ubiquitous; this is evidenced by their detection in all environmental compartments and biota (Aono and Tatsukawa 1997, Norstrom *et al.* 1998, Muir *et al.* 2000). Considering this condition, environmental scientists have done several research works on the residue levels of DDT in water, soil, sediment, vegetables, fishes, other animals and also the fat containing food in different part of the world. The following tables would help to represent the world wide conditions of DDT levels in different compartments of the environment and also biota.

Table 1.2. DDTs in the water samples from different countries

Study area	DDTs (µg L <sup>-1</sup> )	References
Nainital, India	2.13-37.17	(Dua et al.1998)
Dhaka, Bangladesh	0.04-0.16	(Matin et al. 1998)
Hisar, India	6.20–7.06	(Kumari <i>et al.</i> 2007)
Noushehra, South Asia	70.00-400.00	(Jan et al. 2009)
Rawal lake, Pakistan	0.96- 2.87	(Iram et al. 2009)
Lake Burullus, Egyptian Mediterranean sea	0.07-882.60	(Said et al. 2008)
Anzali Wet land, Iran	55.48-180.81	(Javedankherad et al. 2013)
Sao Paulo State, Brazile	0.02-0.58	(Rissato et al. 2007)
Lake Bosomtwi, Ghana	0.07	(Darko <i>et al.</i> 2008)

Table 1.3. DDTs in the soil/sediment samples from different countries

Study area	DDTs (ng g <sup>-1</sup> dw)	Reference
Yamun river, Delhi	17.10-236.60	(Sethi et al. 1999)
Kolleru lake wetland, India	BDL-128,600	(Amaraneni 2006)
Hugli estuary, Sunderban ,Indea	3-119	(Bhattacharya et al. 2003)
Bay of Bengle, India	0.04-4.79	(Rajendran et al. 2005)
District Nagaon, India	166-2288	(Mishra <i>et al</i> . 2012)
Pesticide dumping ground Hydera city, Pakistan	21–21,200	(Alamdar et al. 2014)
Bohai and yellow Sea, China	0.37-1.17	(Ma et al.2001)
Alexandria harbor Egypt	<0.25- 885	(Barakat <i>et al.</i> 2002)
Masan Bay, Korea	0.27-89.20	(Hong et al.2003)
Caspean Sea Russia	0.01-1.90	(De Mora et al. 2001)
Aruwimi Congo River Basin	0.02-0.37	(Verhaert et al.2013)
Qiantang River, East China	1.14-100.2	(Zhou et al. 2006)
Sao Paulo, State Brazile	0.12-11.01	(Rissato et al. 2007)
Ebro River, Spain	9-94	(Cal et al. 2008)
Lake, Bosontwi, Ghana	4.41	(Darko <i>et al.</i> 2008)

<sup>\*</sup>dw- dry weight

Table 1.4. DDTs in the fish samples from different countries

Study area	DDTs (ng g <sup>-1</sup> ww)	References
Cambodia (South East Asia)	0.3-25	(Monirith et al. 1999)
West Coast (Srilanka)	1.3-120	(Guruge and Tanabe 2001)
Korea (Asia)	0.84-27.00	(Yim et al.2005)
Danube River Delta (UK)	179-4829	(Covaci et al. 2006)
Rocky mountain (Canada)	0.17-52	(Demers et al. 2007)
Alpine lakes (Switzerland)	6.6-22	(Schmid et al. 2007)
River Qiantang (East China)	2.65-133.50	(Zhou et al. 2007)
Pearl River Estuary and Day Bay(China)	1.7-462	(Guo et al. 2008, a)
Western Parks (US)	0.16-34	(Ackerman et al. 2008)
Beijing (China)	7.54-88.30	(Li et al. 2008)
Mid-continental great rivers (US)	8.06-9.51	(Blocksom et al. 2010)
Volta, Bosumtwi, Weija Lake (Ghana)	141.13-1126.51	(Adu-Kumi et al. 2010)
Tibetan plateau (Central Asia)	0.84-10.10	(Yang et al. 2010)
Shadegan Marshes (Iran)	32-410	(Davodi et al. 2011)
River Chenab (Pakistan)	8.83-190	(Equani et al. 2013)?
Tamil nadu (India)	0.85-75	(Ramesh et al. 1992).
Ebro river (Spain)	BDL- 2098	(Cal et al. 2008)
Lake Tanganyika, Burundi, Africa	909	(Manirakizaa <i>et al.</i> 2002)
	DDTs (ng g <sup>-1</sup> lw)	
Pearl River Delta (South China)	380-57000	(Sun et al. 2015)
Pongolapoort (South Africa)	5400-6000	(Wepener et al. 2012)

<sup>\*</sup>ww-wet weight, lw-lipid weight

Table 1.5. DDTs in the human and different biota samples from different countries

Study Area	Specimen	DDTs (ng g <sup>-1</sup> lw)	References
Gujrat Islamabad (Pakistan)	Rural mother (Blood)	11	(Ali et al. 2013a,b)
	Rural Children (Blood)	18	
	General population (Blood)	17	
South Africa	Breast milk	9500-18000	(Bouwman et al. 2012)
Hudson Strait (Canadian Arctic)	Beluga Whale (Fat)	520-2521	(Kelly et al. 2008)
Alaska	Killer Whale (Fat)	320000	(Ylitalo et al. 2001)
Bear IS, Norway	Glaucous gull (Plasma)	10245-15076	(Verreault et al.2005c)
South Greenland	Peregrine falcon (egg)	40	(Vorkamp et al.2009)
East Greenland	Polar bear (Fat)	309	(Dietz et al. 2008)
Russia Bering Sea	Stellar sea lions (Blood)	3600-15000	(Myers et al. 2008)
		DDTs (ng g <sup>-1</sup> ww)	
Agra (India)	Vegetables	2.82	(Bhanti and Taneza 2005)
	Vegetables	4-8	(Barriada-Perira <i>et al.</i> 2005)
Tamil Nadu (India)	Crab muscle	6-59	(Ramesh et al. 1999)
Patna	Dolphin muscle	100-5100	(Kannan <i>et al</i> . 1994)
South India	Birds muscle	0.6-3600 (Senthilkumar <i>et a</i> 2001)	
Ghana	Meat	11 (Darko <i>et al.</i> 20	

<sup>\*</sup>ww-wet weight, lw-lipid weight

### 1.7. Organochlorine pesticides (OCPs) in Bangladesh

### 1.7.1. Environmental legislations for OCPs in Bangladesh

According to the Pesticide Ordinance 1971, powers were given to the government in order to make pesticide rules. Pesticide Technical Advisory Committee made the pesticides rules in 1985 which gave the authority to the Director of Plant Protection Wing of Department of Agriculture Extension (DAE) for the registration of pesticides that started in 1986 in the country in exercise of clause 4 and clause 5 of the ordinance. The Pesticide Rules, 1985 provide the registration of manufacturing or importing of pesticides valid for three years (Rahman et al. 1995). Due to the this registration is valid for three years, but unfortunately the practice of assessment is not properly undertaken owing to (Gaston 1986). The implementation of the rule also face disappointing situation as huge amount of toxic and banned pesticides has smuggled from India. According to Parveen and Nakagoshi (2001), the main drawback of the above said regulation is in chapter VII section 33 sub section I (a) which actually provides the provision to state the manufacturer name, formulator name or repacker name in the label even in a case when the certain pesticide is not registered on his/her name. This could make the identification of a respective person very difficult. Taking the advantage of this weak point present in the regulations, the illegal business in the country is going on. The Bangladesh Government signed the Stockholm Convention on 23rd May, 2001. As a signatory, the Government was committed to prepare their National Implementation Plan for POPs, take action for creating awareness regarding consequences of POPs releases and ultimately their elimination from the environment. For National Implementation Plan, the concerned authorities were Department of Environment of Ministry of Environment (ESCO 2005).

# 1.7.2. DDT in Bangladesh

The use of pesticides, including organochlorine compounds, in Bangladesh started during the middle of the 1950s to promote crop production. A factory for production of DDT was built during the 1960s. Bangladesh had small pesticide use until 1970s, farmers were motivated to use pesticide free of cost up to 1974 and at reduced price till 1980 (Rahman 2000). According to statistics from the Government of Bangladesh, consumption of pesticides increased from from 2200 million tons in 1980–82 to 6500

million tons in 1992–94 (Rahman 2005, Rahman and Thapa 1999). According to Rahman *et al.* (1995), 2510 tons of pesticides were used in 1982 and 5150 tons in 1988 which increased to 8000 tons in 1994 and then 16,200 metric tons in 2001, more than doubling in the past decade Matin *et al.* (1998) reported that restricted use of OCPs was allowed by the Registration Authority of Bangladesh in 1990. They also reported that OCPs consumption for public health and malaria control program was low as compared to agricultural usage and 100 tons formulated OCPs were used in 1990 for malaria control programs. Because of the risk to the human health and environment, a number of organochlorine pesticides were banned in Bangladesh in 1993, including DDT. The factory producing DDT was closed down while in 1994, DDT was allowed by public health members for immediate control of plague. However, report says that DDT is still being illegally used in the country (Takada *et al.* 2003).

Table 1.6. DDTs in water, soil, vegetables, fish and human blood samples of Bangladesh

Study area	Samples	DDTs	Unit	References
Chittagong Chemical complex	Water	0.6-3	μg L <sup>-1</sup>	(Mahmud <i>et al.</i> 2015)
	Soil	$1.0\text{-}48.6\text{x}10^2$	mg kg <sup>-</sup>	
Mohonganj River, Mymensing	Fish	4.71-78.81	ng g <sup>-1</sup>	(Hossain et al. 2016)
Fish market of Khulna,	Dry fish	3-878	ng g -1	(Hasan et al. 2014)
Chittagong and Cox's Bazar				
Fish market of Sayedpur and	Fish	14.45-	ng g <sup>-1</sup>	(Hasan et al. 2013)
Cox's Bazar		1249.68		
Asadganj market, Chittagong	Dry fish(winter)	4-250	ug kg <sup>-1</sup>	(Bhuiyan et al.2009)
	Dry fish (rainy- season)	11-1107	ug kg <sup>-1</sup>	
Fish market of Dhaka city	Dry fish	0.03-1	mg kg <sup>-</sup>	(Nahar et al. 2008)
	Vegetables	ND		
	Fish and shrimp	0.03-1	mg kg <sup>-</sup>	
Fish market of Chittagong	Homemade dry fish	ND		
Dhaka and surrounding areas	Human Plasma	3900 (medean)	ng g <sup>-1</sup>	Zamir et al. 2008
Dhaka city	Human blood	Child (2011- 8600)	ng g <sup>-1</sup>	Mamun et al. 2007
		Teenage	ng g -1	
		(860-14900)	fat	
		Adult (1200- 8800)	ng g <sup>-1</sup> fat	

<sup>\*</sup>ND = Non Detectable

# 1.8. Objectives of the present study

Fishes are the most important and preferable food item not only in Bangladesh but also all over the world considering their high nutrient values. Yet at the same time, fishes may contain several contaminants such as organochlorine pollutants taken from the surrounding contaminated environments. Therefore the people should be informed of both the benefits and risk of fish consumption.

The developments of Fisheries sector of Bangladesh are now worldwide recognized. Fishes contribute important roles in the both national and abroad nutrition through high production and huge export in the foreign countries. Therefore it is necessary to have the detail and clear information about their nutrients and contamination profile. In our country, several research works have done on their nutrient evaluation while very few or not so detail works on the toxicological profile of fishes.

From this point of view, the present study investigated the organohalogen residues (specially DDT an its derivatives residues, the most toxic and hazardous persistent pollutants) of different fishes of different seasons. The objectives of the present study are as follows

- To analyze organohalogen pesticide residues from different fishes along the Meghna River in Sonargaon Upazila, Narayangang
- To analyze the seasonal variation of organohalogen residues in fishes from the Meghna River in Sonargaon Upazila
- To correlate organohalogen residues in fishes with selected meteorological parameters i.e. temperature, rainfall and humidity
- To analyze the bioaccumulation of organohalogen residues in fishes at different trophic levels

# 2. MATERIALS AND METHODS

#### 2.1. General

Meghna is one of the most important river in Bangladesh, deteriorating rapidly due to pollution from the wash-out of chemical and fertilizer of agricultural land and the effluents of industries on the bank of the river. The present study aims at determining the organohalogen residues in fishes from Meghna River and also the biological accumulation through the food chain. To fulfil the objectives of this research, the following methodologies were adopted.

#### 2.2. Materials

#### 2.2.1. Chemicals and solvents

Anhydrous magnesium sulphate were purched from Junsei Chemical Co. Ltd., Japan; anhydrous sodium sulphate, sodium chloride (analytical grade) and concentrated H<sub>2</sub>SO<sub>4</sub> (Merck, Germany) were used for this analyses. Acetone, *n*-hexane and ethyl acetate were purchased from SK Chemical Co. Ltd, Republic of Korea.

#### 2.2.2. Standard compounds

The standards of 2,4´-DDT & 4,4´-DDT (99% purity), 4,4´-DDE (99% purity), 4,4´-DDD (99% purity), purchased from Dr. Ehrenstorfer, Germany were used for analysis.

#### 2.2.3. Equipment

Calibrated balance, volumetric flasks and pipettes (calibrated by BSTI) were used for the analysis. All required glass apparatus were cleaned with water using detergent, rinsed about six times with water, then twice with distilled water and finally with redistilled acetone. All glassware was baked at 300°C overnight and stored by covering with aluminum foil prior to use.

#### 2.2.4. Instruments

# 2.2.4.1. Homogenizer

Fish samples were homogenized by normal kitchen blender (Panasonic, China).

# 2.2.4.2. Centrifuge machine

The samples were centrifuged by Hanil Science Industrial Co. Ltd., Model-Combi 514 R or by Heraeus Sepatech (Labofuge A).

# 2.2.4.3. Gas Chromatograph

A Shimadzu-2010 Gas chromatograph with electron capture detector and auto injector (ECD) was used for the determination of pesticide residues in the samples. A HP-5MS quartz capillary column (30 m  $\times$  0.25 mm i.d. and 0.25  $\mu$ m film thickness) from Agilent, USA was used to carry out the separation. Nitrogen was used as the carrier and make up gas. All injections were made in split-less split mode and injection volume was 1  $\mu$ L.



Fig. 2.1. Shimadzu GC-2010

#### **2.2.5.** Methods

### 2.2.5.1. Activation of chemicals

Sodium sulphate and Magnesium sulphate were activated by heating at 300 °C for 8 h in a furnace (GSM 11/8 Hope valley, S336RB, England) then kept out from furnace

and were allowed to cool at room temperature. After that the Sodium sulphate and Magnesium sulphate kept in a desicator.

### 2.2.5.2. Saturation of sulphuric acid

Concentrated sulphuric acid (80 mL, 98%) was taken in a reagent bottle and 20 mL of n-hexane was added to the acid. It was shacked about one minute and kept for 5-6 minutes to separate the two phases. The lower sulphuric acid phase, saturated with n-hexane was collected and stored in amber colored bottle.

### 2.2.5.3. Evaporation

All the evaporations were carried out under reduced pressure using rotary evaporator (Büchi Rotavapor R-114, Germany or Heidolph, Germany) at water bath temperature not exceeding 40°C. Before evaporation of each sample extract, the rotary evaporator was washed for three to four times through evaporation of acetone.

# 2.3. Preparation of standard solutions

### 2.3.1. Preparation of primary standard solutions

The known amount of the certified pesticide standars was dissolved in a definite volume of n-hexane and the concentration of the standard was calculated. Primary stock solutions (100  $\mu$ g/g) of 2,4′-DDT, 4,4′-DDT, 4,4′-DDE and 4,4′-DDD (99% purity) were prepared separately by dissolving 10 mg of each analyte in 100 mL n-hexane. The prepared solutions in 100 mL were labelled indicating name of each of the standard, concentration and the date of preparation. The meniscuses of the solutions were marked with permanent black ink and stored in the freezer (-20 °C) away from the sample storing area until further use.

#### 2.3.2. Preparation of middle and working standard solutions

The primary standard solutions were taken out of the freezer to reach room temperature and checked the meniscus of the layer. If the meniscus of the layer was below the mark then adjusted with n-hexane and vortexed for one minite. Then a definite amount of solution was withdrawn and put a new mark in the stock solution after withdrawing. The withdrawn solution was diluted with the solvent appropriate

for making 20  $\mu$ g/L secondary standard. Then 5  $\mu$ g/L working standards were prepared from the secondary standard by diluting with solvent following same way as mentioned above. The working standard solution (5 $\mu$ g/L) was serially diluted to 2  $\mu$ g/L, 1  $\mu$ g/L in the same procedure.

These solutions were labelled indicating name of the standard, concentration and date of preparation. The meniscuses of the solutions were marked with permanent ink and stored in a freezer (-20°C) away from the pesticide residue laboratory.



Fig. 2.2. Pesticide standard of DDT

### 2.4. Calibration curves

Working standards solutions of DDT and its metabolites were analyzed with GC-ECD and peak areas of corresponding solutions were listed. The calibration curves of each of the standard was prepared by plotting area vs concentration using Microsoft Excel-2010 software and  $r^2$  were found to be 0.9960, 0.9950, 0.9840 and 0.9960 for DDE, DDD, 2,4'-DDT and 4,4'-DDT respectively.

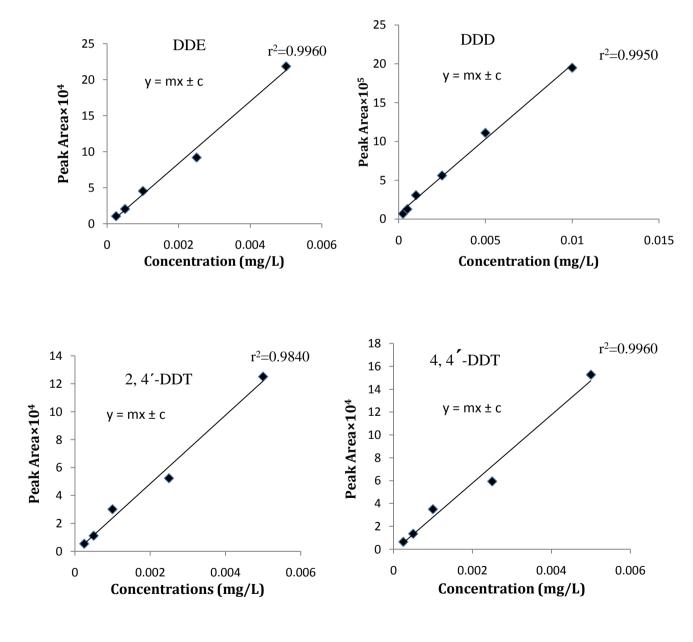


Fig. 2.3. Calibration curves of DDE, DDD, 2,4´-DDT and 4,4´-DDT

### 2.5. Selectivity, Sensitivity and Linearity

Selectivity (or specificity) was assessed by analyzing standard mixture of pesticides, blank matrices and blank matrices spiked with the mixture of pesticides simultaneously and by checking their retention times. Sensitivity of the instruments was assessed by determining limits of detection (LODs) and limits of quantification (LOQs) for each pesticide in each matrix. Linearity was evaluated by constructing calibration curves for each pesticide by injecting standard mixture to GC at 5-8 different concentration levels covering the expected range of pesticides that might be present in the samples.

To determine the LOD, working standard solutions were serially diluted to get desired concentration. The diluted standard solutions were injected one by one until the peak heights of the standards were same to the noise level. The limit of detection (LOD) of the test compounds was determined using a signal-to-noise ratio of 3 with reference to the background noise obtained for the blank sample, whereas the limits of quantification (LOQ) were determined with a signal-to-noise ratio of 10.

# 2.6. Identification and Quantification by GC

The reference standard solutions were injected into the GC-ECD and under the same condition cleaned extract of samples were also injected. Comparing the retention times (retention time of standard and unknown supposed to be same under the identical analytical conditions) of the different peaks of the sample with the retention times of the standard compounds, corresponding residues present in the samples were identified. Quantitative determination was carried out by comparing the peak area of the each DDTs in the sample extract with that of the peak area of the respective DDTs in the external standard solution. For quantification, concentration of the corresponding analytes was found from standard calibration curve taking into consideration that the peak area was in the midpoint of the curves.

### 2.7. Blank experiment

For recovery experiments, control samples were used, which were previously confirmed that they had no pesticide. The control samples were spiked with known

amount of DDTs were extracted follows three replicates followed by respective extraction and clean-up procedure to determine the matrix effect under analysis method. Reagent blank was done following the same extraction procedure and cleaned up method, using only solvent and reagents (in the absence of sample) to make the analysis rational.

### 2.8. Recovery Experiment

The recovery experiments were conducted on uncontaminated control samples by spiking the sample at 3 replicates in three concentration levels. The spiked samples were permitted to equilibrate for 3 h before extraction, in order to allow the pesticide penetrates the matrix. Then the spiked samples were subsequently processed by following the respective extraction and clean-up procedures. The recovery of the each analyte was calculated according to:

$$R = \frac{A_m \times C_{st}}{A_{st} \times C_m} \times \frac{100}{M_{st}}$$

Where R is the recovery (%),  $A_m$  is the peak area of the analyte in the matrix,  $A_{st}$  is the peak area of the analyte in the standard,  $C_m$  is the concentration of the analyte in the matrix,  $C_{sr}$  is the concentration of the analyte in the standard, and  $M_{st}$  is the mass of the analyte in the standard.

#### 2.9. Method Validation

The extraction efficiency of the analytical procedure was evaluated via recovery experiments. Validation of the method was performed in terms of recovery studies before analysis of field samples.

### 2.10. Sample collection and preparation

#### 2.10.1. Selection of sampling site

Fish samples were collected from the Meghna river at Boidyer Bazar of Sonargaon Upazila of Narayanganj District. Boidyer Bazar is the fish landing centre on the bank of the Meghna River at Sonargaon Upazila where around one hundred fishermen bring the fishes caught from nearby the Narayanganj District of Meghna Rriver. The fishes brought there to sell to wholesaler at two times per day, fishes caught at night, brought at 5 a.m. -6 a.m. and caught during morning, brought at 12 a.m. -1 p.m.

Meghna River is one of the major river in Bangladesh, specially famous for its great estuary that discharges the flows of the Ganges-Padma, the Brahmaputra-Jamuna and the Meghna itself. The Meghna has two distinct parts. The Upper Meghna from Kuliarchar to Shatnol is a comparatively small river. The Lower Meghna below Shatnol is one of the largest rivers in the world because of its wide estuary mouth. A larger number of settlements, towns, ports and industries have sprung up on both the banks of the Meghna. Narayangang, Narsingdi, Chandpur, Barisal and Bhola are the district towns that stand on the banks of the Meghna. Kuliarchar, Bhairab Bazar, Chandpur (Puran Bazar), Ramdaspur, Kalupur and Daulatkhan are important riverports and business centres. The Ashuganj thermal power plant and the Fenchuganj fertiliser factory are located on the banks of this river.

### 2.10.2. Selection of fish samples, period and way of sampling

Fish samples of different trophic levels; herbivore, omnivore and carnivore were collected to determine the bioaccumulation through food chain. In case of each fish species, mature fishes were collected. Different variety of fish species were collected for four different seasons. Bangladesh has a tropical monsoon climate with significant variations in rainfall and temperature throughout the country. There are four main seasons: (i) the pre-monsoon or summer during March-May, which has the highest temperatures; (ii) the monsoon or rainy-season during June-September, when the bulk of rainfall occurs; (iii) the post-monsoon or autumn during October-November; (iv) the cool and sunny dry season or winter during December-February (Aquastat 2011).

In each season, samples were collected at the middle part of the season. During rainy-season, twenty-four fish species were collected on 30<sup>th</sup> July 2015; during autumn, twenty-two fish species were collected on 30<sup>th</sup> October, 2015; during winter, twenty-three fish species were collected on 15<sup>th</sup> January, 2016 and during summer, twenty-

two fish species were collected on 15<sup>th</sup> May, 2015. Twenty fish species were common in all four seasons.

Fish species were collected from the fishermen, just after caught by net on the bank of the river at 5 a.m.-7a.m. In case of each small fish, about 1kg fish were collected from five-six different fishermen and then mix together. In case of each large fish 3-5 fishes were collected from different fishermen. The fishes were kept in jip-locked plastic bag and then kept into chill-box at 4 °C. The samples were transported to the laboratory immediately for further analysis. In the laboratory, at first the fish samples were identified by using the morphological characteristics, following Fishbase (2014), Rahman (2005) and Shafi and Quddus (1982). After identification, the measurements of some biological parameters (Length, width and weight) were taken and then the samples were kept in freezer at -20° C in jip-locked plastic bags with proper labeling of identification.

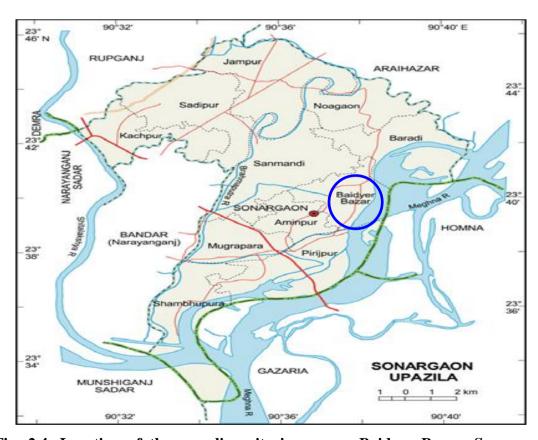


Fig. 2.4. Location of the sampling site in a map, Baidyer Bazar, Sonargaon Upazila

Table 2.1. Name of fish samples collected from Meghna River during rainy-season

No.	Local Name	Scientific Name	Class	Family	Feeding habit
1	Rui	Labeo rohita	Cypriniformes	Cyprinidae	Herbivore
2	Ghainna	Labeo gonius	Cypriniformes	Cyprinidae	Herbivore
3	Bata	Cirrhinus reba	Cypriniformes	Cyprinidae	Herbivore
4	Jat punti	Puntius sophore	Cypriniformes	Cyprinidae	Omnivore
5	Sharpunti	Systomus sarana	Cypriniformes	Cyprinidae	Omnivore
6	Tengra	Mystus vittatus	Cypriniformes	Bagridae	Omnivore
7	Bajari-tengra	Mystus tengra	Cypriniformes	Bagridae	Omnivore
8	Gulsha	Mystus cavasius	Cypriniformes	Bagridae	Omnivore
9	Shing	Heteropnuestes fossilis	Cypriniformes	Heteraopnuestidae	Omnivore
10	Magur	Clarias batrachus	Cypriniformes	Claridae	Omnivore
11	Kachki	Corica soborna	Clupeiformes	Clupeidae	Omnivore
12	Gutum	Lepidocephalus guntea	Cypriniformes	Cobitidae	Omnivore
13	Golda Chingri	Macrobrachium rosenbergii	Melacostraca	ostraca Palaemodnidae	
14	Chanda	Parambassis ranga	Perciformes	Ambassidae	Carnivore
15	Tara baim	Macrognathus aculiatus	Perciformes	Mastacembelidae	Carnivore
16	Boro baim	Mastacembelus armatus	Perciformes	Mastacembelidae	Carnivore
17	Chewa	Pseudapocryptes elongatus	Perciformes	Gobidae	Carnivore
18	Kaikka	Xenentodon cancila	Beloniformes	Belonidae	Carnivore
19	Foli	Notopterus notopterus	Clupeiformes	Notopteridae	Carnivore
20	Meni	Nandus nandus	Perciformes	Perciformes Nandidae	
21	Bele	Glossogobius giuris	Perciformes	Gobidae	Carnivore
22	Poa	Otolithoides pama	Perciformes	Sciaenidae	Carnivore
23	Bacha	Eutropiichthys vacha	Cypriniformes	Schilbeidae	Carnivore
24	Boal	Wallago attu	Cypriniformes	Siluridae	Carnivore
	l .	1	1	l	l

Table 2.2. Name of fish samples collected from Meghna River during autumn

No.	Local Name	Scientific Name	e Class Family		Feeding
					habit
1	Rui	Labeo rohita	Cypriniformes	Cyprinidae	Herbivore
2	Ghainna	Labeo gonius	Cypriniformes	Cyprinidae	Herbivore
3	Bata	Cirrhinus reba	Cypriniformes	Cyprinidae	Herbivore
4	Jat punti	Puntius sophore	Cypriniformes	Cyprinidae	Omnivore
5	Sharpunti	Systomus sarana	Cypriniformes	Cyprinidae	Omnivore
6	Tengra	Mystus vittatus	Cypriniformes	Bagridae	Omnivore
7	Bajari-tengra	Mystus tengra	Cypriniformes	Bagridae	Omnivore
8	Gulsha	Mystus cavasius	Cypriniformes	Bagridae	Omnivore
9	Sing	Heteropnuestes fossilis	Cypriniformes	Heteraopnuestidae	Omnivore
10	Kachki	Corica soborna	Clupeiformes	Clupeidae	Omnivore
11	Golda chingri	Macrobrachium rosenbergii	Melacostraca	Palaemodnidae	Omnivore
12	Khalisha	Trichogaster fasciata	Perciformes	Anabantidae	Omnivore
13	Chanda	Parambassis ranga	Perciformes	Ambassidae	Carnivore
14	Boro Baim	Mastacembelus armatus	Perciformes	Mastacembelidae	Carnivore
15	Chewa	Pseudapocryptes elongatus	Perciformes	Gobidae	Carnivore
16	Foli	Notopterus notopterus	Clupeiformes	Notopteridae	Carnivore
17	Meni	Nandus nandus	Perciformes	Nandidae	Carnivore
18	Shol	Channa striata	Channiformes	Channidae	Carnivore
19	Bele	Glossogobius giuris	Perciformes	Gobidae	Carnivore
20	Poa	Otolithoides pama	Perciformes	Sciaenidae	Carnivore
21	Bacha	Eutropiichthys vacha	Cypriniformes	Schilbeidae	Carnivore
22	Boal	Wallago attu	Cypriniformes	Siluridae	Carnivore

