Dichlorodiphenyltrichloroethane in Environmental Samples and Human Blood of Chittagong Chemical Complex Area

And

Pesticide Residues in Some VegetableSamples

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Submitted by

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AND
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QUALITY ASSURANCE

REFERENCE

Dedicated

To

My father

And my lovely daughters

Lamisa & Raida

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ABSTRACT

Dichlorodiphenyltrichloroethane in Environmental Samples and Human Blood of Chittagong Chemical Complex Area and Pesticide Residues in Some Vegetable Samples

Pesticide is one of the most used components in current agricultural practices for protecting the crops from different kinds of pests. The widespread use of pesticides contaminates soil, water, air and crops. The result of mishandling or overusing of pesticides on rice, vegetables, fruits etc. are the foremost concern in many countries including Bangladesh. Organochlorine pesticides (OCPs), especially DDT was used in Bangladesh for crop manufacture and to abolish vector diseases from early sixties. The only DDT production factory within Chittagong Chemical Complex (CCC) area started in 1966 and soon supply started in the local area. However, due to long persistence in the environment, bioaccumulation, bio magnifications and accumulation to the fatty tissues of human over food chain, the consumption and manufacture of OCPs became restricted worldwide from nineties. The Stockholm Convention identified 12 persistent organic pollutants (POPs) and recently included 13 more, including DDT which are harmful for wildlife and human health and formulated a treaty in 2001 to stop production, usage and elimination of OCs pesticides where Bangladesh is a part of it and has been paying the fees regularly to the secretariat and actively participating in biannual conference (COP). DDT had been emitted in Bangladesh and the factory at CCC area was shut down as a signatory of Stockholm convention. Bangladesh closed down the DDT factory in 1995 without deciding what should happen to the stored DDT in the factory of the CCC area.

This study was shown to monitor the range of dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDE & DDD) in environmental samples (soil, sediment, water and fish) and human blood from areas nearby a closed DDT factory in Bangladesh. Soil, sediment and water samples were collected on 13 July, 2011 from the CCC area in the southern, south western and eastern directions. Fifteen different fish (n=15) samples were collected from a pond in the factory area during June 2016. Thirty human blood (n=30) samples were randomly collected from people (men and women) living inside

and near the factory on June 2014 to determine the level of exposure. DDTs (DDT and its metabolites) from soil and sediment samples were extracted using solvent extraction (SE), water samples by liquid-liquid partitioning, fish samples by solid dispersion method and finally human blood samples by Hovander and coworkers method with slight modification with a mixture of n-hexane: MTBE (1:1) followed by cleaned up using silica gel impregnated with conc. sulphuric acid (2:1 w/w, 1 g). All samples were analyzed by Gas Chromatograph equipped with an Electron Capture Detector (GC-ECD). Linearity's expressed as coefficients (R^2) were ≥0.995. The recoveries were 72–120% and 83–110%, with <15% RSD in soil and water, respectively at two concentration levels. The limit of quantification (LOQ) was 0.0165 mg kg^{-1} in soil and 0.132 µg L^{-1} in water. Higher amounts of DDTs were revealed in the southern (2.2–936×10^2 mg kg^{-1}) or southwestern (86.3– 2067×10^2 mg kg^{-1}) track from the factory than in the eastern track (1.0– 48.6×10^2 mg kg^{-1}). An exemption was the soil sample collected 50 ft (15.24 m) east (2904×10^2 mg kg^{-1}) of the factory. The range of DDTs in the water bodies (0.59–3.01 µg L^{-1}) was approximately equal in all directions. The recovery for fish samples were conducted (n=3) at three concentrations (0.05, 0.1 & 0.2 mg kg^{-1}). The recoveries were 70–105 %, with <16 % RSD. LOD & LOQ was found 0.063 µg kg^{-1} & 0.206 µg kg^{-1} respectively in fish sample. The highest amount of DDT and its metabolites (8.9 µg kg^{-1}) were found in the Shing fish. Boal fish showed small amount of DDTs.

By using internal standard, the recoveries of human blood were 73−108 % (0.05 µg L^{-1}) and 75−98 % (0.025 µg L^{-1}) for CB-53. LOD & LOQ was found 0.025 µg kg^{-1} & 0.083 µg kg^{-1} respectively, in blood sample. The concentration of ∑DDT was in the range (0−1686 µg kg^{-1}) of human blood samples. We established that DDTs might have been discarded randomly around the warehouse after the closing of the factory.

Vegetables are being consumed by the local people of Bangladesh almost every day. Pesticides are being used to protect the crops and there is no guide line about the safe harvesting period of the crops and MRL values for any pesticides in Bangladesh. Studies of dissipation pattern of pesticides in growing crops is necessary which will give a safe harvesting period as well as MRL value after final application. Dissipation pattern of cypermethrin in five different vegetables (tomato, bitter gourd, pumpkin,

eggplant & green chili) were collected February 2016 from the farmer's fields Norundi near Jamalpur district of Bangladesh. For these studies the samples were kept at ambient temperature. Twenty four vegetable samples (snake gourd, ridge gourd, wild ridge gourd & pointed gourd) were also collected from different locations of Bangladesh to analyze the presence of chloropyrifos, cypermethrin, diazinon and fenvelarate residues.

All vegetable samples were extracted by QuEChERS method, cleaned-up by adsorption chromatography technique and analyzed by GC-ECD. Linearity's (R^2) ≥ 0.995 for matrix-matched standard, LOD and LOQ was 0.01 µg kg^{-1} and 0.033 µg kg^{-1} in cypermethrin, respectivly. The recoveries were 82−106 % (RSD ≤ 17 %) at two concentrations (0.25 & 1 mg kg^{-1}) and storage stability was 83% (RSD ≤ 9 %). The MRL of cypermethrin in all vegetables were identified on 0 day samples (2 h after spray). The residue levels went down progressively with days and 74−88% dissipations was observed within 10 days. It was established that cypermethrin residues went lower the MRL value after 1 day of spraying in tomato (143 µg kg^{-1}) & in eggplant (106 µg kg^{-1}) and at 0 day (134 µg kg^{-1}) in bitter gourd (Codex, 2013, 2009). The half-life of cypermethrin was calculated. The most of the vegetable samples were not detrimental to health as all samples had lower the MRL of cypermethrin.

The LOD and LOQ were found 0.8 µg kg^{-1}& 2.64 µg kg^{-1} for diazinon, 0.002 µg $kg^{-1}$and 0.007 µg $kg^{-1}$for chlorpyrifos, 0.01 µg kg^{-1}& 0.033 µg $kg^{-1}$for cypermethrin, and 0.002 µg kg^{-1}& 0.007 µg $kg^{-1}$for fenvalerate, respectivly. The recovery experiments were conducted (n=3) at two concentration levels (0.25 and 0.5 mg kg^{-1}). The average recoveries of the four pesticides in the four vegetables (73 – 115%) with RSD ≤8% Pesticide residues were detected in 40% of the market samples but all were below the MRL values.

Fluxapyroxad is a second-generation carboxamide fungicide that inhibits succinate dehydrogenase of mitochondrial respiratory chain. This study was carried out to assure the safety of fluxapyroxad residues in butter bar (moie) by developing a method and the dissipation pattern was observed under greenhouse conditions from two different treatments (T2 and T3). This experiment was carried out in the laboratory in Republic

of Korea. The leaves which were grown in greenhouse at Naengcheon-ri, Masan-myeon, Gurye-gun, Jeollanam-do, Republic of Korea, from the last week of February until the first week of April, 2015. The method was developed and validated using high performance liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS). The extraction was carried out by the QuEChERS, and then purified with silica solid phase extraction (SPE) cartridge. Correlation coefficient (R^2) of matrix-matched standard was 0.998, LOD was 0.01 µg kg^{-1} and recoveries were 88% & 93% at both concentration 0.5 & 2.5 mg kg^{-1}, respectively with RSD ≤ 10% and storage stability 95±7.04. The method was successfully applied to the experimental field samples, which were collected randomly at 0 to 14 days' post application. In this study, fluxapyroxad was dissipated below the MRL value after 10 days at triple of recommended dose. The rate of disappearance was described to 1^{st} order kinetics with half-life of 2.6 days. The initial residues after application were 11 and 20 µg kg^{-1} on the zero day for T2 and T3 respectively. After 14 days the residues declined to 0.42 and 0.36 mg kg^{-1} for T2 and T3 respectively.

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ABBREVIATIONS

A	Aerosol
ACN	Acetonitrile
ADI	Acceptable Daily Intake
ai	Active ingredient
ANOVA	Analysis of Varience
ARfD	Acute Reference Dose
ATSDR	Agency for Toxic Substances and Disease Registry
ATPase	Adenosine triposphatase
B	Baits
BASF	Badische Anilin und Soda Fabrik
BDL	Below Detection Limit
BEP	Best Environmental Practices
BMC	Benchmark Concentration
BQL	Below Quantification Limit
BRRI	Bangladesh Rice Research Institute
BSTI	Bangladesh Standard Testing Institute
BW	Body weight
C	Concentration
^0C	Degree Celsius
C_{18}	Carbon 18

CAS	Chemical Abstracts Service
CB-53	Tetra Chlorobiphenyl
CCC	Chittagong Chemical Complex
CPA	2,2-dimethyl-3-(2,2-dichlorovinyl) cyclopropane-carboxylic acid
CSF	Cancer Slope Factor
D	Dusts
d.m.	Dry mass
DCM	Dichloromethane
DDT	Dichloro Diphenyl Trichloroethane
DDTs	DDT and its metabolites
DoE	Department of Environment
d-SPE	Dispersive Solid Phase Extraction
DR	Daily consumption Rate
DW	Dry Weight
EA	Ethyl Acetate
EC	Emulsifiable Concentrate
ECD	Electron Capture Detector
EDI	Estimated Daily Intake
EI	Electron Ionization
EPA	Environmental Protection Agency
ESI	Electro Spray Ionisation
EtOAc	Ethyl acetate

EU	European Union
eV	Electron Volt
F	Female
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FID	Flame Ionization Detector
G	Granular
GAP	Good Agricultural Practice
GC	Gas Chromatography
GC-MS	Gas Chromatography with Mass Spectrometer
GDP	Gross Domestic Product
GSM	Grams per Square Meter
h	hour
ha	Hectre
HCH	Hexachlorocyclohexane
HPLC	High Performances Liquid Chromatography
HR	Hazard Ratio
HRI	Hazard Risk Index
HYV	High Yielding Variety
ICSC	International Chemical Safety Cards
IC_{50}	Inhibitory Concentration 50
i.d.	Internal Diameter

i.e.	That is
IUPAC	International Union of Pure and Applied Chemistry
KFDA	Korean Food and Drug Administration
kg	kilogram
L	Liter
LC-MS/MS	Liquid Chromatography With Mass Spectrometer
LC_{50}	Lethal Concentration 50
LD_{50}	Lethal Dose 50
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LSD	Least Significant Difference
M	Male
MCL	Maximum Contaminant Level
ME	Matrix effect
MeCN	Acetonitrile
mg	milligram
mL	milliliter
mm	millimiter
MRL	Maximum Residue Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer

MSD	Mass Selective Detector
MSPD	Matrix Solid-Phase Dispersion
MTBE	tert-butyl methyl ether
μM	Micro miter
NA	Not Analyzed
ND	Not detected
NGO	Non-Government Organization
NPD	Nitrogen Phosphorous Detector
nM	Nano miter
NS	None Significant
2,4'-DDT	1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
OCPs	Organochlorine pesticides
OPPs	Organophosphorus pesticides
4,4'-DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane
4,4'-DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane
4,4'-DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane
PBA	3-Phenoxybenzoic Acid
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-*p*-dioxins
PCDF	Polychlorinated dibenzofurans
PHI	Pre Harvest Interval
PKC	Public Key Certificate

POPs	Persistent Organic Pollutants
ppm	parts per million
PRA	Pesticide Residue Analysis
PSA	Primary Secondary Amine
PTFE	Polytetrafluoroethylene
QA	Quality Assurance
QC	Quality Control
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
rpm	Rotations per minute
RT	Retention Time
S/N	Signal to Noise ratio
SD	Standard Deviation
SE	Solvent Extraction
Si	Silica
SIM	Selected Ion Monitoring
SP	Soluble Powder
SPE	Solid-Phase extraction
SPME	Solid-Phase Micro Extraction
SPSS	Software Package for Statistics and Simulation
SARD	Sustainable Agriculture and Rural Development
T	Treament
$t_{1/2}$	Half-life

TQ	Triple Quadrapole
tr	Trace concentration, LOD < trace < LOQ
UHPLC	Ultra High performances liquid chromatography
UNEP	United Nations Environment Programme
UK	United Kingdom
USA	United States of America
USEPA	U.S. Environmental Protection Agency
VOCs	Volatile Organic Compounds
WHO	World Health Organization
WP	Wettable powder

INTRODUCTION

1.1 Introduction to Pesticides

Pesticides are chemicals used to kill or control pests. They are classified according to their chemical class or intended use. Different pesticides pose varying degrees and types of risk to water quality. It is reported that approximately three million people are poisoned and 200,000 die each year around the world from pesticide poisoning, the majority of them from the developing countries (FAO, 2000). It is also believed that the incidence of pesticide poisoning in developing countries may be greater than reported due to under-reporting, lack of data and misdiagnosis.

Pesticides were proclaimed after the World War II for their several benefits but general global severe practice now poses potential hazards to the environment and human health (Chambers et al., 2001). Organochlorine pesticide suggests a significant group of persistent organic pollutants (POPs), which are hypothetical to be imaginable carcinogens or mutagens as well as endocrine disruptors (Thomas *et al*., 1998; Peter *et al*., 2002). The question of POPs has given rise to a global campaign created by the United Nations Environmental Program (UNEP) to abolish or diminish these health and environment threatening chemicals world-wide. The UNEP has listed 12 such chemicals those are chlorine containing organic compounds of which nine are pesticides (Peter *et al*., 2002). The Stockholm Convention identified 12 persistent organic pollutants (POPs) and recently included 13 more. However, at its fourth meeting held in 2009, the Conference of the Parties (COP) adopted the amendments to annexes A (elimination), B (restriction) and C (unintentional production) of the Stockholm Convention to list nine additional chemicals as persistent organic pollutants, resulting in the "dirty twenty one". Residues and metabolites of many POPs are very stable, with long half-lives in the environment (UNEP, 2002). The manufacture and use of chlorinated pesticides have been banned or restricted in developed countries. Although these bans and restrictions were enacted during the 1970s and 1980s, some developing countries are still using OCPs for agricultural and

public health purposes because of their low cost and versatility in controlling various pests (Xue *et al*., 2006). There is also a scarcity of data on the use of pesticides in the country, a reflection of the deficiency of a mechanism and planning programed in place for chemicals management as well as a low level of understanding of the environmental and public health hazards of pesticide use (Osibanjo *et al*., 2002).