Table 2.3. Name of fish samples collected from Meghna River during winter

No.	Local Name	Scientific Name	Class	Family	Feeding	
					habit	
1	Rui	Labeo rohita	Cypriniformes	Cyprinidae	Herbivore	
2	Ghainna	Labeo gonius	Cypriniformes Cyprinidae		Herbivore	
3	Bata	Cirrhinus reba	Cypriniformes	Cyprinidae	Herbivore	
4	Jat punti	Puntius sophore	Cypriniformes	Cyprinidae	Omnivore	
5	Shar punti	Systomus sarana	Cypriniformes	Cyprinidae	Omnivore	
6	Tengra	Mystus vittatus	Cypriniformes	Bagridae	Omnivore	
7	Bajari-tengra	Mystus tengra	Cypriniformes	Bagridae	Omnivore	
8	Gulsha	Mystus cavasius	Cypriniformes	Bagridae	Omnivore	
9	Shing	Heteropnuestes fossilis	Cypriniformes	Heteraopnuestidae	Omnivore	
10	Magur	Clarias batracus	Cypriniformes	Claridae	Omnivore	
11	Kachki	Corica soborna	Clupeiformes	Clupeidae	Omnivore	
12	Golda Chingri	Macrobrachium rosenbergii	Melacostraca	Palaemodnidae	Omnivore	
13	Chanda	Parambassis ranga	Perciformes	Ambassidae	Carnivore	
14	Tara Baim	Macrognathus aculiatus	Perciformes	Mastacembelidae	Carnivore	
15	Boro baim	Mastacembelus armatus	Perciformes	Mastacembelidae	Carnivore	
16	Chewa	Pseudapocryptes elongatus	Perciformes	Gobidae	Carnivore	
17	Foli	Notopterus notopterus	Clupeiformes	Notopteridae	Carnivore	
18	Meni	Nandus nandus	Perciformes	Nandidae	Carnivore	
19	Shol	Channa striata	Channiformes Channidae		Carnivore	
20	Bele	Glossogobius giuris	Perciformes	Gobidae	Carnivore	
21	Poa	Otolithoides pama	Perciformes	Sciaenidae	Carnivore	
22	Bacha	Eutropiichthys vacha	Cypriniformes	Schilbeidae	Carnivore	
23	Boal	Wallogo attu	Cypriniformes	Siluridae	Carnivore	

Table 2.4. Name of fish samples collected from Meghna River during summer

No.	Local Name	Scientific Name	fic Name Class Family		Feeding
					habit
1	Rui	Labeo rohita	Cypriniformes	Cyprinidae	Herbivore
2	Ghainna	Labeo gonius	Cypriniformes	Cyprinidae	Herbivore
3	Bata	Cirrhinus reba	Cypriniformes	Cyprinidae	Herbivore
4	Jat punti	Puntius sophore	Cypriniformes	Cyprinidae	Omnivore
5	Sharpunti	Systomus sarana	Cypriniformes	Cyprinidae	Omnivore
6	Tengra	Mystus vittatus	Cypriniformes	Bagridae	Omnivore
7	Bajari-tengra	Mystus tengra	Cypriniformes	Bagridae	Omnivore
8	Gulsha	Mystus cavasius	Cypriniformes	Bagridae	Omnivore
9	Shing	Heteropnuestes fossilis	Cypriniformes	Heteraopnuestidae	Omnivore
10	Kachki	Corica soborna	Clupeiformes	Clupeidae	Omnivore
11	Golda chingri	Macrobrachium rosenbergii	Melacostraca	Palaemodnidae	Omnivore
12	Chanda	Parambassis ranga	Perciformes	Ambassidae	Carnivore
13	Boro Baim	Mastacembelus armatus	Perciformes Mastacembelidae		Carnivore
14	Chewa	Pseudapocryptes elongatus	Perciformes	Gobidae	Carnivore
15	Foli	Notopterus notopterus	Clupeiformes	Notopteridae	Carnivore
16	Meni	Nandus nandus	Perciformes	Nandidae	Carnivore
17	Shol	Channa striata	Channiformes	Channidae	Carnivore
18	Gojar	Channa marulius	Channiformes	Channidae	Carnivore
19	Bele	Glossogobius giuris	Perciformes	Gobidae	Carnivore
20	Poa	Otolithoides pama	Perciformes	Sciaenidae	Carnivore
21	Bacha	Eutropiichthys vacha	Cypriniformes	Schilbeidae	Carnivore
22	Boal	Wallago attu	Cypriniformes	Siluridae	Carnivore



Plate 2.1. Sampling site of the Meghna River in different seasons, a. Rainy-season, b. Autumn, c. Winter d. Summer season



Rui (Labeo rohita)



Ghainna (Labeo gonius)



Bata (Cirrhinus reba)



Jat punti (Puntius sophore)



Sharpunti (Systomus sarana)



Tenga (Mystus vittatus)

Plate 2.2.a. Different fishes collected from the Meghna River





Gulsha (Mystus cavaius)



Shing (Heteropnuestus fossilis)



Magur (Clarias batrachus)



Kachki (Corica soborna)



Goldachingri (M. rosenbergii)

Plate 2.2.b. Different fishes collected from the Meghna River



Khalisha(*Trichogaster fasciata*)



Chanda (Parambassis ranga)



Tarabaim (Macrognathus aculiatus)



Borobaim (Mastacembelus armatus)



Chewa (Pseudapocryptes elongatus)



Foli (Notopterus notopterus)

Plate 2.2.c. Different fishes collected from the Meghna River



Meni (Nandus nadus)



Shole (Channa striata)



Gojar (Channa marulius)



Bele (Glossogobius. giuris)



Poa (Otolithoides. pama)



Bacha (Eutropiichthys. vacha)



Boal (Wallago attu)

Plate 2.2.d. Different fishes collected from the Meghna River

# 2.10.3. Sample preparation

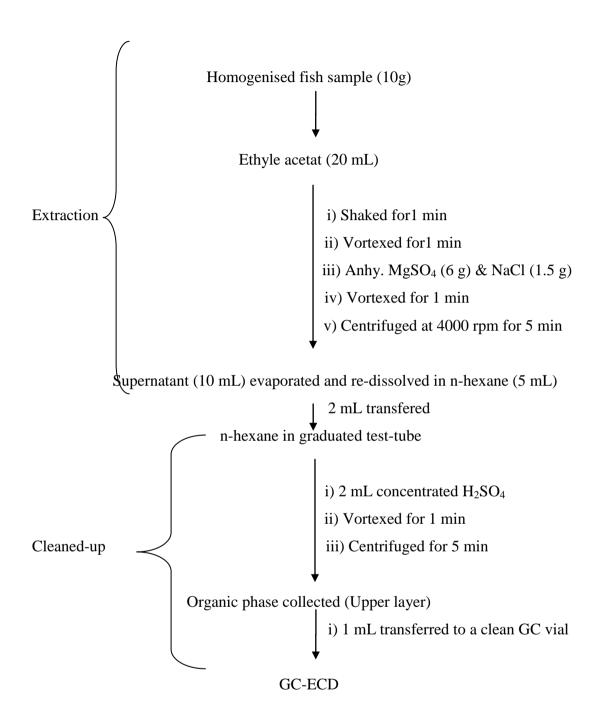
Before extraction the samples were taken out from the freezer and thaw well at room temperature. Then the scales, fins, viscera, gills were removed and washed with clean water. In case of small fish, whole body was grinded to paste and in case of large fishes, bones were removed and the remaining parts were grinded to paste with the help of the blender. The grinded fish samples were then ready for extraction.

### 2.11. Extraction of fish samples

The fish samples were extracted by Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method on following Mastovska *et al.* 2010 with some modifications. 10 g of blended fish sample was taken in a Teflon centrifuge tube (50 mL) and ethylacetate (20 mL) was added and the content was vigorously shaken by hand for 1 min and vortexed for 1 min. Then anhydrous MgSO<sub>4</sub> (6 g) and NaCl (1.5 g) were added and the mixture was again vortexed for 1 min and then centrifuged (4000 rpm for 5 min). Supernatant (10 mL) was taken into a pre-weighted round bottom flask. Ethyle acetate was evaporated (below 40 °C) to dryness by rotary vacuum evaporator. Then the weight of the flask was taken. The lipid weight of the extracted fish sample was got by subtracting the initial weight of round bottom flask from final weight. After evaporation of solvent, re-dissolved in n-hexane (5 mL). The sample extract (2 mL of dissolved 5mL) was kept in a graduated test tube and then cleaned-up. The extraction procedure is demonstrated in Scheme-2.1.

### 2.12. Cleaned-up of fish samples

The 2mL extract in a graduated test tube was treated with 2 mL concentrated sulphuric acid (saturated with n-hexane). The test tube was vortexed for 1 minute and then centrifuge for 5 minutes. Supernatant (1 mL) was taken using pipette and kept into GC vial and analyzed by GC connected with an electron capture detector (ECD). The clean-up procedure is demonstrated in Scheme 2.1.



Scheme 2.1. Extraction and cleand-up method of Fish samples



1. Sample collection



2. Samples transported to Lab. immediately



3. Identification and take measurement



4. Samples kept in freezer



5. Sample preparation



6. Blended samples ready for extraction

Plate 2.3. Different steps prior to extraction





1. Extraction

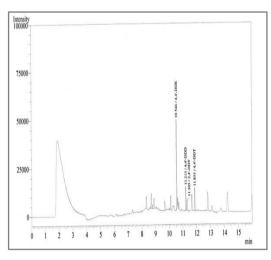




2. Cleaned-up



3. Analysis by GC-ECD



4. Chromatogram of DDTs residues

Plate 2.4. Different steps of analysis of DDTs residues

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# 2.13. Analysis of Organohalogen residues by GC-ECD

A Gas Chromatograph (GC-2010 Shimadzu) coupled with Electron Capture detector, (GC-ECD) was used for analysis. Separations were performed on HP-5 quartz capillary column (30 m long x 250 μm *i.d.*; 0.25 μm film thicknesses), nitrogen was used as carrier (column flow 1.92 mL/min.) as well as make up gas. The injector and detector temperatures were set at 220 °C and 290 °C, respectively. All injections were made in split-less/ split mode and injection volume was 1μL. The oven temperature was programmed as: initial temperature of 120 °C hold for 1 minute; increased at 20 °C min<sup>-1</sup> to 280 °C; hold for 4 min. Identifications of the organochlorine compounds analyte samples were done by comparing retention time of corresponding certified standard samples and quantification by using external calibration curves of the corresponding reference standard.

Table 2.5. Column oven temperature program

Initial Temperature : 120 °C					
Total program time: 16 m	in				
Rate(C/min)	Temperature(°C)	Hold time(min)			
	120	1.00			
20.0	280.0	4.00			

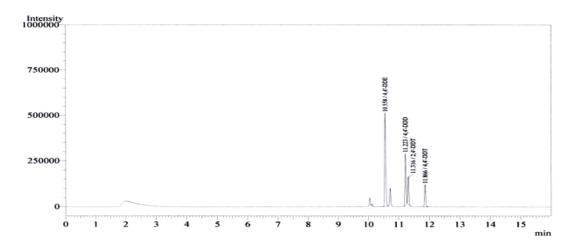


Fig. 2.5. Chromatogram of standards

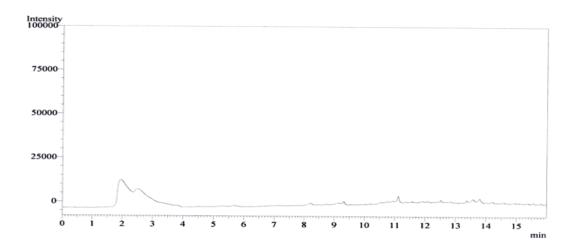


Fig. 2.6. Chromatogram of control sample

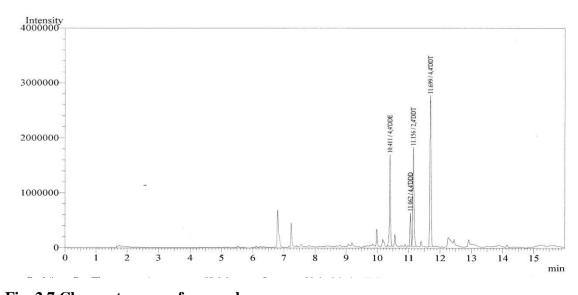


Fig. 2.7.Chromatogram of a sample

# 2.14. Recovery, LOD and LOQ (Method Validation)

In order to make standard calibration curves for DDE, DDD, 2,4′-DDT and 4,4′-DDT stock solution of standard reference certified samples were serially diluted to obtain 12 different concentrations. The linearity of the method was well demonstrated over concentration range of 0.00025-1.00 mg/L with an  $R^2$  (regression coefficient) value in the range of 0.9840- 0.9960 (Table 2.6). The method was validated in terms of three replicates experiments. The percentage recoveries for fish samples were found to be 88-92 %, 101-113%, 76-104 % and 70-90 % for DDE, DDD, 2,4′-DDT and 4,4′-DDT respectively (Table 2.6), which were in the range 70-120 and acceptable for samples according to standard methodology. From the calibration curve the LOD and LOQ that are given in Table 2.6.

Table 2.6. Retention times (RT), Regression Coefficients ( $\mathbb{R}^2$ ), Limit of Detection (LOD), Limit of Quantification (LOQ) for DDTs in fish and prawn samples

Residues	RT (min)	Linearity (R <sup>2</sup> )	LOD	LOQ
DDE	10.363	0.9960	0.0625 ng g <sup>-1</sup>	0.2063 ng g <sup>-1</sup>
DDD	11.012	0.0050		
DDD	11.012	0.9950		
2,4´-DDT	11.099	0.9840		
4,4´-DDT	11.637	0.9960		

For recovery experiment control fish sample (Cultured Rui fish) was spiked separately with known amount of certified four standards at 3 different concentration levels (0.05, 0.10, 0.20  $\mu$ g/mL or mg/kg). extraction and cleaned up were done following similar procedure as described in section 2.11 and 2.12. Present recovery and their RSD were calculated and presented in Table 2.7.

Table 2.7. Data of the recovery experiments

Standards	Spiked Level	(%) Recovery	RSD (%)
	(mg/kg)		
DDE	0.05	88.67	3.02
	0.10	92.55	7.13
	0.20	92.24	2.76
DDD	0.05	101.76	16.34
	0.10	101.32	6.17
	0.20	113.83	1.54
2,4 -DDT	0.05	104.89	9.29
	0.10	87.89	8.30
	0.20	76.25	2.05
4,4 -DDT	0.05	90.78	15.50
	0.10	70.10	0.78
	0.20	71.64	3.64

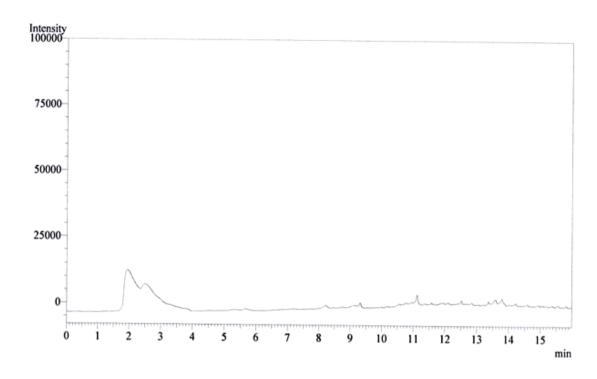


Fig. 2.8. Chromatogram of a control sample

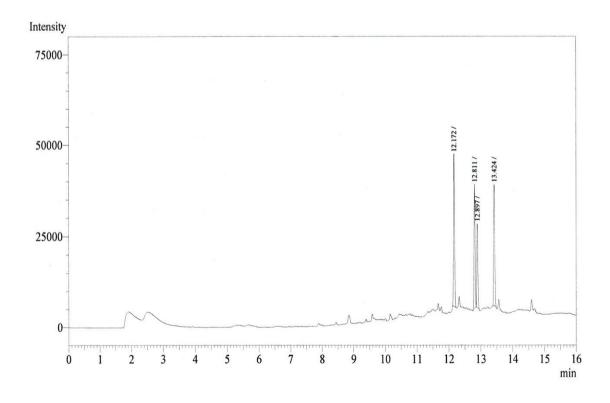


Fig. 2.9. Chromatogram of standards in a recovery

# 2.15. Meteorological data of the sampling site

The accumulation of organohalogen residues in fishes has some relations to ambient temperature, rainfall and humidity. Therefore these meteorological data are needed to predict the influencing factors of DDTs accumulations in the present study. These data were collected from the Climate division, Meteorological department of Bangladesh. The monthly and seasonally data (March-December 2015, June-September 2015, October-November 2015 and January-February 2016) of Ambient bulk temperature, total rainfall and humidity of sampling site during sample collection were shown in Table 2.8.

Table 2.8. Average values of ambient temperature, rainfall, humidity of sampling site during the sample collection

Month	Season	Temperature (monthly)	Temperature (seasonally)	Rainfall (monthly)	Rainfall (seasonally)	Humidity (monthly)	Humidity (seasonally)
		<sup>0</sup> C	<sup>0</sup> С	mm	mm	%	%
March	Summer	26.3	26.97	4	118.33	52	63.67
April	-	27.9	-	166		68	
May	_	26.7	-	185		71	
June	Rainy- season	29.3	28.97	375	434.75	77	78.75
July	Scason	28.4		623		81	
August		29.2	-	395		79	
September	-	29.0	-	346	_	78	
October	Autumn	27.7	26.1	51	51	73	71
November		24.5	-	0		69	
December	Winter	20.4	21.1	1	5.67	68	66.33
January		18.9	-	3		68	
February	-	24.0	-	13		63	

#### 2.16. Human health risk estimation

Health risk assessment of consumers from the intake of pesticides contaminated fish was characterized by using health risk index (HI). The estimated HIs were obtained by dividing the Estimated Daily Intake (EDI) by their corresponding values of Acceptable Daily Intakes (ADI) by WHO/FAO (FAO/WHO 2010) as shown by the equation;

HI = EDI / ADI

When the HI is less than 1, the food concerned is considered acceptable. If it is greater than 1, the food concerned is considered a risk to the consumer (Darko and Akoto 2008, Akoto *et al.* 2015).

#### Estimated Daily Intake (EDI)

The EDIs of OCPs expressed as nanogram per kilogram body weight per day (ng/kg bw/d) were calculated as follows:

$$EDI = (C \times DR) / BW$$

Where C is the measured concentration of OCPs ng g<sup>-1</sup>, DR is average daily consumption rate of fish (g day<sup>-1</sup>) and BW is body weight (kg).

#### 2.17. Statistical analysis of data

Statistical analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) followed by Tukey's HSD and LSD post hoc tests, were conducted for multiple comparison to test for significant differences between organohalogen residues of different seasons and different feeding modes. The relationship between residue levels with lipid contents, temperature, humidity and rainfall were assessed using pearson correlation. In all cases, the level of significance was set at 5% ( $p \le 0.05$ ).

Principle Component Analysis (PCA) was carried out on STATA 12 software to explore the relationship between residues and fishes of different modes. To identify the classification of species that indicates their similarity based on DDTs residues, Dendrogram for Agglomerative Hierarchial Clustering was performed using SPSS.

# 3. RESULTS AND OBSERVATIONS

# 3.1. Analysis of residual DDT and its metabolites in fish and prawn samples of different seasons from the Meghna River

Samples were collected periodically from four different seasons given in the Table 2.1- Table 2.4. The samples were immediately wrapped with aluminium foil, put into a chill box with ice and transported to the laboratory on the same day. All the collected fish samples were identified and some morphometric data were taken. The samples were then stored in a freezer at a temperature below -20°C temperature until analysis. Before extraction, the fish tissue was made bone free, chopped and blended. The samples were extracted and cleaned up following the procedures described in Scheme 2.1, analyzed for the presence of residual DDT and its metabolites; DDE, DDD, 2,4′ DDT and 4,4′ DDT. The samples after cleaned up were analyzed by GC-ECD for the residual amounts of DDTs (DDT and its metabolites). The results of residual amounts of DDTs are given in the section 3.1.1 - 3.1.12.

#### 3.1.1. Biological Parameters of fish and prawn samples for different seasons

Twenty-four, twenty-two, twenty-three and twenty-two different fishes and prawn species were studied during rainy-season, autumn, winter and summer respectively for the study. In each season only one species was prawn and remaing others were finfish species. In case of four different seasons, twenty species were found commonly to all seasons. In all seasons, the collected fish and prawn samples were mature and of maximum size. The length, width, weight, and sample number of samples of rainy-season, autumn, winter and summer are given in the Table 3.1, 3.2, 3.3 and 3.4 respectively.

In rainy-season, among the twenty-four analysed fish samples, the largest fish was the Boal (length was 70.49 cm, width was 12.88 cm) while the smallest fish was Kachki (length was 1.81 cm, width was 0.50 cm). The highest weight was recorded in Rui

(1800.59 g) and lowest in Kachki (0.74 g). Three fishes were herbivore, nine fishes and one prawn were omnivore an eleven fishes were carnivore.

In autumn, among the twenty-two analysed fish samples, the largest fish was the Boal (length was 77.49 cm, width was 15.28 cm) while the smallest fish was Kachki (length was 1.92 cm, width was 0.72 cm). The highest weight was recorded in Rui (4050.69 g) and lowest in Kachki (0.74 g). Three fishes were herbivore, eight fishes and one prawn were omnivore and ten fishes were carnivore.

In winter, among the twenty-three analysed fish samples, the largest fish was the Boal (length was 72.49 cm, width was 15.28 cm) while the smallest fish was Kachki (length was 2.92 cm, width was 0.72 cm). The highest weight was recorded in Rui (1900.59 g) and lowest in Kachki (0.82 g). Three fishes were herbivore, eight fishes and one prawn were omnivore an eleven fishes were carnivore.

In summer, among the twenty-two analysed fish samples, the largest fish was the Boal (length was 76.89 cm, width was 12.98 cm) while the smallest fish was Kachki (length was 3.29 cm, width was 0.73 cm). The highest weight was recorded in Rui (4600.59 g) and lowest in Kachki (0.84 g). Three fishes were herbivore, seven fishes and one prawn were omnivore an eleven fishes were carnivore.

Table 3.1. Average length, width, weight and sample number of fish and prawn samples collected during rainy-season

No.	Common Name	Scientific Name	Length	Width	Weight	n=
1	Rui	Labeo rohita	( <b>cm</b> ) 40.90	( <b>cm</b> ) 12.98	(g) 1800.59	1
	CI.	7.7	20.40	0.20	870.00	4
2	Ghainna	Labeo gonius	30.48	8.38	870.00	4
3	Bata	Cirrhinus reba	14.63	2.73	30.11	10
4	Jatpunti	Puntius sophore	7.57	3.37	10.49	25
5	Sharpunti	Systomus sarana	22.33	6.53	144.24	8
6	Tengra	Mystus vittatus	7.80	1.30	5.48	25
7	Bajari-tengra	Mystus tengra	2.27	1.00	1.33	40
8	Gulsha	Mystus cavaius	14.77	2.40	18.44	20
9	Shing	Heteropnuestes fossilis	17.13	2.70	37.40	20
10	Magur	Clarias batrachus	17.17	3.47	48.10	15
11	Kachki	Corica soborna	1.80	0.50	0.74	250
12	Gutum	Lepidocephalus guntea	3.23	1.70	5.40	10
13	Golda Chingri	Macrobrachium rosenbergii	26.98	4.27	179.68	35
14	Chanda	Parmbassis ranga	4.43	1.67	2.71	20
15	Tara baim	Macrognathus aculiatus	4.83	1.83	17.27	25
16	Boro baim	Mastacembelus armatus	38.32	4.33	192.60	5
17	Chewa	Pseudapocryptes elongatus	22.17	2.13	33.60	15
18	Kaikka	Xenentodon cancila	19.73	1.53	61.31	15
19	Foli	Notopterus notopterus	19.47	6.73	67.30	8
20	Meni	Nandus nandus	14.27	4.47	50.71	15
21	Bele	Glossogobius giuris	24.30	5.23	147.99	8
22	Poa	Otolithoides pama	23.20	5.27	121.47	10
23	Bacha	Eutropiichthys vacha	22.93	6.65	51.46	6
24	Boal	Wallago attu	70.49	12.28	1002.22	2

Table 3.2. Average length, width, weight and sample number of fish and prawn samples collected during autumn

No	Name	Scientific Name	Length (cm)	Width (cm)	Weight (g)	n=
1	Rui	Labeo rohita	60.45	19.51	4050.69	3
2	Ghainna	Labeo gonius	24.48	7.18	840.00	3
3	Bata	Cirrhinus reba	15.98	3.65	32.11	10
4	Jat punti	Puntius sophore	8.97	4.27	10.49	35
5	Sharpunti	Systomus sarana	24.83	7.23	148.25	15
6	Tengra	Mystus vittatus	7.80	1.30	5.48	35
7	Bajari-tengra	Mystus tengra	2.27	1.00	1.33	45
8	Gulsha	Mystus cavaius	16.77	3.15	18.44	15
9	Shing	Heteropnuestes fossilis	15.33	3.26	39.54	15
10	Kachki	Corica soborna	1.92	0.72	0.74	200
11	Golda Chingri	Macrobrachium rosenbergii	18.28	3.81	179.68	10
12	Khalisha	Trychogaster fasciata	10.12	4.56	15.12	20
13	Chanda	Pseudambassis ranga	4.10	2.54	3.57	40
14	Boro baim	Mastacembelus armatus	40.53	5.67	267.98	3
15	Chewa	Pseudapocryptes elongatus	24.57	2.56	35.60	30
16	Foli	Notopterus notopterus	18.27	7.23	65.30	8
17	Meni	Nandus nandus	15.27	4.27	48.21	15
18	Shol	Channa striata	43.39	9.48	790.68	3
19	Bele	Glossogobius giuris	22.35	5.43	150.99	8
20	Poa	Otolithoides pama	21.20	4.67	119.37	8
21	Bacha	Eutropiichthys vacha	21.53	5.98	48.96	6
22	Boal	Wallago attu	77.49	15.28	1552.82	3
	1	1	1	i	i	

Table 3.3. Average length, width, weight, and sample number of fish and prawn samples during winter

No	Common Name	Scientific Name	Length (cm)	Width (cm)	Weight (g)	n=
1	Rui	Labeo rohita	42.89	13.78	1900.59	3
2	Ghainna	Labeo gonius	25.48	7.18	780.90	3
3	Bata	Cirrhinus reba	12.63	2.13	27.11	12
4	Jatpunti	Puntius sophore	7.77	3.45	10.89	20
5	Shar punti	Systomus sarana	21.35	6.23	134.24	10
6	Tengra	Mystus vittatus	6.98	1.25	5.22	35
7	Bajari-tengra	Mystus tengra	2.20	0.97	1.19	40
8	Gulsha	Mystus cavaius	14.23	2.34	17.24	15
9	Shing	Heteropnuestes fossilis	18.13	2.98	38.45	20
10	Magur	Clarias batracus	16.87	3.39	47.25	19
11	Kachki	Corica soborna	2.92	0.72	0.82	222
12	Golda Chingri	Macrobrachium rosenbergii	27.12	4.27	186.68	8
13	Chanda	parambassis ranga	4.23	1.60	2.67	40
14	Tara Baim	Macrognathus aculiatus	5.23	1.89	17.67	15
15	Boro baim	Mastacembelus armatus	40.32	5.12	198.34	5
16	Chewa	Pseudapocryptes elongatus	21.34	2.23	32.60	30
17	Foli	Notopterus notopterus	18.27	6.98	69.12	6
18	Meni	Nandus nandus	13.37	4.56	52.71	15
19	Shol	Channa striata	37.59	8.38	789.98	3
20	Bele	Glossogobius giuris	23.23	5.13	140.29	8
21	Poa	Otolithoides pama	21.20	4.67	120.17	8
22	Bacha	Eutropiichthys vacha	23.13	6.85	51.90	6
23	Boal	Wallago attu	72.49	15.28	1412.22	3
	l				1	

Table 3.4. Average length, width, weight and feeding habit of fish and prawn samples collected during summer

No	Name	Scientific Name	Length (cm)	Width (cm)	Weight (g)	n=
1	Rui	Labeo rohita	62.90	19.98	4600.59	3
2	Ghainna	Labeo gonius	32.48	9.78	856.56	3
3	Bata	Cirrhinus reba	15.63	3.53	30.11	10
4	Jat punti	Puntius sophore	7.87	4.13	11.79	30
5	Sharpunti	Systomuss sarana	26.53	6.96	146.64	15
6	Tengra	Mystus vittatus	6.98	2.13	6.14	35
7	Bajari-tengra	Mystus tengra	5.69	1.52	1.78	40
8	Gulsha	Mystus cavaius	19.81	3.81	22.54	12
9	Sing	Heteropnuestes fossilis	16.43	3.17	40.23	12
10	Kachki	Corica soborna	3.29	0.73	0.84	200
11	Golda chingri	Macrobrachium rosenbergii	20.58	3.77	182.18	8
12	Chanda	Pseudambassis ranga	4.13	1.57	3.27	40
13	Boro Baim	Mastacembelus armatus	40.32	5.00	204.60	3
14	Chewa	Pseudapocryptes elongatus	20.67	2.56	34.23	35
15	Foli	Notopterus notopterus	20.14	6.83	70.13	8
16	Meni	Nandus nandus	15.65	5.20	51.87	12
17	Shol	Channa striata	46.39	7.45	800.68	3
18	Gajar	Channa marulius	68.56	11.56	2025.34	3
19	Bele	Glossogobius giuris	22.43	4.78	150.19	8
20	Poa	Otolithoides pama	21.32	5.89	125.47	7
21	Bacha	Eutropiichthys vacha	23.19	6.98	54.26	6
22	Boal	Wallago attu	76.89	12.98	1502.22	3

#### 3.1.2. Lipid contents of fish and prawn samples of different seasons

## 3.1.2.1. Lipid contents during rainy-season

In rainy-season, the lipid contents (%) of different fish and prawn species ranged from 0.53  $\pm$  0.01% in Boal to 13.98  $\pm$  1.50% in Bacha (Table 3.5). Considering the amount of lipid the chronology is Boal < Kaikka <Golda-chingri < Kachki < Bele < Poa < Meni < Shing < Chewa < Foli < Chanda < Borobaim < Tarabaim < Bajari-tengra < Ghainna < Gutumn < Gulsha < Bata < Sharpunti < Tengra < Jatpunti < Magur < Rui. Among the analyzed fishes, Gulsha, Bata, Sharpunti, Tengra, Jatpunti, Magur, Rui and Bacha contained higher amount of lipid (4.67  $\pm$  0.29% to 13.98  $\pm$  1.50 %) while Chanda, Barabaim, Tarabaim, Bajari-tengra, Ghainna and Gutumn contained medium amount of lipid (2.65  $\pm$  0.05 % to 4.56  $\pm$  0.11 %) and Boal, Kaikka, Golda-chingri, Kachki, Bele, Poa, Meni, Shing , Chewa and Foli contained lower amount of lipid (0.53  $\pm$  0.03 % to 1.98  $\pm$  0.07 %) are shown in Fig. 3.1 where red coloured bar reported for higher, green for medium and blue for lower amount of lipid.