1.2 Background

Bangladesh is an insignificant and increasing country loaded with almost insufferable pressure of human population. But, the nonstop process of expansion and development consumes the limited land area (Wiki. 2013). Agriculture plays a vital role in overall budget of the country, donating with approximately 20% of the GDP. More than 70% of the inhabitants depend on agriculture. Therefore, it is essential to promote high resilient agricultural crop variations needful widespread use of fertilizers and pesticides. Pesticide was first introduced in Bangladesh in 1951 (Rahman *et al*., 1997).

Dichlorodiphenyltrichloroethane (DDTs) are harmful for wildlife and human health and formulated a treaty in 2001 to stop production, usage and elimination of OCs pesticides. Bangladesh is a part of it and has been paying the fees regularly to the secretariat and actively participating in biannual conference (COP). In early sixties, DDT used to be imported to Bangladesh to increase crop production and to eradicate vector diseases. After independence organochlorine pesticides including DDTs was available for the farmers in local market to ensure food production. DDT had been banned in 34 countries and was also severely restricted in other 34 countries. DDT had been expelled in Bangladesh and the factory at CCC area was shut down as a signatory of Stockholm convention. In 1993 the use of DDT had been banned in Bangladesh (U. Haque *et.al*; 2009). So Bangladesh closed down the DDT factory in 1995 without deciding what should happen to the stored DDT in the factory of the CCC area.

In an effort to supernumerary the persistent organochlorine pesticide, agricultural sectors have unconcerned towards organophosphate pesticides. However,

organophosphate pesticides are generally much more toxic to vertebrates compared to other classes of insecticides even though they can rapidly abolish in the environment.

In the ever-growing tendency of environmental-concerned society, it is seeming that many countries are starting to apply strict environmental regulations in almost every aspect associated with human life (Sunarso and Ismadji, 2009). The cumulative worldwide essential for food burdens a higher agricultural output, which can only be attained by a widespread use of pesticides. Unfortunately pesticides contaminate the environment through intensive or inappropriate use. Although organochlorine insecticides like DDTs, lindane, aldrin or dieldrin for example have been expelled years ago in many countries based on their mutagenic, carcinogenic and endrocrine disrupting properties, they still can be found in environmental samples due to their persistence and lipophilic properties (Lesueur *et al*., 2008). The grade of threats depends on the amount of pesticides on crops and their poisonousness.

Pesticide, used in crop production has been suspected of being a major contribution to environmental pollution. There are extensive and growing concerns of pesticide over-use, relating to a number of proportions such as contamination of ground water, surface water, soils and food, and the consequent impacts on wildlife and human health (McLaughlin and Mineau, 1996). A greater problem lies in the bioaccumulation of pesticides in beneficial organisms like fish. Residues in food pose to consumers if the maximum residue limit (MRL) set by Food and Agriculture Organization (FAO) and World Health Organization (WHO) is exceeded (Pingali and Roger, 1995). For evaluating safety to concern about human health and environment pesticide application must be come in regulations. So governments and international organizations have compiled and published a list of pesticides, which includes their tolerances and maximum residue limits (MRLs) (Codex Alimentarius Commission, 2010; KHIDI, 2011). However, food safety and soil quality are sometimes under threat due to the application of fertilizer and pesticides. (Karim, *et al*., 1994).

The primary regulatory standard employed to control pesticide residues in food is the MRL. The MRL has been defined as “the maximum concentration of pesticide residue that is legally permitted or recognized as acceptable on a food, agricultural

commodity, or animal feed" (Holland, 1996). The MRL is intended primarily as a check that use of pesticide is occurring according to authorized labels and Good Agricultural Practice (GAP). Detection of residues at or below the MRL implies that label directions and GAP have been properly followed. MRLs are not set on the basis of toxicology data, but once proposed based on GAP they must be evaluated for safety. This generally accomplished through a risk assessment process that compares dietary intakes estimated from expected residues concentrations in foods consumed with the relevant health-related regulatory endpoints, the acceptable daily intake (ADI) and the acute reference dose (ARfD) (Solecki *et al*., 2005).

Every pesticide has a Pre Harvest Interval (PHI) to dissipate below the MRL established for that crop. PHI means difference between the date of final pesticides application and harvest. Due to lack of education, farmers of our country do not follow the prescribed dosage and use pesticides at any stage of crop and harvest without following the time of PHI (Handa *et al.*, 1999). That is the main concerns over the possibility of excessive residues remain in crops, vegetables etc. Therefore, international organizations has compiled and published a list of pesticides with their MRLs in food and food commodities (Codex Alimentarius Commission, 2010). It is important to find out MRL value and dissipation patterns of pesticide on crops for producing safe food and protecting human health and environment. The dissipation rate of a pesticide after application is a useful tool for the assessment of the behavior of pesticide residues in each crop (Omirou *et al*., 2009).

Pesticides are commonly used to increase crop production and quality by controlling pests and diseases. However, extensive application of pesticides poses potential risks to human, animals, and the environment (Philp, 2003). The residues of pesticides may persist in food and food commodities with substantial hazards due to the unwise and indiscriminate application on crops. The degree of hazards depends on the amount and toxicity of the pesticides that are using on crops. The intake of the pesticides by human even in trace amounts creates bad effects on health (Handa *et al*., 1999). For evaluating safety to concern about human health and environment pesticide application must be come in regulations.

1.3 Pesticide and Pest

Pests are those organisms like weeds, insects, bacteria, fungi, viruses and animals, which adversely affect our way of life. Pests can reduce the quantity and quality of produced food by lowering production and they destroy stored food. They compete with humans for food and affect health and the way of life. They are also cause of major cause of land degradation. Their activity greatly increases the cost of farming (Miller, 2004).

Pesticides can be derived from plants (*e.g.* pyrethrin, neem or azadirachta from neem seed oil) or they can be chemically manufactured (*e.g.* DDT, 2,4-D, Acephate, Aclonifen, Cyprodilin etc.). Natural products or biological agents (*e.g*. pathogens, pheromones) and genetically engineered pesticides such as toxin-producing *Bacillus thuringiensis* are also used (Creanshaw, 2010).

Chemical insecticides are usually contact, stomach or fumigant poisons. Contact poisons may have immediate or delayed effects after physical contact with a pest. Fumigants, which may initially have the form of a solid, liquid or gas, but they kill pests in a gaseous state (Cornell Univ. Pest. Safe. Edu. Prog. 2007).

1.3.1. A Short History of Pesticide

At the ancient time the farmed crops suffered from pests and diseases causing a large loss in yield with consequence of famine for the population. Still today even though advances in agricultural sciences but losses due to pests and diseases range from 10-90%, with an average of 35 to 40%, for all potential food and fiber crops (Peshin, 2002). Pesticides have been used to protect crops from pest attack since ancient times (Unsworth, 2010).

So it was a great motivation to find the ways of overcoming the problems caused by pests and diseases. The first recorded insecticides were sulpher compounds to control insects and mites that were used by Sumerians about 4500 years ago. About 3200 years ago the Chinese were using mercury and arsenical compounds for controlling

body lice (History of Pesticides, 2008). Copper sulfate and hydrated lime called Bordeaux mixture proved to be an effective fungicide and became widely used between 1860 and 1942. Pyrethrum derived from the dried flowers of *Chrysanthemum cinerariaefolium* "Pyrethrum daisies", has been used as an insecticide for over 2000 years (Pyrethrum.html. 2012). Nitrophenols, chlorophenols, creosote, naphthalene and petroleum oils were used for fungal and insect pests. Ammonium sulphate and sodium arsenate were used as herbicides.

The growth in synthetic pesticides such as DDT, BHC, aldrin, dieldrin, endrin, chlordane, parathion, captan and 2,4-D accelerated in the 1940s. These products were effective and inexpensive and DDT was the most popular, because of its broad-spectrum activity (History of Pesticides, 2008; Delaplane, 2000; Unsworth, 2010). Many persons predicted pesticides coupled with high-yield plant types, chemical fertilizers, irrigation technology, and mechanization would be a "Green Revolution" that would create an abundance of food for the world. The publication of *Silent Spring* by Rachel Carson in 1962 describes how DDT can enter the food chain, accumulate in the fatty tissue of all animals, including humans, and cause cancer and genetic damage. She concluded that DDT and other pesticides had irreversibly harmed birds and animals and negatively affected the world's food supply. Considering the adverse effects of halogenated pesticides 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" (www.pops.int).

1.3.2 Organochlorine Pesticides

Organochlorine pesticides are insecticides composed primarily of carbon, hydrogen, and chlorine. They break down slowly and can remain in the environment long after application and in organisms long after exposure. DDT (Dichlorodiphenyl trichloroethane) is one of the most infamous organochlorine pesticides. Promoted as a "cure all" insecticide in the 1940s, DDT was widely used in agricultural production around the world for many years. It was also the chemical of choice for mosquito

control; until the 1960s, trucks sprayed DDT in neighborhoods across the U.S. DDT was also the primary weapon in the global "war against malaria" during this period, and continues to be used for malaria control in a handful of countries. (chemicalbodyburden, 2013).

1.3.3 Green Pesticides

Green pesticides, also called **ecological pesticides**, pesticides are derived from organic sources (Wikipedia, 2013b). Green pesticides refer to all types of nature-oriented and beneficial pest control materials that contribute to reduce the pest population and increase food production. They are safe and eco-friendly. They are more compatible with the environment components than synthetic pesticides. Eminent scientists have highlighted the importance of green pesticides (Ignacimuthu and Jayaraj, 2005).

1.3.4 Modern Pesticides

In modern times, some sophisticated compounds, which are very carefully researched to ensure their effectiveness against target organisms, are safe to the environment and can be used without hazards to the operators or consumers are used as pesticides. These pesticides are called modern pesticides. Some of the modern pesticides are diazinon, malathion, cypermethrin, chlorpyrifos, acephate, fenitrothion, quinalphos and fenvalerate etc.

These pesticides belong to different classes of chemical action. Synthetic compounds predominate among them, especially derivatives of phosphoric, phosphorothioic acids. Pyrethroid pesticides are also modern pesticides. Basically the representatives of the same class are characterized by common specific properties and a single mechanism of their action on an organism (Ghosh, 1998). Organophosphate pesticides have increased in use, because they are less damaging to the environment and they are less persistent than organochlorine pesticides (Jaga and Dharmani, 2003). Many of these have been developed to target specific biochemical reactions within the target

organisms, e.g. an enzyme necessary for photosynthesis within a plant or a hormone required for normal development in an insect. Modern chemicals are much safer, more specific and friendlier to environment than the older products they have replaced.

1.3.4.1 Organophosphorus

Organophosphate insecticides have low persistence in the environment. They break down rapidly and therefore, do not cause any long term hazards. However these compounds are highly toxic in nature. Apart from killing non-target insects, they also harm the human and the wild life. Further due to their instability they have to be applied at frequent intervals. Therefore, they are not always economically viable.

Among various pesticide classes, OPP group is the most widely used class of agricultural pesticides. In recent years, many studies have proved OPPs to be mutagenic, carcinogenic, cytotoxic, genotoxic, teratogenic and immunotoxic. Some of the pesticides belonging to OPPs group are also known for their interferences in reproduction especially in males. Few studies reported that the exposure of OPPs had posed a direct potential risk to human health by inhibiting acetyl cholinesterase and modifying cholinergic signaling. OPPs bind to the enzyme acetyl cholinesterase and to disrupt nerve function which further result in paralysis and death (Sharma *et al.*, 2010).

1.3.4.2 Carbamates

Carbamates insecticides are intermediate in behaviour in comparison to organochlorine-hydrocarbons and organophosphates. They are less toxic than organophosphates and less persistent than organochlorine hydrocarbons. Examples include carbaryl and methiocarb.

1.3.4.3 Pyrethroids

The pyrethroids are the most recent group of insecticides. Chronologically, pyrethroids constitute the fourth major group of insecticides developed (after organochlorines, organophosphates and carbamates). They are structural analogues of pyrethrum, the naturally occurring insect repellent compound present in chrysanthemum. Pyrethroids have two important advantages: They are neither persistent nor toxic. Therefore, despite their high cost, they account for about one-third of world insecticides use. Example includes pyrethrum, deltamethrin. They are non-systemic (in plants) contact and stomach poisons to many insects and arachnids with an additional anti-feeding action. The compounds act on the central and peripheral nervous system of target insects at very low doses (Roberts and Hutson, 1999). Synthetic pyrethroids are effective against a wide spectrum of pest. They are widely used as pest control agents in agricultural production because of their selective insecticidal activity, rapid biotransformation and excretion by the mammalian catabolic system and their non-persistence in the environment.

Pyrethroids are a class of hydrophobic insecticide very easily degrade in the natural environment. The two main routes of degradation, photo-degradation and biodegradation, are often superimposed. Pyrethroids developed for use in agriculture are much more photo stable than the natural pyrethrins or early synthetic derivatives but they are still sensitive to sunlight, which triggers many alterations such as isomerisation or ester cleavage. The basic pathways of pyrethroid metabolism include hydrolysis of the central ester linkage and oxidation of both acid and alcohol moieties. The rate of these metabolic transformations and the nature of the metabolites depend mainly on the organism involved. Birds and mammals metabolise and excrete more rapidly than insects and fishes. In plants, initial metabolic processes are identical to those known in animals. In soil, pyrethroids undergo the same type of transformation, hydrolysis and oxidation. Neither degradation products nor intact parent compounds are leached.

1.3.5 Continuing Development and Alternatives

New pesticides are being developed, including biological and botanical derivatives and alternatives that are thought to reduce health and environmental risks. A compound that kills organisms by virtue of specific biological effects rather than as a broader chemical poison is termed as a **Bio-pesticide**. Bio-pesticides are more likely to be bio-degradable and they specifically interfere with the adsorption of food from the guts of some insects but are harmless to mammals (Rahman *et al.*, 1995). Biological pesticides based on entomopathogenic fungi, bacteria and viruses cause disease in the pest species can also be used (Miller, 2004).

Integrated Pest Management (IPM), the use of multiple approaches to control pests, is becoming widespread and has been used with success in countries such as Indonesia, China, Bangladesh, the US, Australia, and Mexico (Miller, 2004). IPM attempts to recognize the more widespread impacts of an action on an ecosystem, so that natural balances are not upset (Daly *et al*., 1998).

In addition, applicators are being encouraged to consider alternative controls and adopt methods that reduce the use of chemical pesticides. Alternatives to pesticides are available and include methods of cultivation such as polyculture (growing multiple types of plants), crop rotation, planting crops in areas where the pests that damage them do not live, time of planting according to when pests will be least problematic, and use of trap crops that attract pests away from the real crop and use of biological controls, such as pheromones and microbial pesticides, and genetic engineering, and methods of interfering with insect breeding. Interfering with insects' reproduction can be accomplished by sterilizing males of the target species and releasing them, so that they mate with females but do not produce offspring (Miller, 2004). This technique was first used on the screwworm fly in 1958 and has since been used with the medfly, the tsetse fly, and the gypsy moth (SP-401 Skylab, 2007). However, this can be a costly, time consuming approach that only works on some types of insects (Miller, 2004).These methods are becoming increasingly popular and often are safer than traditional chemical pesticides.

1.3.6 Types of Pesticides

There are multiple ways of classifying pesticides

According to chemical composition:

- **Inorganic compounds:** mercury, fluorine, barium, sulphur, copper, chlorates and borates.
- **Organic compounds:** organochlorine, organophorous, derivatives of carbonic, thio and di-thio acid, carbamides etc.
- **Synthetic and biological pesticides (biopesticides):** pyrethrines, bacterial and fungal preparations, antibiotics and phytocides.

According to function of different type of pest control:

- **Algicides** or **algaecides** for the control of algae
- **Avicides** for the control of birds
- **Bactericides** for the control of bacteria
- **Fungicides** for the control of fungi and oomycetes
- **Herbicides** (*e.g.* glyphosate) for the control of weeds
- **Insecticides** (*e.g.* organochlorines, organophosphates, carbamates, and pyrethroids) for the control of insects - these can be ovicides (substances that kill eggs), larvicides (substances that kill larvae) or adulticides (substances that kill adults)
- **Miticides** or **acaricides** for the control of mites
- **Molluscicides** for the control of slugs and snails
- **Nematicides** for the control of nematodes
- **Rodenticides** for the control of rodents
- **Virucides** for the control of viruses (*e.g.* H5N1)

According to mode of application:

- **Anti-fouling agents:** Kills or repel organisms that attach to under water surfaces such as boat bottoms.
- **Attractants:** Attracts pests (for example to lure an insect or rodent to a trap).

- **Repellants:** Repel pests including insects (such as mosquitoes) and birds.
- **Insect growth regulators:** Disrupt the molting, maturity from pupal stage to adult or other life processes of insects.
- **Plant growth regulators:** Substances (excluding fertilizers and other plant nutrients) that alter the expected growth, flowering or reproduction of plants.
- **Desiccant:** Promote drying of living tissues.
- **Defoliants:** Cause leaves or other foliage to drop from a plant, usually to facilitate harvest.

1.3.7 Formulation

Formulation is the term used to describe the physical state of a pesticide and determines how it will be applied. The effective use of pesticide to control pests, plant diseases and weeds not only depends on their toxicity but also to a considerable extent on the form of pesticide. It also improves the properties of a chemical for handling, storage, application and safety. Common formulations are:

i) The active ingredient is mixed with an oil base i.e. forming an emulsion which is diluted with water (**emulsifiable concentrate; EC or E**) for application,

ii) A liquid, can be mixed with water to form a suspension in a spray tank (**flowable; For L**),

iii) The active ingredient is made into coarse particles with inert material like fired clay particles (**granules; G**),

iv) The active ingredient is combined with a fine powder look like dusts and mix with water (**wettable powders; WP**),

v) The active ingredient is added to an edible or attractive substance and are often used to control slugs, snails, ground-dwelling insects, and rodents (**baits; B**)

vi) An active ingredient in powder form is dissolved in water (**Soluble powders; SP**). (Cress, 1990)

Pesticides are also used for house hold pests; these are very low-concentrate solutions, usually applied as a fine spray or mist generally sold in aerosol cans for mosquito (**aerosols; A**), and made by adding the active ingredients to a fine, inert powder or talc (**dusts; D**).

1.3.8 Importance of Pesticide Use

Pesticides can save farmers' money by preventing crop losses to insects and other pests and they ensure a plentiful supply and variety of high quality, wholesome food at a reasonable price, nutritious food free from harmful organisms and blemishes (WRI, 1998-99). In the US, farmers get an estimated fourfold return on money they spend on pesticides (Kellogg *et al*., 2000). One study found that not using pesticides reduced crop yields by about 10% (Kuniuki, 2001). Another study, conducted in 1999, found that a ban on pesticides in the United States may result in a rise of food prices, loss of jobs, and an increase in world hunger (FAO, 1998). Pesticides are used in grocery stores and food storage facilities to manage rodents and insects that infest food such as grain. They are used to control harmful organisms. For example, they are used to kill mosquitoes that can transmit potential deadly diseases like west Nile virus, yellow fever, and malaria. They can also kill bees, wasps or ants that can cause allergic reactions. Insecticides can protect animals from illnesses that can be caused by parasites such as fleas (Purdue.*edu*, 2007). Pesticides can prevent sickness in humans that could be caused by moldy food or diseased produce.

However, DDT use is not always effective, as resistance to DDT was identified in Africa as early as 1955, and by 1972 nineteen species of mosquito worldwide were resistant to DDT. A study for the World Health Organization in 2000 from Vietnam established that non-DDT malaria controls were significantly more effective than DDT use (afronets.com 2007). The ecological effect of DDT on organisms is an example of bioaccumulation. Again, a study (October 2007) has linked breast cancer from exposure to DDT prior to puberty (sustainableproduction.org, 2007). Poisoning may also occur due to use of DDT and other chlorinated hydrocarbons by entering the

human food chain when animal tissues are affected. Symptoms include nervous excitement, tremors, convulsions or death.

Each use of a pesticide carries some associated risk. Proper pesticide use decreases these associated risks to a level deemed acceptable by pesticide regulatory agencies. Uncontrolled pests such as termites and mold can damage structures such as houses (Purdue.*edu*, 2007).

At present, pesticides are indispensable tools for the protection of economically important crops from damage by weeds, insects, fungi, and nematodes, etc. in modern agriculture. Up to 40 percent of the world's potential crop production is already lost annually because of the effects of weeds, pests and diseases. These crop losses would be doubled if existing pesticide uses were abandoned. Because the use of pesticides improves crop yields and quality, maintains food prices in check, the consumers can get good quality food in cheap rate.

Pesticides allow consumers to consume high-quality product that is free of insect blemishes and insect contamination. It reduces or in some cases, eliminates, insect damage allow the consumer to purchase high-quality product free of insect fragments. Pest control products protect our families, pets, and communities from mosquitoes, ticks, rodents, bedbugs, and cockroaches, and help prevent health issues associated with these pests. Pesticide products are also used to control household pests such as termites, roaches, ants, rats and other pests.

1.3.9 Effects of Pesticide Use

1.3.9.1 Environmental Effects

Pesticide use raises a number of environmental concerns. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments, and food (Miller, 2004). Pesticide drift occurs when pesticides suspended in the air as particles are carried by wind to other areas, potentially contaminating them. Pesticides are one of the causes

of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination.

Non target organisms, including predators and parasites of pests, can also be affected by chemical application. The reduction of these beneficial organisms can result in changes in the natural biological balances. Losses of honey bees and other pollinating insects can also be a problem (FAO, 1998).

1.3.9.2 Health Effects

Pesticides can present danger to consumers, onlookers or workers during manufacture, transport or during and after use (US EPA, 2007).

Farmers and Workers

There have been many studies of farmers with the goal of determining the health effects of pesticide exposure (McCauley *et al.* 2006). The World Health Organization and the UN Environment Programed estimate that each year, 3 million workers in agriculture in the developing world experience severe poisoning from pesticides, about 18,000 of whom die (Miller, 2004). According to one study, as many as 25 million workers in developing countries may suffer mild pesticide poisoning yearly (Jeyaratnam, 1990).

These are associated with acute health problems for workers that handle the chemicals, such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems (Ecobichon, 1996). Additionally, many studies have indicated that pesticide exposure is associated with long-term health problems such as respiratory problems, memory disorders, dermatologic conditions (Arcury *et al*., 2003; O'Malley, 1997), cancer (Daniels *et al*., 1997), depression (Beseler *et al*., 2008), neurological deficits (Kamel *et.al*. 2003; Firestone *et. al*., 2005), miscarriages, and birth defects (Engel *et al*., 2000; Cordes and Foster, 1988; Das *et al*., 2001; Eskenazi *et al.* 1999; García, 2003; Moses, 1989; Schwartz *et al*., 1986; Stallones and Beseler, 2002; Strong *et al*., 2004; Van Maele-Fabry and Willems, 2003). Summaries

of peer-reviewed research have examined the link between pesticide exposure and neurologic outcomes and cancer, perhaps the two most significant things resulting in organophosphate-exposed workers (Alavanja *et al*., 2004; Kamel and Hoppin, 2004).

Impact on Humans

Pesticides used to control pests on food crops are dangerous to people who consume those foods. The Bhopal disaster occurred when a pesticide producing plant released 40 tons of methyl isocyanate (MIC) gas, a chemical intermediate in the synthesis of some carbamate pesticides. The disaster immediately killed nearly 3,000 people and ultimately caused at least 15,000 deaths (BBC News, 1984). In China, an estimated half million people are poisoned by pesticides each year, 500 of whom die (Lawrence, 2007).

Children have been found to be especially susceptible to the harmful effects of pesticides (Noyes, 2007). A number of research studies have found higher instances of brain cancer, leukemia and birth defects in children with early exposure to pesticides according to the Natural Resources Defense Council, U.S.A. (NRDC, 1998). Peer-reviewed studies now suggest neurotoxic effects on developing animals from organophosphate pesticides at recommended tolerable levels, including fewer nerve cells, lower birth weights, and lower cognitive scores. Some scientists think that exposure to pesticides in the uterus may have negative effects on a fetus that may manifest as problems such as growth and behavioral disorders or reduced resistance to pesticide toxicity later in life (Lorenz, 2006). Pyrethrins, insecticides commonly used in common bug killers, can cause a potentially deadly condition if breathed in (Young, 1986).

1.3.10 Potential Adverse Consequences of Pesticides

Pesticides can produce negative health impacts, both private and social (Antle and Pingali, 1994). For example, when farm workers are exposed to dangerous pesticides, this exposure can reduce farm productivity through effects on farmer health. In some

cases, health costs from pesticide exposure may completely counteract the benefits derived from yield improvements (Rola and Pingali 1993). Excessive application of pesticide also can be ecologically damaging. Low dosages can lead to the development of resistance in target populations and pesticide runoff can reduce the productivity of aquatic ecosystems.

Pesticide use in crop production has been suspected to cause environmental pollution. There are widespread and growing concerns of pesticide over-use, relating to a number of dimensions such as contamination of ground water, surface water, soils and food, and the consequent impacts on wildlife and human health (McLaughlin and Mineau, 1996). The usual practice of draining paddy water into irrigation canals may cause river and lake contamination. Residues carried by the water can be taken up by non-target flora and fauna, leach into soil, and possibly contaminate groundwater or potable water. (Perveen, 2001)

Biodiversity is declining due to the effect of pesticide and fertilizer use. Population of native fish species (Channa spp., Heteropneustes clarias, and Anabas testudineus) is now endangered and the traditional rice-fish systems have disappeared. The bird and other small wild animals are in threat of wide spread because of the use of pesticides in rice and vegetables (Perveen, 2001). The reduction of beneficial organism or species can result in changes in the biodiversity of an area and affect natural biological balances.

1.3.11 Persistence

Persistence describes how long a pesticide remains active. Half-life is one of the terms of measure of persistence. The half-life of a substance is the time required for that substance to degrade to one-half its original concentration and it is not an absolute factor. The factors that influence the persistence of pesticides are the characteristics of the pesticide, including its over-all stability either as parent compound or metabolites, its volatility, solubility, formulation, and the method and site of application. It is also depend on environmental factors, particularly temperature, precipitation (humidity)

and air movement (wind).The other factors depend on the properties of the plant or soil characteristics influencing the persistence of pesticides in plants include the plant species involved, the nature of the harvested crop, the structure of the cuticle, the stage and rate of growth and the general condition of the plant. Corresponding soil characteristics are the soil type and structure, its organic matter content, clay content, acidity or alkalinity, mineral ion content and degree of aggregation and its microbial population. Of these factors, the most important seem to be related to the chemical stability and physical characteristics of the pesticide; its stability exerting the greatest influence, otherwise volatility being more important in soil and solubility in plants. In general, the longer a pesticide persists in the environment, the more likely it is to move from one place to another and be a potential source of pollution (Rahman and Alam, 1997; Aziz, 2005).

So **Persistant organic pollutants** are organic chemical substances, possess some physical and chemical properties in a particular combination. Therefore once released into the environment, they:

- remain intact for exceptionally long periods of time (many years).
- 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 humans, biomagnified through the 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).

During the survey conducted by the World Bank in 2003, the use of two POPs were encountered, heptachlor and endrin. As can be seen in **Figure 1** below, the presence of POPs were found in the districts of Comilla, Chittagong, Dhaka, Rajshahi and Mymensingh.

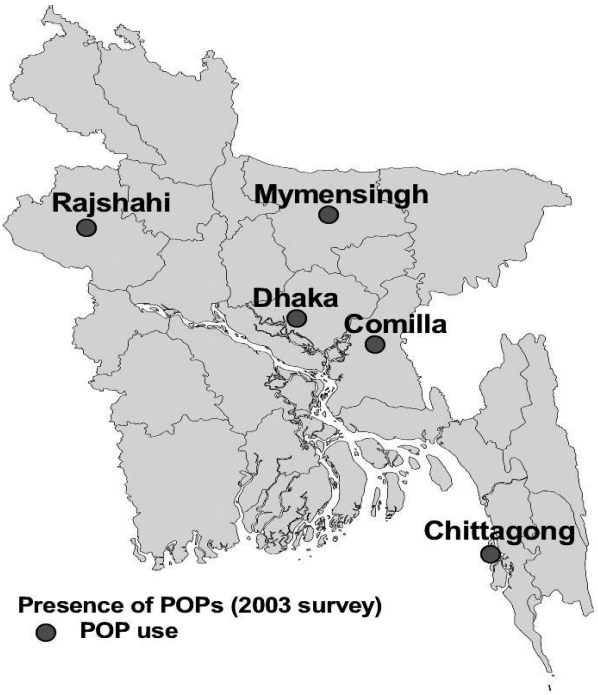


Fig.-1.1: Prevalence of Persistent Organic Pollutants, 1999-2000

1.3.12 Toxicity

The toxicity of a pesticide is its capacity or ability to cause injury or illness and determined by subjecting test animals to varying dosages of the active ingredient (a.i.) and each of its formulated products. The active ingredient is the chemical component in the pesticide product that controls the pest. The two types of toxicity are acute and chronic. Acute toxicity of a pesticide refers to the chemical's ability to cause injury to a person or animal from a single exposure, generally of short duration and is determined by examining the dermal toxicity, inhalation toxicity, and oral toxicity of test animals. In addition, eye and skin irritation are also examined and is measured as the amount or concentration of a toxicant required to kill 50 percent of the animals in a test population. This measure is usually expressed as LD_{50} (lethal dose 50) or LC_{50} (lethal concentration 50) and it is recorded in milligrams of pesticide per kilogram of body weight (mg/kg b.w.) of the test animal or in parts per million (ppm). The chronic toxicity of a pesticide is determined by subjecting test animals to long-term exposure

to the active ingredient. Any harmful effects that occur from small doses repeated over a period of time are termed as chronic effects. Some of the suspected chronic effects from exposure to certain pesticides include birth defects, production of tumors, blood disorders, and neurotoxic effects (nerve disorders).There is no term to express chronic toxicity. Pesticides are divided into four categories on the basis of their relative toxicity are given in **Table-1.1** and also summarized values of LD_{50} and LC_{50} for each route of exposure for the four toxicity categories and their associated signal word (Rahman and Alam, 1997).