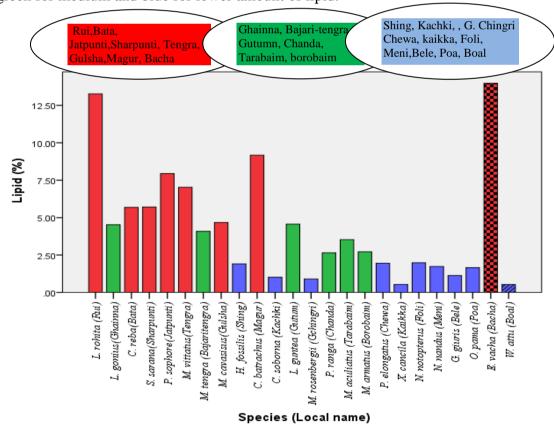


Fig. 3.1. Bar diagram of lipid content (%) of fish and prawn samples from the Meghna River during rainy-season

Table 3.5. Lipid content (%) of fish and prawn samples from the Meghna River during rainy-season

No.	Name	Scientific name	Lipid (%)	
			Mean ± SD	RSD
1	Rui	Labeo rohita	13.27±0.64	4.85
2	Ghainna	Labeo gonius	$4.52 \pm 0.41$	9.09
3	Bata	Cirrhinus reba	$5.68 \pm 0.27$	3.07
4	Jat punti	Puntius sophore	$7.94 \pm 0.22$	2.8
5	Sharpunti	Systomus sarana	$5.71 \pm 0.25$	4.34
6	Tengra	Mystus vittatus	$7.03 \pm 0.26$	3.76
7	Bajari-tengra	Mystus tengra	$4.08 \pm 0.23$	12.00
8	Gulsha	Mystus cavasius	$4.67 \pm 0.29$	6.28
9	Shing	Heteropnuestes fossilis	$1.91 \pm 0.01$	0.21
10	Magur	Clarias batrachus	9.17 ± 1.47	16.02
11	Kachki	Corica soborna	$1.01 \pm 0.15$	15.2
12	Gutum	Lepidocephalus guntea	$4.56 \pm 0.11$	2.32
13	Golda Chingri	Macrobrachium rosenbergii	$0.90 \pm 0.07$	7.63
14	Chanda	Parambassis ranga	$2.65 \pm 0.05$	1.71
15	Tara baim	Macrognathus aculiatus	$3.53 \pm 0.13$	3.62
16	Boro baim	Mastacembelus armatus	$2.71 \pm 0.17$	6.14
17	Chewa	Pseudapocryptes elongatus	$1.95 \pm 0.03$	0.42
18	Kaikka	Xenentodon cancila	$0.53 \pm 0.10$	2.67
19	Foli	Notopterus notopterus	$1.98 \pm 0.07$	3.34
20	Meni	Nandus nandus	1.73 ±0.15	8.73
21	Bele	Glossogobius giuris	$1.13 \pm 0.02$	1.69
22	Poa	Otolithoides pama	$1.65 \pm 0.01$	0.43
23	Bacha	Eutropiichthys vacha	$13.98 \pm 1.50$	9.63
24	Boal	Wallago attu	$0.53 \pm 0.03$	5.75

<sup>\*</sup>SD = Standard Deviation, RSD= Relative Standard Deviation

## 3.1.2.2. Lipid contents during autumn

In autumn season, the mean lipid contents (%) of different fish and prawn samples ranged from  $0.51 \pm 0.06\%$  in Boal to  $14.43 \pm 0.64\%$  in Rui (Table 3.6) and the increased in the following order: Goldga-chingri < Shole < kachki < Chanda < Bele < Chewa < Foli < Poa < Shing < Meni < Bajari-tengra < Ghainna < Khalisha < Borobaim < Bata < Gulsha < Tengra < Jatpunti < Sharpunti < Bacha < Rui. Among the analyzed fishes, Borobaim, Bata, Gulsha, Tengra, Jatpunti, Sharpunti, Bacha, Rui contained higher amount of lipid ( $5.52 \pm 0.16\%$  to  $14.43 \pm 0.64\%$ ) while Foli, Poa, Shing, Meni, Bajari-tengra, Ghainna, Khalish contained medium amount of lipid ( $2.30 \pm 0.30\%$  to  $4.95 \pm 0.70\%$ ) and Boal, Goldachingri, Shole, Kachki, Chanda, Bele and Chewa contained lower amount of lipid ( $0.51 \pm 0.06\%$  to  $2.01 \pm 0.09\%$ ) are shown in Fig. 3.2 where red coloured bar reported for higher, green for medium and blue for lower amount of lipid.

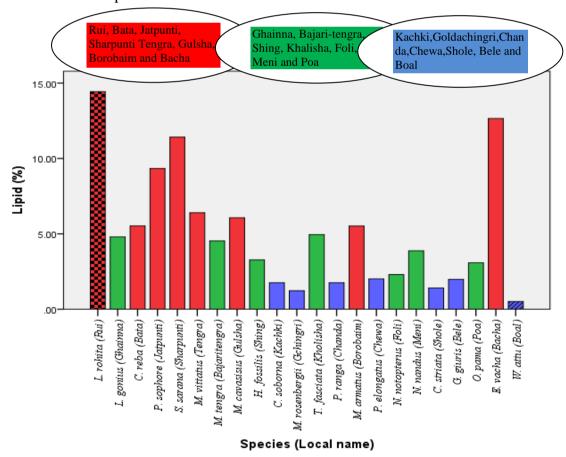


Fig. 3.2. Bar diagram of lipid content (%) of fish and prawn samples from the Meghna River during autumn

Table 3.6. Lipid content (%) of fish and prawn samples from the Meghna River during autumn

No.	Name	Scientific Name	Lipid (%)				
			Mean ± SD	RSD			
1	Rui	Labeo rohita	$14.43 \pm 0.64$	4.46			
2	Ghainna	Labeo gonius	$4.80 \pm 0.38$	7.98			
3	Bata	Cirrhinus reba	$5.53 \pm 0.33$	5.93			
4	Jat punti	Puntius sophore	$9.34 \pm 0.16$	1.74			
5	Sharpunti	Systomus sarana	11.42 ± 1.03	9.04			
6	Tengra	Mystus vittatus	$6.40 \pm 0.17$	2.64			
7	Bajari-tengra	Mystus tengra	$4.53 \pm 0.35$	9.11			
8	Gulsha	Mystus cavsius	$6.07 \pm 0.28$	4.68			
9	Shing	Heteropnuestes fossilis	$3.27 \pm 0.22$	6.56			
10	Kachki	Corica soborna	$1.76 \pm 0.03$	1.47			
11	Golda Chingri	Macrobrachium rosenbergii	$1.23 \pm 0.12$	14.05			
12	Khalisha	Trychogaster fasciata	$4.95 \pm 0.70$	14.13			
13	Chanda	Parambassis ranga	$1.76 \pm 0.37$	20.91			
14	Boro baim	Mastacembelus armatus	$5.52 \pm 0.16$	2.88			
15	Chewa	Pseudapocryptes elongatus	$2.01 \pm 0.09$	4.26			
16	Foli	Notopterus notopterus	$2.30 \pm 0.30$	13.18			
17	Meni	Nandus nandus	$3.88 \pm 0.03$	0.75			
18	Shol	Channa striata	$1.41 \pm 0.15$	10.32			
19	Bele	Glossogobius giuris	$1.98 \pm 0.13$	5.98			
20	Poa	Otolithoides pama	$3.08 \pm 0.62$	6.98			
21	Bacha	Eutropiichthys vacha	$12.65 \pm 0.76$	12.44			
22	Boal	Wallogo attu	$0.51 \pm 0.06$	11.42			

<sup>\*</sup>SD = Standard Deviation, RSD= Relative Standard Deviation

## 3.1.2.3. Lipid contents during winter

In the winter, the mean lipid contents (%) of different fish and prawn species ranged from  $0.41\pm0.07$  in Shole to  $13.66\pm0.56\%$  in Rui (Table 3.7.). Considering the value the chronology is Shole < Chewa < Boal < Golda-chingri < Tarabaim < Chanda < Kachki < Foli < Bele < Shing < Poa < Borobaim < Meni < Bajari tengra < Ghainna < Gulsha < Tengra < Magur < Bata < Sharpunti < Jatpunti < Bacha < Rui. In the analyzed fishes Rui, Bacha, Jatpunti, Sharpunti, Bata, Magur, Tengra, Gulsha contained higher amount of lipid  $(6.07\pm0.28\%$  to  $13.66\pm0.56\%$ ), Ghainna, Bajaritengra, Meni and Borobaim contained medium amount  $(3.49\pm0.21\%$  to  $4.60\pm0.39\%$ ) and Poa, Shing, Bele, Foli, Kachki, Chanda, Tarabaim, Goldachingri, Boal, Chewa and Shole contained lower amount of lipid  $(0.41\pm0.07\%$  to  $2.39\pm0.56\%$ ) are shown in Fig. 3.3 where red coloured bar reported for higher, green for medium and blue for lower amount of lipid.

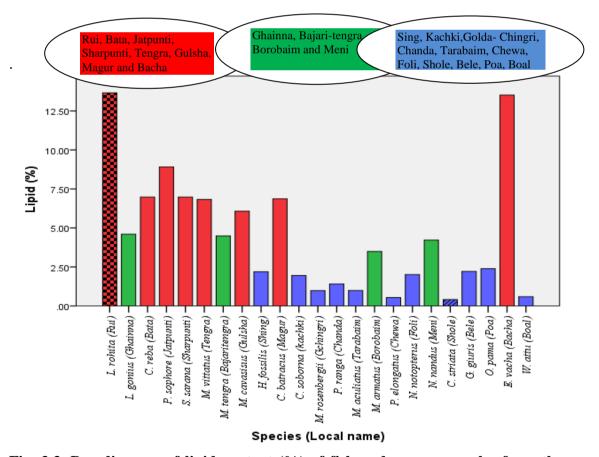


Fig. 3.3. Bar diagram of lipid content (%) of fish and prawn samples from the Meghna River during winter

Table 3.7. Lipid contents (%) of fish and prawn samples from the Meghna River during winter

No.	Common Name	Scientific name	Lipid (%)					
			Mean ± SD	RSD				
1	Rui	Labeo rohita	$13.66 \pm 0.56$	9.80				
2	Ghainna	Labeo gonius	$4.60 \pm 0.39$	8.58				
3	Bata	Cirrhinus reba	$6.98 \pm 0.76$	12.76				
4	Jat punti	Puntius sophore	$8.91 \pm 0.54$	6.04				
5	Shar punti	Systomus sarana	$6.98 \pm 0.13$	13.09				
6	Tengra	Mystus vittatus	$6.83 \pm 0.17$	2.49				
7	Bajari-tengra	Mystus tengra	$4.49 \pm 0.20$	4.55				
8	Gulsha	Mystus cavasius	$6.07 \pm 0.28$	4.28				
9	Shing	Heteropnuestes fossilis	$2.19 \pm 0.18$	8.01				
10	Magur	Clarias batracus	$6.87 \pm 1.50$	21.76				
11	Kachki	Corica soborna	$1.95 \pm 0.13$	6.44				
12	Golda Chingri	Macrobrachium rosenbergii	$0.98 \pm 0.06$	1.18				
13	Chanda	Parambassis ranga	$1.41 \pm 0.06$	4.18				
14	Tara Baim	Macrognathus aculiatus	$0.99 \pm 0.12$	1.52				
15	Boro baim	Mastacembelus armatus	$3.49 \pm 0.21$	6.02				
16	Chewa	Pseudapocryptes elongatus	$0.54 \pm 0.04$	7.85				
17	Foli	Notopterus notopterus	$2.01 \pm 0.29$	5.78				
18	Meni	Nandus nandus	$4.22 \pm 0.08$	1.96				
19	Shol	Channa striata	$0.41 \pm 0.07$	16.50				
20	Bele	Glossogobius giuris	$2.21 \pm 0.12$	2.76				
21	Poa	Otolithoides pama	$2.39 \pm 0.56$	8.90				
22	Bacha	Eutropiichthys vacha	$13.52 \pm 1.37$ $10.1$					
23	Boal	Wallogo attu	$0.59 \pm 0.03$	4.23				

<sup>\*</sup>SD = Standard Deviation, RSD= Relative Standard Deviation

## 3.1.2.4. Lipids contents during summer

In summer season, the mean lipid contents (%) of different fish and prawn species ranged from 0.12  $\pm$  0.01% in Boal to 15.57  $\pm$  0.01% in Bacha (Table 3.8.). Considering the value the chronology is Boal < Gojar < Shole < Golda chingri < Foli < Chewa < Bele < Kachki < Chanda < Shing < Borobaim < Poa < Meni < Ghainna < Gulsha < Sharpunti < Tengra< Bajari-tengra < Bata < Jatpunti < Rui < Bacha. Among the analyzed fishes, Gulsha, Sharpunti, Tengra, Bajari-tengra, Jatpunti, Rui, Bacha contained higher amount of lipid (6.67  $\pm$  0.13% to 15.57  $\pm$  0.14%) while Chanda, Shing, Borobain, Meni, Ghainna contained medium amount of lipid (2.88  $\pm$  0.01% to 4.59  $\pm$  0.03%) and Boal, Gojar, Shole, Goldachingri, Foli, Chewa, Bele and Kachki contained lower amount of lipid (0.12  $\pm$  0.01% -1.99  $\pm$  0.15%) shown in Fig. 3.4 where red coloured bar reported for higher, green for medium and blue for lower amount of lipid.

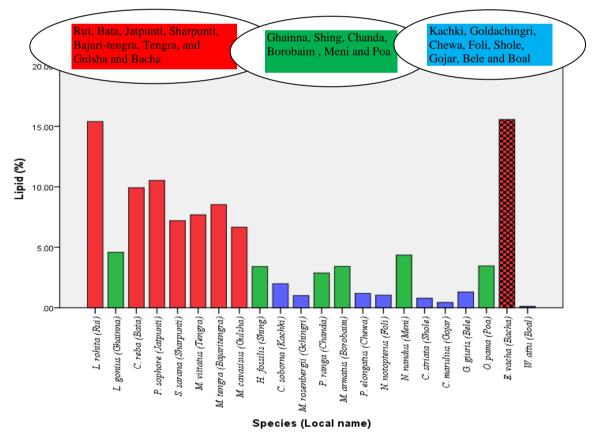


Fig. 3.4. Bar diagram of lipid content (%) of fish and prawn samples from the Meghna River during summer

Table 3.8. Lipid content (%) of fish and prawn samples from the Meghna River during summer

No.	Name	Scientific name	Lipid ((%)				
			Mean ± SD	RSD			
1	Rui	Labeo rohita	$15.40 \pm 0.01$	0.77			
2	Ghainna	Labeo gonius	$4.59 \pm 0.34$	7.48			
3	Bata	Cirrhinus reba	9.93 ± 1.24	2.53			
4	Jat punti	Puntius sophore	$10.53 \pm 0.15$	4.28			
5	Sharpunti	Systomus sarana	$7.20 \pm 0.01$	0.06			
6	Tengra	Mystus vittatus	$7.69 \pm 0.11$	2.98			
7	Bajari-tengra	Mystus tengra	$18.53 \pm 0.58$	3.12			
8	Gulsha	Mystus cavaius	$6.67 \pm 0.13$	2.02			
9	Sing	Heteropnuestes fossilis	$3.41 \pm 0.01$ 3				
10	Kachki	Corica soborna	$1.99 \pm 0.15$	7.33			
11	Golda chingri	Macrobrachium rosenbergii	$1.01 \pm 0.01$	1.43			
12	Chanda	Parambassis ranga	$2.88 \pm 0.10$	3.28			
13	Boro Baim	Mastacembelus armatus	$3.42 \pm 0.02$	0.45			
14	Chawa	Pseudapocryptes elongatus	$1.19 \pm 0.01$	1.08			
15	Foli	Notopterus notopterus	$1.04 \pm 0.05$	4.76			
16	Meni	Nandus nandus	$4.36 \pm 0.19$	16.6			
17	Shol	Channa striata	$0.78 \pm 1.01$	129			
18	Gojar	Channa marulius	$0.44 \pm 0.03$	7.58			
19	Bele	Glossogobius giuris	$1.31 \pm 0.16$	5.10			
20	Poa	Otolithoides pama	$3.46 \pm 0.02$	0.61			
21	Bacha	Eutropiichthys vacha	$15.57 \pm 0.14$ 1.				
22	Boal	Wallago attu	$0.12 \pm 0.01$	5.24			

<sup>\*</sup>SD = Standard Deviation, RSD= Relative Standard Deviation

#### 3.1.3. DDT and its metabolite residues in four different seasons

#### 3.1.3.1. DDT and its metabolites during rainy-season

The mean concentrations of DDE, DDD, 2,4' DDT and 4,4' DDT residues in the fishes and prawn species during rainy-season are presented in the Table 3.9.

The concentrations of DDE residue ranged from  $0.58 \pm 0.01$  ng g<sup>-1</sup> in Ghainna to  $10.32 \pm 0.83$  ng g<sup>-1</sup> in Meni. The concentrations of DDD residue ranged from  $0.71 \pm 0.09$  ng g<sup>-1</sup> in Bajari-tengra to  $170.83 \pm$  ng g<sup>-1</sup> in Bacha. The mean concentrations of 2,4′-DDT residue ranged from BDL (Below detection limit) in Poa to  $21.65 \pm 2.81$ ng g<sup>-1</sup> in Gulsha. The concentrations of 4,4′-DDT residue ranged from  $0.17 \pm 0.05$  ng g<sup>-1</sup> in Kaikka to  $22.11 \pm 0.87$  ng g<sup>-1</sup> in Meni. The compositional profile of DDTs in fish and prawn samples showed the contribution of 4,4′-DDT (3.44-58.49%), 2,4′-DDT (4.85-89.37%), DDD (0.001-72.04%) and DDE (2.17-64.25%) during rainy-season. DDD (55.54%) was the major contributor in fish and prawn samples in this season and followed by 4,4′-DDT (16.10%), DDE (14.36%) and 2,4′-DDT(14.00%) (Fig. 3.5.).

### 3.1.3.2. DDT and its metabolites during autumn

The mean concentrations of DDE, DDD, 2,4′ DDT and 4,4′ DDT residues in twenty-two different fish and prawn species during autumn are presented in the Table 3.10.

The concentrations of DDE residue ranged from  $4.22\pm0.39$  ng g<sup>-1</sup> in khalisha to  $164.14\pm4.21$  ng g<sup>-1</sup> in Bacha. The concentrations of DDD residue ranged from  $3.27\pm0.63$  ng g<sup>-1</sup> in Shing to  $53.87\pm5.39$  ng g<sup>-1</sup> in Bacha. The value of 2,4'-DDT residue ranged from BDL in Bata to  $31.19\pm3.13$  ng g<sup>-1</sup> in Bacha. The concentrations of 4,4'-DDT residue ranged from  $0.06\pm0.003$  ng g<sup>-1</sup> in Bata to  $22.32\pm0.86$  ng g<sup>-1</sup> in Bacha. The compositional profile of DDTs in fish and prawn samples showed the contribution of 4,4'-DDT(15.12-89.29%), 2,4'-DDT(15.12-89.29%), and 15.12-89.29%).

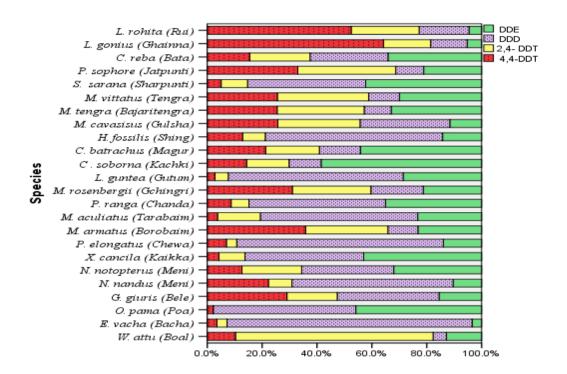


Fig. 3.5. Composition profile (%) of DDTs in fish and prawn samples during rainy-season

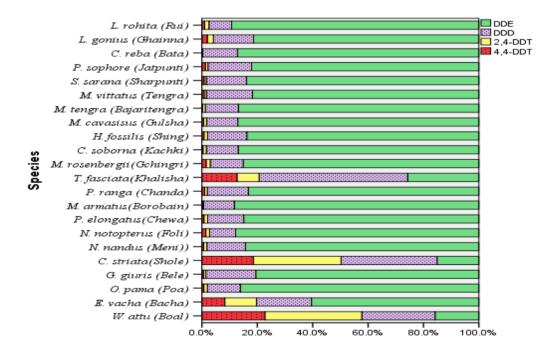


Fig. 3.6. Composition profile (%) of DDTs in fish and prawn samples during autumn

Table 3.9. Level of different organohalogen pesticide residues of fishes collected during rainy season (March-August, 2015) from the Meghna River. (Values express as mean  $\pm$  SD in ng g<sup>-1</sup> in f.w., n = 3 replicates)

Number	Name	Scientific name	DDE		DDD		2,4'-DDT		4,4' - DDT		∑DDTs	
			Mean ± SD	RSD								
1	Rui	Labeo rohita	$0.62 \pm 0.02$	3.75	$2.45 \pm 0.20$	8.25	$3.34 \pm 0.21$	5.88	7.08 ±0.02	0.24	$13.48 \pm 0.07$	0.50
2	Ghainna	Labeo gonius	$0.58 \pm 0.01$	1.78	$1.47 \pm 0.13$	9.01	$1.89 \pm 0.17$	9.41	$7.08 \pm 0.20$	0.24	$11.02 \pm 0.13$	1.14
3	Bata	Cirrhinus reba	$2.27 \pm 0.08$	3.62	$1.89 \pm 0.27$	14.32	$1.46 \pm 0.14$	9.78	$1.03 \pm 0.05$	4.39	$6.65 \pm 0.40$	6.06
4	Jat punti	Puntius sophore	$2.62 \pm 0.17$	6.35	$1.26 \pm 0.06$	4.48	4.42 ±0.15	3.32	$4.09 \pm 0.48$	11.80	$12.39 \pm 0.63$	5.06
5	Sharpunti	Systomus sarana	$4.86 \pm 0.09$	1.87	$4.95 \pm 0.41$	8.17	$1.11 \pm 0.15$	13.41	$0.58 \pm 0.12$	20.31	$11.50 \pm 0.33$	2.84
6	Tengra	Mystus vittatus	$2.26 \pm 0.12$	5.22	$0.85 \pm 0.12$	13.75	$2.51 \pm 0.41$	16.15	1.93 ±0.26	13.51	$7.55 \pm 0.87$	11.53
7	Bajari-tengra	Mystus tengra	$2.38 \pm 0.07$	2.91	$0.71 \pm 0.09$	13.43	$2.29 \pm 0.06$	2.62	$1.84 \pm 0.14$	7.87	$7.22 \pm 0.36$	5.03
8	Gulsha	Mystus cavasius	$8.37 \pm 2.42$	28.9	$23.69 \pm 6.21$	26.20	$21.65 \pm 2.81$	12.97	$18.62 \pm 4.16$	2.12	$72.33 \pm 7.47$	10.32
9	Shing	Heteropnuestes fossilis	10.08 ±0.87	8.62	$45.86 \pm 2.83$	6.18	$5.87 \pm 0.94$	16.08	$9.12 \pm 1.45$	15.91	$70.93 \pm 2.43$	3.43
10	Magur	Clarias batrachus	$5.65 \pm 0.92$	16.22	$1.91 \pm 0.35$	18.29	$2.51 \pm 0.25$	10.13	$2.72 \pm 0.34$	12.39	12.79 ± 1.21	9.49
11	Kachki	Corica soborna	$1.55 \pm 0.08$	4.99	$0.31 \pm 0.21$	0.31	$0.41 \pm 0.03$	6.73	$0.38 \pm 0.05$	12.79	$2.64 \pm 0.35$	13.38
12	Gutum	Lepidocephalus guntea	$3.11 \pm 0.24$	7.76	6.94±0.10	1.42	$0.52 \pm 0.12$	6.04	$0.31 \pm 0.08$	13.80	$10.87 \pm 0.51$	3.39
13	Golda Chingri	Macrobrachium rosenbergii	$1.18 \pm 0.11$	9.06	$1.06 \pm 0.02$	2.25	1.59 ±0.33	20.66	$1.72 \pm 0.15$	8.54	$5.55 \pm 0.29$	3.87
14	Chanda	Parambassis ranga	$3.07 \pm 0.07$	2.39	$4.36 \pm 0.11$	2.55	$0.57 \pm 0.01$	0.48	0.76 ±0.01	0.96	$8.76 \pm 0.03$	0.34
15	Tara baim	Macrognathus aculiatus	$5.25 \pm 1.11$	11.53	$12.9 \pm 2.02$	15.62	3.52 ±0.35	9.92	$0.84 \pm 0.16$	19.19	$22.52 \pm 2.63$	10.20
16	Boro baim	Mastacembelus armatus	$7.55 \pm 0.09$	11.91	$3.57 \pm 0.19$	3.58	$9.76 \pm 2.03$	20.80	$11.66 \pm 0.90$	7.70	$32.54 \pm 3.72$	11.42
17	Chewa	Pseudapocryptes elongatus	$6.74 \pm 0.65$	9.66	$36.36 \pm 3.29$	9.07	1.83 ±0.10	5.69	$3.36 \pm 0.54$	15.97	48.29 ± 4.12	8.59
18	Kaikka	Xenentodon cancila	$1.72 \pm 0.12$	7.07	1.73 ±0.48	4.73	$0.38 \pm 0.60$	16.12	$0.17 \pm 0.05$	8.89	4.00 ±0.61	4.59
19	Foli	Notopterus notopterus	$1.75 \pm 0.42$	16	1.83 ±0.12	6.44	$1.19 \pm 0.05$	3.91	$0.69 \pm 0.01$	1.62	$5.45 \pm 0.34$	3.01
20	Meni	Nandus nandus	$10.32 \pm 0.83$	8.07	58.3±2.01	3.44	$8.53 \pm 1.19$	13.94	$22.11 \pm 0.87$	3.93	99.23 ± 2.75	2.77
21	Bele	Glossogobius giuris	$7.72 \pm 1.05$	13.67	$18.46 \pm 3.11$	16.84	$9.13 \pm 1.66$	19.76	$14.45 \pm 1.51$	10.48	49.77 ± 4.04	8.12
22	Poa	Otolithoides pama	$8.02 \pm 0.06$	0.79	$9.09 \pm 0.40$	4.38	nd		$0.38 \pm 0.01$	3.18	$17.50 \pm 0.65$	3.66
23	Bacha	Eutropiichthys vacha	$6.58 \pm 1.02$	15.44	170.83 ± 29.31	17.16	$7.05 \pm 0.67$	4.99	$6.68 \pm 0.43$	6.51	191.14 ± 31.18	16.31
24	Boal	Wallago attu	$2.17 \pm 0.04$	1.92	$0.82 \pm 0.15$	19.13	$12.19 \pm 0.21$	9.46	$1.74 \pm 0.19$	11.13	16.91 ± 0.54	7.77
Range		•	0.58 - 10.32		0.71 -170.83		nd - 21.65		0.17 - 22.11		2.64 - 191.14	

<sup>\*</sup>f.w.- Fresh weight, nd- Non detectable, SD-Standard deviation, RSD- Relative standard deviation

Table 3.10. Level of different organohalogen pesticide residues of fishes collected during autumn (October-November 2015) from the Meghna River. (Values express as mean  $\pm$  SD in ng g<sup>-1</sup> in f.w., n=3 replicates)

No.	Name	Scientific name	DDE		DDD		2,4'- DDT		4,4' - DDT		∑DDTs	
			Mean ± SD	RSD								
1	Rui	Labeo rohita	$41.28 \pm 6.04$	14.62	$3.77 \pm 0.74$	19.5	$0.74 \pm 0.10$	13.67	$0.44 \pm 0.06$	13.25	$46.24 \pm 6.75$	14.60
2	Ghainna	Labeo gonius	$25.85 \pm 0.99$	3.84	$4.60 \pm 0.38$	8.16	$0.70 \pm 0.08$	11.06	$0.62 \pm 0.11$	17.55	$31.78 \pm 0.83$	2.60
3	Bata	Cirrhinus reba	27.64 ± 2.62	9.49	$4.00 \pm 0.24$	6.1	nd		$0.06 \pm 0.003$	5.54	31.70 ± 2.87	9.01
4	Jat punti	Puntius sophore	57.06 ± 0.77	1.34	$10.94 \pm 0.25$	2.33	$0.65 \pm 0.05$	7.36	$0.86 \pm 0.17$	19.50	$69.50 \pm 0.62$	0.89
5	Sharpunti	Systomus sarana	50.79 ± 2.73	5.38	$8.81 \pm 1.12$	12.7	$0.48 \pm 0.10$	20.58	$0.46 \pm 0.10$	20.99	$60.54 \pm 4.04$	6.67
6	Tengra	Mystus vittatus	64.80 ± 17.08	26.35	$13.23 \pm 1.47$	11.1	$0.66 \pm 0.13$	20.36	$0.60 \pm 0.12$	15.90	79.28 ± 18.38	23.18
7	Bajari-tengra	Mystus tengra	43.91 ± 2.27	5.16	$6.04 \pm 0.38$	0.38	$0.49 \pm 0.03$	5.18	$0.13 \pm 0.01$	11.38	50.57 ± 2.67	5.28
8	Gulsha	Mystus cavasius	53.56 ± 8.46	15.80	$6.89 \pm 0.68$	9.85	0.64 ±0.08	12.88	$0.40 \pm 0.09$	21.62	61.48 ±8.78	14.28
9	Shing	Heteropnuestes fossilis	19.36 ± 2.52	13.03	$3.27 \pm 0.63$	19.18	$0.30 \pm 0.06$	18.76	$0.17 \pm 0.02$	11.61	23.11 ± 1.98	8.56
10	Kachki	Corica soborna	37.51 ± 2.24	5.96	$4.98 \pm 0.25$	5.11	$0.51 \pm 0.08$	14.83	$0.17 \pm 0.02$	12.18	43.16 ± 2.01	4.67
11	Golda chingri	Macrobrachium rosenbergii	44.37 ± 4.16	9.38	$6.20 \pm 0.95$	15.3	$0.79 \pm 0.11$	14.26	$0.77 \pm 0.01$	1.19	52.13 ± 3.48	6.68
12	Khalisha	Trichogaster fasciata	$4.22 \pm 0.39$	9.28	8.81 ± 1.16	13.2	$1.31 \pm 0.03$	2.17	$2.08 \pm 0.34$	16.55	$16.42 \pm 1.90$	11.57
13	Chanda	Paarambassis ranga	34.15 ± 3.05	8.93	$6.09 \pm 0.96$	15.8	$0.39 \pm 0.04$	10.03	$0.40 \pm 0.02$	6.10	41.03 ± 4.03	9.81
14	Boro baim	Mastacembelus armatus	49.88 ± 4.75	9.52	$6.26 \pm 0.67$	10.6	$0.22 \pm 0.03$	13.60	$0.12 \pm 0.12$	12.01	56.49 ± 5.41	9.58
15	Chewa	Pseudapocryptes elongatus	$46.06 \pm 3.98$	8.64	$7.04 \pm 0.64$	9.14	$0.73 \pm 0.01$	13.27	$0.40 \pm 0.04$	9.42	54.24 ± 4.72	8.70
16	Foli	Notopterus notopterus	$51.19 \pm 6.02$	11.80	$5.48 \pm 1.60$	20	$0.78 \pm 0.11$	14.32	$0.82 \pm 0.01$	1.19	58.26 ± 7.29	12.50
17	Meni	Nandus nandus	63.30 ± 2.29	4.62	$10.46 \pm 1.33$	12.71	$0.83 \pm 0.15$	17.85	$0.50 \pm 0.12$	24.50	$75.08 \pm 4.45$	5.93
18	Shol	Channa striata	$8.79 \pm 2.19$	24.91	$20.13 \pm 4.73$	20	$18.38 \pm 3.94$	21.41	$10.83 \pm 2.26$	20.81	58.13 ± 8.14	14.01
19	Bele	Glossogobius giuris	63.85 ± 6.99	10.80	14.31 ± 1.35	9.46	$0.66 \pm 0.09$	15.37	$0.44 \pm 0.01$	1.57	$80.12 \pm 7.82$	9.76
20	Poa	Otolithoides pama	$53.47 \pm 3.62$	6.78	$7.39 \pm 0.92$	12.09	$0.78 \pm 0.09$	12.09	$0.42 \pm 0.04$	9.18	62.06 ± 4.18	6.73
21	Bacha	Eutropiichthys vacha	164.14 ±4.21	2.56	$53.87 \pm 5.93$	11.01	31.19 ±3.13	10.03	$22.32 \pm 0.86$	3.84	271.5 ±6.17	2.27
22	Boal	Wallago attu	11.33 ±0.74	6.54	$18.95 \pm 0.69$	3.67	25.02 ± 4.53	18.10	$16.24 \pm 2.09$	12.86	71.55 ± 7.70	10.76
Range	•	<b>.</b>	4.22 -164.14	1	3.27 – 53.87		nd-31.19	ı	0.06-22.32	1	16.42 – 271.5	

<sup>\*</sup>f.w.- Fresh weight, nd- Non detectable, SD-Standard deviation, RSD- Relative standard deviation

## 3.1.3.3. DDT and its metabolites during winter

The mean concentrations of DDE, DDD, 2,4′ DDT and 4,4′ DDT residues in twenty-three different fish and prawn species during winter are presented in the Table 3.11.