Table-1.1: Toxicity categories for active ingredients

	Toxicity category			
Routes of exposure	**I**	**II**	**III**	**IV**
Oral LD_{50}	Up to and including 50 mg/kg	50–500 mg/kg	500–5,000 mg/kg	>5,000 mg/kg
Inhalation LC_{50}	Up to and including 0.2 mg/l	0.2–2 mg/l	2–20 mg/l	>20 mg/l
Dermal LD_{50}	Up to and including 200 mg/kg	200–2,000 mg/kg	2,000–20,000 mg/kg	>20,000 mg/kg
Eye Effects	Corrosive corneal Opacity not reversible; persisting within 7 days	Corneal opacity reversible; within 7 days	No corneal opacity irritation reversible within 7 days	No irritation irritation for 7 days
Skin Effects	Corrosive	Severe irritation at 72hours	Moderate irritation at 72 hours	Mild or slight irritation at 72 hours
Signal Word	DANGER WARNING	CAUTION	CAUTION	POISION

1.3.13 Uses of Pesticides in Bangladesh

Bangladesh is predominantly an agricultural country with over population and agriculture plays an important role in the lives of its people. The use of pesticides, in Bangladesh started during the middle of the 1950s to promote crop production (Rahman and Alam, 1997). Major crops of the country are rice, wheat, pulses, jute, oilseed, vegetables, potatoes, sugarcane, cotton and tea of which rice accounts for 80% of the total cultivated area. The warm and humid climatic conditions of the country increased modern high yielding varieties of crops and more use of chemical fertilizers are highly favorable for development and multiplication of pests and diseases. The estimated loss in yields due to attacks from pest and diseases annually ranges from 15 to 25 percent (Aziz, 2005). Usually farmers get information about pesticides from traders. More than 47% of farmers in Bangladesh use more pesticides than needed to protect their crops, according to a recent survey of 820 boro (a variety of rice), potato, bean, eggplant, cabbage, sugarcane and mango growers (Rahman, 2002).

In the absence of detailed information on pesticide use at the farm level for all areas of Bangladesh. A recent World Bank farm-level pesticide survey conducted in 2003 (Meisner and Dasgupta, 2004).

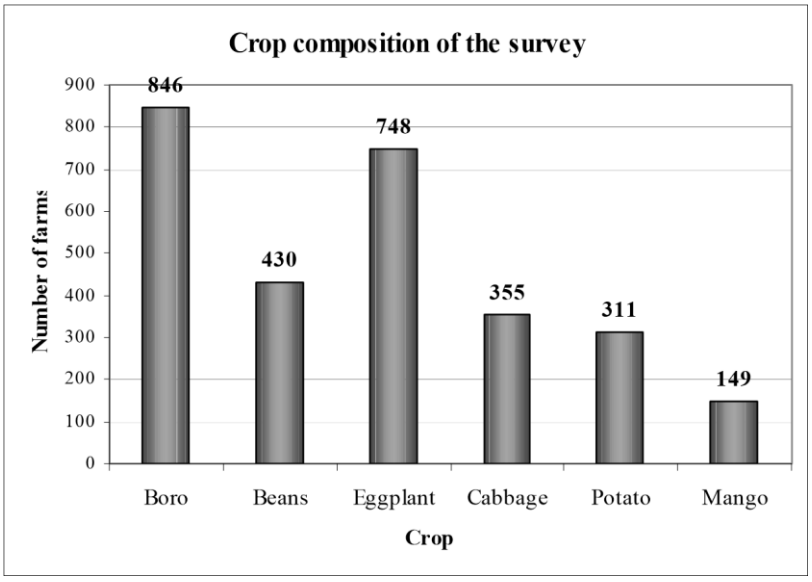


Fig.-1.2: Crop composition of the World Bank survey, (2003).

The survey sample was also scattered among several districts to capture any possible differences in production or pesticide use. The survey covered the following districts: Bogra, Chapainawabgunj, Rajshahi and Rangpur (Rajshahi division); Chittagong and Comilla (Chittagong division); Jessore (Khulna division); and Kishoreganj, Munshiganj, Narsingdi and Mymensingh (Dhaka division) (Rasul and Thapa, 2003; Hossain 1988) (**Figure 3**). Among the various production and input information collected, the survey contained information on the type and application amounts of pesticides used for each crop.

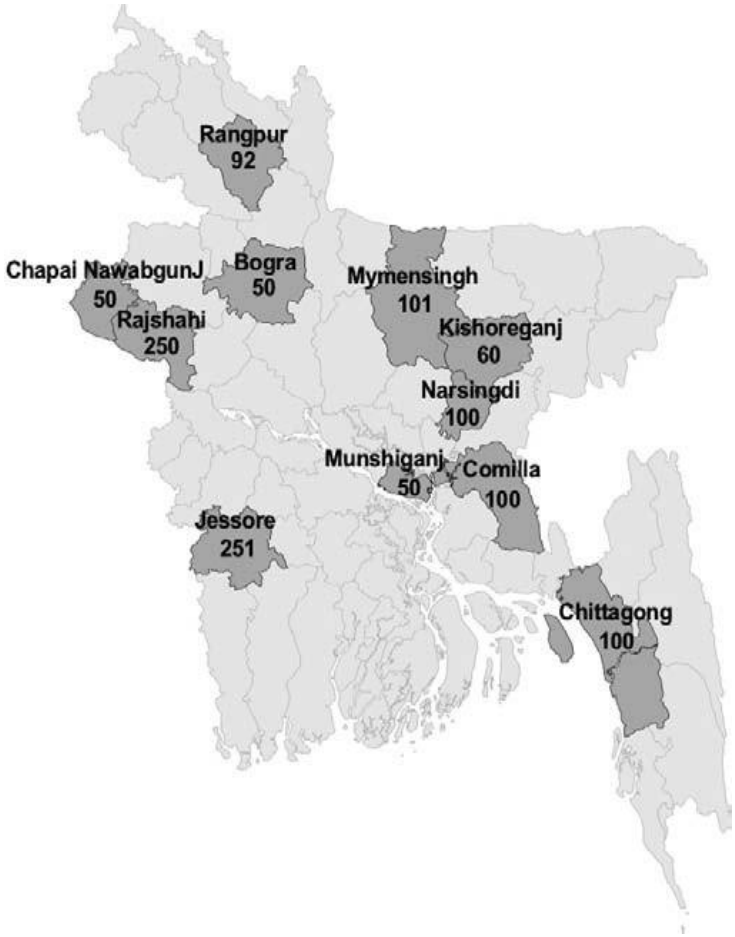
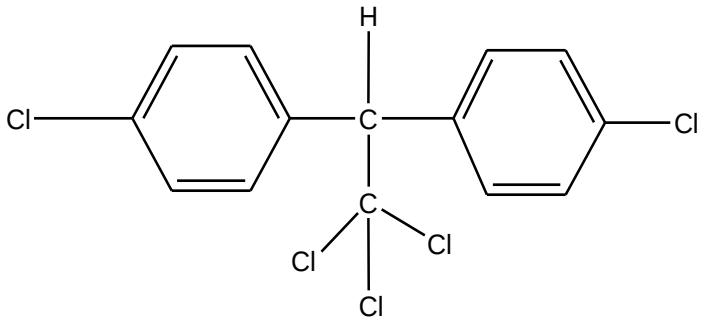


Fig.-1.3: Geographical distribution of the survey (sample size indicated)

1.4 DDT



4,4/- DDT

Fig.-1.4: Structure of *4,4′* DDT

DDT is perhaps the most infamous of the POPs. It was initially used during the Second World War to protect troops and civilians from malaria, typhus and vector-borne diseases. After the war, DDT was widely used for agriculture and disease control (Smith, 1991: IARC, 1974). It has a strong persistence in soil. Being the earliest, well known and one of the most widely used pesticides, DDT caused widespread contamination of water and soil resources, resulting in serious health effects in humans and animals (ATSDR, 2002). Its half-life is ~15 years (PULSE, 2009). Food is the primary route of exposure to DDT and its metabolites. They are fat soluble and accumulate well in adipose tissues and other organs of the body. In 1995, DDT has been banned in 34 countries and severely restricted in an additional 34 countries (Ritter *et al*., 1995). WHO Acceptable Daily Intake (ADI) allowance (0.02 mg/kg bw) (IPCS and IARC, 2009) and the Maximum Residue Limit (MRL) value is 0.05mg/kg (http//www, 2009). Estimated lethal dose for human is 500 mg/kg (ICPS, 1976).

1.4.1 Isomers and Related Compounds

The term "**total DDT**" is often used to refer to the sum of all DDT related compounds (*4,4′*-DDT, *2,4′*-DDT, DDE, and DDD) in a sample (Wiki.).

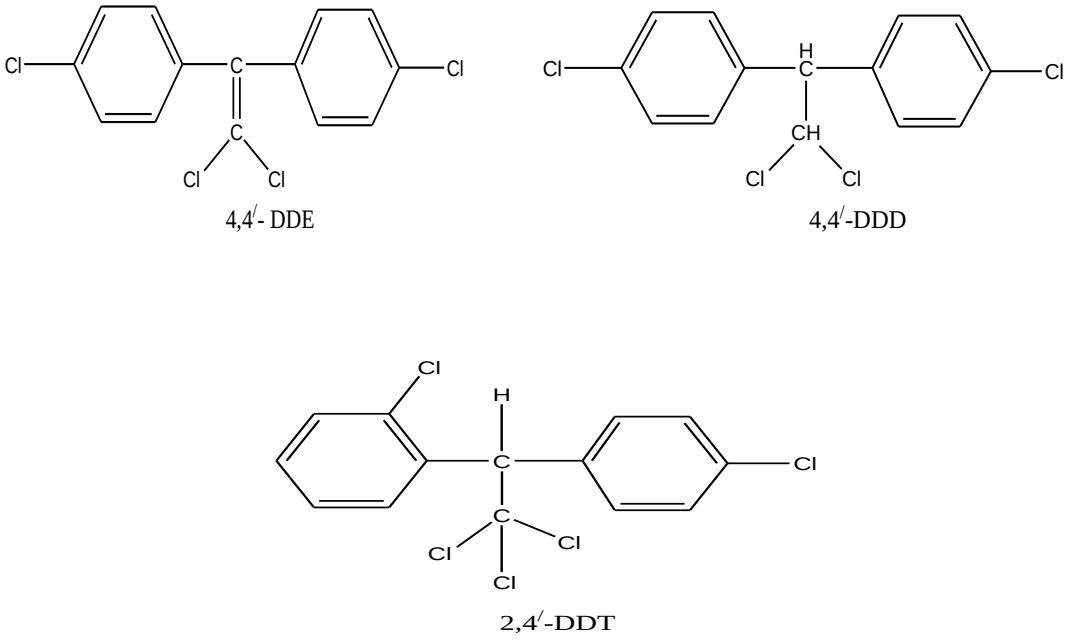


Fig.-1.5: Structure of DDE, DDD and *2,4′* DDT

1.4.2 Properties of DDT

DDT is a colorless crystalline substance, which is practically insoluble in water but highly soluble in fats and most organic solvents. It has potent insecticidal properties, which kills by opening sodium channels in insect neurons, causing the neuron to fire spontaneously. This leads to uncontrolled spamming and eventual death.

1.4.3 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 (van Wendel *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 DDT would possibly persist throughout the life span (Smith, 1991).

1.4.3.1 Acute Toxicity of DDT

DDT is moderately to slightly toxic to mammalian species via the oral route. Reported oral LD_{50}s range from 113 to 800 mg/kg in rats (1-3); 150-300 mg/kg in mice. Toxicity will vary according to formulation. DDT is readily absorbed through the gastrointestinal tract, with increased absorption in the presence of fats.

Acute effects likely in humans due to low to moderate exposure may include nausea, diarrhea, increased liver enzyme activity, irritation (of the eyes, nose or throat), disturbed gait, malaise and excitability; at higher doses, tremors and convulsions are possible. While adults appear to tolerate moderate to high ingested doses of up to 280

mg/kg, a case of fatal poisoning was seen in a child who ingested one ounce of a 5% DDT: kerosene solution. (pmep.cce.cornell.edu, 2013)

1.4.3.2 Chronic Toxicity of DDT

DDT has caused chronic effects on the nervous system, liver, kidneys, and immune systems in experimental animals. Effects on the nervous system observed in test animals include: tremors in rats at doses of 16-32 mg/kg/day over 26 weeks.

The main effect on the liver seen in animal studies was localized liver damage. This effect was seen in rats given 3.75 mg/kg/day over 36 weeks, rats exposed to 5 mg/kg/day over 2 years and dogs at doses of 80 mg/kg/day over the course of 39 months. Kidney damage was also seen in rats at doses of 10 mg/kg/day over 27 months.

Persons eating fish contaminated with DDT or its metabolites may also be exposed via bioaccumulation of the compound in fish. Adverse effects on the liver, kidney and immune system due to DDT exposure have not been demonstrated in humans in any of the studies which have been conducted to date (pmep.cce.cornell.edu, 2013).

1.4.3.3 Effects of DDT in Reproductive System

There is evidence that DDT causes reproductive effects in test animals. In rats, oral doses of 7.5 mg/kg/day for 36 weeks resulted in sterility. It is thought that many of these observed effects may be the result of disruptions in the endocrine (hormonal) system.

Available epidemiological evidence from two studies does not indicate that reproductive effects have occurred in humans as a result of DDT exposure. No associations between maternal blood levels of DDT and miscarriage or premature

rupture of fetal membranes were observed in two separate studies (pmep.cce.cornell.edu, 2013).

1.4.3.4 Teratogenic Effects of DDT

There is evidence that DDT causes teratogenic effects in test animals as well. In a two-generational study of rats, 10 mg/kg/day resulted in abnormal tail development. It seems unlikely that teratogenic effects will occur in humans due to DDT at likely exposure levels (pmep.cce.cornell.edu, 2013).

1.4.3.5 Mutagenic Effects of DDT

The evidence for mutagenicity and genotoxicity is contradictory. In only 1 out of 11 mutagenicity assays in various cell cultures and organisms did DDT show positive results. Results of in vitro and in vivo genotoxocity assays for chromosomal aberrations indicated that DDT was genotoxic in 8 out of 12 cases, and weakly genotoxic in 1 case.

In humans, blood cell cultures of men occupationally exposed to DDT showed an increase in chromosomal damage. Thus it appears that DDT may have the potential to cause genotoxic effects in humans, but does not appear to be strongly mutagenic (pmep.cce.cornell.edu, 2013).

1.4.3.6 Carcinogenic Effects of DDT

The evidence regarding the carcinogenicity of DDT is equivocal. It has been shown to cause increased tumor production (mainly in the liver and lung) in test animals such as rats and mice in some studies but not in others. In rats, liver tumors were induced in three separate studies at doses of 12.5 mg/kg/day over periods of 78 weeks to life, and thyroid tumors were induced at doses of 85 mg/kg/day over 78 weeks.

The available epidemiological evidence regarding DDT's carcinogenicity in humans, when taken as a whole, does not suggest that DDT and its metabolites are carcinogenic in humans at likely dose levels (pmep.cce.cornell.edu, 2013).

1.4.3.7 Neurochemical Effects of DDT

The main target of DDT is the sodium ion channel. DDT delays the closure and prevents full opening of the channels in excited cells, leading to hyper excitability. DDT also reduces potassium permeability through pores, inhibits neuronal ATPase's, particularly the Ca^{2+}-ATPase and Na/K-ATPase's, which are important for the neuronal repolarization, and inhibits the ability of calmodulin to transport Ca^{2+} (Echobichon, 1996). In agreement, (Kodavanti *et al*., 1996) showed that DDT at relatively low concentrations (IC_{50}, 4-5 μM) inhibits Ca^{2+} uptake in mitochondria *in-vitro*. Involvement of calcium may influence brain PKC activity and. (Bagchi *et al*. 1997) reported increased brain PKC activity *in-vivo* in DDT exposed rats (40 mg/kg/body weight) and also in PC12 cells *in-vitro* (50-200 nM). Exposure to DDT at doses inducing tremors (25-100 mg/kg) also revealed region specific alterations in the levels of the rat brain biogenic amines, such as serotonin and noradrenalin (Hudson *et al*., 1985; Hong *et al*., 1986). Similarly, DDT has been shown to increase the levels of the amino acid neurotransmitters, glutamate and aspartate in brainstorm, and to stimulate release of acetylcholine at high concentrations (Hudson *et al*., 1985; Morio *et al*., 1985). The effects on neurotransmitters have been attributed to DDT's effect on sodium channels, promoting release of neurotransmitters (Hong *et al*., 1986). Eriksson and coworkers found that DDT decreases the levels of the muscarinic cholinergic receptors in the brain of mice administered a single dose of DDT (0.5 mg/kg) (Eriksson *et al*., 1984; Eriksson and Nordberg, 1986; Johansson *et al*., 1995). The nervous system is the major target of acute DDT exposure (RFI, 2001). Chronic exposure leads to neurological, hepatic, renal and immunological effects.

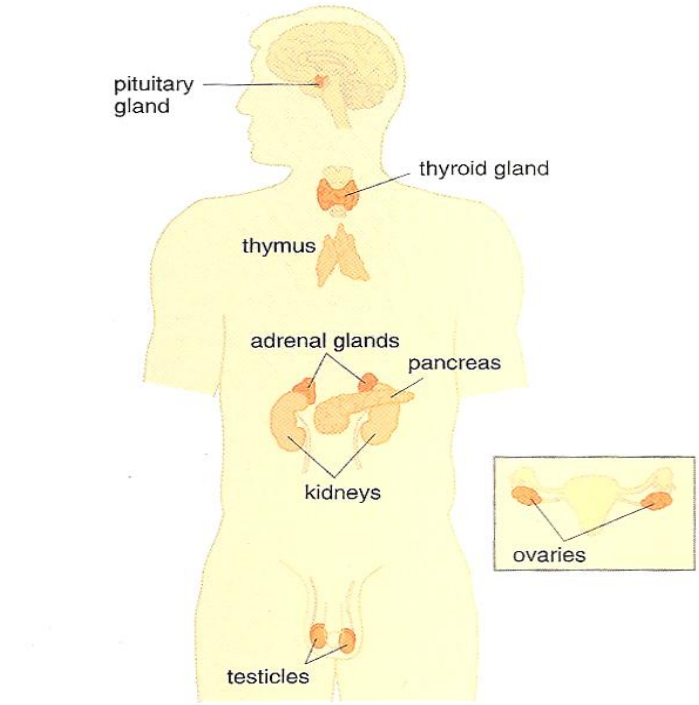


Fig.-1.6: Endocrine and related organs of human

1.4.4 Ecological Effect

1.4.4.1 Effects on Birds

DDT may be slightly toxic to practically non-toxic to birds. Reported dietary LD50s range from greater than 2,240 mg/kg in mallard, 841 mg/kg in Japanese quail and 1,334 mg/kg in pheasant. In birds, exposure to DDT occurs mainly through the food web through predation on aquatic and/or terrestrial species having body burdens of DDT, such as fish, earthworms and other birds.

There has been much concern over chronic exposure of bird species to DDT and effects on reproduction, especially eggshell thinning and embryo deaths. There is evidence that synergism may be possible between DDT's metabolites and organophosphate (cholinesterase-inhibiting) pesticides to produce greater toxicity to the nervous system and higher mortality (pmep.cce.cornell.edu, 2013).

1.4.4.2 Effects on Fish Species

DDT is very highly toxic to fish species as well. Reported 96-hour LC50s are less than 10 µg/L in coho salmon (4.0 µg/L), rainbow trout (8.7 µg/L), northern pike (2.7 µg/L), black bullhead (4.8 µg/L), bluegill sunfish (8.6 µg/L), largemouth bass (1.5 µg/L), and walleye (2.9 µg/L). The reported 96-hour LC_{50s} in fathead minnow and channel catfish are 21.5 µg/L and 12.2 µg/L respectively. Observed toxicity in Coho and Chinook salmon was greater in smaller fish than in larger. It is reported that DDT levels of 1 ng/L in Lake Michigan were sufficient to affect the hatching of Coho salmon eggs.

In addition to acute toxic effects, DDT may bioaccumulation significantly in fish and other aquatic species, leading to long-term exposure. This occurs mainly through uptake from sediment and water into aquatic flora and fauna, and also fish. Fish uptake of DDT from the water will be size-dependent with smaller fish taking up relatively more than larger fish. A half- time for elimination of DDT from rainbow trout was estimated to be 160 days.

The reported bio concentration factor for DDT is 1,000 to 1,000,000 in various aquatic species and bioaccumulation may occur in some species at very low environmental concentrations (pmep.cce.cornell.edu, 2013).

1.4.5 Fate in Humans & Animals

DDT is very slowly transformed in animal systems. Initial degrades in mammalian systems are DDE and DDD, which are very readily stored in fatty tissues. These compounds in turn are ultimately transformed into bis (dichlorodiphenyl) acetic acid (DDA) via other metabolites at a very slow rate. DDA, or conjugates of DDA, are readily excreted via the urine.

Blood samples collected in the latter half of the 1970s showed that blood levels were declining further, but DDT or metabolites were still seen in a very high proportion of

the sample. Levels of DDT or metabolites may occur in fatty tissues (e.g. fat cells, the brain, etc.) at levels of up to several hundred times that seen in the blood. DDT or metabolites may also be eliminated via mother's milk by lactating women (pmep.cce.cornell.edu, 2013).

1.4.6 Environmental Fate of Pesticides

When a pesticide is released into the environment, whether through an application, a disposal or a spill, it influenced by many processes (Fishel, 1997). It may be taken up by a plant or ingested by insects, worms or microorganisms in the soil. It may stick to the soil particles or dissolve in water and move down through the soil to the water table. It may vaporize and enter the atmosphere or break down through microbial and chemical pathways into other less toxic compounds. Pesticides may dissolve in rain or irrigation water (Rao *et al*., 1983; Buttler *et al*., 1998). These processes determine a pesticide's persistence and movement and its ultimate fate. Pesticides fate processes fall into three major types namely adsorption, transfer and degradation (Fishel, 1997). All these processes are governed by the physic-chemical properties of pesticide and site on which it is applied (Buttler *et al*., 1998; Linde, 1994; Kerle *et al*., 2007).

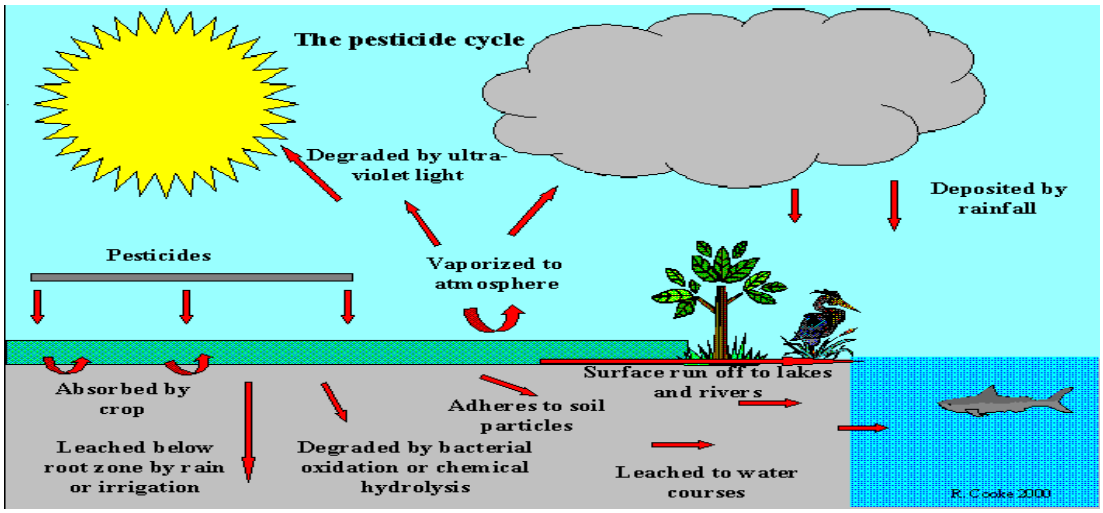


Fig.-1.7: The pesticide cycle

1.4.6.1 Pesticides Adsorption

Pesticide adsorption is the binding of pesticides to soil or sediment particles (Linde, 1994; Devlin *et al*., 2008; Sprague, 2012). Pesticides adsorption often occurs because of the attraction between a chemical and soil or sediment particles (Fishel, 1997). The mechanisms which operate when pesticides adsorb include strong or weak ionic attraction, hydrophobic attraction, and hydrogen bondings. Factors which control pesticide adsorption includes polarity of pesticide itself, amount of moisture in soil or sediment, soil PH, organic matter content and soil texture (Fishel, 1997; Linde, 1994; Sprague, 2012).

Wet soils tend to adsorb less pesticide than dry soils because water molecules compete with the pesticides for the binding sites (Fishel, 1997; Jansma and Linders, 1995). A polar pesticide will be very water soluble and tend not to be adsorbed onto soil. Pesticides that are non-polar tend to be pushed out of water and onto soils which contain non polar carbon material (Linde, 1994). For pesticides that are weak acids or bases, adsorption is influenced by the pH of the soil; however pesticides that are not ionisable are generally not affected by pH (Linde, 1994). Some pesticides such as paraquat and glyphosate bind tightly to the soil while others bind only weekly and are readily desorbed or released back into the solution (Fishel, 1997).

The amount of organic matter in soil is the greatest factor influencing the amount of pesticides adsorbed. This is because organic matter is non polar and has a relatively light negative charge. Most pesticides are non-polar and will be attracted to the lightly charged surface (Linde, 1994). Besides, soils high in organic matter or clay are more adsorptive than sandy soils because clay or organic soil has more particle surface area or more sites into which pesticides can bind. Therefore sandy soil increase pesticides mobility while clay soil reduces pesticides mobility (Fishel, 1997).

1.4.6.2 Pesticides Transfer Processes

Transfer processes include processes that move the pesticide away from the target (Navarro et al., 2007). These processes include volatilization, spray drift, runoff, leaching, absorption and crop removal (Fishel, 1997; Navarro *et al*., 2007; Singh and Walker, 2006). Pesticides transfer process depends on its solubility in water, adsorption (retention) by soil and its persistence. It's also influenced by environmental and site characteristics including weather, topography, canopy, ground cover; and soil organic matter, soil texture and structure (Kerle *et al*., 2007; Estevez *et al.*, 2008).

1.4.6.3 Pesticide Runoff

Runoff is the movement of pesticides in water over a sloping surface. The pesticides are either mixed in the water or bound to eroding soil. Runoff can also occur when water is added to a field faster than it can be absorbed into the soil. Pesticides may move with runoff as compounds dissolved in the water or attached to soil particles. The amount of pesticide runoff depends on the slope, the texture of the soil, the soil moisture content, the amount and timing of a rain event (irrigation or rainfall) and the type of pesticide used (Kerle *et al*., 2007; Sprague, 2012).

1.4.6.4 Leaching

Leaching is the removal of soluble materials by water passing through the soil (Buttler *et al*., 1998; Bicki, 1989; Kerle *et al*., 1996). Leaching occurs downward, upward, or sideways. The factors influencing leaching of pesticides depend on both, the characteristics of the soil and pesticide. These include water solubility of the pesticide, soil structure and texture as well as the amount and persistence of pesticide adsorption to soil particles (Chilton *et al*., 1994; Holland and Sinclair, 2004).

Highly water soluble pesticides tend to move easier through the soil profile into groundwater (Kerle *et al.*, 1996; Tharp, 2012). Pesticides which degrade easily the opportunity of leaching to occur is limited, on the other hand for pesticides which degrade slowly the chance of leaching to occur is maximum (Cardeal, 2011). Soil with high organic matter increases the capacity for adsorption of pesticides hence reduces leaching (CES. Univ Alaska, 2011). Leaching can be increased when a rain-event occurs shortly after spraying (Estevez *et al*., 2008; CES. Univ Alaska, 2011).

1.4.6.5 Pesticide Absorption or Uptake

Absorption or uptake is the movement of pesticides into and within the plant and animals (Fishel, 1997; Burner *et al*., 1997). Most pesticides break down once they are absorbed. Pesticide residues may be broken down or remain inside the plant or animal and be released back into the environment when the animal dies or as the plant decays. Some pesticides stay in the soil long enough to be absorbed by plants grown in a field years later. They may damage or leave residues in future crops (Tiryaki and Temur, 2010).