The concentrations of DDE ranged from  $1.83 \pm 0.06$  ng g<sup>-1</sup> in Tarabaim to  $48.16 \pm 5.99$  ng g<sup>-1</sup> in Bele. The concentrations DDD ranged from BDL in Goldachingri to  $81.42 \pm 15.01$ ng g<sup>-1</sup> in Bacha. The mean concentrations 2, 4′- DDT ranged from 0.16  $\pm 0.07$  ng g<sup>-1</sup>ng/g in Boal to  $19.65 \pm 3.94$  ng g<sup>-1</sup> in Borobaim. The concentrations 4, 4′- DDT ranged from  $0.13 \pm 0.01$  ng g<sup>-1</sup> in Shole to  $13.76 \pm 2.28$ ng g<sup>-1</sup> in Bacha.

The compositional profile of DDTs in fish and prawn samples showed the contribution of 4,4'-DDT(12.22-97.05%), 2,4'-DDT (0.001-65.15%), DDD (0.87-38.75%) (Fig. 3.7.) In winter the major contributor is DDE (68.80%) and followed by DDD(18.23%), 2,4'-DDT(7.63%) and 4,4'-DDT(5.33%).

#### 3.1.3.4. DDT and its metabolites summer

The mean concentrations of DDE, DDD, 2,4′ DDT and 4,4′ DDT residues in twenty-two different fish and prawn species during summer are presented in the Table 3.12.

The concentrations of DDE ranged from  $20.54 \pm 3.34$  ng g<sup>-1</sup> in Boal to  $476.53 \pm 48.16$ ng g<sup>-1</sup> in Bacha. The concentrations of DDD ranged from  $17.22 \pm 0.45$  ng g<sup>-1</sup> in Shing to  $329.71 \pm 4.08$  ng g<sup>-1</sup> in Bacha. The concentrations of 2 ,4' DDT ranged from  $45.0 \pm 5.80$  ng g<sup>-1</sup> in Kachki to  $427.22 \pm 3.63$  ng g<sup>-1</sup> in Poa. The concentrations of 4,4' DDT ranged from  $55.49 \pm 2.28$  ng g<sup>-1</sup> in Shing to  $625.39 \pm 32.88$  ng g<sup>-1</sup> in Gojar.

The compositional profile of DDTs in fish and prawn samples showed the contribution of 4,4'-DDT(4.73-29.05%), 2,4'-DDT(8.25-31.21%), DDD (11.93-40.85%) and DDE (29.51-65.12%) (Fig. 3.8). In this season, the major contributor is 4,4'-DDT(40.23%) and followed by 2,4'-DDT(27.59%), DDE (18.60%) and DDD (13.58%).

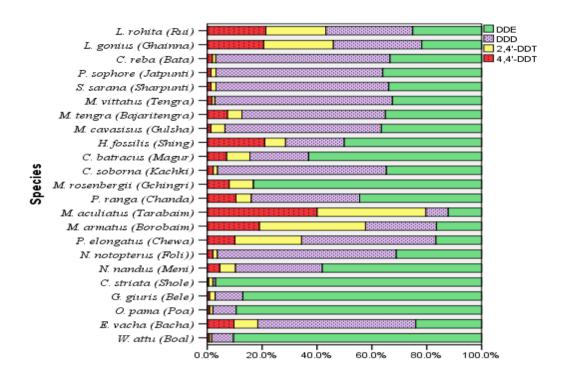


Fig. 3.7. Composition profile (%) of DDTs in fish and prawn samples during winter

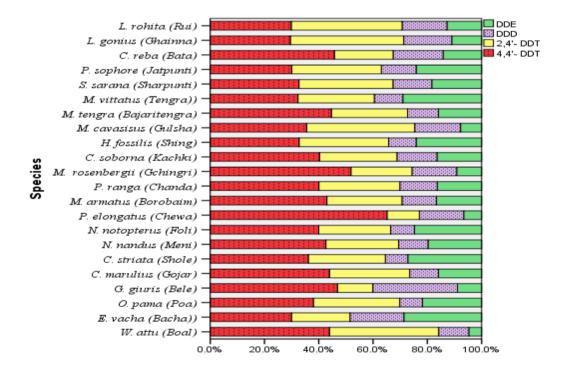


Fig. 3.8. Composition profile (%) of DDTs in fish and prawn samples during summer

Table 3.11. Level of different organohalogen pesticide residues of fishes collected during winter (December 2015-February 2016) from the Meghna River. (Values express as mean  $\pm$  SD in ng g<sup>-1</sup> in f.w., n= 3 replicates)

No.	Name	Scientific name	DDE		DDD	2,4'-DDT		4,4'-DDT		∑DDTs		
			Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD
1	Rui	Labeo rohita	$4.61 \pm 0.36$	4.89	$5.81 \pm 0.28$	4.88	4.01 ± 0.77	18.80	$3.92 \pm 0.75$	19.06	$18.35 \pm 2.06$	11.20
2	Ghainna	Labeo gonius	$3.37 \pm 0.43$	12.89	$4.97 \pm 0.36$	7.25	$3.92 \pm 0.35$	9.001	$3.16 \pm 0.32$	10.23	15.43 ± 0.78	5.04
3	Bata	Cirrhinus reba	$5.51 \pm 0.49$	15.51	$10.46 \pm 0.32$	3.04	$0.21 \pm 0.07$	7.16	$0.30 \pm 0.05$	17.19	$16.48 \pm 0.82$	4.73
4	Jat punti	Puntius sophore	$5.99 \pm 0.32$	5.28	$10.10 \pm 1.09$	10.78	$0.31 \pm 0.04$	11.15	$0.22 \pm 0.05$	21.02	16.62 ± 1.35	8.11
5	Shar punti	Sytomus sarana	5.06 ± 0.16	3.15	$9.40 \pm 0.26$	2.78	0.29 ±0.01	1.33	$0.19 \pm 0.02$	9.24	14.94 ± 0.36	2.39
6	Tengra	Mystus vittatus	$6.13 \pm 0.22$	6.13	$12.19 \pm 0.32$	2.63	$0.21 \pm 0.07$	6.78	$0.30 \pm 0.01$	1.77	$18.83 \pm 0.44$	2.41
7	Bajari-tengra	Mystus tengra	$8.08 \pm 1.48$	18.34	$12.00 \pm 1.78$	14.84	$1.22 \pm 0.07$	5.88	$1.69 \pm 0.42$	24.65	22.98 ± 3.19	13.88
8	Gulsha	Mystus cavasius	$7.06 \pm 0.89$	12.58	$10.99 \pm 1.29$	11.8	$0.99 \pm 0.29$	18.78	$0.25 \pm 0.02$	9.50	19.28 ± 1.96	10.53
9	Shing	Heteropnuestes fossilis	$2.11 \pm 0.38$	18.19	$0.90 \pm 0.02$	2.76	$0.32 \pm 0.05$	16.14	$0.88 \pm 0.16$	17.85	$4.21 \pm 0.32$	7.68
10	Magur	Clarias batracus	$6.29 \pm 0.19$	2.99	$2.13 \pm 0.22$	10.36	$0.85 \pm 0.11$	12.49	$0.70 \pm 0.09$	12.52	$9.97 \pm 0.24$	2.31
11	Kachki	Corica soborna	$3.87 \pm 0.27$	6.85	$6.85 \pm 0.48$	7.03	$0.17 \pm 0.01$	6.69	$0.24 \pm 0.05$	8.27	$11.13 \pm 0.82$	7.34
12	Golda Chingri	Macrobrachium rosenbergii	$3.23 \pm 0.66$	18.65	nd		$0.34 \pm 0.04$	10.68	$0.31 \pm 0.03$	8.81	$3.88 \pm 0.60$	15.52
13	Chanda	Paraambassis ranga	$6.03 \pm 0.61$	10.05	$5.36 \pm 0.36$	6.67	$0.75 \pm 0.04$	5.55	$1.42 \pm 0.03$	1.82	$13.57 \pm 0.85$	6.30
14	Tara Baim	Macrognathus aculiatus	$1.83 \pm 0.06$	3.01	$1.22 \pm 0.11$	8.69	$5.93 \pm 0.31$	5.21	$6.00 \pm 0.51$	8.56	$14.98 \pm 0.88$	5.90
15	Boro baim	Mastacembelus armatus	$8.35 \pm 0.99$	11.86	$13.10 \pm 1.98$	5.14	$19.65 \pm 3.94$	20.05	9.61 ± 1.31	4.64	50.70 ± 5.24	10.33
16	Chewa	Pseudapocryptes elongatus	$4.16 \pm 0.50$	11.98	12.17± 2.89	23.75	$6.04 \pm 1.01$	16.70	$2.52 \pm 0.40$	13.61	24.90 ± 2.01	8.07
17	Foli	Notopterus notopterus	$3.23 \pm 0.43$	13.31	$6.75 \pm 0.32$	4.67	$0.17 \pm 0.01$	2.70	$0.21 \pm 0.04$	16.87	$10.36 \pm 0.68$	6.61
18	Meni	Nandus nandus	9.01 ± 1.47	16.36	$4.90 \pm 0.09$	1.77	$0.88 \pm 0.06$	6.28	$0.71 \pm 0.06$	7.89	15.49 ± 1.37	8.83
19	Shol	Channa striata	26.30 ± 4.22	16.06	$0.25 \pm 0.06$	23.42	$0.42 \pm 0.04$	10.57	$0.13 \pm 0.01$	4.64	27.11 ± 4.31	15.88
20	Bele	Glossogobius giuris	$48.16 \pm 5.99$	12.44	$5.57 \pm 1.02$	18.29	$1.17 \pm 0.11$	9.60	$0.43 \pm 0.06$	14.21	55.33 ± 6.97	12.59
21	Poa	Otolithoides pama	39.67 ± 4.68	11.79	$3.77 \pm 0.68$	18.01	$0.52 \pm 0.07$	13.16	$0.37 \pm 0.05$	12.58	44.33 ± 4.77	10.77
22	Bacha	Eutropiichthys vacha	34.06 ±3.39	9.96	81.42 ±15.01	18.38	12.33 ±1.77	14.32	13.76 ±2.28	16.59	141.57 ± 10.24	7.23
23	Boal	Wallago attu	$16.68 \pm 0.06$	3.60	$1.45 \pm 0.21$	14.31	$0.16 \pm 0.003$	1.94	$0.14 \pm 0.02$	13.01	$18.44 \pm 0.76$	4.10
Range	•	•	1.83 - 48.16	•	nd-81.42	•	0.16 -19.65		0.13 – 13.76	•	3.88 141.57	•

<sup>\*</sup>f.w.- Fresh weight, nd- Non detectable, SD-Standard deviation, RSD- Relative standard deviation

Table 3.12. Level of different organohalogen pesticide residues of fishes collected during summer (March - May 2015) from the Meghna River. (Values express as mean  $\pm$  SD in ng g<sup>-1</sup> in f.w., n = 3 replicates)

No.	Name	Scientific name	DDE		DDD		2,4'-DDT		4,4'-DDT		∑DDTs	
			Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD	Mean ± SD	RSD
1	Rui	Labeo rohita	32.01 ± 3.71	11.58	$41.42 \pm 0.65$	1.56	$102.28 \pm 2.95$	2.88	$74.68 \pm 8.97$	12.01	$250.40 \pm 9.17$	3.66
2	Ghainna	Labeo gonius	25.81 ± 2.28	3.84	41.42 ± 5.11	11.06	97.81 ± 2.80	2.87	69.10 ± 2.14	3.10	$234.15 \pm 0.65$	0.28
3	Bata	Cirrhinus reba	43.90 ±2.69	6.14	57.02 ± 2.51	4.41	66.96 ± 4.86	7.26	141.53 ± 10.72	7.58	309.42 ± 20.36	6.58
4	Jat punti	Puntius sophore	68.86 ± 8.81	12.79	$36.72 \pm 5.22$	14.23	94.35 ± 6.69	7.09	85.78 ± 15.83	18.45	285.71 ± 15.30	5.36
5	Sharpunti	Systomus sarana	49.49 ± 2.33	4.70	$38.45 \pm 6.03$	15.68	93.23 ± 5.10	5.47	88.02 ± 12.08	13.72	269.19 ± 15.81	5.87
6	Tengra	Mystus vittatus	$138.01 \pm 4.72$	3.42	49.86 ± 1.61	3.23	133.81 ± 4.59	3.43	153.44 ± 3.94	2.57	475.11 ± 11.67	2.46
7	Bajari-tengra	Mystus tengra	92.59 ± 3.24	3.50	66.42 ± 4.29	6.47	163.54 ± 19.67	12.03	259.90 ± 5.401	2.08	582.44 ± 32.29	5.54
8	Gulsha	Mystus cavasius	31.42 ± 6.42	20.43	$68.55 \pm 12.07$	17.60	161.32 ±18.82	11.67	143.83 ± 11.83	8.23	405.12 ± 48.42	11.95
9	Sing	Heteropnuestes fossilis	40.86 ± 3.87	9.48	$17.22 \pm 0.45$	2.59	55.88 ± 1.78	3.19	$55.49 \pm 2.28$	4.12	169.45 ± 8.11	4.79
10	Kachki	Corica soborna	$25.91 \pm 0.52$	2.02	23.23 ± 1.45	6.24	45.01 ± 5.80	12.88	63.42 ± 2.77	4.37	$157.58 \pm 1.15$	0.73
11	Golda-chingri	Macrobrachium rosenbergii	$30.65 \pm 0.24$	0.77	$54.93 \pm 0.24$	0.44	74.79 ± 1.07	1.44	172.70 ± 1.21	0.70	333.07 ± 2.70	0.81
12	Chanda	Parambassis ranga	25.91 ± 4.88	18.84	$21.84 \pm 1.70$	7.80	47.25 ± 4.73	10.01	$63.42 \pm 2.77$	4.37	158.43 ± 5.72	3.61
13	Boro Baim	Mastacembelus armatus	$185.84 \pm 8.19$	4.40	$142.60 \pm 18.90$	13.25	$309.20 \pm 7.28$	2.35	480.60 ± 7.19	1.50	1118.24 ± 34.74	3.11
14	Chawa	Pseudapocryptes elongatus	46.82 ± 1.57	3.35	$116.07 \pm 2.24$	1.93	84.73 ± 2.48	2.93	462.36 ± 8.41	1.82	709.98 ± 11.07	1.56
15	Foli	Notopterus notopterus	$10403 \pm 2.25$	2.16	37.11 ± 1.56	4.2	111.35 ± 6.103	5.48	168.23 ± 4.70	2.79	420.72 ± 13.96	3.32
16	Meni	Nandus nandus	173.01 ± 5.93	3.43	$94.87 \pm 8.42$	8.88	233.95 ± 18.27	7.80	372.91 ± 45.03	12.07	874.74 ± 31.15	3.56
17	Shol	Channa striata	210.31 ± 8.25	3.92	$63.76 \pm 3.14$	4.92	219.46 ± 4.93	2.25	279.07 ± 14.87	5.33	772.59 ± 26.21	3.39
18	Gojar	Channa marulius	226.20 ± 11.24	4.97	150.92 ± 1.19	0.79	419.98 ± 36.69	8.74	625.39 ± 32.88	5.26	$1422.48 \pm 77.83$	5.47
19	Bele	Glossogobius giuris	34.61 ± 1.55	4.47	121.09 ± 1.13	0.94	50.45 ± 4.24	8.41	$181.84 \pm 1.80$	0.99	387.99 ± 8.52	2.20
20	Poa	Otolithoides pama	292.78 ± 1.95	0.67	$113.16 \pm 5.70$	5.04	427.22 ± 3.63	0.85	$510.60 \pm 3.60$	0.71	1343.77 ± 6.43	0.48
21	Bacha	Eutropiichthys vacha	476.53 ± 48.16	10.11	329.71 ± 4.08	1.24	$357.86 \pm 70.30$	19.6	496.80 ± 62.16	12.51	$1660.89 \pm 157.9$	9.51
22	Boal	Wallago attu	20.54 ±3.34	16.28	48.23 ±6.73	13.96	174.74 ±17.14	9.81	190.74 ± 8.81	4.62	443.24 ± 35.72	8.23
Rang	ge	•	20.54 - 476.53	•	17.22 – 329.71	•	45.01 – 427.22	•	55.49 - 625.39	•	157.58 – 1660.89	•

<sup>\*</sup>f.w.- Fresh weight, nd- Non detectable, n-3, Replicate number is three, SD-Standard deviation, RSD- Relative standard deviation

## 3.1.4. Total DDTs residues of fishes and prawn of different seasons

## 3.1.4.1. Total DDTs ( $\sum$ DDTs) residues during rainy-season

The mean concentrations of total DDTs ( $\Sigma$ DDTs) residue (ng g <sup>-1</sup> ww) in fishes and prawn during rainy-season are shown in Table. 3.13. In this season  $\Sigma$ DDTs ranged from 2.64  $\pm$  0.35 ng g <sup>-1</sup> in Kachki to 191.14  $\pm$  31.18 ng g <sup>-1</sup> in Bacha and increased in the following order: Kachki < Kaikka < Foli < G.Chingri < Bata < Boal < Bajaritengra < Tengra < Chanda < Gutumn < Ghainna < Sharpunti < Jatpunti < Magur < Rui < Tarabaim < Poa < Borobaim < Chewa < Bele < Shing < Gulsha< Meni < Bacha. For fish consumption, European Union (Binelli and Provini, 2004) has established a Maximum admissible concentration (MAC) of 50 ng g <sup>-1</sup> ww for  $\Sigma$ DDTs. Considering the valueof MAC, Gulsha, Shing, Meni, Bele and Bacha contained higher amount of  $\Sigma$ DDTs (>50 ng g <sup>-1</sup> ww) while the remaining others contained lower (<50 ng g <sup>-1</sup> ww) amount are shown in Fig. 3.9 where brown bar represents higher and green for lower amount.

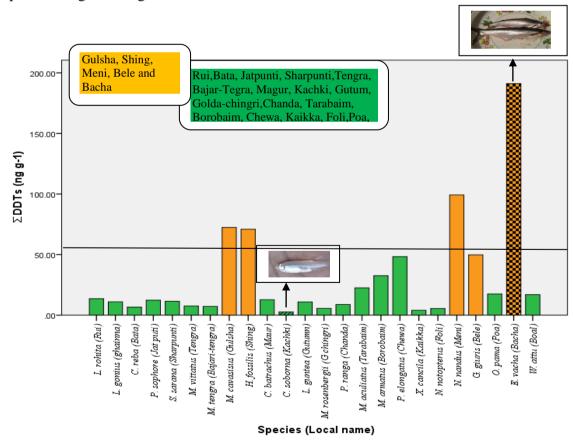


Fig. 3.9. Bar diagram of total DDTs ( $\sum$ DDTs) residues in fish and prawn samples from the Meghna River during rainy-season

Table 3.13.  $\sum$ DDTs residues in fish and prawn samples from the Meghna River during rainy-season (values express as mean  $\pm$  SD in ng g<sup>-1</sup> in wet weight- w.w.)

No.	Name	Scientific name	∑DDTs (ng g <sup>-1</sup> in	$\sum$ DDTs (ng g <sup>-1</sup> in ww)		
			Mean ± SD	RSD		
1	Rui	Labeo rohita	$13.48 \pm 0.07$	0.50		
2	Ghainna	Labeo gonius	$11.02 \pm 0.13$	1.14		
3	Bata	Cirrhinus reba	$6.65 \pm 0.40$	6.06		
4	Jat punti	Puntius sophore	$12.39 \pm 0.63$	5.06		
5	Sharpunti	Systomus sarana	$11.50 \pm 0.33$	2.84		
6	Tengra	Mystus vittatus	$7.55 \pm 0.87$	11.53		
7	Bajari-tengra	Mystus tengra	$7.22 \pm 0.36$	5.03		
8	Gulsha	Mystus cavasius	$72.33 \pm 7.47$	10.32		
9	Shing	Heteropnuestes fossilis	$70.93 \pm 2.43$	3.43		
10	Magur	Clarias batrachus	12.79 ± 1.21	9.49		
11	Kachki	Corica soborna	$2.64 \pm 0.35$	13.38		
12	Gutum	Lepidocephalus guntea	$10.87 \pm 0.51$	3.39		
13	Golda Chingri	Macrobrachium rogenbergii	$5.55 \pm 0.29$	3.87		
14	Chanda	Parambassis ranga	$8.76 \pm 0.03$	0.34		
15	Tara baim	Macrognathus aculiatus	$22.52 \pm 2.63$	10.20		
16	Boro baim	Mastacembelus armatus	$32.54 \pm 3.72$	11.42		
17	Chewa	Pseudapocryptes elongatus	$48.29 \pm 4.12$	8.59		
18	Kaikka	Xenentodon cancila	4.00 ±0.61	4.59		
19	Foli	Notopterus notopterus	$5.45 \pm 0.34$	3.01		
20	Meni	Nandus nandus	99.23 ± 2.75	2.77		
21	Bele	Glossogobius giuris	49.77 ± 4.04	8.12		
22	Poa	Otolithoides pama	$17.50 \pm 0.65$	3.66		
23	Bacha	Eutropiichthys vacha	191.14 ± 31.18	16.31		
24	Boal	Wallago attu	$16.91 \pm 0.54$	7.77		

# 3.1.4.2. Total DDTs ( $\sum$ DDTs) residues in autumn

The mean concentrations of total DDTs ( $\Sigma$ DDTs) residue (ng g<sup>-1</sup> ww) in fishes and prawn during autumn are shown in Table. 3.14.  $\Sigma$ DDTs ranged from 16.42 ± 1.90 ng g<sup>-1</sup> in Kalisha to 271.50 ± 6.17 ng g<sup>-1</sup> in Bacha and increased in the following order : Kalisha < Shing < Bata < Ghainna < Chanda < Kachki < Rui < Bajaritengra < Goldachingri < Chewa < Borobaim < Shole < Foli < Sharpunti < Gulsh < Poa < Jatpunti < Boal < Meni < Tengra < Bele < Bacha. The  $\Sigma$ DDTs residues in most of the samples during autumn were higher than rainy-season. Jatpunti, Sharpunti, Tengra, Bajari-Tengra, , Gulsha, Goldachingri, Borobaim,Chewa, Foli, Meni, Shole, Bele, Poa, Boal and Bacha contained higher amount of  $\Sigma$ DDTs residues (>50 ng g<sup>-1</sup>) while Rui, Ghainna, Bata, Shing, Kachki, Khalisha and Chanda contained lower (<50 ng g<sup>-1</sup>) shown in Fig.3.10 where brown bar represents higher and green for lower amount.

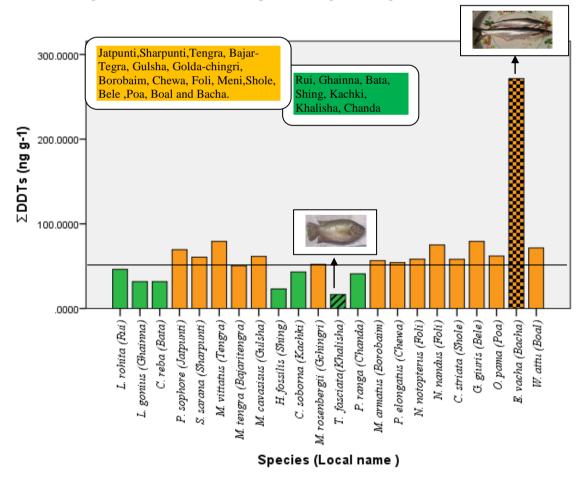


Fig. 3.10. Bar diagram of total DDTs ( $\sum$ DDTs) residues in fish and prawn samples from the Meghna River during autumn

Table 3.14. Total DDTs ( $\sum$ DDTs) residues in fish and prawn samples from the Meghna River during autumn (values express as mean  $\pm$  SD in ng g<sup>-1</sup> in w. w.)

No.	Name	Scientific name	$\sum$ DDTs (ng g <sup>-1</sup> in ww)		
			Mean ± SD	RSD	
1	Rui	Labeo rohita	46.24 ± 6.75	14.60	
2	Ghainna	Labeo gonius	$31.78 \pm 0.83$	2.60	
3	Bata	Cirrhinus reba	$31.70 \pm 2.87$	9.01	
4	Jatpunti	Puntius sophore	69.50 ± 0.62	0.89	
5	Sharpunti	Systomus sarana	$60.54 \pm 4.04$	6.67	
6	Tengra	Mystus vittatus	79.28 ± 18.38	23.18	
7	Bajari-tengra	Mystus tengra	50.57 ± 2.67	5.28	
8	Gulsha	Mystus cavasius	61.48 ± 8.78	14.28	
9	Shing	Heteropnuestes fossilis	23.11 ± 1.98	8.56	
10	Kachki	Corica soborna	43.16 ± 2.01	4.67	
11	Golda Chingri	Macrobrachium rosenbergii	52.13 ± 3.48	6.68	
12	Khalisha	Trychogaster fasciata	16.42 ± 1.90	11.57	
13	Chanda	Parambassis ranga	41.03 ± 4.03	9.81	
14	Boro baim	Mastacembelus armatus	56.49 ± 5.41	9.58	
15	Chewa	Pseudapocryptes elongatus	54.24 ± 4.72	8.70	
16	Foli	Notopterus notopterus	58.26 ± 7.29	12.50	
17	Meni	Nandus nandus	$75.08 \pm 4.45$	5.93	
18	Shol	Channa striata	58.13 ± 8.14	14.01	
19	Bele	Glossogobius giuris	$80.12 \pm 7.82$	9.76	
20	Poa	Otolithoides pama	62.06 ± 4.18	6.73	
21	Bacha	Eutropiichthys vacha	271.50 ± 6.73	2.27	
22	Boal	Wallago attu	$71.55 \pm 7.70$	10.76	

# 3.1.4.3. Total DDTs ( $\sum$ DDTs) residues during winter

The mean concentrations of total DDTs ( $\Sigma$ DDTs) residue (ng g<sup>-1</sup> ww) in fishes and prawn during winter are shown in Table. 3.15.  $\Sigma$ DDTs ranged from 3.88  $\pm$  0.60 ng g<sup>-1</sup> in Golda Chingri to 141.57  $\pm$  10.24 ng g<sup>-1</sup> in Bacha and considering the residue levels the chronology is Gloda chingri < Sing < Magur < Foli < Kachki < Chanda < Sharpunti < Tarabaim < Ghainnna < Meni < Jatpunti < Bata < Rui < Boal < Tengra < Gulsha < Bajari-tengra < Chewa < Shole < Poa < Bacha. The  $\Sigma$ DDTs residues in the overall samples in winter lower than rainy-season and autumn. Among the analyzed samples, Borobaim, Bele and Bacha contained higher amount of  $\Sigma$ DDTs (>50 ng g<sup>-1</sup> ww) while the remaining others contained lower amount (<50 ng g<sup>-1</sup> ww) are reported in Fig 3.11, brown coloured bar represents higher and green for lower amount.

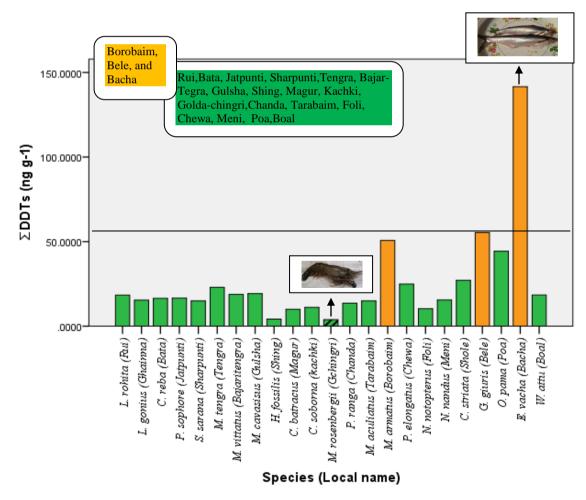


Fig. 3.11. Bar diagram of total DDTs ( $\sum$ DDTs) residues in fish and prawn samples from the Meghna River during winter season

Table 3.15. Total DDTs ( $\sum$ DDTs) residues in fish and prawn samples from the Meghna River during winter (values express as mean  $\pm$  SD in ng g- $^1$  in w. w.)