The most important factor governing sorption and movement within the plant is the solubility of the pesticide in water (Kerle *et al.*, 2007; Burner *et al*., 1997). The content of the surrounding soil is also important to the plant uptake. For non-polar pesticides the volume of organic matter is particularly important. Other factors such as pH and clay and microbial activity are more important as the polarity of the pesticide increases (Burner *et al*., 1997). Plant uptake of pesticides prevents runoff or leaching (Kerle *et al*., 2007).

1.4.7 Pesticide Degradation Processes

Degradation or transformation of pesticides is the breaking down pesticides into simpler molecules than the parent compound, they can be none or less toxic

compounds and, in some cases, they are also toxic and more persistence than the parent compound for example the degradation of endosulfan to endosulfan sulfate (Cardeal, 2011; Vargas, 1975; NRCS, 1998; Shivaramaiah and Kennedy, 2006). Pesticides can be degraded by chemical (chemical degradation), sunlight (photo degradation) or by microbial activity (biological degradation) The rate of degradation depends on pesticide chemistry, environmental conditions, distribution between foliage and soil, as well as temperature, soil and water pH. The degradation of pesticides can occur in plants, animals, soil and water; or it can take place upon exposure to ultra-violet (UV) radiation (Kerle *et al*., 2007).

1.4.7.1 Chemical Degradation of Pesticides

Chemical degradation is the breakdown of a pesticide by processes not involving a living organism. Chemical processes generally occur in water or atmosphere and follow one of the three reactions: oxidation, reduction, hydrolysis, and photolysis. The rate and type of chemical reactions that occur are influenced by the adsorption, temperatures, pH, moisture and physical chemical properties of the pesticide (Fishel, 1997; Linde, 1994).

1.4.7.2 Biological Degradation of Pesticides

Pesticides biotransformation or biodegradation is the process by which pesticide substances are broken down into smaller compounds by the enzymes produced by living microbial organisms (Porto et al., 2011). The degradation of pesticides through microbial metabolic process may involve a three-phase process. In Phase I transformation, the initial properties of a parent compound are transformed through oxidation, reduction, or hydrolysis to generally produce a more water-soluble and usually a less toxic product than the parent. The second phase involves conjugation of a pesticide or pesticide metabolite to a sugar amino acid, or glutathione, which increases the water solubility and reduces toxicity compared with the parent pesticide.

Generally, Phase II metabolites have little or no phytotoxicity and may be stored in cellular organelles. The third phase involves conversion of Phase II metabolites into secondary conjugates, which are also nontoxic (Hernandez *et al.*, 2011; Chaplain *et al*., 2011; Eard *et al.*, 2003). The processes responsible for biotransformation of pesticides include biodegradation, co-metabolism and synthesis (Chaplain *et al.*, 2011).

Biodegradation process, the microbial organisms transform the substance through metabolic or enzymatic processes this occurs when microorganisms use pesticides as a food substrate. The microorganisms participating in biodegradation include fungi, bacteria and other microorganisms that use pesticides as their substrate (Chaplain *et al*., 2011).

Synthesis includes conjugation and oligomerization. Pesticides are transformed into compounds with chemical structures more complex than those of the parent compounds. During conjugation, a pesticide (or one of its transformation products) is linked to hydrophilic endogenous substrates, resulting in the formation of methylated, acetylated, or alkylated compounds, glycosides, or amino acid conjugates. These compounds can be excreted from the living cells, or stored. During oligomerization, a pesticide combines with itself, or with other xenobiotic residues (proteins, soil organic residues). Consequently, they give high-molecular weight compounds, which are stable and often incorporated into cellular components or soil constituents (soil organic matter) (Chaplain *et al.*, 2011).

The rate of microbial degradation depends highly on the amount and nature of pesticides present in the soil, the microbial population in the soil and soil conditions that favors microbial activities, such as warm temperature, favorable pH, adequate soil moisture, aeration and high organic matter content (Chaplain et al., 2011).

1.4.8 Bioaccumulation in Fish

An important process through which chemicals can affect living organisms is bioaccumulation. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment (extoxnet.orst.edu, 2013). POPs are highly hydrophobic chemicals that may accumulate in fishes depending on their lipid content. Knowledge regarding bioaccumulation and the levels of chemicals in biota is a prerequisite to understand the adverse effects of the chemicals on ecosystems (Franke *et al*., 1994). POPs may accumulate in fishes via various pathways, for example, via direct uptake from water through gills or skin (bio-concentration), ingestion of particulate matter from water (ingestion) and/or consumption of contaminated food (bio-magnification).

Normally all these processes occur in varying degrees of combinations in all fishes. Even if acute or chronic effects are not detected in toxicity tests, accumulation of POPs in fish tissues should be regarded as a hazard criterion because some effects may be recognized only at a later stage of life, may be multigenerational (*e.g*. impact of PCBs on the egg hatching success (Tillitt *et al*., 1992), or may manifest themselves only in higher members of the food web. Even contaminant concentrations in fishes may vary depending on species, age groups, reproductive status and various other parameters.

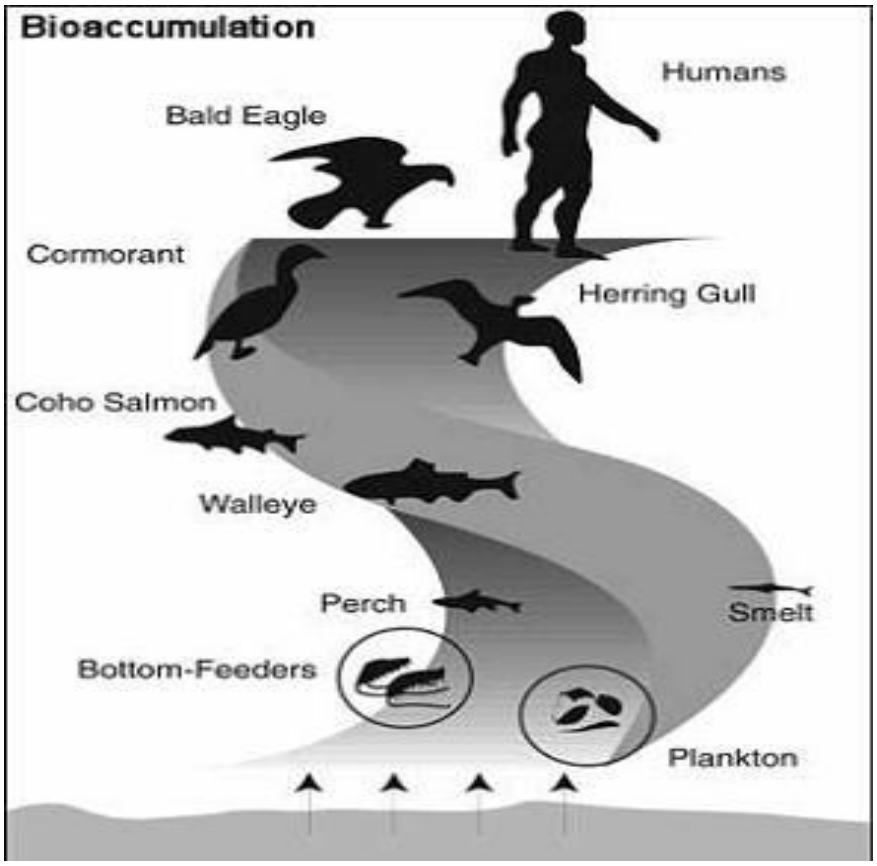


Fig.-1.8: Bioaccumulation of DDTs

1.4.9 Bioconcentration in Fish

Bioconcentration is the specific bioaccumulation process by which the concentration of a chemical in an organism becomes higher than its concentration in the air or water around the organism (extoxnet.orst.edu, 2013). Bio concentration is the specific bioaccumulation process by which the concentration of a chemical is an organism becomes higher than its concentration in the air or water around the organism for fish and other aquatic animals, bio concentration after uptake through the gills (sometimes the skin) is usually the most important bioaccumulation process.

1.4.10 Biomagnification in Fish

Biomagnification, also known as bioamplification or biological magnification, is the increase in concentration of a substance that occurs in a food chain.

Biological magnification often refers to the process whereby certain substances such as pesticides or heavy metals move up the food chain, work their way into rivers or lakes, and are eaten by aquatic organisms such as fish, which in turn are eaten by large birds, animals or humans. The substances become concentrated in tissues or internal organs as they move up the chain.

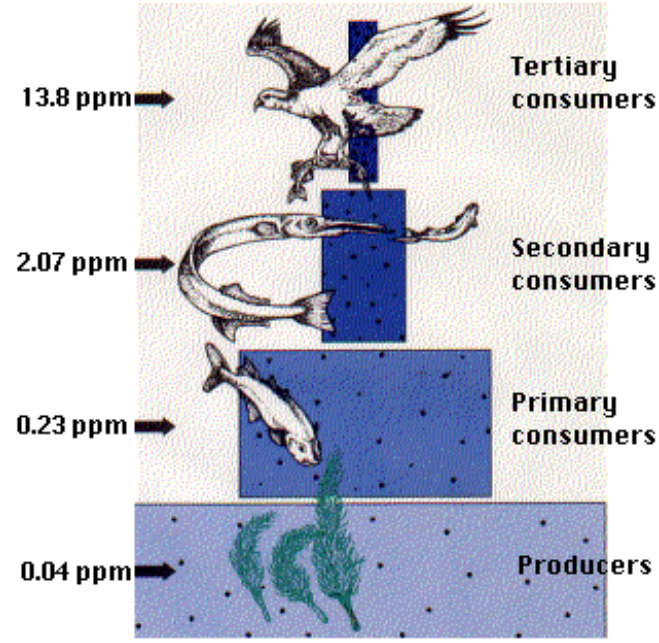


Fig.-1.9: Biomagnification of DDTs

The following is an example showing how bio-magnification takes place in nature: An anchovy eats zoo-plankton that has tiny amounts of mercury that the zoo-plankton has picked up from the water throughout the anchovies lifespan. A tuna eats many of these anchovies over its life, accumulating the mercury in each of those anchovies into its body. If the mercury stunts the growth of the anchovies, that tuna is required

to eat more little fish to stay alive. Because there are more little fish being eaten, the mercury content is magnified. (Wiki/Bio magnification, 2013).

1.4.11 DDTs in Different Countries

DDTs 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^{-1})	References
Nainital, India	2.13-37.17	(Dua *et al*.1998)
Dhaka	0.04-0.16	(Matin *et al.* 1998)
Hisar, India	6.20–7.06	(Kumari *et al.* 2007)
Noushehra	70-400	(Jan *et al.* 2009)
Rawal lake, Pakistan	0.96- 2.87	(Iram *et al*. 2009)
Lake Burullus, Egyptian Mediterranean sea	0.07-882.6	(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 *et al*. 2008)

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

Study area	DDTs (ng g^{-1}) (dw)	Reference
Yamun river, Delhi	17.10-236.6	(Sethi *et al.* 1999)
Kolleru lake wetland, India	BDL-128,600	(Amaraneni 2006)
Hugli estuary, Sunderban ,India	3-119	(Bhattacharya *et al.* 2003)
Bay of Bengle, India	0.04-4.79	(Rajendran *et al.* 2005)
District Nagaon, India	166-2288	(Mishra *et al*. 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 *et al*. 2002)
Masan Bay, Korea	0.27-89.2	(Hong *et al*.2003)
Caspean Sea, Russia	0.01-1.9	(De Mora *et al*. 2001)
Aruwimi Congo River, Basin	0.023-0.37	(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 *et al*. 2008)

*dw- dry weight

Table-1.4: DDTs in the fish samples from different countries

Study area	DDTs (ng g^{-1}) (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)	0.84-27.0	(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)	2.65-133.5	(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)	7.54-88.3	(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)	0.84-10.1	(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	909.1±42.5	(Manirakizaa *et al.* 2002)
	DDTs (ng g^{-1}) (lw)	
Pongolapoort (South Africa)	5400-6000	(Wepener *et al.* 2012)
Pearl River Delta (South China)	380-57000	(Sun *et al.* 2015)

*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^{-1})(lw)	References
Gujrat Islamabad (Pakistan)	Rural mother (Blood)	11	(Ali *et al.* 2013a,b)
	Rural Children (Blood)	17.5	(Ali *et al.* 2013a,b)
	General population (Blood)	17	(Ali *et al.* 2013a,b)
South Africa	Breast milk	9500-18000	(Bouwman *et al.* 2012)
Hudson Strait (Canadial Arctic)	Beluga Whale (Fat)	520-2521	(Kelly *et al.* 2008a)
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.* 2004)
Russia Bering Sea	Stellar sea lions (Blood)	3600-15000	(Myers *et al*. 2008)
		DDTs (ng g^{-1}) (ww)	
Agra (India)	Vegetables	2.82	(Bhanti and Taneza 2005)
	Vegetables	4.13-8.44	(Barriada-Perira *et al.* 2005)
Tamil Nadu (India)	Crab muscle	5.8-59	(Ramesh *et al.* 1999)
Patna	Dolphin muscle	100-5100	(Kannan *et al.* 1994)
South India	Birds muscle	0.6-3600	(Senthilkumar *et al.* 2001)
Ghana	Meat	10.82	(Darko *et al.* 2007)

*ww-wet weight, lw-lipid weight

1.4.12 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. 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, vegetable, fish and human blood samples of Bangladesh

Study area	Samples	DDTs	Unit	References
Chittagang Chemical comples	Water	0.59-3.01	µg L^{-1}	(Mahmud *et al.* 2015)
Chittagang Chemical comples	Soil	1.0-48.6x10^2	mg kg^{-1}	(Mahmud *et al.* 2015)
Fish market of Dhaka city	Dry fish	0.03-1.2	mg kg^{-1}	(Nahar *et al.* 2008)
Fish market of Chittagong	Homemade dry fish	ND		(Nahar *et al.* 2008)
Fish market of Dhaka city	Vegetables	ND		(Nahar *et al.* 2008)
Fish market of Dhaka city	Fish and shrimp	0.03-1.2	mg kg^{-1}	(Nahar *et al.* 2008)
Fish market of Khulna, Chittagong and Cox's Bazar	Dry fish	2.81 to 877.82	ng g^{-1}	(Hasan *et al.* 2014)
Asadganj market, Chittagong (winter)	Dry fish	3.6-250.8	ug kg^{-1}	(Bhuiyan *et al.*2009)
Asadganj market, Chittagong (rainy-season)	Dry fish	11.1-1107.4	ug kg^{-1}	(Bhuiyan *et al.* 2009)
Fish market of Sayedpur and Cox's Bazar	Fish	14.454-1249.686	ng g^{-1}	(Hasan *et al.* 2013)
Mohonganj River, Mymensing	Fish	4.71-78.81	ng g^{-1}	(Hossain *et al.* 2016)
Dhaka city	Human blood		ng g^{-1}	(Zamir, *et al.* 2008)
Dhaka city	Human blood	1200-8800	ng g^{-1}	(Mamun, *et al.* 2007)
	Marine fish			(Shoeb, *et al.* 2016)

1.5 Cypermethrin, A Synthetic Pyrethroid

Cypermethrin is a synthetic compound primarily used as an insecticide. It acts as a fast-acting neurotoxin in insects. It is easily degraded on soil and plants but can be

effective for weeks when applied to indoor inert surfaces. Exposure to sunlight, water and oxygen will accelerate its decomposition. It is a synthetic pyrethroid (Abou-awad and El-banhawy, 1985).

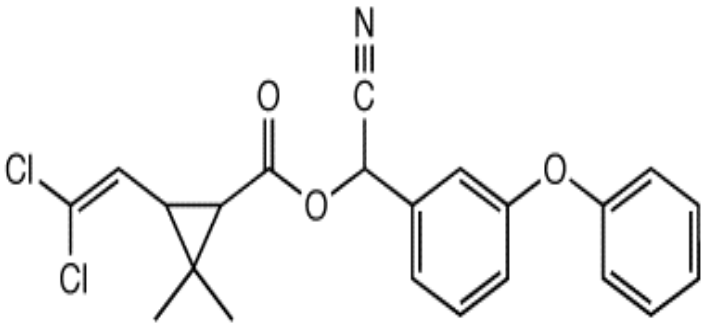


Fig.-1.10: Structure of cypermethrin

Cypermethrin was initially synthesized in 1974 and first marketed in 1977 as a highly active synthetic pyrethroid insecticide, effective against a wide range of pests in agriculture, public health, and animal husbandry. In agriculture, its main use is against foliage pests and certain surface soil pests, such as cutworms, but because of its rapid breakdown in soil, it is not recommended for use against soil-borne pests below the surface (Ahmed et al., 1985).

1.5.1 Physical Properties of Cypermethrin

Most technical grades of cypermethrin contain more than 90% of the active material. The material varies in physical form from a brown-yellow viscous liquid to a semi-solid (Tomlia, 14th ed.). Cypermethrin has a very low vapour pressure and solubility in water, but it is highly soluble in a wide range of organic solvents. Cypermethrin is highly stable to light and at temperatures below 220 °C. It is more resistant to acidic than to alkaline media, with an optimum stability at pH 4.

1.5.2 Environmental Levels and Residues in Food

Cypermethrin is formulated as emulsifiable concentrates (100 and 250 g/litre), ultra-low-volume concentrate (10 - 50 g/litre), wettable powder (125 g/kg), and animal dip concentrate (5 - 15%).

Cypermethrin is used in a wide range of crops. In general, the maximum residue limits are low, ranging from 0.05 to 2.0 mg/kg in the different food commodities. The residues will be further reduced during food processing. In food of animal origin, residues may range between 0.01 and 0.2 mg/kg product. Residues in non-food commodities are generally higher, ranging up to 20 mg/kg product [FAO/WHO (1982a); FAO/WHO (1985c)].

Some of the maximum residue limits (MRL) values of cypermethrin in different foods according to Codex Alimentarius Commission (FAO, 1986) are shown in **Table-1.7.**

Table-1.7: Codex limits for cypermethrin residues

Commodity	Maximum Residue Limit (mg kg^{-1})
Brassica leafy vegetables	1.0
Citrus	2.0
Lettuce	2.0
Oil seeds except peanuts	0.2
Peas	0.05
Root and tuber vegetables	0.05
Tomatoes	0.5
Wheat grain	0.2
Carcass meat (carcass fat)	0.2
Meat products	0.2
Eggs	0.05
Milk (whole milk)	0.01

Acceptable Daily Intake (ADI) of cypermethrin = 0.05 mg/kg body weight

1.5.3 Environmental Fate of Cypermethrin

1.5.3.1 Breakdown in Soil and Groundwater

Cypermethrin has a moderate persistence in soils. Under laboratory conditions, cypermethrin degrades more rapidly on sandy clay and sandy loam soils than on clay soils, and more rapidly in soils low in organic material (US EPA, 1989). In aerobic conditions, its soil half-life is 4 days to 8 weeks. It photodegrades rapidly with a half-life of 8 to 16 days. Cypermethrin is also subject to microbial degradation under aerobic conditions. Cypermethrin is not soluble in water and has a strong tendency to adsorb to soil particles (Kidd and James, 1991).

1.5.3.2 Breakdown in Water

In neutral or acid aqueous solution, cypermethrin hydrolyzes slowly, with hydrolysis being more rapid at pH 9 (basic solution). Under normal environmental temperatures and pH, cypermethrin is stable to hydrolysis with a half-life of greater than 50 days and to photodegradation with a half-life of greater than 100 days. In pond waters and in laboratory degradation studies, pyrethroid concentrations decrease rapidly due to sorption to sediment, suspended particles and plants. Microbial degradation and photodegradation also occur (Muir *et al*., 1985; Agnihotri, 1986).

1.5.3.3 Breakdown in Vegetation

When applied to strawberry plants, 40% of the applied cypermethrin remained after one day, 12% remained after three days, and 0.5% remained after seven days, with a light rain occurring on day 3. When cypermethrin was applied to wheat, residues on the wheat were 4 ppm immediately after spraying and declined to 0.2 ppm 27 days later. No cypermethrin was detected in the grain. Similar residue loss patterns have been observed on treated lettuce and celery crops (Ruzo and Casida, 1980).

1.5.4 Degradation of Cypermethrin

Cypermethrin is a modern pesticide and it can undergo photo degradation, microbial degradation and biological degradation. The *trans*- isomer is more degradable than the *cis*-isomer. The *trans*- isomer shows a much shorter half-life.

1.5.4.1 Photodegradation

Cypermethrin is one of the pyrethroids which are more light-stable. No loss of solid cypermethrin was detected exposing it to sunlight for 30 h. When exposed in methanol solution to light of wavelength > 290 nm for about 2 days, 55% of cypermethrin was recovered, the reaction quantum yield at 300 nm in methanol was low (Lauren and Henzel, 1977). Cypermethrin is more susceptible to radiation of lower wavelengths; under ultra-violet radiation, 90% of cypermethrin on a glass petri dish was decomposed after 3 days, but only 45% was decomposed after 3 days when the cypermethrin was deposited on grass and placed under an UV-lamp (Takahashi *et al*., 1985). The most important photo degradation products are; 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane-carboxylic acid **(CPA)**, 3-phenoxybenzoic acid **(PBA)**, the amide analogue of cypermethrin and various phenoxybenzyl derivatives, such as the alcohol, aldehyde, and acid (Day and Leahey, 1980; Leahey, 1979). These metabolites further degrade to smaller fractions. In studies on the 2 metabolites (PBA and CPA), PBA was quicker to degrade than CPA (Miyamoto and Mikami, 1983).

1.5.4.2. Biological Degradation

Cypermethrin degrades relatively quickly in soils, primarily by biological processes involving cleavage of the ester linkages, to give the two main degradation products, CPA and PBA. These products are themselves subsequently mineralized. There is also evidence for the formation, as an intermediate, of the amide of the intact molecule and occasionally the 4-hydroxy phenoxy analogue. Neither of the latter

products appears to persist in the soil (Sakata et al., 1986; Roberts and Standen, 1981).

1.5.4.3 Toxicity of Cypermethrin

Cypermethrin is a moderately toxic material by dermal absorption or ingestion. Symptoms of high dermal exposure include numbness, tingling, and itching, burning sensation, loss of bladder control, seizures, and possible death. Pyrethroids like cypermethrin may adversely affect the central nervous system. Symptoms of high-dose ingestion include nausea, prolonged vomiting, stomach pains, and diarrhea which progresses to convulsions, unconsciousness, and coma. Cypermethrin is a slight skin or eye irritant, and may cause allergic skin reactions (US NLM, 1995; Waller, 1988).

1.5.4.4. Cypermethrin in Bangladesh

Cypermethrin is now indiscriminately used by the farmers in Bangladesh. Farmers and workers of Bangladesh spraying pesticides in crop fields are extremely susceptible to various diseases as the occupation is maximum often done without taking any safety measures; they absorb the toxic item ignorant in different ways, including inhalation. Most farmers of Bangladesh spray pesticides without wearing masks, gloves and other proper clothes (Gain, P. *et al*., 1998). Even, many farmers often blow air from the mouth through the spraying pipe to make it clear (Daily Star, 2010).

Table-1.8: Cypermethrin in vegetable samples of Bangladesh

Study area	Samples	Cypermethrin	Unit	References
BSMRAU, Gazipur	Egg plant	0.03-3.16	µg L^{-1}	S Rahman *et al.*,2015
BSMRAU, Gazipur	Soil	0.50-1.98	mg kg^{-1}	S Rahman *et al.*,2015
BSMRAU, Gazipur	Bean	0.17-2.98	mg kg^{-1}	Mithun Paul *et al.*, 2016
BARI, Gazipur	Cauliflower	0.02	mg kg^{-1}	Sheheli Islam *et al.*, 2009
BARI, Gazipur	Tomato	0.01-0.55	mg kg^{-1}	Nahar *et al.*, 2012
Jessore	Yard long bean (Borboti)	0.56	mg kg^{-1}	M. S. Ahmed *et al.*, 2016
Savar	Tomato	BDL-0.27	mg kg^{-1}	M.A.Z. Chowdhury *et al.*, 2013
Dhaka City	Brinjal	0.03	mg kg^{-1}	Md. S Hossain *et al.*, 2013
Dhaka City	Tomato	0.5	mg kg^{-1}	Md. S Hossain *et al.*, 2013
Dhaka City	Ladies finger	0.5	mg kg^{-1}	Md. S Hossain *et al.*, 2013
Manikganj	Water	BDL-0.25	µg L^{-1}	S Bhattacharjee *et al.*, 2012
Narshindhi	Egg plant	ND-0.53	mg kg^{-1}	M. W. Islam *et al.*, 2014
Narshindhi	Country bean	ND-0.26	mg kg^{-1}	M. W. Islam *et al.*, 2014
Narshindhi	Cauliflower	ND	mg kg^{-1}	M. W. Islam *et al.*, 2014

1.6 Fluxapyroxad, A fungicide

Fluxapyroxad is a broad-spectrum, pyrazole carboxamide fungicide registered for uses on a wide range of crops (cereal grains, legume vegetables, oil seed crops, peanuts, pome fruit, stone fruit, root and tuber vegetables, fruiting vegetables and cotton) (EPA, 2012).

Fluxapyroxad, a second-generation, 3-(difluoromethyl)-1-methyl-N-(3′,4′,5′-trifluoro[1,1′-biphenyl]-2-yl)-1H-pyrazole-4-caroxamide, a new active ingredient developed by BASF Corporation in UK in 2012 to control a broad spectrum of fungal

diseases. It is in the process of being registered in China for cucumbers and tomatoes (WU Xiao-hu *et al*.2014). It inhibits succinate dehydrogenase in complex II of mitochondrial respiratory chain. Results in inhibition of mycelial growth within the fungus target species (EPA, 2012). Its molecular weight 381.3, molecular formula $C_{18}H_{12}F_5N_3O$ and melting point 157°C.

Fluxapyroxad is formulated as an emulsifiable concentrate (EC) or suspension concentrate (SC) and is foliar applied or used as a seed treatment (EPA 2012).

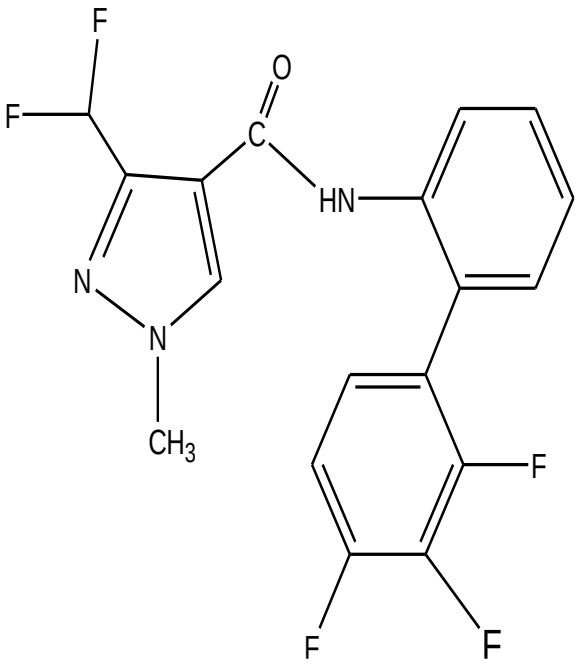


Fig.-1.11: Structure of fluxapyroxad

1.6.1 Environmental Fate

Fluxapyroxad has been demonstrated to persist for a long period in soils (half-lives ranging from 213 to 1,827 days) (EAP2012).

1.6.2 Toxicology and Exposure

EPA's screening models generate high-end, conservative exposure estimates for active ingredients and toxicologically significant degrades. Model inputs include annual usage at maximum use rates, maximum treated acres, maximum food residues, peak runoff and drift scenarios, etc. Some proposed products, application rates and use scenarios' are not relevant to Minnesota (EPA, 2012).

1.6.3 Degradation

Fluxapyroxad (parent) is the only residue of concern in drinking water. One major degrade was identified: (3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid), which has a lower toxicity than the parent and will be present in low concentrations relative to parent in drinking water (EPA,2012).

1.7 Name and Structures of Some Common Pesticides

1.7.1 Diazinon

The CAS name for diazinon is O, O-diethyl O-[6- methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate and the International Union of Pure and Applied Chemistry (IUPAC) name is O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate (Tomlin, 2006).

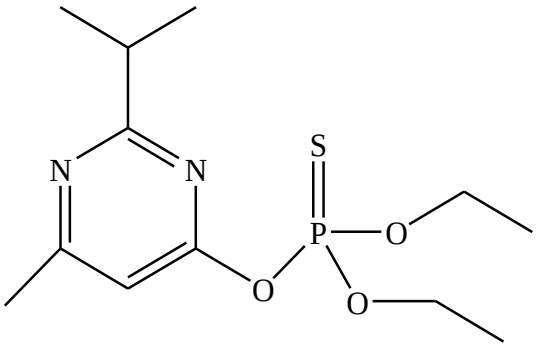


Fig.-1.12: Structure of diazinon

1.7.2 Chlorpyrifos

The CAS name for chlorpyrifos is O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate and the International Union of Pure and Applied Chemistry (IUPAC) name is O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate (Tomlin, 2006).