No.	Name	Scientific name	$\sum$ DDT (ng g <sup>-1</sup> in ww)		
			Mean ± SD	RSD	
1	Rui	Labeo rohita	$18.35 \pm 2.06$	11.20	
2	Ghainna	Labeo gonius	$15.43 \pm 0.78$	5.04	
3	Bata	Cirrhinus reba	$16.48 \pm 0.82$	4.73	
4	Jat punti	Puntius sophore	$16.62 \pm 1.35$	8.11	
5	Shar punti	Systomus sarana	$14.94 \pm 0.36$	2.39	
6	Tengra	Mystus vittatus	$18.83 \pm 0.44$	2.41	
7	Bajari-tengra	Mystus tengra	22.98 ± 3.19	13.88	
8	Gulsha	Mystus cavasius	61.48 ±8.78	14.28	
9	Shing	Heteropnuestes fossilis	23.11 ± 1.98	8.56	
10	Magur	Clarias batracus	$9.97 \pm 0.24$	2.31	
11	Kachki	Corica soborna	$11.13 \pm 0.82$	7.34	
12	Golda Chingri	Macrobrachium rosenbergii	$3.88 \pm 0.60$	15.52	
13	Chanda	Parambassis ranga	$13.57 \pm 0.85$	6.30	
14	Tara Baim	Macrognathus aculiatus	$14.98 \pm 0.88$	5.90	
15	Boro baim	Mastacembelus armatus	50.70 ± 5.24	10.33	
16	Chewa	Pseudapocryptes elongatus	24.90 ± 2.01	8.07	
17	Foli	Notopterus notopterus	$10.36 \pm 0.68$	6.61	
18	Meni	Nandus nandus	$75.08 \pm 4.45$	5.93	
19	Shol	Channa striata	27.11 ± 4.31	15.88	
20	Bele	Glossogobius giuris	55.33 ± 6.97	12.59	
21	Poa	Otolithoides pama	44.33 ± 4.77	10.77	
22	Bacha	Eutropiichthys vacha	141.57 ± 10.24	7.23	
23	Boal	Wallago attu	$18.44 \pm 0.76$	4.10	

## 3.1.4.4. Total DDTs ( $\Sigma$ DDTs) residues during summer

The mean concentrations of total DDTs ( $\Sigma$ DDTs) residue (ng g<sup>-1</sup> ww) in fishes and prawn during summer are reported in Table. 3.16.  $\Sigma$ DDTs ranged from 157.58 ± 1.15 ng g<sup>-1</sup> in Kachki to 1660.89 ± 157.9 ng g<sup>-1</sup> in Bacha and increased in the following : Kachki < Chanda < Shing < Ghainna < Sharpunti < Rui < Jatpunti < Bata < Goldachingri < Bele < Gulsha < Foli < Boal < Tengra < Bajari-tengra < Chewa < Shole < Meni < Borobaim < Poa < Gojar < Bacha. The DDTs residues in all the samples during summer were much higher (> 50 ng g<sup>-1</sup>) than other three seasons are shown in Fig.3.12.

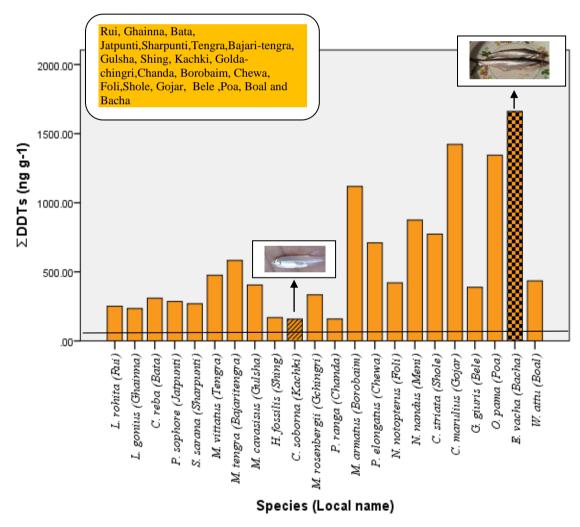


Fig. 3.12. Bar diagram of total DDTs (∑DDTs) residue in fish and prawn samples from the Meghna River during summer

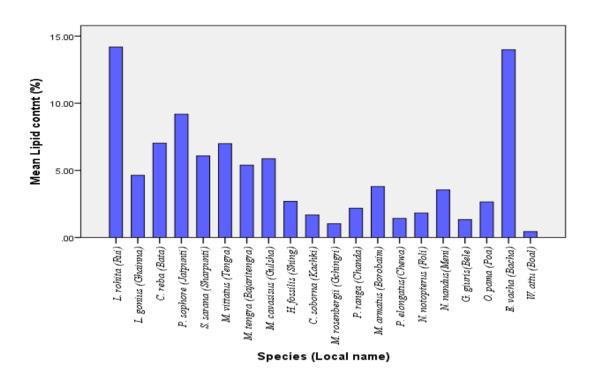
Table 3.16. Total DDTs ( $\sum$ DDTs) residues in fish and prawn samples from the Meghna River during summer (values express as mean  $\pm$  SD in ng g<sup>-1</sup> in w. w.)

No	Name	Scientific name	$\sum$ DDT (ng g <sup>-1</sup> in ww)			
			Mean ± SD	RSD		
1	Rui	Labeo rohita	$250.40 \pm 9.17$	3.66		
2	Ghainna	Labeo gonius	$234.15 \pm 0.65$	0.28		
3	Bata	Cirrhinus reba	$309.42 \pm 20.36$	6.58		
4	Jat punti	Puntius sophore	285.71 ± 15.30	5.36		
5	Sharpunti	Systomus sarana	<i>Systomus sarana</i> 269.19 ± 15.81			
6	Tengra	Mystus vittatus	475.11 ± 11.67	2.46		
7	Bajari-tengra	Mystus tengra	582.44 ± 32.29	5.54		
8	Gulsha	Mystus cavasius	$405.12 \pm 48.42$	11.95		
9	Sing	Heteropnuestes fossilis	$169.45 \pm 8.11$	4.79		
10	Kachki	Corica soborna	157.58 ± 1.15	0.73		
11	Golda chingri	Macrobrachium rosenbergii	$333.07 \pm 2.70$	0.81		
12	Chanda	Parambassis ranga	$158.43 \pm 5.72$	3.61		
13	Boro Baim	Mastacembelus armatus	1118.24 ± 34.74	3.11		
14	Chewa	Pseudapocryptes elongatus	709.98 ± 11.07	1.56		
15	Foli	Notopterus notopterus	420.72 ± 13.96	3.32		
16	Meni	Nandus nandus	874.74 ± 31.15	3.56		
17	Shol	Channa striata	772.59 ± 26.21	3.39		
18	Gojar	Channa marulius	1422.48 ± 77.83	5.47		
19	Bele	Glossogobius giuris	$387.99 \pm 8.52$	2.20		
20	Poa	Otolithoides pama	1343.77 ± 6.43	0.48		
21	Bacha	Eutropiichthys vacha	$1660.89 \pm 157.9$	9.51		
22	Boal	Wallago attu	443.24 ± 35.72	8.23		

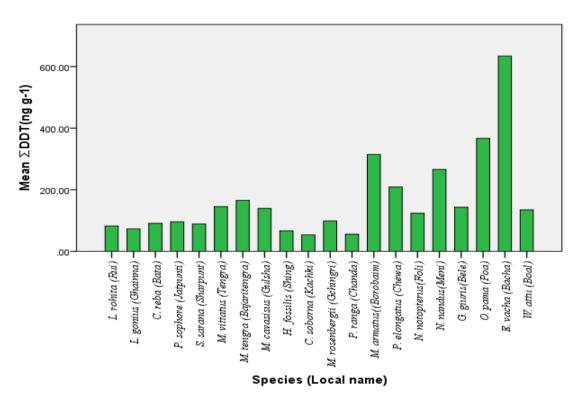
#### 3.1.5. Total mean of lipid contents and total DDTs concentrations of four seasons

Among different analyzed fish and prawn species, twenty species were common for all seasons. In case of that twenty species, the average lipid contents of four seasons are shown in Table 3.17 and Fig.3.13.a. Rui exhibited highest lipid content with an average of 14.19% and Boal exhibited lowest content with an average of 0.44 %. Considering these values the chronology is Boal (0.44) < Goldachingri (1.03) < Bele (1.33) < Chewa (1.42) < Kachki (1.68) < Foli (1.82) < Chanda (2.18) < Poa (2.65) < Shing (2.69) < Meni (3.55) < Borobaim (3.79) < Bujuri-Tengra (5.39) < Gulsha (5.87) < Sharpunti (6.08) < Tengra (6.99) < Bata (7.03) < Jatpunti (9.18) < Bacha (13.93) < Rui.(14.19).

The average total  $\Sigma$ DDTs concentrations of four different seasons of the commonly found twenty fishes are shown in Table 3.17 and Fig. 3.13.b. Bacha exhibited the highest  $\Sigma$ DDTs concentration with an average of 634.16 ng g<sup>-1</sup> while Kachki exhibited the lowest with an average of 53.63 ng g<sup>-1</sup>. Considering these values the chronology is Kachki (53.63 ng g<sup>-1</sup>) < Chanda (55.45) < Shing (66.92) < Ghainna (73.11) < Rui (82.17) < Sharpunti (89.11) < Bata (91.10) < Jatpunti (96.11) < Goldachingri (98.66) < Foli (123.71) < Boal (135.04) < Gulsha (139.55) < Bele (143.31) < Tengra (145.20) < Bajari-tengra (165.81) < Chewa (209.35) < Meni (266.14) < Borobaim (314.50) < Poa (366.92) < Bacha (634.16 ng g<sup>-1</sup>).



a



b

Fig. 3.13. Bar diagram of (a) mean of total lipid contents and (b) mean of total  $\sum$ DDTs residue in fishes and prawn sampls during four different seasons

Table 3.17. Average lipid content and  $\Sigma DDTs$  residues of fish and prawn samples of different seasons

No. Name		Scientific name	Average lipid (%)	Average ∑DDTs (ng g <sup>-1</sup> )	
1	Rui	Labeo rohita	14.19	82.17	
2	Ghainna	Labeo gonius	4.63	73.11	
3	Bata	Cirrhinus reba	7.03	91.10	
4	Jat punti	Puntius sophore	9.18	96.11	
5	Shar punti	Systomus sarana	6.08	89.11	
6	Tengra	Mystus vittatus	6.99	145.20	
7	Bajari-tengra	Mystus tengra	5.39	165.81	
8	Gulsha	Mystus cavasius	5.87	139.55	
9	Shing	Heteropnuestes fossilis	2.69	66.92	
10	Kachki	Corica soborna	1.68	53.63	
11	Golda Chingri	Macrobrachium rosenbergii	1.03	98.66	
12	Chanda	Parambassis ranga	2.18	55.45	
13	Boro baim	Mastacembelus armatus	3.79	314.50	
14	Chewa	Pseudapocryptes elongatus	1.42	209.35	
15	Foli	Notopterus notopterus	1.82	123.71	
16	Meni	Nandus nandus	3.55	266.14	
17	Bele	Glossogobius giuris	1.33	143.31	
18	Poa	Otolithoides pama	2.65		
19	Bacha	Eutropiichthys vacha	13.93	634.16	
20	Boal	Wallago attu	0.44	135.04	

## 3.1.6. The hierarchical cluster analysis (Dendogram)

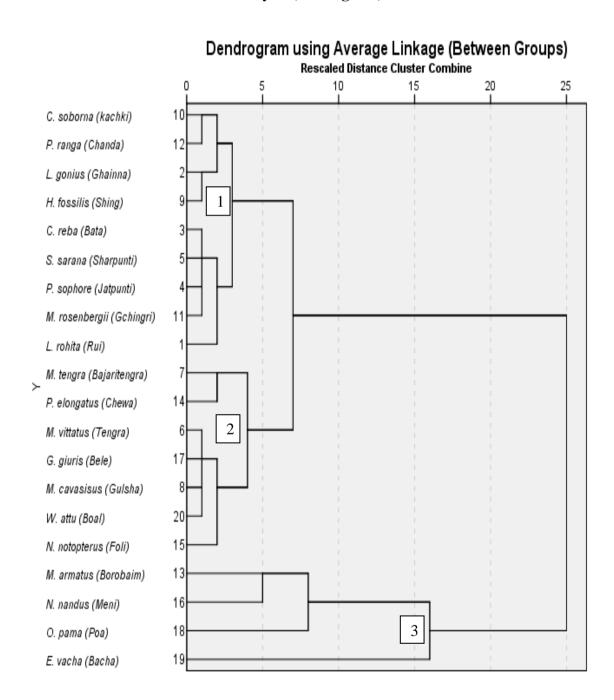


Fig. 3.14. Hierarchical dendrogram for 20 fishes and prawn represented by total DDTs residues

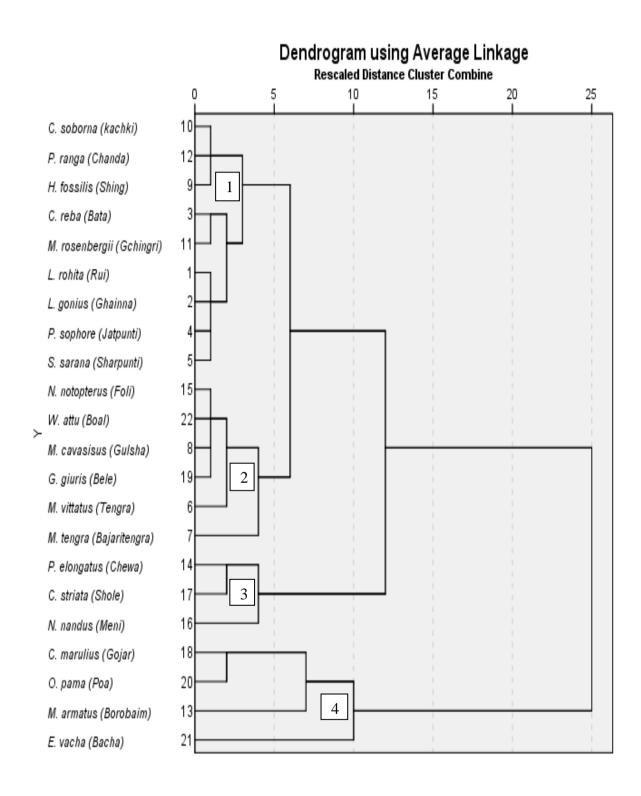


Fig. 3.15. Hierarchical dendrogram for fishes and prawn of summer season represented by total DDTs residues

#### The hierarchical cluster analysis (Dendogram)

The hierarchical cluster analysis (Ward's method applying Pearson correction) of fishes and prawn species using average linkage between groups and square Euclidean distance which uncover the similarities or dissimilarities between species depending on organohalogen residues. Two cluster analyses was done, one for the fishes commonly found in all seasons and second for the fishes of summer season as contained maximum amount of DDTs residues (Fig. 3.14. and Fig. 3.15 respectively).

The dendogram of Fig. 3.14. grouped the species into three big clusters with subgroups. The first cluster contains all herbivores and the omnivores that mostly feed on plant; Kachki, Chanda, Ghainna, Shing, Bata, Sharpunti, Jatpunti, Gurachingri and Rui. The second cluster contains omnivores that mostly feed on animals and the carnivores of low fat; Bajari-tengra, Chewa, Tengra, Bele, Gulsha, Boal and Foli. The third cluster contains bottom feeder and highly carnivorous; Borobaim, Meni, Poa and Bacha.

The dendogram of Fig. 3.15. grouped the species into four clusters with subgroup. The first cluster contains herbivores and omnivores of mostly plant feeder; Kachki, Chanda, Shing, Bata, Gurachingri, Rui, Ghainna, Jatpunti and Sharpunti. The second cluster contains omnivores of mostly animal feeder and carnivores with low fat; Foli, Boal, Gulsha, Bele, Tengra and Bajari-terngra. The third cluster contains highly carnivores; Chewa, Shole and Meni. The fourth cluster contains also highly carnivores and bottom feeder Gojar, Poa, Borobaim and Bacha.

#### 3.1.7. Total DDTs ( $\Sigma$ DDTs) concentrations in herbivore, omnivore and carnivore

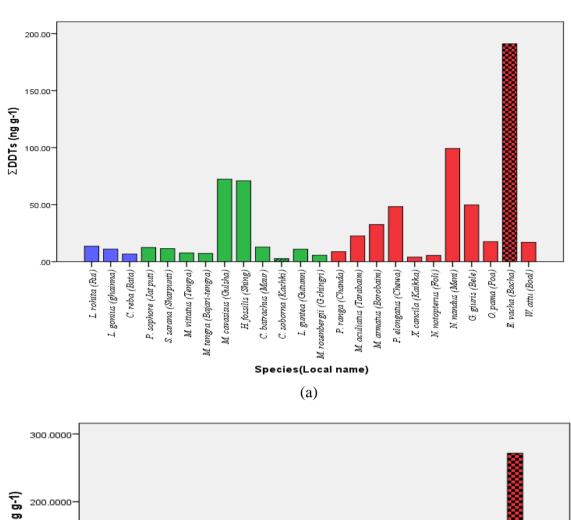
During rainy-season, the concentrations of total DDTs residue in herbivore fishes ranged from of  $11.02 \pm 0.13$  ng g<sup>-1</sup> in Ghainna to  $13.48 \pm 0.07$  ng g<sup>-1</sup> in Rui, in case of omnivores the ranged from  $2.64 \pm 0.35$  ng g<sup>-1</sup> in Kachki to  $72.33 \pm 7.47$  ng g<sup>-1</sup> in Gulsha and in case of carnivores the range was  $4.00 \pm 0.61$  ng g<sup>-1</sup> in Kaikka to 191.14  $\pm$  31.81 ng g<sup>-1</sup> in Bacha (Fig. 3.16.a.). The mean concentration of total DDTs for all the herbivores, omnivores and carnivores in this season were 10.38, 21.38 and 45.10 ng g<sup>-1</sup>.respectively.

During autumn, in case of herbivore fishes the concentrations of total DDTs residue ranged from  $31.70 \pm 2.87$  ng/g in Bata to  $46.24 \pm 6.75$  ng g<sup>-1</sup> in Rui, in case of omnivores the range was  $16.42 \pm 1.90$  ng g<sup>-1</sup> in kalisha to  $79.28 \pm 18.38$  ng g<sup>-1</sup> in Tengra and in case of carnivores the range was  $41.03 \pm 4.03$  ng g<sup>-1</sup> in Meni to  $271.50 \pm 6.71$  ng g<sup>-1</sup> in Bacha (Fig. 3.16.b.). The mean concentration of total DDTs for all the herbivores, omnivores and carnivores in this season were 36.37, 47.34, 96.00 ng g<sup>-1</sup>.respectively.

During winter, in case of herbivore fishes the concentrations of total DDTs residue ranged from  $15.43 \pm 0.78$  ng g<sup>-1</sup> in Ghainna to  $18.35 \pm 2.06$  ng g<sup>-1</sup> in Rui, in case of omnivores the range was  $3.88 \pm 0.60$  ng g<sup>-1</sup> in Goldachingri to  $22.98 \pm 3.19$  ng g<sup>-1</sup> in Bajari-Tengra and in case of carnivores the range was  $10.36 \pm 0.68$  ng g<sup>-1</sup> in Fole to  $141.57 \pm 10.24$  ng g<sup>-1</sup> in Bacha (Fig.3.17.a.). The mean concentration of total DDTs for all the herbivores, omnivores and carnivores in this season were 16.75, 20.32 and 43.30 ng g<sup>-1</sup>.respectively.

During summer, in case of herbivore fishes the concentrations of total DDTs residue ranged from  $234.15 \pm ng g^{-1}$  in Bata to  $309.42 \pm ng g^{-1}$  in Ghainna, in case of omnivores the range was  $157.58 \pm ng g^{-1}$  in Kachki to  $582.44 \pm ng g^{-1}g$  in Tengra and in case of carnivores the range was  $158.43 \pm ng g^{-1}$  Chanda to  $1660.89 \pm ng g^{-1}$  in Bacha (Fig.3.17.b.). The mean lipid contents for all the herbivores, omnivores and carnivores in this season were 264.64, 334.71, 845.83 ng/g.respectively.

One way ANOVA with Tukey HSD and LSD tests showed significant differences in total DDTs between herbivore, omnivore and carnivore fishes (f = 3.098,  $p \le .050$ ).



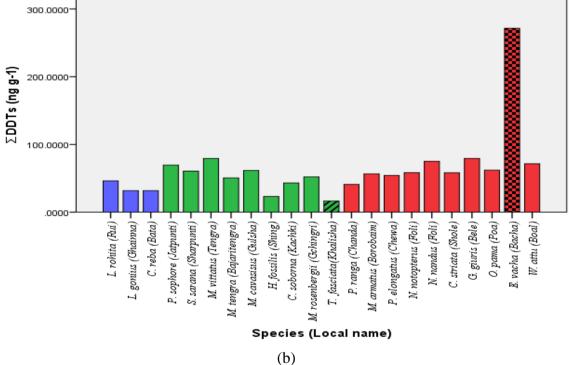
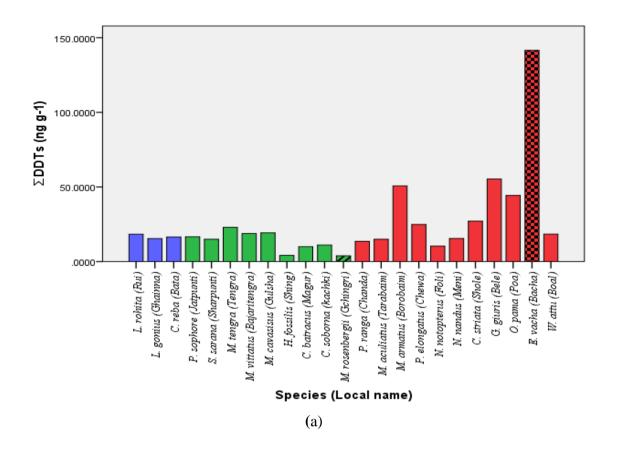


Fig. 3.16. Bar diagram of total DDTs residue in fish and prawn samples from the Meghna River during (a) rainy- season and (b) autumn



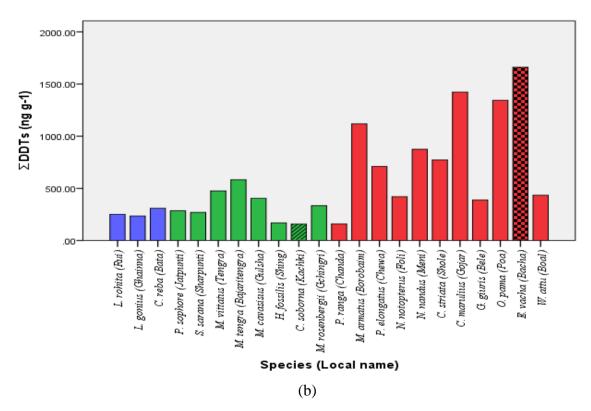


Fig. 3.17. Bar diagram of total DDTs residue in fish and prawn samples from the Meghna River during (a) winter and (b) summer

## 3.1.8. Multivariate statistical analysis (Principle Component Analysis-PCA)

On the basis of correlation matrix, a Euclidean biplot was obtained using the first two axis of PCA (Fig. 3.18.). The first axis explained 68.10% of total variance and second axis accounted for 24.6% of variance.

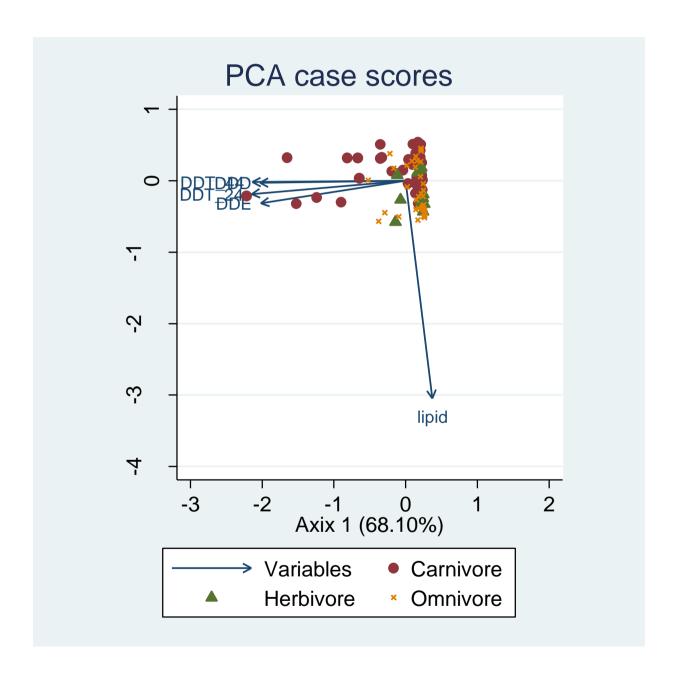


Fig. 3. 18. PCA on the basis of feeding habit of fish species.

# 3.1.9. Correlation between DDTs residues with lipid, rainfall, temperature and humidity

Table 3.18. Pearson correlation matrix between DDTs, lipid and some meteorological parameters

	Rainfall.	Temperature	Humidity	Lipid	DDE	DDD	2,4'- DDT	4,4'-DDT	∑DDTs
Rainfall	1	.684**	.955**	047	187	.010	060	057	080
Temperature		1	.525**	.040	.243*	.338*	.379**	.380**	.368**
Humidity			1	070	318**	161	276**	273**	285**
Lipid				1	.210*	.275**	.090	038	.129
DDE					1	.774**	.835**	.785**	.897**
DDD						1	.756**	.791**	.861**
2,4'-DDT							1	.940**	.967**
4,4'-DDT								1	.970**
∑DDTs									1

<sup>\*\*</sup> Correlation is highly significant at the level 0.01 level \* correlation is significant at the level 0.05 level.

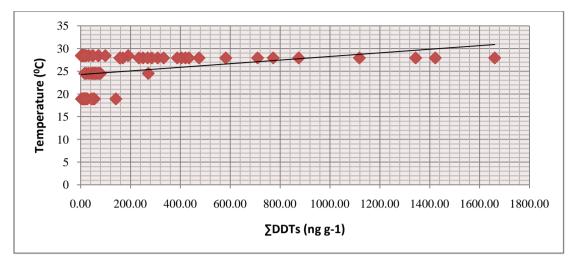


Fig. 3.19. Correlation between  $\sum$ DDTs residues in fishes with temperature

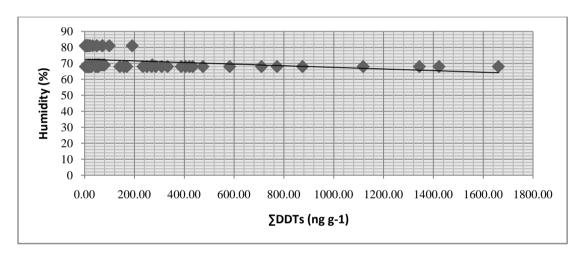


Fig. 3.20. Correlation between  $\sum$ DDTs residues in fishes with humidity

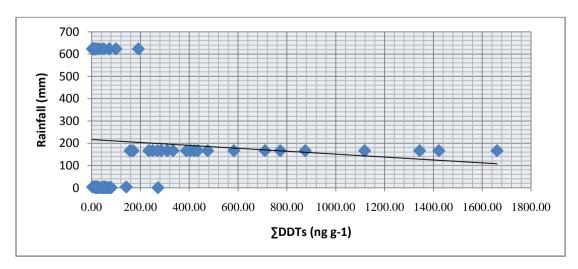


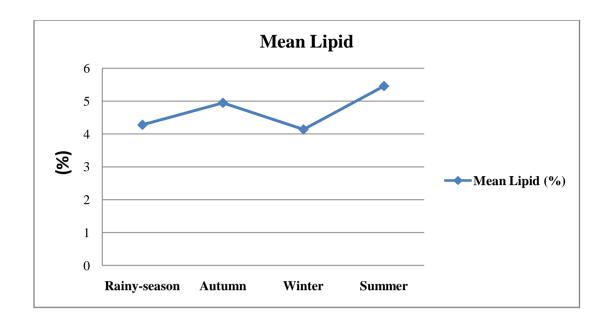
Fig. 3.21. Correlation between  $\sum$ DDTs residues in fishes with rainfall

### 3.1.10. Seasonal variations of lipid contents and DDTs concentrations

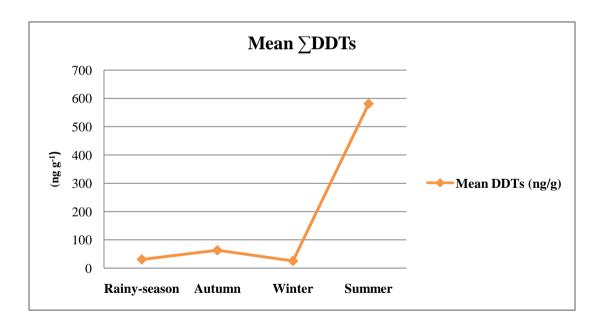
Total DDTs (∑DDTs) concentrations vary significantly between species for all seasons (p<0.01). One way ANOVA with Tukey HSD and LSD tests showed highly significant differences in total DDTs concentrations of fishes and prawn between seasons (p<0.01) where the summer season showed highly significant differences between rainy-season, autumn and winter (p<0.01 in all cases; summer vs rainy-season-p=0.000, summer vs autumn-p=0.000 and summer vs winter-p=0.000) while there were no significant difference in ∑DDTs concentrations among rainy-season, autumn and winter (p>0.05 in all cases; rainy-season vs autumn-p=1.00, rainy-season vs winter-p=0.555, autumn vs winter-p=.559). The lipid contents in fishes and prawn showed variation between seasons but there is no significant differences (p>0.05).

In case of lipid content, 55% species showed the highest lipid contents in summer and lowest in rainy-season. The mean values of total lipid contents of all samples for each seasons shown in Fig. 3.22.a. Considering these values the chronology is rainy-season (4.28%) < winter (4.14%) < autumn (4.95%) < summer (5.46%).

In case of  $\Sigma$ DDTs concentrations 70% species showed the highest amount in summer and lowest in rainy-season. The mean values of total  $\Sigma$ DDTs concentrations in all samples for the seasons shown in Fig. 3.22.b. Considering these values the chronology is winter (25.60 ng g<sup>-1</sup>) < rainy-season (30.88 ng g<sup>-1</sup>) < autumn (63.34 ng g<sup>-1</sup>) < summer (580.72 ng g<sup>-1</sup>).



(a)



(b)

Fig. 3.22. (a) Mean values of total lipid contents (b) Mean values of total  $\Sigma$ DDTs concentrations of total fishes and prawn samples of four different seasons

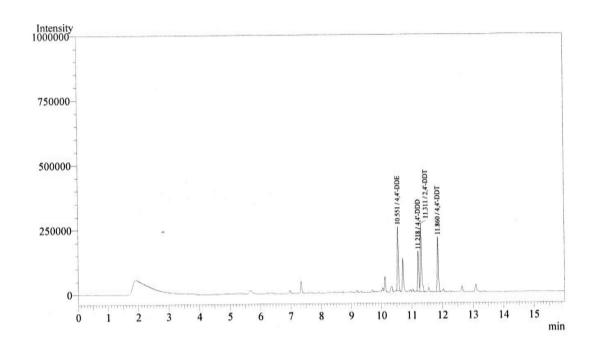


Fig. 2.23.a. Chromatogram of a sample in rainy season

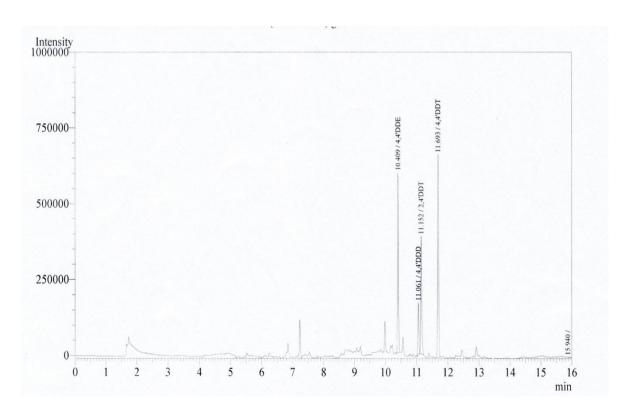


Fig. 2.23.b. Chromatogram of a sample in autumn

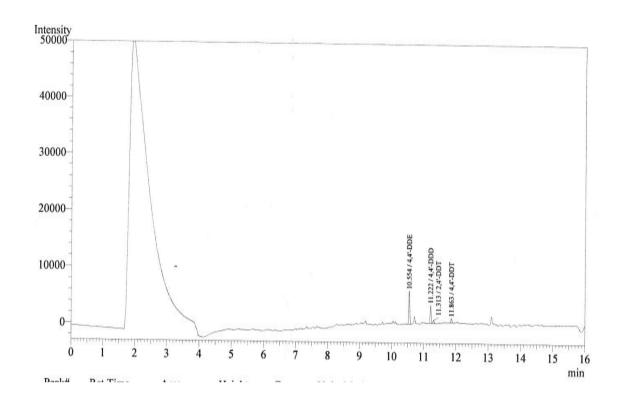


Fig. 2.23.c. Chromatogram of a sample in winter

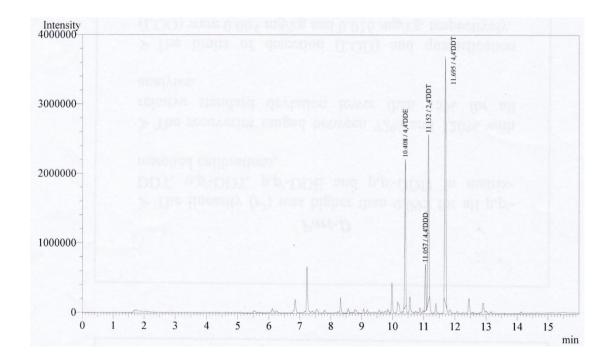


Fig. 2.23.d. Chromatogram of a sample in summer

#### 3.1.11. Recent or historical use of DDT

The (DDE+DDD)/DDTs ratio can be used to establish whether its input occurred recently or in the past, and also whether degradation of DDT is significant or not. If the ratios were less than 0.5, DDT can be used as fresh input instead of degraded as historical resides (Yu *et al.*2011). In this study, there were 38.46% of the total samples of four seasons having values of (DDE+DDD)/DDTs lower than 0.5 and 61.54% having values upper than 0.5. (Fig. 3.24.). The values of the (DDE+DDD)/DDTs for fishes of four seasons are given in the Table 3.19.

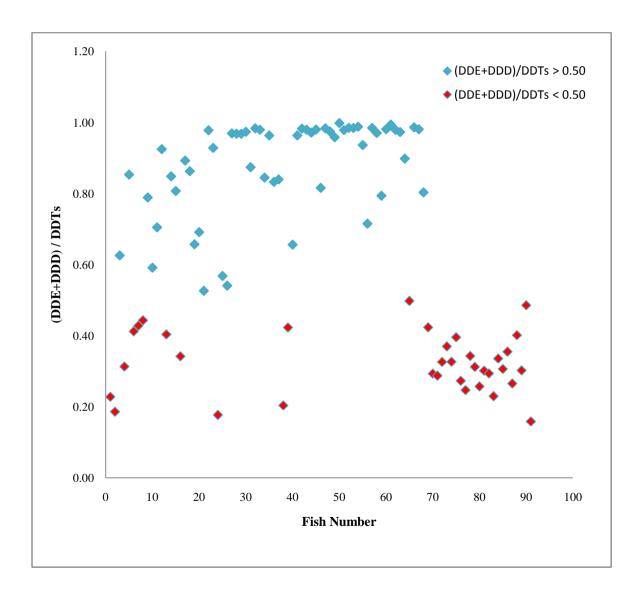


Fig. 3.24. Ratios of (DDE+DDD)/DDTs) in different fish and prawn samples from the Meghna River

Table 3.19. Ratios of (DDE+DDD)/DDTs) in different fish and prawn samples

Fishes	(DDE+DDD) / DDTs in rainy- season	(DDE+DDD) / DDTs in autumn	(DDE+DDD) / DDTs in winter	(DDE+DDD) / DDTs in summer
Rui	0.23	0.97	0.57	0.29
Ghainna	0.19	0.96	0.54	0.29
Bata	0.63	1.00	0.97	0.33
Jatpunti	0.31	0.98	0.97	0.37
Sharpunti	0.85	0.98	0.97	0.33
Tengra	0.41	0.98	0.87	0.40
Bajari- tengra	0.43	0.99	0.98	0.27
Gulsha	0.44	0.98	0.94	0.25
Shing	0.79	0.98	0.71	0.34
Magur	0.59		0.84	
Kachki	0.70	0.98	0.97	0.31
Gutum	0.92			
Golda Chingri	0.40	0.97	0.83	0.26
Khalisha		0.79		
Chanda	0.85	0.98	0.84	0.30
Tara baim	0.81		0.20	
Boro baim	0.34	0.99	0.40	0.29
Chewa	0.89	0.98	0.66	0.23
Kaikka	0.86			
Foli	0.66	0.97	0.96	0.34
Meni	0.69	0.90	0.98	0.31
Shole		0.50	0.98	0.35
Gojar				0.27
Bele	0.53	0.99	0.97	0.40
Poa	0.98	0.98	0.98	0.30
Bacha	0.93	0.80	0.82	0.49
Boal	0.18	0.42	0.98	0.16

#### 3.1.12. Human health risk

Health risk assessment of consumers from the intake of pesticides contaminated fish was characterized by using Health risk Index (HI). The estimated HIs were obtained by dividing the Estimated Daily Intake (EDI) by their corresponding values of Acceptable Daily Intakes (ADI) by WHO/FAO (2010) as shown by the equation;

HI = EDI / ADI

When the HI is less than 1, the food concerned is considered acceptable. If it is greater than 1, the food concerned is considered a risk to the consumer (Darko and Akoto 2008; Akoto *et al.* 2015).

Estimated Daily Intake (EDI)

The EDIs of OCPs expressed as nanogram per kilogram body weight per day (ng/kg bw/d) were calculated as follows:

$$EDI = (C \times DR) / BW$$

Where C is the measured concentration of OCPs ng g<sup>-1</sup>, DR is average daily consumption rate of fish (g day<sup>-1</sup>) and BW is body weight (kg) which was set at 60 kg (WHO, 2010). The average daily consumption rate was derived form fisheries resource survey of Bangladesh, 2013-14 and the value is 52.88 g/day (NFW 2015). The values of EDI of fishes and prawn samples of four seasons are given in the Table 3.20.

Acceptable Daily Intake (ADI)

ADI represents the daily concentration below which there is a high probability of no adverse health effect. It is an estimate of the residue that can be ingested by a person daily over an extended period of time without suffering deleterious effects. ADI is expressed by body mass per kilogram per day which was set at 10000 ng /kg bw/d

The hazard indices presented in Table 3.21. All the detected residues of DDTs were <1.

Table 3.20. EDI of different fish and prawn samples

Fishes	EDI (ng/kg bw/d) of fishes in rainy-season	EDI(ng/kg bw/d) of fishes in autumn	EDI (ng/kg bw/d) of fishes in winter	EDI (ng/kg bw/d) of fishes in summer
Rui	11.88	40.75	16.17	220.69
Ghainna	9.71	28.01	13.60	206.36
Bata	5.86	27.94	14.52	272.70
Jatpunti	10.92	61.25	14.65	251.81
Sharpunti	10.14	53.36	13.17	237.25
Tengra	6.65	69.87	16.60	418.73
Bajari-tengra	6.36	44.57	20.25	513.32
Gulsha	63.75	54.18	16.99	357.05
Shing	62.51	20.37	3.71	149.34
Magur	11.27		8.79	
Kachki	2.33	38.04	9.81	138.88
Gutum	9.58			
Golda Chingri	4.89	45.94	3.42	293.55
Khalisha		14.47		
Chanda	7.72	36.16	11.96	139.63
Tara baim	19.85		13.20	
Boro baim	28.68	49.79	44.68	985.54
Chewa	42.56	47.80	21.95	625.73
Kaikka	3.53			
Foli	4.80	51.35	9.13	370.79
Meni	87.45	66.17	13.65	770.94
Shole		51.23	23.89	680.91
Gojar				1253.68
Bele	43.86	69.86	48.76	341.95
Poa	15.42	54.70	39.07	1184.31
Bacha	168.46	239.30	124.77	1463.80
Boal	14.90	63.06	16.25	382.71

Table 3.21. HI of different fish and prawn samples

Fishes	HI of fishes in rainy-season	HI of fishes in autumn	HI of fishes in winter	HI of fishes in summer
Rui	0.001	0.004	0.002	0.022
Ghainna	0.001	0.003	0.001	0.021
Bata	0.001	0.003	0.001	0.027
Jatpunti	0.001	0.006	0.001	0.025
Sharpunti	0.001	0.005	0.001	0.024
Tengra	0.001	0.007	0.002	0.042
Bajari-tengra	0.001	0.004	0.002	0.051
Gulsha	0.006	0.005	0.002	0.036
Shing	0.006	0.002	0.000	0.015
Magur	0.001		0.001	
Kachki	0.000	0.004	0.001	0.014
Gutum	0.001			
Golda Chingri	0.000	0.005	0.000	0.029
Khalisha		0.001		
Chanda	0.001	0.004	0.001	0.014
Tara baim	0.002		0.001	
Boro baim	0.003	0.005	0.004	0.099
Chewa	0.004	0.005	0.002	0.063
Kaikka	0.000			
Foli	0.000	0.005	0.001	0.037
Meni	0.009	0.007	0.001	0.077
Shole		0.005	0.002	0.068
Gojar				0.125
Bele	0.004	0.007	0.005	0.034
Poa	0.002	0.005	0.004	0.118
Bacha	0.017	0.024	0.012	0.146
Boal	0.001	0.006	0.002	0.038

## 4. DISCUSSION

### 4.1. DDT and metabolites in fish and prawn samples

The chemical analyses showed that DDT and its derivatives were detected in variable quantities in all species through out the year indicating their widespread contamination in the aquatic environment of Meghna river system. The potential route and possible sources of DDT contamination of aquatic ecosystems are the surface runoff as a result off the possible illegal use of the pesticides in surrounding areas and also atmospheric deposition (Bouwman *et al.* 2008, Bornman *et al.* 2007). Therefore, the wide detection of DDTs in analysed fish and prawn species may be related to the extensive applications of DDT in the surrounding environments. According to the district statistics huge amount of food grains, vegetables, banana trees were cultivated on the bank of the Meghna River at Sonargaon Upazila (NDS 2013). So there may be possible illegal use of DDT as pesticide in crop production on the bank of the river which could be washing out into the adjacent waterbody and DDT residues were evident from the analysed fishes. Reports from fishes of other river and common fish markets also indicate higher level of DDT residues (Nahar *et al.* 2008, Hossain *et al.* 2016).

Significant differences of DDTs levels were found among species in each season. The results imply that the bioconcentration of DDTs in fish is species-specific due to their ecological characteristics such as feeding habits and habitats. Moreover lipid content, dietary consumption, metabolism rate and excretion rate are all the factors of primary importance in explaining body burden of DDTs. Considering the average concentrations of total DDTs (\subsetengtheapDTs) residue of four seasons, the twenty fish and prawn species that analysed in all seasons showed the chronology of Kachki (Corica soborna) < Chanda (Parambassis ranga) < Shing (Heteropnuestes fossilis) < Ghainna (Labeo gonius) < Rui (Labeo rohita) < Systomus sarana (Sharpunti) < Bata (Cirrhinus reba) < Jatpunti (Puntius sophore) < Goldachingri (Macrobrachium rosenbergii) < Foli (Notopterus notopterus) < Boal (Wallago attu) < Gulsha (Mystus cavasius) < Bele (Glossogobius giuris) < Tengra (Mystus vittatus) < Bajari-tengra

(Mystus tengra) < Chewa (Pseudapocrypter elongates) < Meni (Nandus nandus) < Borobaim (Mastacembelus armatus) < Poa (Otolithoides pama) < Bacha (Eutropiichthys vacha). The other fishes that analysed in one or two seasons showed the chronology, Kaikka (Xenentodon cancila) < Khalisha (Trychogaster fasciata) < Gutum (Lepidocephalus guntea) < Magur (Clarius batrachus) < Tarabaim (Macrognatus aculiatus) < Shole (Channa striata) < Gojar (Channa marulius). Therefore, among these twenty-seven analyzed species, Rui (L. rohita), Ghainna (L. gonius), Bata (C. reba), Jatpunti (P. sophore), Sharpunti (S. sarana), Shing (H. fossilis), Magur (C. batrachus), Kachki (C. soborna), Goldachingri (M. rosenbergii), Gutum (L. guntea), Khalisha (T. fasciata), Kaikka (X. cancila), Tarabaim (M. aculeatus) and Boal (W. attu) conained lower amount of DDTs residue. While Tengra (M. vittatus), Bajari-tengra (M. tengra), Gulsha (M. cavasius), Borobaim (M. armatus), Chewa (P. elongatus), Meni (N. nandus), Bele (G. giuris), Poa (O. pama) and Bacha (E. vacha) tissue contained higher amont of total DDTs residues.

Trophic position and lipid content of aquatic organisms are reliable predictors of OCP concentrations in aquatic ecosystem (Kidd *et al.* 2000, Crosly *et al.* 1998). The present analysis showed that Kachki, Chanda and Shing fish contained low amount of lipid contents. Kachki fish mainly feed on phytoplankton and zooplankton while Shing feed on insects and plant materials due to their omnivorous nature (Shafi and Quddus 1982). Chanda is a carnivorous fish feed on zooplankton and larvae. As these three fishes occupy lower position in the food chain (may be just after the herbivore) and together with their low lipid contets may be related to their low DDTs residues.

Rui, Ghainna and Bata fish mainly feed on phytoplankton and algae due to their herbivorous nature (Fishbase 2014, Shafi and Quddus 1982). The present study showed that these fishes contained higher amount of lipid. Although containing higher lipid, they occupy the second trophic level just after the producer may be responsible for containing lower amount of DDTs residue. Similarly herbivore fish *Sarotherofon galilaeus* contained lower concentrations of organochlorine residues as it feed on phytoplankton and algae (Akoto *et al.* 2016).

Jatpunti and Sharpunti mainly feed on algae, plant material and small amount insects due to omnivorous nature (Fishbase 2014, Shafi and Quddus 1982) while contained higher lipid contents may be responsible for comperatively lower DDTs accumulation but higher than Kachki, Shing, Magur, Chanda, Rui, Ghainna and Bata.

Only prawn species is Goldachingri contained lower amount of DDTs residue may be related to their low lipid content. Moreover Goldachingri is an omnivorous species mainly feed on plankton, Diatoms, Copepods and small crustaceans. Similar result was reported by Nahar *et al.* (2008).

Foli (*N. notopterus*) and Boal (*W. attu*) also contained lower amount of DDTs residues. Foli is a carnivore fish feed on insects, prawns, nematods, aquatic weed and bottom sands (Kiran and Waghray, 1996). Although it occupy in the higher trophic level but contained low amount of lipid content may be responsible for lower amount of DDTs residues. Boal is a highly carnivorous fish but contained low DDTs residues as it contained very low amount of lipid content moreover it's exretion rate is high. According to ASTRD 2002 DDT leaves the body mostly in Urine. So there may be relation between it's exscretion rate, lipid content and low DDTs residue. Similar findings repoted in the Boal fish from othe rivers and common fish market (Hossain *et al.* 2016, Nahar *et al.* 2008). Deribe *et al.* 2011 also reported that African big barb (*Barbus itermedius*) is positioned at the higher trophic level but contained low concentration of DDTs compare to others at the same trophic level due to its lower relative lipid content.

It is well established that the extent of accumulation of organochlorine compounds is greater in fish that have high lipid content (Muir *et al.* 1990). Because of lipophilic nature, organohalogen compounds can biomagnify and bioaccumulate in carnivore fish which as they are generally positioned at the top of trophic level in an ecosystem (Deribe *et al.* 2011, Zhou *et al.* 2007). Tengra, Bajari-tengra and Gulsha are omnivorous fishes feed mainly on small fishes, insects, moluscks and little amount of algae and plant material (Chaklader *et al.* 2014, Gupta *et al.* 2014) together with higher amount of lipid contents may be the cause of containing higher DDTs residues.

Chewa (*P. elongatus*) depends on shrimps and non-shrimp crustaceans mainly include copepods, crab larvae, mysids and amphipods predominantly as it is carnivore (Rahman *et al.* 2016). Meni (*N. nadus*) is a bottom and column feeder and feed mainly on small fish, prawn, fish fry, chironomid and insect larvae and predominantly carnivorous fish (Mustafa *et al.* 1980). Bele (*G. giuris*) is carnivore and cannibalistic in nature. Food items mainly consist of fish, crustaceans, insects, zooplankton and on the other hand considerable time of the year a recognizable proportion of food composed of juvenile of bele (Hossain *et al.* 2016). Poa (*O. pama*) is highly carnivore fish with predatoy in nature in both juvenile and adult life. It feeds mainly on small fishes, fish fry and bottom dwelling invertebrates (Manojkumar and Acharia 1990). The Shole (*C. striata*) and Gojar (*C. marulius*) feed on small sized fish, frog, snake, insects, earthworm and tadpole larva due to their highly carnivorous and predatory in nature (Amin *et al.* 2014).

From the above discussion on the food and feeding habits of these carnivorous fish species, it is clear that these fishes depend on diverse food items cover several trophic levels which make them as top consumers in the aquatic body. According to ATSDR (2002) and Connell (1995), persistent lipophilic organic compounds bioaccumulate and biomagnify with increasing trophic levels. Similar trends of DDT accumulation to higher levels in fishes of higher trophic levels observed, reaching levels thousand times higher than in water and organisms at lower trophic level. Therefore, the higher amount of DDTs residues in Chewa, Meni, Shole, Gojar, Bele, Poa, Shole and Gojar in the present study are in accordance with their higher trophic position in food chain. Similarly the significant higher concentration of DDTs were also found in carnivorous species African catfish (Clarias gariepinus) from the lake Koka, Ethoipia, marine fish snakefish from Natuna Island, South China sea and fresh water catfish (Clarias anguillaris) from Tono reservoir of Ghana (Derib et al. 2011, Hao et al. 2014 and Akoto et al. 2016). Similarly the carnivore and predator fishes like silver catfish, (Schilbe intermedius) in Tono reservoir of Ghana, contained higher concentration of organochlorine residues (Akoto et al. 2016).

Borobaim (*M. armatus*) contained higher amount of DDTs which may be attributed as it is a bottom-dwelling carnivorous habits and inhabits in muddy tunnel which may increase its exposure to DDTs residue at the bottom sediment. After entering into the aquatic habitat, DDT settle down and deposited into the bottom sidiments may be related to the higher DDT accumulation in sediment feeder fishes. Similar finding, relatively higher concentrations of organochlorine pesticide residues were also found in bottom-feeding species in Oueme River catchment in the Repablic of Benin (Pazou *et al.* 2006) and bottom-feeding marine fish from China and the United States (Xia *et al.* 2012, Morgan and Lohmann 2010, Sun *et al.* 2014). Kent and Johnson (1979) also reported that bottom feeder catfish, *Clarius anguillaris* contained highest level of OC pesticides in the Americal Fall Reservoir. However, data from the National Contaminant Biomonitoring Program in major US Rivers and Great lakes found no differences between OC pesticide residues in bottom feeders and predatory fish. (Caldas *et al.* 1999).

From the present analysed fish and prawn species, Bacha fish (*E. vacha*) contained highest amount of DDTs residues in all seasons. Several factors may be responsible for its higher DDTs accumulation. Bacha contained much higher amount of lipid content. According to Shafi and Quddus (1982), Abbas (2010), Gupta and Benergi (2016), in adult stage, Bacha fish is a carnivorous and piscivorous fish feed on small fishes, juvenile of taki and other large fishes, mollusks, crustaceans and annelids etc. Their feeding intensity is also higher as they are voracious. Therefore the high lipid content together with piscivorous and voracious nature of Bacha may be responsible for its higher DDTs accumulation in tissue. Connell (1995) observed the same findings and concluded that fatty carnivorous fish species are expected to have higher concentration of POPs than lean fish in the same trophic level.

#### 4.2. Composition profiles of DDT and its metabolites

When deposition in water, DDT will bind to particles in water, settle and be deposited in the sediment. Then, it is taken up by small organisms and fish (ASTRD 2002). After deposition in water, 4, 4'- DDT and 2,4'- DDT are broken down to DDD and DDE by sunlight and by the micro-organisms (Ssebugere *et al.* 2009, Rao *et al.* 2014).

From this study, the maximum percentage of 4,4' DDT (40.2% of the sum of DDT) reported in summer season followed by rainy-season, winter and autumn, this may indicting the recent input of DDT prior to summer season. While DDD and DDE showed lower percentage in summer and rainy-season but increasing to the next two seasons. It could be happened that during summer, DDT deposited to the water-body as before i.e. during winter season huge amount of vegetables cultivated on the bank of the river where pesticide may be used that persist in the soil which could be runoff in the river through the short summer rain season. After introduce into the waterbody the DDTs then enter the food chain, enters into the fish body and converted to DDD and DDE within next three seasons. Micro-organisms and oxygenase enzymes present in the fish body may be responsible from rapid convertion of 4,4'- DDT to DDD and DDE. As we know, metabolic functions through oxygenase (enzyme) in different organisms specially in fish has a central role in converting 4,4'- DDT into DDE (Muralidharan et al. 2009). According to Binelli and Provini 2003 and Kongwick et al. 2006, DDT metabolis faster in fish where its half life is approximately eight months in fish.

# 4.3. Understanding differences in DDTs residues among fishes of different feeding habits and lipid contents

A wide range of DDTs concentrations was observed in different fishes. As stated earlier in countries other than Bangladesh, the lipid content and trophic positions influence the accumulation of contaminatant concentrations in fishes (Singh and singh 2008, Arnot and Gobas 2004, Canbana and Rusmussen 1994, Pastor *et al.*1996). This study focused on the DDTs concentration in relation to the trophic position of fishes and also relationship to their lipid contents. In this respect, statistical analysis showed significant differences in total DDTs between fishes of different trophic levels; herbivore, omnivore and carnivore fishes. Moreover Pearson correlation between lipid contents with DDT and its derivatives showed significant direct positive correlations between lipid contents with DDE and DDD other than DDT. It could be attributed that although DDT is lipophilic but in the fish body DDT rapidly breakdown to DDE and DDD and then stored to fish fat. Similar relationships between DDE and lipid

content was found in *Schilbe marmoratus* fish in Congo river basin, reported by Verhaet *et al.* (2013).

Through out the seasons, the mean DDTs residual amount for different trophic positioned fishes; herbivores, omnivores and carnivores showed the chronology as carnivore > omnivore > herbivore. The DDTs accumulation found to be increased with higher trophic levels. Similar bioaccumulation of organochrine compounds reported in plankivore fish Enjunguri (*Haprochromis nigripinnis*) from lake Edward, Uganda displayed no detectable levels of DDTs (Ssebugere *et al.* 2009), in omnivore Silver fish (*Rastreneobola argentea*) from lake Victoria and lake Kyoga in Uganda displayed lower DDTs residues (Nnamuyomba *et al.*, 2014) and in carnivore Tiger fish (*Hydrocynus vittatus*) from the Luvuvhu River of South Africa displayed the highest OCP bioaccumulation (Gerber *et al.* 2014). Similarly, Verhaert *et al.* (2013) also reported significant increase of DDT with increasing trophic levels in cases fishes from Itaimbiri, Aruwimi and Lomami river basins.

Some exception is that all species of higher trophic levels did not contained higher residue levels. The omnivore fishes, Kachki and Shing contained residual levels lower than that of some herbivore species while the carnivore fishes, Chanda and Boal contained residue levels lowere than that of some omnivore species. This may be related to their lower lipid contents that mentioned before. Similar findings was reported carnivore fish Boal fish of common fish market contained low amount of DDTs residues (Nahar *et al.* 2008). On the otherhand the herbivore fish Rui contained much higher lipid content but its residue level was lower which could be attributed to its lower trophic level. Besides if in culture condition the contamination will be less. In addition age is to be another factor to accumulation.

Therefore, it could not be said that all fishes of higher lipid contents contained higher residues level or all fishes of higher trophic levels contained higher. Both the lipid contents and (or) the trophic position were the predictor of concentrations of organohalogen residues, also reported in the fishes from subarctic lakes in Yukon Terrritoy (kidd *et al.* 1998, 2000). Generally fatty fishes of higher trophic levels

contained higher DDTs residues than the lean fishes of lower trophic levels reported from the fishes from Qiantang River in East China (Kong *et al.* 2005, Zhou *et al.* 1999, 2007).

# 4.4. Grouping of fishes on the basis of total DDTs concentration using dendogram

To have a clear idea about the grouping of fishes and prawn on the basis of DDTs concentrations, dendogram was constructed using the raw data of total DDTs residues. The two dendograms, first one classifies the fishes and prawn that commonly found in all seasons according to their average annual DDTs accumulations. While the second dendogram classifies the fishes and prawn of summer season only according to their total DDTs accumulations of the season. The second dendogram was conducted on summer season because of the species contained maximum DDTs concentrations in this season.

The first dendogram grouped species into three big clusters with subgroups. The first cluster group contains Kachki, Chanda, Ghainna, Shing in one subgroup and Bata, Sharpunti, Jatpunti, Golda-chingri and Rui in other subgrpup that shows the lower DDTs accumulations. Therefore this Cluster includes all herbivores (Rui, Ghainna and Bata that occupy second trophic level just after the producer), five omnivores (Kachki, Shing, Sharpunti, Jatpunti and Goldachingri that contain low lipid conternt and occupy lower trophic levels as feed mainly on plant materials, planktons etc), one carnivore fish (Chanda that contains low lipid content and occupy lower trophic level as feed mainly on zooplankton). The second Cluster includes Bajaritengra, Chewa in one subgroup and Tengra, Bele, Gulsha, Boal and Foli in other subgroup that shows higher DDTs accumulation. Therefore, this Cluster containes three omnivores (Bajaritengra, Tengra and Gulsha that have higher lipid contents and occupying higher trophic levels that feed mainly on animals; insects, small fishes etc.) and four carnivores (Chewa, Bele, Boal and Foli which have much lower lipid contents and occupy higher trophic level that feed small fishes, mollusks, insects and crustaceans etc.). The Cluster 3 group includes the species of four carnivore fishes Boro baim, Meni, Poa and Bacha that shows much higher DDTs accumulations. In this case

Borobaim mainly feed on bottom sediment, Meni and Poa are highly carnivorous and predatory in nature and Bacha is also highly carnivorous, voracious and containe higher lipid content than the others.

The second dendogram grouped species into four big Clusters with subgroups which is more or less similar to the first one. The Cluster 1 group shows the lower DDTs accumulation, the Cluster 2 and 3 groups show higher while the Cluster 4 shows much higher DDT accumulations. The Cluster 1 group contains just like the first dendogram, all herbivores, five omnivores that have low lipid conternt and occupy lower trophic levels, one carnivore fish that contains low lipid and occupy lower trophic level as feed mainly on zooplankton. The Cluster 2 group includes three omnivores that have higher lipid content and and occupying higher trophic levels that feed mainly on animals; insects, small fishes etc. and three carnivores that have much lower lipid contents and occupy higher trophic level that feed small fishes, mollusks, insects and crustaceans etc. The Cluster 3 group includes the species of three carnivore fishes that are predatory in nature and shows higher DDTs accumulations. The Cluster 4 group contains four carnivores in which case one is bottom sediment feeder, two highly carnivorous and predatory in nature and one is also highly carnivorous, voracious and contained much higher lipid content.

From the cluster analysis it is easy to identify the groups of fishes with lower, medium and higher DDTs residues. Therefore from above discussion, it could be said that lower residues may be found in herbivores, lean and plant based omnivores and lean and zooplankton based carnivores; medium or higher residues may be found in fatty and animal based omnivores, lean and lower carnivores while the fishes with much higher residues may include bottom feeder carnivores, predators and fatty top carnivores.

From the dendogram, it is clear that lipid contents, trophic positions and feeding habit all together influence the accumulation of pesticide residues in fishes.

# **4.5. DDTs residues and different feeding mode through Principal Component Analysis (PCA)**

From the principal component analysis (PCA), all metabolites of DDTs were strongly correlated with the first axis, suggested agricultural run-off as an important source of pollution in the study area. Three distinct groups are formed on the basis of trophic levels of fish species (herbivore, omnivore and carnivore). The maximum concentrations of DDTs were recorded in group carnivores that were relatively higher than omnivores and carnivores. Trophic positions and lipid contents are reliable predictors of organohalogen concentrations in aquatic ecosystem (Kidd *et al.* 2000). DDTs can biomagnify and bioaccumulate in carnivore fish as they are generally positioned at the top of trophic level (Deribe *et al.* 2011, Zhou *et al.* 2007). A significant high concentrations of DDTs in muscle of carnivore fish species has been reported in contrast to other fresh water fish species with various feeding habits by Robinson *et al.* (2016) and Kong *et al.* (2005). Therefore it has been suggested that bioaccumulation of OHs is species-specific in fish due to their ecological features e.g. habitat and feeding modes.

#### 4.6. Meteorological parameters that influence DDTs concentrations

Temperature and humidity affect the toxic potential of environmental pollutants like DDT, has been suggested by Baetjer (1968). In the present study, DDT and its residues showed significant positive correlation with ambient temperature where highest concentrations of DDTs residues in species observed in summer season that had higher ambient temperature and lowest concentrations observed in winter that had lowest temperature. The results were such that higher ambient temperatures enhance the percutaneous absorption of chemicals, has been reported by Suskind (1977) and also the bioconcentration factor or amount accumulated has been shown to increase with temperature for DDT (Cember *et al.* 1978, Boudou *et al.* 1980, Reinert *et al.* 1974). Similar findings also reported by Phillips (1980) that the solubility of several organochlorine insecticides in water increases with ambient water temperature, leading to greater uptake and possibly higher toxicity to aquatic biota and study with DDT and mosquito fish, and rainbow trout support this.

The other parameter, humidy showed significant negetive correlation with DDTs residues that is concentratons of DDTs residues increases with decresing humidity. That might be due to the volatile nature of DDT. As the lower humidity exerts the evaporation rate to higher and which increases DDT burden in the air and finally atmospheric diposition of DDT into aquatic habitat. Baetjer (1968) reported the effect of humidity on toxic chemical.

Rainfall is the additional important parameter to influence the DDTs accumulations. In the present study, rainfall showed negative correlation but not significant effective. The highest bulk rainfall occurred in rainy-season showed lower concentrations of residues. This might be due to dilution effect of contaminants in the aquatic body. But the lowest rainfall in winter season did not show highest concentration of residues because other physiological and biological factors influence together on DDTs accumulations.

In the environment the meteorological factors persist together. Therefore it could not expect that high temperature cause higher accumulation or higher humidity and rainfall cause lower accumulation in all time. In the present study such condition also shown in average highest temperature was in rainy-season but contained lower concerntrations of DDTs and lowest rainfall in winter but contained lower residues level as not expected. Therefore it could be said that temperature, humidity and rainfall have the combined effects on DDTs accumulation. In addition, for instance low metabolism in fishes in cold weather reduce the accumulation of toxic chemicals.

#### 4.7. Seasonal distribution of DDTs

Concentration of total DDTs in fish and prawn species varied greately with the seasons. The species in summer season showed the highest mean concentration and in winter showed lowest concentration where the chronology is summer > autumn > rainy-season > winter and the ANOVA results confirmed that there was indeed a significant differences between seasons ( LSD test,  $p \le 0.01$ ) showed that the concentrations of summer seasons varied significantly with other three seasons. Similar results were observed by the Ntow (2005) that season had large and

significant effects on the varience for concentrations of DDTs and fishes accumulated greater pesticide residues during summer season. In the present study, the alarming condition is that during summer season the mean concentration of total DDTs was significantly higher than other three seasons which was around ten times greater than autumn and around twenty times greater than rainy-season and winter. Several factors and conditions may be responsible for this alarming result.

First factor is food intake. Food intake in one of the main route by which fish can accumulate POPs in their natural aquatic habitat (Holden *et. al.* 1996). Fish shows high rate of feeding intensity during summer or premonsoon month may be due to extra energy requirement for building up of gonads (Abbas 2010, Chaklader *et. al.* 2014, Khan *et al.* 1988, Serajuddin *et al.* 1988). Moreover the higher levels of DDTs in fish generally may be ascribed to increase feeding in insect larvae, coarse vegetable matters and sediments associated particles that had accumulated the pesticides over time. As before mentioned during summer and premonsoon the river water shows highest concentration of DDTs and the concentration of DDTs may be several thousand times grater in fish body than the surrounding water because of its bioconcentration and bioaccumulation nature (ATSDR, 2002). So it is clear that the highest concentration of DDTs in river water and highest feeding intensity together with may be responsible for much higher concentration of DDTs in fish tissue during summer.

Second factor is the rainfall. After winter or dry season, the short rainfall of summer and premonsoon season may washout DDTs from the adjacent field to the river that was previously exposed. As the water level was comperatively lower during premonsoon, the runoff DDTs would make the river water contaminated with highest concentration in that season. Similar result was reported for the waterbody of river Sio, river Nzoi and Lake Victoria of Kenya. Where DDT concentration in water of these rivers and lake were highest in short rain season than heavy rain and dry season (Wangdiga and Jumba 2002)

Third is temperature. Rate of residue accumulation and bioconcentration in fish increases with temperature (Kidwell *et al.* 1990). The highest ambient temperature reported in premonsoon may also increase higher DDTs accumulation in fish body during this season. Upadhi and Wokoma in 2012 repoated the same findings that high temperatue increased the higher pesticide residues accumulations in fish tissue in Elechi Creek, Nigeria Delta, Nigeria.

The fourth and well known factor is lipid contents. The highest mean lipid content of fishes reported in premonsoon prior to spawning also reported similar findings in some fishes by Vollenweider *et al.* 2011. Higher lipid content may also responsible for higher DDTs accumulation in the season.

From the above discussion, it is clear that rainfall, food intake, ambient temperature and lipid contents may influence in DDT accumulation. The combined effects that increase its concentration at cumulative rate in fish body. Therefore the mean DDTs concentration of fish and prawn species was much higher in summer season than other seasons. The result of ANOVA with Tukey HSD and LSD statistical test also confirmed that the mean DDTs concentration of fish and prawn species in summer season showed significant differences with other three seasons.

Higher residue levels also detected during autumn compare to rainy and winter season may be attributed to high feeding intensity and concentrated pollutants due to small volume of water in riverbases. During autumn, the fish takes food highly for recovery energy that they loss through spawning and also food availability is much in this season. Therefore higher feeding intensity in autumn may be responsible for higher DDTs accumulation. On the otherhand, as there are two main routes by which fish can bioaccumulate POPs in their natural aquatic habitat: from water via gill and body surface and from the diet (Burreau *et al.* 2004, Campbell *et al.* 2000 and Holden *et al.* 1966). So much pollutant could be entered in the fish body from the surrounding water with high concentration.

On the other hand low residue levels detected during rainy-season compare to the summer and autumn. This could be attributed to dilution effects based on large volumes of rainwater. A similar finding, lower concentration of persitant organis pollutents was also observed in the Lake Victoria of Kenya during wet season (Wandiga and Jamba, 2002). However fresh water fishes spawn mostly during rainy-season, July-september (Rahman 2005, Shafi and Quddus 1982). Organohalogen compounds are transferred from muscle tissue to the gonads along with the fat during the final stage of gonad maturation (Frantzen *et al.* 2011). Therefore, through spawning DDTs may be decreased in fish body during rainy-season. Maternal transfer of organohalogen compounds to offspring via gonads has been shown for oviparous fish species such as zebrafish and salmonoids (Russell *et al.* 1999, Heiden *et al.* 2005, Nyholm *et al.* 2008). Moreover feeding intensity of fishes is lower during spawing season (Jhingaran 1961, Desai 1970, Bhatnagar and Karamchandani 1979, Fatima and Khan 1991) may be related to lower DDTs accumulation

Lowest residues levels also detected during winter could be attributed by the lower feeding intensity (Abbas 2010), lower lipid content and also lower ambient temperature. During winter season, the feeding intensity of fishes is very much low due to scarcity of food, moreover they loss of stored lipid contents through metabolism to supply energy for physiological activities. In the season the ambient temperature was lowest may also be related to lower DDTs accumulation.

## 4.8. Comparison with previous work

Comparison with results from other water bodies of Bangladesh is difficult because all the relevant studies have been done with species collected from different ecological habitat, small sizes of the samples and also samples collected in a single season or randomly from market etc. Furthermore, the results found are expressed in different units. In a recent study (Hossain *et al.* 2016) on twenty two different fish species of Kangsha and Titas river, the concentrations of DDTs in several fish species were similar to concentrations that were found in the present study while the range of concentration (4.71-78.81 ng g<sup>-1</sup>) was similar to range of rainy and winter season's samples of the present study. The concentration levels of DDTs (0.02-1.4 mg/kg or

20-1400 ng g<sup>-1</sup>) reported by Nahar *et al.* (2008), were also closely similar to the present study. On the otherhand, most of the rearch on DDTs concentration in our country, were held on dry fishes for the direct and excessive use of this contaminant in dry fish where the concentration levels were higher to the present study reported by Bhuiyan *et al.* (2009), Hasan *et al.* (2013) and Hasan *et al.* (2014)

The results of the present investigation are comparable with other studies done in the world. For example, Cal *et al.* 2008 found that the levels of DDT and its metabolites residues in fishes from Cinca river, a tributary of Ebro river of Spain were higher (nondetected to 2098 µg kg<sup>-1</sup> or ng g<sup>-1</sup>) than the residue levels that analysed in the present study. Concentration levels of DDTs residues in fishes of Midcontinental great river (US), alpine lake (Switzerland), rocky mountains (Canada), Tibetan plateau (Central Asia) were reported in lower levels (Blocksom *et al.* 2010, Schemid *et al.* 2007, Demers *et al.* 2007 and Yang *et al.* 2010) than the present study.

As mentioned above, in most of the studies where concentration levels analysed only for one or two seasons while the present study includes four seasons as in, our country four seasons are distinct and the meteorological, physical, biological and many other factors vary with these seasons which directly or idirectly affects the residue levels. Therefore it could be said that the present study shows the actual status of contaminant levels in fishes throughout the year which is quite difficult to compare with other study with limited data.

### 4.9. Detection of DDT exposure time from the ratio of (DDE+DDD)/DDT

Metabolites of some organochlorine pesticides such as DDT have different concentration ratios in the environment, thereby indicating different contamination sources. Specific ratios of parent and metabolites of organochlorine pesticides compounds have been widely used to identify past and present input application into the environment (Walker *et al.* 1999).

The ratio of (DDE+DDD)/DDT is a helpful tool in revaling the significance of the degradation of DDT and to evaluate the current or past use of DDT in the region (Yu

et al. 2011). The ratio higher than 0.5 indicates past input of DDT while lower indicates recent input of DDT. In the present study, 38.46% samples having value of (DDE+DDD)/DDT lower than 0.5 ratios. These result indicated that the presence of new DDT inputs in the environments of Meghna river and flood plains of Sonargaon area. Fresh inputs of DDTs were also reported in India (Chourasiya et al. 2014) Uganda (Sseburgene et. al. 2009), South China (Hao et al. 2014, Sun et al. 2014, Zhao et al. 2009) and in Brazil (Rissato et al. 2007) etc.

Moreover tropical data on POPs shows rapid degradation patterns, low residual levels and wide distribution because the tropics are characterized by different radiation throughout the year, a high load of microorganisms, tropical rain and various soil types (Wandiga *et al.* 2001, Peters *et al.* 2001).

#### 4.10. Tolerance limits

Food consumption is the main exposure route for organochlorinated pollutants for the general population, and fish and fishery products seem to be the main contributors to the total dietary consumption of these pollutants (Schnitzler *et al.* 2011). Therefore, fish consumption can be a major source of human exposure to OCPs in Bangladesh, and the exposure levels depend on the lipid content of the fish and the amount of fish consumed.

No regulations by Bangladesh agriculture or health agencies are available regarding maximum OCP limits in fish and other food. To roughly evaluate the potential environmental risk of organochlorine residues, concentrations of selected analytes in tissues of the present study were compared in with the maximum residue limits (MRLs) recommended for human consumption by various agencies.

Maximum Residue Level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly. Good Agricultural Practice will ensure a concern safety to human health. The concentration of organochlorine pesticides obtained in various food samples were compared with MRLs, set by the various international agencies such as Food and Agricultural

Organisation/World Health Organisation (FAO/WHO 2012). The values found in fish tissue in four different seasons are below the MRL value of 5000 ng g<sup>-1</sup> w.w. for consumption set by Codex Alimentarius Commission of FAO/WHO (2012). On the other hand, the European Union (Binelli and Provini, 2004) has established a maximum admissible limit of 50 ng g<sup>-1</sup> ww for DDTs, in the present study, 20.83% species of rainy-season, 68.18% of autumn, 13.04% in winter and 100% in summer exceeded this limit. Moreover, European Union Directive 1999/788 has established the maximum acceptable limit of 200 ng g<sup>-1</sup> ww for human consumption (Robinson *et al.*2016). Only 4.55% species of autumn and 86.36% of summer samples exceeded this limit. However, 4.55% species of autumn and 36.36% of summer are above concentrations associated with reproductive toxicity in several species of fish (500 ng g<sup>-1</sup> w.w.; Jarvinen and Ankley 1999).

#### 4.11. Human Health risk estimates

From the obtained concentrations of DDTs residues, dietary exposure and health risks were calculated for adult. The estimated daily intake and Health Risk Index (HI) were calculated for the contaminant of each fishes. The HI showed that total DDTs residues in all fishes recorded less than 1 (HI<1).

The statistic of daily food intake of an adult person of our country reveals that fish rank fourth after rice, cereal and vegetables which contribute about 4% of the total daily food consumption in weight (HIES 2010). Although detail study was lacking about the DDTs residues in our food items, some previous works reported the detection of the contaminants in some food such as chicken, dry fish and vegetables (Nahar *et al.* 2008). Therefore it can be said that although the HI of the studied fishes are less than 1 but toghther with other food items of daily meal, total HI would be greater than 1. This shows that there is health risk associoated with lifetime consumption of the studied fishes, togther with other contaminated foods.

Similar findings also reported by the Nuapia *et al.* (2016) that the values of HI of fishes were <1 but together with vegetables and meat the values were >1 and that associated with human health hazards.

# 5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

## **5.1. SUMMERY**

Pesticide residue problem is an environmental hazard and becoming serious focus for human health. Organohalogen pesticides such as DDT and its metabolites are of great concern to the environmental scientists for several decades, due to their persistence, bioaccumulation, long-range transport, toxicity and adverse effects on environment and human health including reproduction and birth defects, immune system dysfunction, endocrine disruptions and canceR. The intensive cultivation of the agriproducts depends upon the use of fertilizer, pesticides, insecticides, fungicides and herbicides. About 25% of these compounds pass to the nearby water-body and act as a pollutant sources for fish and other aquatic organisms. Fishes are used extensively for environmental monitoring because they uptake contaminants directly from water and food. Generally the ability of the fish to metabolize organohalogen is moderate; therefore, contaminants load in fish are well reflective of the state of pollution in surrounding environment (Matin et al. 1998). Thus in view of multidimensional impacts of pesticide in fish, environment and human, the scope of the present study is to asses some organohalogen pesticide residues in fishes of Bangladesh. In this study, the concentrations of organohalogen pesticide residues; DDT and its metabolites DDE, DDD, 2,4-DDT and 4,4-DDT were investigated in different fish and prawn species of Meghna river

Different fish and prawn species of different trophic levels were collected from Meghna river at Boidyer Bazar of Sonargaon Upazila of Narayanganj District. Samples were collected periodically from four different seasons; rainy-season, autum, winter and summer. The samples were immediately wrapped with aluminium foil, put into a chill box with ice and transported to the laboratory on the same day. After identification and taking some physiological data, the samples were then stored in a freezer at a temperature below -20°C temperature until analysis. Before extraction, the fish tissue was made bone free, chopped and blended. The samples were extracted

following QuEChERS method using ethyle acetate as solvent and cleaned up using sulphuric acid. The cleaned extracts were analyzed by GC-ECD for the residual amounts of DDTs (DDT and its metabolites). The calibration curve of serially diluted certified DDTs (DDE,DDD, 2,4' DDT and 4,4' DDT) standard solution showed peak area with a very linear correlation ( $r^2$  =0.996,  $r^2$ =0.995,  $r^2$ =0.985 and  $r^2$ =0.996 respectively). LOD and LOQ were found to be 0.0625 ng g<sup>-1</sup>, 6.25 ng g<sup>-1</sup> respectively. The percent of the recovery DDTs in spiked control Rui fish samples at different levels (0.05, 0.10 and 0.20 ng g<sup>-1</sup>) The percentage recoveries for fish samples were found to be 88-92 % for DDE , 101-113% for DDD, 76-104 % for 2,4'-DDT and 70-90 % for 4,4'-DDT.

## 5.1.1. Lipid contents of fish and prawn samples of different seasons

The lipid contents (%) of different fish and prawn species ranged from  $0.53 \pm 0.01\%$  in Boal to  $13.98 \pm 1.50\%$  in Bacha during rainy season,  $0.51 \pm 0.06\%$  in Boal to  $14.43 \pm 0.64\%$  in Rui during autumn,  $0.41 \pm 0.07$  in Shole to  $13.66 \pm 0.56\%$  in Rui during winter and  $0.12 \pm 0.01\%$  in Boal to  $15.40 \pm 0.01\%$  in Bacha during summer. Variation of lipid contents is species specific. As DDT accumulates in the fat body of organisms due to its lipophilic nature, lipid contents of the fishes also studied due to analyse their relationsphip.

## 5.1.2. Total DDTs residues of fishes and prawn of different seasons

The mean concentrations of  $\Sigma$ DDTs residue (ng g <sup>-1</sup> ww) in fishes and prawn during rainy-season ranged from 2.64  $\pm$  0.35 ng g <sup>-1</sup> in Kachki to 191.14  $\pm$  31.18 ng g <sup>-1</sup> in Bacha, 16.42  $\pm$  1.90 ng g <sup>-1</sup> in Kalisha to 271.50 $\pm$ 6.17 ng g <sup>-1</sup> in Bacha during autumn, 3.88  $\pm$  0.60 ng g <sup>-1</sup> in Golda Chingri to 141.57  $\pm$  10.24 ng g <sup>-1</sup> in Bacha during winter and 157.58  $\pm$  1.15 ng g <sup>-1</sup> in Kachki to 1660.89  $\pm$  157.9 ng g <sup>-1</sup> in Bacha during summer. Considering the mean concentrations of DDTs residue of four seasons, Rui, Ghainna, Bata, Jatpunti, Sharpunti, Shing, Magur, Kachki, Goldachingri, Gutum, Khalisha, Kaikka, Tarabaim and Boal conained lower amount of DDTs residue While Tengra, Bajari-tengra, Gulsha, Borobaim, Chewa, Meni, Bele, Poa and Bacha contained higher amont of DDTs residues.

The mean concentration of total DDTs for all the herbivores, omnivores and carnivores were 10.38, 21.38 and 45.10 ng g<sup>-1</sup> during rainy-season, 36.37, 47.34, 96.00 ng g<sup>-1</sup>.during autumn, 16.75, 20.32 and 43.30 ng g<sup>-1</sup>.during winter and 264.64, 334.71, 845.83 ng g<sup>-1</sup> during summer. Therfore in every season on the basis of average total DDTs residues the chronlolgy was herbivore < omnivore < carnivore. One way ANOVA with Tukey HSD and LSD Post Hoc tests showed significant differences in total DDTs between herbivore, omnivore and carnivore fishes.

In the present study, fishes with lower DDTs residues may be attributed to their lower position in food chain or low lipid content and sometimes both of these. As mentioned above, Rui, Ghainna and Bata contained lower DDTs residues may be related to their lower position in foodchain that feed on plant materials while the lower residue levels of Kachki, Chanda, Shing fish and Goldachingri may be related to their lower lipid content and positioned in lower trophic levels that mainly feed on zooplankton and plant materials.

Jatpunti and Sharpunti contained comperatively lower DDTs residues but higher than Kachki, Shing, Magur, Chanda, Rui, Ghainna and Bata may be attributed to their higher lipid content and feeding habit also that mainly feed on algae, plant material and small amount insects due to omnivorous nature

Foli and Boal also contained lower amount of DDTs residues. Although they occupy in the higher trophic level but contained low amount of lipid content may be responsible for lower amount of DDTs residues.

Tengra, Bajari-tengra and Gulsha contained higher DDTs residues may be related to their both higher lipid content and higher trophic positions as feed mainly on small fishes, insects, moluscks and little amount of algae and plant material due to their omnivorous nature.

Chewa, Meni, Bele, Poa, Shole, Gojar and Bacha contained much higher amount of DDTs residues. All these fishes are highly carnivorous, voracious, predatory in nature,

and top consumer as feed on small fish, juvenile of large fish, frog, snake, insects, earthworm and tadpole larva etc. due to their highly carnivorous and predatory in nature, which could be related to their higher DDTs accumulation. While Borobaim contained higher residues due to their bottom dwelling carnivorous nature.

Fishes are classified into different cluster on the basis of DDTs residues through dendogram. From the cluster analysis, it is clear that herbivore fishes, lean and plant based omnivores and lean, zooplankton based carnivores belong to same group, contained lower DDTs residues; fatty and animal based omnivores, lean and lower carnivores contained medium or higher DDTs residues while bottom feeder carnivores, predators and fatty top carnivore fishes belong to same group that contained much higher residues.

Therefore, it is clear that lipid contents, trophic positions and feeding habit all together influence the accumulation of pesticide residues in fishes.

## 5.1.3. Meteorological parameters and DDTs concentrations

In the present study together with biological factors the meteorological parameters (temperature, humidity and rainfall) are also considered to asses the accumulation of DDTs in fish body. From the analysis of distribution pattern of contaminats in fish and prawn tissues, it is clear that both biological and analytical factors are important to interpret DDTs bioaccumulation.

In the present study, DDTs residues showed significant positive correlation with ambient temperature and significant negative correlation with humidity. Rainfall is other important parameter to influence the DDTs accumulations showed negative correlation but not significant.

Therefore all these physical factors are also important to predict the DDTs level but they do not work singlely as persist together in the environment and influence together to accumulation DDTs.

#### **5.1.4.** Seasonal distribution of DDTs concentrations

Lipid contents and  $\Sigma$ DDTs concentrations vary significantly between species for all seasons. One way ANOVA with Tukey HSD and LSD Post Hoc tests showed highly significant differences in total DDTs ( $\Sigma$ DDTs) concentrations of fishes and prawn between seasons (p<0.01) where the summer season showed highly significant variations between rainy-season, autumn and winter. The biological and meteorological factors that influence DDTs accumulation, vary greately with seasons may be attributed to the significant seasonal variation of residues.

In case of  $\Sigma$ DDTs concentrations 70% species showed the highest amount in summer and lowest in rainy-season. Considering the mean values DDTs residues for seasons the chronology is winter (25.60 ng/g) < rainy-season (30.88 ng/g) < autumn (63.34 ng/g) < summer (580.72 ng/g).

During summer season, the mean concentration of  $\sum DDTs$  was significantly higher than other three seasons which was around ten times greater than autumn and around twenty times greater than rainy-season and winter. Several factors and conditions influence together to higher accumulation. The factors are higher food intake, high ambient temperature and lipid contents, short rain in summer may influence together in DDT accumulation that increase its concentration at cumulative rate in fish body.

Higher residue levels also detected during autumn compare to rainy-season and winter may be attributed to high feeding intensity for recovery energy that they loss through spawning in rainy-season and also food availability is much in this season and concentrated pollutants due to small volume of riverwater.

On the other hand low residue levels detected during rainy-season compate to the summer and autumn. This could be attributed to dilution effects based on large volumes of rainwater. Rainy-season is the spawning season of fishes therefore, fishes transfer DDTs residues to offspring via gonads. Moreover feeding internsity of fishes is lower during spawing season may be related to lower DDTs accumulation

Lowest residues levels also detected during winter could be attributed to lower feeding intensity, lower lipid content and also lower ambient temperature. During winter season, the feeding internsity of fishes is very much low due to scarcity of food, moreover they loss stored lipid contents through metabolism to supply energy for physiological activities. In this season the ambient temperature was lowest may also be related to lower DDTs accumulation.

#### **5.1.5.** Tolerance limits

The values found in fish tissue in four different seasons are below the MRL value for consumption set by Codex Alimentarius Commission of FAO/WHO (2012) but exceeded the maximum admissible limit and maximum acceptable limit set by other international agencies. However, in some cases the concentration levels exceeded the limit that may be associated with reproductive toxicity in several species of fish.

#### 5.1.6. Present and historical use of DDT

From the ratio of (DDE+DDD)/DDT, it can be evaluated the current or past use of DDT in the region. In the present study, analyzing the ratios indicates the evidence of both current and historical use of DDT as pesticides in the neighbouring environments of Meghna river and flood plains of Sonargaon area. Therefore, it can be said that DDT is still exposed to the environments in the country.

#### 5.1.7. Human health risk

As the residue levels exceeded some tolerance limits together with their continuous exposure make these contaminants as major concer to human health hazard. From this point of view the human health risk was estimated for the studied fish species.

Hazard Index (HI) is used to assess the health risk of consumers from the intake of pesticides contaminated fish. The estimated HIs is obtained by dividing the Estimated Daily Intake (EDI) by their corresponding values of Acceptable Daily Intakes (ADI) by WHO/FAO (FAO/WHO 2010). If the value of HI is greater (>) than 1 that would be associated to health risk.

All the detected HIs of DDTs residues for studied fish and prawn species were <1. Though the HIs are less than 1 but toghther with other food items of daily meal, total HI would be greater than 1. This shows that there is health risk associoated with lifetime consumption of the studied fishes, togther with other contaminated foods.

## 5.2. CONCLUSION

Organohalogen compounds especially dichlorodiphenyltrichloroethanes (DDTs) become a worldwide concern, despite controls on their manufacturing, importation and agricultural or vector practices. Through their lipophilicity and persistence, the toxic chemicals and their residues may concentrate in the fatty tissues of animals leading to environmental persistence, bioconcentration and biomagnification through the food chain. Because these chemicals are toxic to living organisms, increased accumulation in the food chain may pose serious health hazards to human and wildlife.

From this comprehensive analysis of DDT and its metabolites (4,4-DDT, 2,4-DDT,DDD and DDE) in reverine fishes and prawn species of different feeding habits at different seasons, demonstrating that the meteorological and biological parameters influencing the contamination, lead to the environmental risk of fish species for human health issues. The widespread year round occurance of DDTs in all fishes and prawn species confirms the recent use of this illegal compound of potential contaminant to fish and environment. Moreover the significant levels of DDT metabolites indicating the past use of the parent compound (DDT).

The levels of total DDTs residues in most cases exceeded the prescribed maximum admissible and acceptable limits set by different international agencies; European Union and FAO/WHO, though below the limit of Maximum Residue Level (MRL) for consumption set by Codex Alimentarius Commission of FAO/WHO (2012). It is predicted that these residues may be originated from present and also past activities in agricultural practices. The continuous consumption of contaminated fishes throughout the year with significant amount of the toxic eliments may pose human health hazards. From the estimated Hazarad Index (HI) of studied fishes, it can be said that the fishes together with other contaminations in food further cause complex hazard issues. To prevent this kind of health disaster, it is essential to formulate a regular monitoring practice on the residues of the key components of human food chain. Further policy forming and awareness building on this issue is of prime need.

## **5.3. RECOMMENDATIONS**

- Since fish is common and essential part of our diet, this study showed serious concern on food born pesticide contaminant like illegal DDT in fish tissue
- The control of illegal agrochemicals is must, as well the strong monitoring and legal control is necessary
- More understanding on agrochemicals instruction in food chart and the food safety issues is essential
- As DDT is not produced locally, stopping illegal entry of these long persistent substances in the country is of great importance for human health issues.

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# Appendix-1. One way Analysis of Variance (ANOVA) for significance test of DDE, DDD and 2,4-DDT residues in samples between and within four seasons

### **ANOVA**

#### DDE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	145566.942	3	48522.314	11.336	.000
Within Groups	372392.581	87	4280.374		
Total	517959.523	90			

### **ANOVA**

### DDD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	72274.570	3	24091.523	14.546	.000
Within Groups	144088.995	87	1656.195		
Total	216363.565	90			

## **ANOVA**

### 2,4'-DDT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	406812.361	3	135604.120	38.767	.000
Within Groups	304322.681	87	3497.962		
Total	711135.042	90			

# Appendix-2. One way Analysis of Variance(ANOVA) for significance test of 4,4-DDT, total DDTs residues and lipid contents in samples between and within four seasons

#### **ANOVA**

#### 4,4'-DDT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	882774.209	3	294258.070	38.866	.000
Within Groups	658678.436	87	7571.017		
Total	1541452.645	90			

### ANOVA

### Total DDTs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4839080.946	3	1613026.982	31.944	.000
Within Groups	4393079.732	87	50495.169		
Total	9232160.678	90			

### ANOVA

## Lipid contents

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.444	3	8.481	.465	.708
Within Groups	1588.144	87	18.255		
Total	1613.588	90			

# Appendix-3. Post Hoc tests (Tukey HSD and LSD) for significance test of DDE residues in samples between and within four seasons

#### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: DDE

•	(I) Season	(J) Season	Mean	Std. Error	Sig.	95% Confide	ence Interval
			Difference (I-			Lower Bound	Upper Bound
-			J)		•		
		Autumn season	-11.94975	19.09063	.923	-61.9555	38.0560
	Rainy season	Winter season	-43.86629	19.31091	.113	-94.4491	6.7165
		Summer season	-103.56947*	19.31091	.000	-154.1523	-52.9867
		Rainy season	11.94975	19.09063	.923	-38.0560	61.9555
	Autumn season	Winter season	-31.91654	19.51066	.364	-83.0225	19.1895
Talaaa HCD		Summer season	-91.61972 <sup>*</sup>	19.51066	.000	-142.7257	-40.5137
Tukey HSD		Rainy season	43.86629	19.31091	.113	-6.7165	94.4491
	Winter Season	Autumn season	31.91654	19.51066	.364	-19.1895	83.0225
		Summer season	-59.70318 <sup>*</sup>	19.72625	.017	-111.3739	-8.0325
	Summer season	Rainy season	103.56947*	19.31091	.000	52.9867	154.1523
		Autumn season	91.61972*	19.51066	.000	40.5137	142.7257
		Winter season	59.70318*	19.72625	.017	8.0325	111.3739
		Autumn season	-11.94975	19.09063	.533	-49.8944	25.9949
	Rainy season	Winter season	-43.86629*	19.31091	.026	-82.2488	-5.4838
		Summer season	-103.56947*	19.31091	.000	-141.9520	-65.1869
		Rainy season	11.94975	19.09063	.533	-25.9949	49.8944
	Autumn season	Winter season	-31.91654	19.51066	.105	-70.6961	6.8630
LSD		Summer season	-91.61972*	19.51066	.000	-130.3993	-52.8402
LSD		Rainy season	43.86629*	19.31091	.026	5.4838	82.2488
	Winter Season	Autumn season	31.91654	19.51066	.105	-6.8630	70.6961
		Summer season	-59.70318*	19.72625	.003	-98.9112	-20.4951
		Rainy season	103.56947*	19.31091	.000	65.1869	141.9520
	Summer season	Autumn season	91.61972*	19.51066	.000	52.8402	130.3993
		Winter Season	59.70318*	19.72625	.003	20.4951	98.9112

st. The mean difference is significant at the 0.05 level.

# Appendix-4. Post Hoc tests (Tukey HSD and LSD) for significance test of DDD residues in samples between and within four seasons

### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: DDD

	(I) Season	(J) Season	Mean	Std. Error	Sig.	95% Confiden	ce Interval
			Difference (I-J)			Lower Bound	Upper Bound
	_	Autumn season	7.34047	11.87504	.926	-23.7649	38.4458
	Rainy season	Winter season	4.35053	12.01207	.984	-27.1137	35.8148
		Summer season	-61.69629*	12.01207	.000	-93.1606	-30.2320
		Rainy season	-7.34047	11.87504	.926	-38.4458	23.7649
	Autumn season	Winter season	-2.98994	12.13631	.995	-34.7797	28.7998
T 1 110D		Summer season	-69.03676*	12.13631	.000	-100.8265	-37.2470
Tukey HSD		Rainy season	-4.35053	12.01207	.984	-35.8148	27.1137
	Winter season	Autumn season	2.98994	12.13631	.995	-28.7998	34.7797
		Summer season	-66.04682*	12.27042	.000	-98.1878	-33.9058
		Rainy season	61.69629*	12.01207	.000	30.2320	93.1606
	Summer season	Autumn season	69.03676*	12.13631	.000	37.2470	100.8265
		Winter Season	66.04682*	12.27042	.000	33.9058	98.1878
		Autumn season	7.34047	11.87504	.538	-16.2625	30.9434
	Rainy season	Winter season	4.35053	12.01207	.718	-19.5248	28.2258
		Summer season	-61.69629*	12.01207	.000	-85.5716	-37.8210
		Rainy season	-7.34047	11.87504	.538	-30.9434	16.2625
	Autumn season	Winter season	-2.98994	12.13631	.806	-27.1122	21.1323
1 00		Summer season	-69.03676*	12.13631	.000	-93.1590	-44.9145
LSD		Rainy season	-4.35053	12.01207	.718	-28.2258	19.5248
	Winter Season	Autumn season	2.98994	12.13631	.806	-21.1323	27.1122
		Summer season	-66.04682*	12.27042	.000	-90.4356	-41.6580
		Rainy season	61.69629*	12.01207	.000	37.8210	85.5716
	Summer season	Autumn season	69.03676*	12.13631	.000	44.9145	93.1590
		Winter season	66.04682*	12.27042	.000	41.6580	90.4356

 $<sup>\</sup>ensuremath{^{*}}.$  The mean difference is significant at the 0.05 level.

# Appendix-5. Post Hoc tests (Tukey HSD and LSD) for significance test of 2,4'-DDT residues in samples between and within four seasons

### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: 2,4'-DDT

	(I) Season	(J) Season	Mean	Std. Error	Sig.	95% Confide	nce Interval
			Difference (I-			Lower Bound	Upper
			J)				Bound
		Autumn season	1.69384	17.25786	1.000	-43.5112	46.8989
	Rainy season	Winter season	-1.03561	17.45700	1.000	-46.7623	44.6911
		Summer season	-155.91346 <sup>*</sup>	17.45700	.000	-201.6401	-110.1868
		Rainy season	-1.69384	17.25786	1.000	-46.8989	43.5112
	Autumn season	Winter season	-2.72945	17.63756	.999	-48.9291	43.4702
Tukey HSD		Summer season	-157.60730*	17.63756	.000	-203.8069	-111.4077
Tukey HSD		Rainy season	1.03561	17.45700	1.000	-44.6911	46.7623
	Winter season	Autumn season	2.72945	17.63756	.999	-43.4702	48.9291
		Summer season	-154.87785*	17.83246	.000	-201.5880	-108.1677
		Rainy season	155.91346 <sup>*</sup>	17.45700	.000	110.1868	201.6401
	Summer season	Autumn season	157.60730*	17.63756	.000	111.4077	203.8069
		Winter season	154.87785*	17.83246	.000	108.1677	201.5880
		Autumn season	1.69384	17.25786	.922	-32.6080	35.9957
	Rainy season	Winter season	-1.03561	17.45700	.953	-35.7333	33.6621
		Summer season	-155.91346*	17.45700	.000	-190.6111	-121.2158
		Rainy season	-1.69384	17.25786	.922	-35.9957	32.6080
	Autumn season	Winter season	-2.72945	17.63756	.877	-37.7860	32.3271
LSD		Summer season	-157.60730*	17.63756	.000	-192.6639	-122.5507
LSD		Rainy season	1.03561	17.45700	.953	-33.6621	35.7333
	Winter season	Autumn season	2.72945	17.63756	.877	-32.3271	37.7860
		Summer season	-154.87785*	17.83246	.000	-190.3218	-119.4339
		Rainy season	155.91346*	17.45700	.000	121.2158	190.6111
	Summer season	Autumn season	157.60730*	17.63756	.000	122.5507	192.6639
		Winter season	154.87785*	17.83246	.000	119.4339	190.3218

st. The mean difference is significant at the 0.05 level.

# Appendix-6. Post Hoc tests (Tukey HSD and LSD) for significance test of 4,4-DDT residues in samples between and within four seasons

### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: 4,4'-DDT

	(I) Season	(J) Season	Mean	Std. Error	Sig.	95% Confide	ence Interval
			Difference (I-J)			Lower Bound	Upper Bound
		Autumn season	2.94293	25.38964	.999	-63.5624	69.4483
	Rainy season	Winter season	1.22932	25.68260	1.000	-66.0434	68.5020
		Summer season	-228.65705 <sup>*</sup>	25.68260	.000	-295.9298	-161.3843
		Rainy season	-2.94293	25.38964	.999	-69.4483	63.5624
	Autumn season	Winter season	-1.71362	25.94825	1.000	-69.6822	66.2549
T 1 110D		Summer season	-231.59998*	25.94825	.000	-299.5685	-163.6314
Tukey HSD		Rainy season	-1.22932	25.68260	1.000	-68.5020	66.0434
	Winter Season	Autumn season	1.71362	25.94825	1.000	-66.2549	69.6822
		Summer season	-229.88636*	26.23498	.000	-298.6060	-161.1667
		Rainy season	228.65705*	25.68260	.000	161.3843	295.9298
	Summer season	Autumn season	231.59998*	25.94825	.000	163.6314	299.5685
		Winter Season	229.88636*	26.23498	.000	161.1667	298.6060
		Autumn season	2.94293	25.38964	.908	-47.5217	53.4076
	Rainy season	Winter season	1.22932	25.68260	.962	-49.8176	52.2763
		Summer season	-228.65705 <sup>*</sup>	25.68260	.000	-279.7040	-177.6101
		Rainy season	-2.94293	25.38964	.908	-53.4076	47.5217
	Autumn season	Winter season	-1.71362	25.94825	.947	-53.2886	49.8613
I GD		Summer season	-231.59998*	25.94825	.000	-283.1749	-180.0250
LSD		Rainy season	-1.22932	25.68260	.962	-52.2763	49.8176
	Winter Season	Autumn season	1.71362	25.94825	.947	-49.8613	53.2886
		Summer season	-229.88636 <sup>*</sup>	26.23498	.000	-282.0312	-177.7415
		Rainy season	228.65705 <sup>*</sup>	25.68260	.000	177.6101	279.7040
	Summer season	Autumn season	231.59998*	25.94825	.000	180.0250	283.1749
		Winter season	229.88636*	26.23498	.000	177.7415	282.0312

 $<sup>\ ^{*}.</sup>$  The mean difference is significant at the 0.05 level.

# Appendix-7. Post Hoc tests (Tukey HSD and LSD) for significance test of total DDTs residues in samples between and within four seasons

### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: Total DDTs

	(I) Season	(J) Season	Mean	Std. Error	Sig.	95% Confide	nce Interval
			Difference (I-			Lower Bound	Upper
			J)				Bound
		Autumn season	.02750	65.56987	1.000	-171.7255	171.7805
	Rainy season	Winter Season	-39.32205	66.32647	.934	-213.0569	134.4128
		Summer season	-549.83626 <sup>*</sup>	66.32647	.000	-723.5711	-376.1015
		Rainy season	02750	65.56987	1.000	-171.7805	171.7255
	Autumn season	Winter season	-39.34955	67.01251	.936	-214.8814	136.1823
T 1 110D		Summer season	-549.86376 <sup>*</sup>	67.01251	.000	-725.3956	-374.3319
Tukey HSD		Rainy season	39.32205	66.32647	.934	-134.4128	213.0569
	Winter season	Autumn season	39.34955	67.01251	.936	-136.1823	214.8814
		Summer season	-510.51421*	67.75301	.000	-687.9857	-333.0427
		Rainy season	549.83626 <sup>*</sup>	66.32647	.000	376.1015	723.5711
	Summer season	Autumn season	549.86376 <sup>*</sup>	67.01251	.000	374.3319	725.3956
		Winter Season	510.51421*	67.75301	.000	333.0427	687.9857
		Autumn season	.02750	65.56987	1.000	-130.2997	130.3547
	Rainy season	Winter season	-39.32205	66.32647	.555	-171.1531	92.5090
		Summer season	-549.83626 <sup>*</sup>	66.32647	.000	-681.6673	-418.0052
		Rainy season	02750	65.56987	1.000	-130.3547	130.2997
	Autumn season	Winter season	-39.34955	67.01251	.559	-172.5442	93.8451
LSD		Summer season	-549.86376 <sup>*</sup>	67.01251	.000	-683.0584	-416.6691
LSD		Rainy season	39.32205	66.32647	.555	-92.5090	171.1531
	Winter Season	Autumn season	39.34955	67.01251	.559	-93.8451	172.5442
		Summer season	-510.51421*	67.75301	.000	-645.1807	-375.8478
		Rainy season	549.83626 <sup>*</sup>	66.32647	.000	418.0052	681.6673
	Summer season	Autumn season	549.86376 <sup>*</sup>	67.01251	.000	416.6691	683.0584
		Winter season	510.51421*	67.75301	.000	375.8478	645.1807

<sup>\*.</sup> The mean difference is significant at the 0.05 level.

# Appendix-8. Post Hoc tests (Tukey HSD and LSD) for significance test of lipid content in samples between and within four seasons

### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: Lipid content

	(I) Season	(J) Season	Mean	Std. Error	Sig.	95% Confidence	e Interval
			Difference (I-J)			Lower Bound	Upper
							Bound
		Autumn season	.14025	1.24671	.999	-3.1254	3.4059
	Rainy season	Winter season	66265	1.26109	.953	-3.9659	2.6406
		Summer season	-1.17992	1.26109	.786	-4.4832	2.1234
		Rainy season	14025	1.24671	.999	-3.4059	3.1254
	Autumn season	Winter season	80291	1.27414	.922	-4.1404	2.5346
Tukey HSD		Summer season	-1.32018	1.27414	.729	-4.6576	2.0173
Tukey HSD		Rainy season	.66265	1.26109	.953	-2.6406	3.9659
	Winter season	Autumn season	.80291	1.27414	.922	-2.5346	4.1404
		Summer season	51727	1.28822	.978	-3.8916	2.8571
		Rainy season	1.17992	1.26109	.786	-2.1234	4.4832
	Summer season	Autumn season	1.32018	1.27414	.729	-2.0173	4.6576
		Winter season	.51727	1.28822	.978	-2.8571	3.8916
		Autumn season	.14025	1.24671	.911	-2.3377	2.6182
	Rainy season	Winter season	66265	1.26109	.601	-3.1692	1.8439
		Summer season	-1.17992	1.26109	.352	-3.6865	1.3266
		Rainy season	14025	1.24671	.911	-2.6182	2.3377
	Autumn season	Winter season	80291	1.27414	.530	-3.3354	1.7296
LSD		Summer season	-1.32018	1.27414	.303	-3.8527	1.2123
LSD		Rainy season	.66265	1.26109	.601	-1.8439	3.1692
	Winter season	Autumn season	.80291	1.27414	.530	-1.7296	3.3354
		Summer season	51727	1.28822	.689	-3.0777	2.0432
		Rainy season	1.17992	1.26109	.352	-1.3266	3.6865
	Summer season	Autumn season	1.32018	1.27414	.303	-1.2123	3.8527
		Winter season	.51727	1.28822	.689	-2.0432	3.0777

# Appendix-9. One way Analysis of Variance (ANOVA) for significance test of DDE, DDD and 2,4-DDT residues in samples between and within different feeding habits

#### **ANOVA**

DDE

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	34277.769	2	17138.884	3.118	.049
Within Groups	483681.755	88	5496.384		
Total	517959.523	90			

### ANOVA

DDD

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17109.357	2	8554.678	3.778	.027
Within Groups	199254.208	88	2264.252		
Total	216363.565	90			

### **ANOVA**

2,4'-DDT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	28816.442	2	14408.221	1.858	.162
Within Groups	682318.601	88	7753.620		
Total	711135.042	90			

Appendix-10. One way Analysis of Variance (ANOVA) for significance test of 4,4-DDT, total DDTs residues and lipid contents in samples between and within different feeding habits

### **ANOVA**

4,4'-DDT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	86670.033	2	43335.016	2.621	.078
Within Groups	1454782.612	88	16531.621		
Total	1541452.645	90			

#### **ANOVA**

#### Total DDTs

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	607187.647	2	303593.824	3.098	.050
Within Groups	8624973.031	88	98011.057		
Total	9232160.678	90			

#### **ANOVA**

Lipid contents

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	306.879	2	153.439	10.333	.000
Within Groups	1306.709	88	14.849		
Total	1613.588	90			

## Appendix-11. Post Hoc tests (Tukey HSD and LSD) for significance test of DDE residues in samples between and within different feeding habits

#### **Post Hoc Tests**

#### **Multiple Comparisons**

Dependent Variable: DDE

	(I) Food habit	(J) Food habit	Mean	Std. Error	Sig.	95% Confiden	ce Interval
			Difference (I-J)			Lower Bound	Upper Bound
	Herbivore	Omnivore	-9.04193	24.80063	.929	-68.1669	50.0830
	Helbivoic	Carnivore	-45.15477	24.14435	.153	-102.7151	12.4056
Tukey HSD	0 :	Herbivore	9.04193	24.80063	.929	-50.0830	68.1669
	Omnivore	Carnivore	-36.11284	16.79159	.086	-76.1441	3.9185
	Carnivore	Herbivore	45.15477	24.14435	.153	-12.4056	102.7151
	Carnivore	Omnivore	36.11284	16.79159	.086	-3.9185	76.1441
	Herbivore	Omnivore	-9.04193	24.80063	.716	-58.3280	40.2441
	Helbivoic	Carnivore	-45.15477	24.14435	.065	-93.1366	2.8271
LSD	0 :	Herbivore	9.04193	24.80063	.716	-40.2441	58.3280
2.52	Omnivore Omnivore	Carnivore	-36.11284 <sup>*</sup>	16.79159	.034	-69.4826	-2.7431
	C :	Herbivore	45.15477	24.14435	.065	-2.8271	93.1366
	Carnivore	Omnivore	36.11284 <sup>*</sup>	16.79159	.034	2.7431	69.4826

<sup>\*.</sup> The mean difference is significant at the 0.05 level.

### Homogeneous Subsets

DDE

	Food habit	N	Subset for alpha = 0.05
			1
	Herbivore	12	17.7875
T-1 HCDab	Omnivore	35	26.8294
Tukey HSD <sup>a,b</sup>	Carnivore	44	62.9423
	Sig.		.110

a. Uses Harmonic Mean Sample Size = 22.283.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

# Appendix-12. Post Hoc tests (Tukey HSD and LSD) for significance test of DDD residues in samples between and within feeding habits

#### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: DDD

	(I) Food habit	(J) Food habit	Mean Difference	Std. Error	Sig.	95% Confidence	e Interval
			(I-J)			Lower Bound	Upper
							Bound
	II 1:	Omnivore	-1.28429	15.91793	.996	-39.2328	36.6642
	Herbivore	Carnivore	-28.38318	15.49671	.165	-65.3275	8.5611
Tulou HCD	0 :	Herbivore	1.28429	15.91793	.996	-36.6642	39.2328
Tukey HSD	Omnivore	Carnivore	-27.09890*	10.77744	.036	-52.7924	-1.4054
	<b>.</b>	Herbivore	28.38318	15.49671	.165	-8.5611	65.3275
	Carnivore	Omnivore	27.09890*	10.77744	.036	1.4054	52.7924
	Herbivore	Omnivore	-1.28429	15.91793	.936	-32.9178	30.3493
	пеготуоге	Carnivore	-28.38318	15.49671	.070	-59.1796	2.4133
I CD	0 :	Herbivore	1.28429	15.91793	.936	-30.3493	32.9178
LSD Omnivore	Carnivore	-27.09890*	10.77744	.014	-48.5168	-5.6810	
		Herbivore	28.38318	15.49671	.070	-2.4133	59.1796
	Carnivore	Omnivore	27.09890 <sup>*</sup>	10.77744	.014	5.6810	48.5168

<sup>\*.</sup> The mean difference is significant at the 0.05 level.

#### **Homogeneous Subsets**

#### DDD

	Food habit	N	Subset for alpha = 0.05	
			1	
	Herbivore	12	14.9400	
	Omnivore	35	16.2243	
Tukey HSD <sup>a,b</sup>	Carnivore	44	43.3232	
	Sig.		.120	

a. Uses Harmonic Mean Sample Size = 22.283.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

## Appendix-13. Post Hoc tests (Tukey HSD and LSD) for significance test of 2,4'-DDT residues in samples between and within different feeding habits

#### **Post Hoc Tests**

### **Multiple Comparisons**

Dependent Variable: 2,4'-DDT

	(I) Food habit	(J) Food habit	Mean	Std. Error	Sig.	95% Confider	ce Interval
			Difference (I-J)			Lower Bound	Upper
							Bound
	- II 1:	Omnivore	-1.36229	29.45619	.999	-71.5861	68.8616
	Herbivore	Carnivore	-36.61370	28.67671	.412	-104.9792	31.7519
Tulcay HCD	0	Herbivore	1.36229	29.45619	.999	-68.8616	71.5861
Tukey HSD	Omnivore	Carnivore	-35.25141	19.94369	.187	-82.7974	12.2945
	Carnivore	Herbivore	36.61370	28.67671	.412	-31.7519	104.9792
	Carnivore	Omnivore	35.25141	19.94369	.187	-12.2945	82.7974
	Herbivore	Omnivore	-1.36229	29.45619	.963	-59.9003	57.1757
	Herorvoie	Carnivore	-36.61370	28.67671	.205	-93.6026	20.3752
LSD	Omnivara	Herbivore	1.36229	29.45619	.963	-57.1757	59.9003
LSD	SD Omnivore	Carnivore	-35.25141	19.94369	.081	-74.8853	4.3825
	G .	Herbivore	36.61370	28.67671	.205	-20.3752	93.6026
	Carnivore	Omnivore	35.25141	19.94369	.081	-4.3825	74.8853

### Homogeneous Subsets

2.4'-DDT

		2,4 DD1	
	Food habit	N	Subset for alpha = 0.05
			1
	Herbivore	12	23.6100
Tukov USD <sup>a,b</sup>	Omnivore	35	24.9723
Tukey HSD <sup>a,b</sup>	Carnivore	44	60.2237
	Sig.		.352

a. Uses Harmonic Mean Sample Size = 22.283.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

# Appendix-14. Post Hoc tests (Tukey HSD and LSD) for significance test of 4,4'-DDT residues in samples between and within different feeding habits

#### **Post Hoc Tests**

#### **Multiple Comparisons**

Dependent Variable: 4,4'-DDT

	(I) Food habit	(J) Food habit	Mean	Std. Error	Sig.	95% Confider	nce Interval
			Difference (I-J)			Lower Bound	Upper
							Bound
Herbivore	II 1:	Omnivore	-4.88514	43.01124	.993	-107.4244	97.6541
	Carnivore	-65.31795	41.87307	.268	-165.1437	34.5078	
Talaaa HCD	Tulcay HSD Omniyona	Herbivore	4.88514	43.01124	.993	-97.6541	107.4244
Tukey HSD	Omnivore	Carnivore	-60.43281	29.12131	.101	-129.8583	8.9927
	Ci	Herbivore	65.31795	41.87307	.268	-34.5078	165.1437
	Carnivore	Omnivore	60.43281	29.12131	.101	-8.9927	129.8583
	Herbivore	Omnivore	-4.88514	43.01124	.910	-90.3610	80.5907
	пеготуоге	Carnivore	-65.31795	41.87307	.122	-148.5319	17.8960
I CD	O	Herbivore	4.88514	43.01124	.910	-80.5907	90.3610
LSD Omnivore	Ommvore	Carnivore	-60.43281*	29.12131	.041	-118.3053	-2.5603
	Ci	Herbivore	65.31795	41.87307	.122	-17.8960	148.5319
	Carnivore	Omnivore	60.43281*	29.12131	.041	2.5603	118.3053

<sup>\*.</sup> The mean difference is significant at the 0.05 level.

#### Homogeneous Subsets

4,4'-DDT

4,4-001								
	Food habit	N	Subset for alpha = 0.05					
			1					
	Herbivore	12	25.7500					
Tukey HSD <sup>a,b</sup>	Omnivore	35	30.6351					
Tukey HSD*	Carnivore	44	91.0680					
	Sig.		.213					

a. Uses Harmonic Mean Sample Size = 22.283.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

# Appendix-15. Post Hoc tests (Tukey HSD and LSD) for significance test of total DDTs residues in samples between and within different feeding habits

#### **Post Hoc Tests**

#### **Multiple Comparisons**

Dependent Variable: Total DDTs

	(I) Food habit	(J) Food habit	Mean	Std. Error	Sig.	95% Confide	ence Interval
			Difference (I-J)			Lower Bound	Upper Bound
	-	Omnivore	-16.57364	104.72776	.986	-266.2456	233.0983
Herbivore	Herbivore	Carnivore	-175.46961	101.95642	.203	-418.5347	67.5955
Tl HCD	TI HOD O	Herbivore	16.57364	104.72776	.986	-233.0983	266.2456
Tukey HSD	Omnivore	Carnivore	-158.89596	70.90726	.070	-327.9396	10.1476
		Herbivore	175.46961	101.95642	.203	-67.5955	418.5347
	Carnivore	Omnivore	158.89596	70.90726	.070	-10.1476	327.9396
	Herbivore	Omnivore	-16.57364	104.72776	.875	-224.6981	191.5508
	пеничне	Carnivore	-175.46961	101.95642	.089	-378.0866	27.1474
I CD	0 :	Herbivore	16.57364	104.72776	.875	-191.5508	224.6981
LSD	LSD Omnivore	Carnivore	-158.89596 <sup>*</sup>	70.90726	.028	-299.8092	-17.9827
		Herbivore	175.46961	101.95642	.089	-27.1474	378.0866
	Carnivore	Omnivore	158.89596*	70.90726	.028	17.9827	299.8092

<sup>\*.</sup> The mean difference is significant at the 0.05 level.

#### **Homogeneous Subsets**

**Total DDTs** 

	Food habit	N	Subset for alpha = 0.05
			1
	Herbivore	12	82.0875
T-l HCD <sup>a,b</sup>	Omnivore	35	98.6611
Tukey HSD <sup>a,b</sup>	Carnivore	44	257.5571
	Sig.		.153

a. Uses Harmonic Mean Sample Size = 22.283.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

## Appendix-16. Post Hoc tests (Tukey HSD and LSD) for significance test of lipid content in samples between and within different feeding habits

#### Post Hoc Tests

### **Multiple Comparisons**

Dependent Variable: Lipid contens

	(I) Food habit	(J) Food habit	Mean	Std. Error	Sig.	95% Confide	nce Interval
			Difference (I-J)			Lower Bound	Upper Bound
Herbivore	** 1.	Omnivore	3.29240*	1.28906	.033	.2193	6.3655
	Carnivore	5.49174 <sup>*</sup>	1.25495	.000	2.4999	8.4835	
	Herbivore	-3.29240 <sup>*</sup>	1.28906	.033	-6.3655	2193	
ukey HSD	Omnivore	Carnivore	2.19934*	.87277	.036	.1186	4.2800
	a ·	Herbivore	-5.49174 <sup>*</sup>	1.25495	.000	-8.4835	-2.4999
	Carnivore	Omnivore	-2.19934*	.87277	.036	-4.2800	1186
	Herbivore	Omnivore	3.29240 <sup>*</sup>	1.28906	.012	.7307	5.8541
	Helbivole	Carnivore	5.49174*	1.25495	.000	2.9978	7.9857
I CD	0	Herbivore	-3.29240 <sup>*</sup>	1.28906	.012	-5.8541	7307
LSD	LSD Omnivore	Carnivore	2.19934*	.87277	.014	.4649	3.9338
	<b>G</b> :	Herbivore	-5.49174 <sup>*</sup>	1.25495	.000	-7.9857	-2.9978
	Carnivore	Omnivore	-2.19934*	.87277	.014	-3.9338	4649

<sup>\*.</sup> The mean difference is significant at the 0.05 level.

#### **Homogeneous Subsets**

Lipid

Enplu									
	Food habit	N	Subset for	r alpha = 0.05					
			1	2					
Tukey HSD <sup>a,b</sup>	Carnivore	44	3.1241						
	Omnivore	35	5.3234						
	Herbivore	12		8.6158					
	Sig.		.143	1.000					

a. Uses Harmonic Mean Sample Size = 22.283.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Appendix-17. Pearson correlation matrix between DDT and it metabolites, total DDTs, lipid and some meteorological parameters

## **Correlations**

		Temperature	rainfall	Humidity %	lipid	DDE	DDD	2,4'-DDT	4,4'-DDT	Total DDTs
	Pearson Correlation	1	.684**	.525**	.040	.243*	.338**	.379**	.380**	.368**
Temperature	Sig. (2-tailed)		.000	.000	.707	.020	.001	.000	.000	.000
	N	91	91	91	91	91	91	91	91	91
	Pearson Correlation	.684**	1	.955***	047	187	.010	060	057	080
rainfall	Sig. (2-tailed)	.000		.000	.660	.076	.923	.572	.594	.451
	N	91	91	91	91	91	91	91	91	91
	Pearson Correlation	.525**	.955**	1	070	318**	161	276**	273**	285**
Humidity %	Sig. (2-tailed)	.000	.000		.507	.002	.126	.008	.009	.006
	N	91	91	91	91	91	91	91	91	91
	Pearson Correlation	.040	047	070	1	.210*	.275**	.090	.038	.129
lipid	Sig. (2-tailed)	.707	.660	.507		.045	.008	.395	.722	.222
	N	91	91	91	91	91	91	91	91	91
	Pearson Correlation	.243*	187	318**	.210*	1	.774**	.835**	.785**	.897**
DDE	Sig. (2-tailed)	.020	.076	.002	.045		.000	.000	.000	.000
	N	91	91	91	91	91	91	91	91	91
	Pearson Correlation	.338**	.010	161	.275**	.774**	1	.756**	.791**	.861**
DDD	Sig. (2-tailed)	.001	.923	.126	.008	.000		.000	.000	.000
	N	91	91	91	91	91	91	91	91	91
2,4'-DDT	Pearson Correlation	.379**	060	276***	.090	.835**	.756**	1	.940**	.967**

	Sig. (2-tailed)	.000	.572	.008	.395	.000	.000		.000	.000
	N	91	91	91	91	91	91	91	91	91
	Pearson Correlation	.380**	057	273**	.038	.785**	.791**	.940**	1	.970**
4,4'-DDT	Sig. (2-tailed)	.000	.594	.009	.722	.000	.000	.000		.000
	N	91	91	91	91	91	91	91	91	91
	Pearson Correlation	.368**	080	285**	.129	.897**	.861**	.967**	.970**	1
Total DDTs	Sig. (2-tailed)	.000	.451	.006	.222	.000	.000	.000	.000	
	N	91	91	91	91	91	91	91	91	91

<sup>\*\*.</sup> Correlation is significant at the 0.01 level (2-tailed).

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<sup>\*</sup> Correlation is significant at the 0.05 level (2-tailed)

# Appendix-18 Hierarchical dendrogram for 20 fishes and prawn represented by DDTs residues obtained by Ward's hierarchical clustering method

#### **Proximities**

## Case Processing Summary<sup>a</sup>

Cases								
Va	ılid	Mis	sing	Total				
N	Percent	N	Percent	N	Percent			
20	100.0%	0	0.0%	20	100.0%			

a. Euclidean Distance used

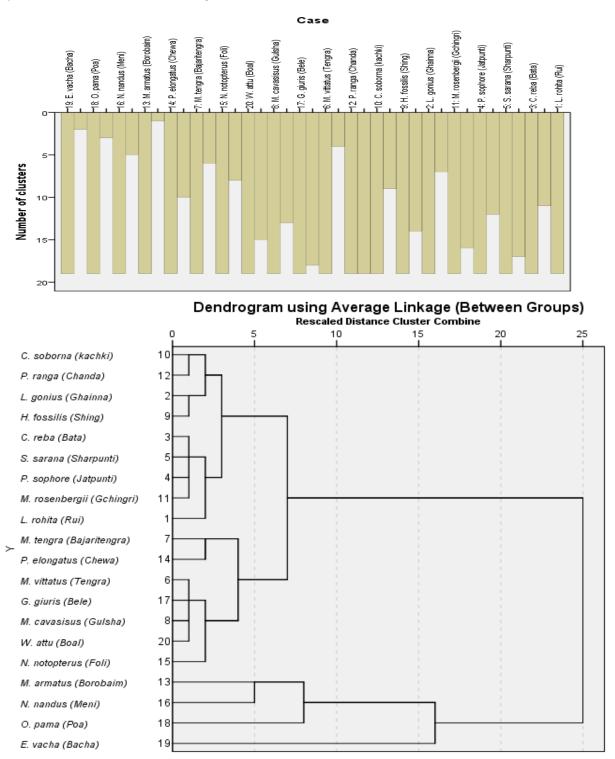
#### Cluster

### **Average Linkage (Between Groups)**

#### **Agglomeration Schedule**

Stage	Cluster C	ombined	Coefficients	Stage Cluster	First Appears	Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	10	12	.016	0	0	11
2	6	17	.017	0	0	7
3	3	5	.018	0	0	8
4	4	11	.023	0	0	8
5	8	20	.040	0	0	7
6	2	9	.055	0	0	11
7	6	8	.062	2	5	12
8	3	4	.065	3	4	9
9	1	3	.103	0	8	13
10	7	14	.135	0	0	14
11	2	10	.138	6	1	13
12	6	15	.152	7	0	14
13	1	2	.260	9	11	16
14	6	7	.321	12	10	16
15	13	16	.432	0	0	17
16	1	6	.618	13	14	19
17	13	18	.684	15	0	18
18	13	19	1.358	17	0	19
19	1	13	2.190	16	18	0

## Hierarchical dendrogram for 20 fishes and prawn represented by DDTs residues obtained by Ward's hierarchical clustering method



# Appendix-19 Hierarchical dendrogram for fishes and prawn of summer season represented by DDTs residues obtained by Ward's hierarchical clustering method

#### **Proximities**

Case Processing Summary<sup>a</sup>

Cases								
	Valid	Mis	sing	Total				
N	Percent	N	Percent	N	Percent			
2	2 100.0%	0	0.0%	22	100.0%			

a. Euclidean Distance used

#### Cluster

### **Average Linkage (Between Groups)**

**Agglomeration Schedule** 

Stage	Cluster C	ombined	Coefficients		First Appears	Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	10	12	.002	0	0	2
2	9	10	.026	0	1	14
3	15	22	.031	0	0	8
4	1	2	.037	0	0	9
5	4	5	.037	0	0	9
6	8	19	.039	0	0	8
7	3	11	.054	0	0	10
8	8	15	.070	6	3	12
9	1	4	.080	4	5	10
10	1	3	.139	9	7	14
11	14	17	.142	0	0	15
12	6	8	.143	0	8	16
13	18	20	.178	0	0	18
14	1	9	.269	10	2	17
15	14	16	.302	11	0	20
16	6	7	.358	12	0	17
17	1	6	.476	14	16	20
18	13	18	.600	0	13	19
19	13	21	.830	18	0	21
20	1	14	1.045	17	15	21
21	1	13	2.232	20	19	0

Hierarchical dendrogram for fishes and prawn of summer season represented by DDTs residues obtained by Ward's hierarchical clustering method