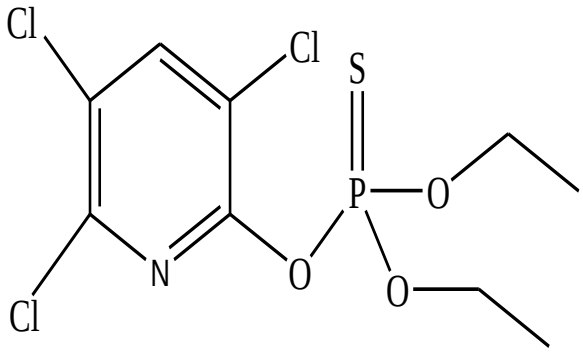


Fig.-1.13: Structure of chlorpyrifos

1.7.3 Fenvalerate

Chemical Abstract Name: cyano (3-phenoxyphenyl) methyl-4-chloro-α-(1-methylethyl) bezeneacetate (CAS number: 51630-58-1).

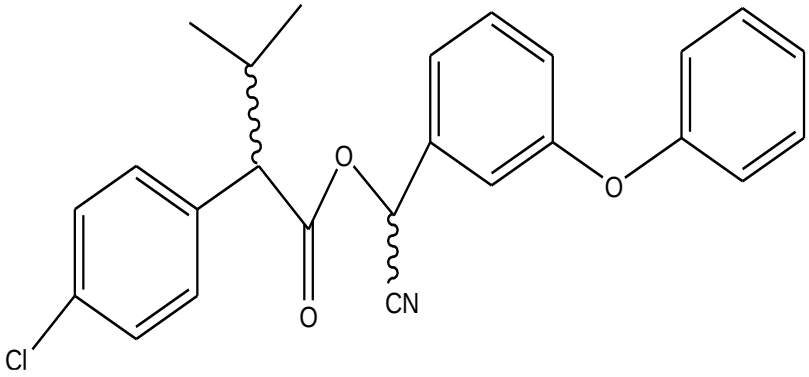


Fig.-1.14: Structure of fenvalerate

1.8 The Way of Reducing the Use of Pesticides

By using suitable methods of cultivation, biological pest controls (such as pheromones and microbial pesticides), genetic engineering and methods of interfering with insect breeding might reduce the usage of pesticides. Application of composted yard waste has also been used as a way of controlling pests. These methods are becoming increasingly popular and often are safer than traditional chemical pesticides. In addition, EPA is registering reduced-risk conventional pesticides in increasing numbers.

Cultivation practices include poly-culture (growing multiple types of plants), crop rotation planting crops in areas where the pests that damage them do not live, time of planting according to when pests will be least problematic, and use of trap crops that attract pests away from the real crop. In the U.S., farmers have had success controlling insects by spraying with hot water at a cost that is about the same as pesticide spraying. Release of other organisms that fight the pest is another example of an alternative to pesticide use. These organisms can include natural predators or parasites of the pests. Biological pesticides based on entomoapthogenic fungi, bacteria and viruses cause disease in the pest species can also be used (Banglapedia, 2010).

1.9 Analytical Methods for Monitoring of Pesticide in Food and Environment

The practice of pesticides in agriculture has improved after World War II in mandate to proliferation the world food production. Since then there had been noticeable improvement of different types of pesticides going to various groups. The use of pesticides and additional environmental pollution due to industrial discharges during the production of pesticides have resulted in occurrence of residues of these chemicals and their metabolites in every component of environment, i.e. air, water and soil along with that in the crops, vegetables and fruits (Bai *et al*., 2006).

Pesticides are frequently applied in different kinds of fruits and vegetables during the entire period of growth and sometimes even at the ripening stage to get better yield

and quality. These pierce into the vegetables, which may become toxic to humans (Kumari et al., 2004). It is assessed that over 1000 compounds are applied to agricultural crops in order to control undesirable weeds, molds, insects and pests. Many scientists have estimated the pesticide residues in various fruits, vegetables and environmental samples and reported the occurrence of pesticide residues to be even more than MRL values recommended by European Union (EU), Codex Alimentarius Commission. The content of pesticides in various fruits and crops does not only depend on the sprayed amount over them but also on the content present in soil or water used for irrigation.

1.9.1 Extractions

Extraction methods of pesticides vary from matrix to matrix (solid, semisolid, liquid) and samples to samples (vegetables, soil, water, fruits etc.) as well as chemical nature of the targeted pesticides (polar, nonpolar, heat and light sensitive). It is important that recovery is within acceptable levels. Extraction methods involve solvent extraction and solid phase dispersion for solid matrices, liquid-liquid extraction for liquid samples and solid-phase extraction (SPE) for pre-concentration and clean-up (reversed phase, bonded-phase and normal phase) (Åkerblom 1995).

The methods which include higher amount of environmental non- responsive solvents changed into a number of environmental responsive methods. Among them, solid-matrix solid-phase dispersion (MSPD) and the Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method (Anastassiades *et al*., 2003) are well reported. Modern trends have also added the use of nominal amounts of sample, making methods safer (or “green”), limit sample preparation or analysis time and decrease manual labor without losing recovery or precision.

1.9.2 Clean-up

After the assortment of a suitable extraction technique, most of the resulting extracts need to be cleaned by solid phase extraction (SPE), dispersive solid-phase extraction

(DSPE). Sulphuric acid treatment is also used for the clean-up many of halogenated compounds.

Other clean-up methods such as florisil, silica gel, charcoal etc. are also being used. With the introduction of QuEChERS by Anastassiades, *et al*., the popularity of using DSPE cleanup techniques increased (Anastassiades *et al*., 2003) where primary secondary amine (PSA) stationary phase is used. Gravimetric analysis showed that PSA removed more matrix co-eluents than -NH_2 and Alumina.

1.9.3 Instrumentation

Gas chromatography (GC) couple with electron captured detector (ECD) is the most commonly used instrument for analysis of non-polar and semi-polar compounds. For polar and non-volatile compounds, high performances liquid chromatography (HPLC) with ultraviolet (UV-Vis/PDA) and fluorescence detectors are used. One of the advantages of GC over HPLC is that a large number of compounds can be separated by GC with capillary column. Recently, gas chromatography with mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS) and liquid chromatography with tandem mass (LC-MS/MS) are being frequently used for the analysis and confirmation of compounds in different matrices.

1.10 Maximum Residue Limit

The Maximum residue limit (MRL) has been demarcated as "the maximum concentration of a residue that is officially allowed or documented as acceptable in or on a food or agricultural commodity or animal feedstuff" (FAO code of conduct, 2002). The MRL is reflected mainly as a patterned that use of pesticide is going on according to approved tags and Good agricultural practice (GAP). It implies that label directions and GAP have been properly followed when residues found at or below the MRL.

Every pesticide has a Pre Harvest Interval (PHI) for residues to dispel below the MRL eminent for that crop. Pre Harvest Interval (PHI) means, the interval of time between the last application of pesticide and the safe harvesting of comestible crops for immediate consumption. Food products become only safe after pre-harvest interval and it differs from pesticide to pesticide and crop to crop. The amount and rate of dissipations depends on the nature of the pesticides, crop, way of application and various environmental conditions under which the crops are grown.

1.11 Factors of Dissipation of Pesticides

Dissipation pattern of a specific pesticide be contingent on its physical and chemical properties and also on the climatic condition of the country where the application is done. The dissipation pattern of pesticides also depends on other aspects like the applied dose and formulation, application parameters, the number of application, the species cultivated, sun light etc.

1.12 Pesticides in the Context of Bangladesh

Bangladesh is primarily an agricultural country with above population and agriculture shows a vital role in the lives of its people. The use of pesticides, in Bangladesh started during the middle of the 1950s to stimulate crop production (Rahman and Alam, 1997). Major crops of the country are rice, wheat, pulses, jute, oilseed, vegetables, potatoes, sugarcane, cotton and tea of which rice accounts for 80% of the total cultivated area. The warm and humid climatic conditions of the country increased modern high yielding varieties of crops and more use of chemical fertilizers are highly favorable for expansion and growth of pests and diseases. The expected loss in yields due to attacks from pest and diseases annually ranges from 15 to 25 percent (Aziz, 2005). Both misuse and exploitation of insecticides may lead to the loss of efficiency of insecticides due to the development of resistance and could cause human health hazards and environmental pollution. Unsuitable selection of

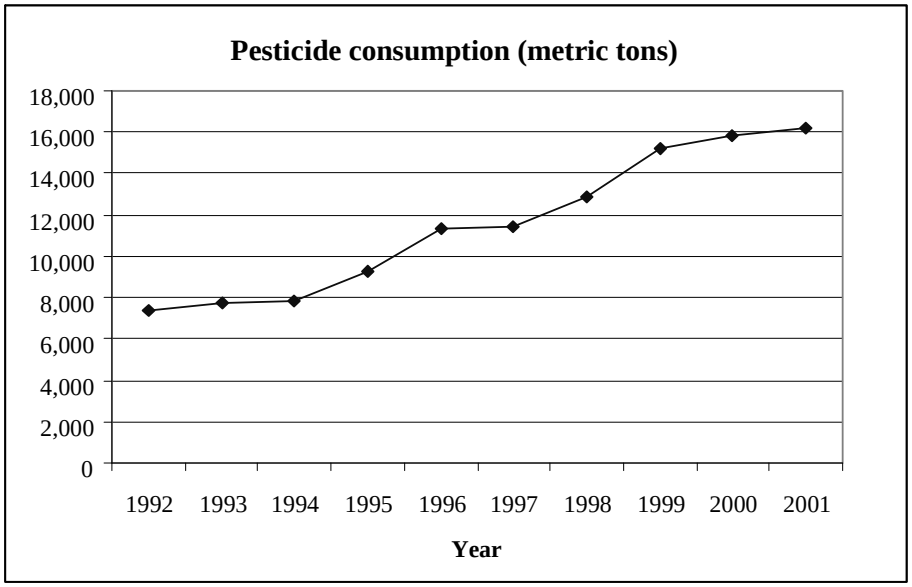
insecticides and doses, indecorous spray setting up and insufficient spray coverage may source to the fiasco in controlling insect pests.

Pesticide as agricultural contribution was introduced in Bangladesh in 1957 and mainly DDT and HCH was circulated by the Government to the farmers free of cost until 1973.There were two reasons the pesticides become very widespread to the farmers; firstly rapid and noticeable effect on pest and secondly, lower cost. The funding was reduced to 50% in 1974 and in 1979 it was completely inhibited (Islam, 2000). As a result at first pesticide use waned and again gradually increased then in 1999 the amount reached 15000 metric tons.

In Bangladesh the harvesting strength is higher; each year three crops per field may be produced, and pesticides are used for each crop. Therefore, the health and environment are continuously exposed to pesticides. Moreover, most of the farmers do not take any safety measure during application and storage of pesticides. Farmers are, therefore, more exposed than other people. Farmers do not even know the toxicity and hazardous effect of pesticides. Use of broad spectrum pesticides pose serious risk to the environment, leading to diminishing bio-diversity, hampering the growth of aquatic habitats, disrupting natural pest control, reducing earthworms, causing toxicity in soil, developing resistance among target pests and creating potential hazards to human health (Ibiayo, 2006). Indiscriminate use of pesticides is very common in Bangladesh, although there are Pesticide Acts and Rules (The Environment Court Act, 2000). However, in most cases the legislation are not strictly followed, which may result in the gradual increase in the risk to humans, animals, fish, birds and the environment.

The Government of Bangladesh also promotes the use of pesticides to expand its agricultural frontiers and increase output per acre of land (Hossain, M. 1988). As a result, pesticide use in general is increasing. According to statistics from the Government of Bangladesh, consumption of pesticides increased from 7,350 metric tons in 1992 to 16,200 metric tons in 2001, more than doubling in the past decade (CRAIG MEISNER, 2004). Perhaps of even greater concern than the absolute quantity of pesticides is the trend in the composition of pesticides currently in use in

Bangladesh. Insecticides and fungicides account for 97% of pesticide use and have registered a steady increase over the years.



Source : Department of Plant Protection Wing, Bangladesh

Fig.-1.15: Trends in pesticide use in Bangladesh, (1992-2001).

1.13 Justification for the Study

Pesticides are known to be noxious to man (Ademoroti, 1996). The use of pesticides introduces some risks to the environment, the degree of risk depending upon the pesticide persistence, mobility, non-target toxicity and volume of use. The toxicity level of a pesticide also depends on the deadliness of the chemical, the length of exposure, the health status of the recipient and the route of entry into the body.

Residues of these toxic chemicals found in water, sediments and aquatic biota pose a risk to aquatic organisms, predators and humans. OCPs act as central nervous system stimulants in aquatic fishes. In order to minimize the health risks from the ingestion of food contaminated with these chemicals, environmental protection agencies and public health authorities, including the WHO, have set MRL for OCPs in water, fishes and shellfishes (UNEP/FAO/WHO, 1988). Pollution by persistent chemicals is potentially harmful to the organisms at higher trophic levels in the food chain. The

aquatic organisms like fish are able to accumulate several fold higher concentration of pesticide residues than the surrounding water (Siddiqui *et al*., 2005). Research efforts indicate that more than 80% of the total intake of pesticide residues in human beings is through the food chain (Trotter and Dickerson, 1993; Martinez *et al*., 1997). It has been reported that the consumption of contaminated fishes is one of the important pathways of human exposure to OCPs (Mwevura *et al*., 2002; Zhou *et al*., 2007; Muralidharan *et al*., 2008). Indeed, studies have related the presence of organochlorine residues in breast milk to the consumption of contaminated fishes (Fitzgerald *et al*., 2001). In early sixties, DDT was imported to Bangladesh for plant protection to eradicate vector diseases and DDT factory was established at Chittagong Chemical Complex (CCC) area. Stockholm Convention urges restriction, reduce use of POPs and eliminate to the stockpiles. Bangladesh is a signatory of the convention and has being paying the fees regularly to the secretariat and actively participating in biannual conference (COP). As a signatory of Stockholm convention use of DDT had been banned in Bangladesh and the factory at CCC area was shut down. It was unclear what happened to the stockpile DDT in the godown of the CCC area.

As the farmers are not properly educated and aware about the toxicity of the chemicals overdosing and not maintaining PHI are common scenario in the country. There are few laboratories in the country that are competent to analyze pesticides residues in agricultural foods. To consume the safe food, pesticide residue should be below the maximum residue limit (MRL), jointly established by FAO and the WHO called the ‘Codex Alimentarius Commission’.

1.14 Aim of the Study

This research is a contamination monitoring study aimed at investigating the occurrence, concentration and distribution of DDTs in environmental samples and human blood in CCC people with a view to assessing the current state of contamination of the study area and exposure to these toxicants and priority pollutants. The another investigation was to determine the dissipation pattern of cypermethrin and fluxapyroxad on vegetable samples. These data are needed for

establishing proper management practices in terms of harvest times and waiting periods to ensure food safety and environmental sustainability in vegetables production systems in the humid tropics.

1.15 Objective of the Present Work

Therefore, the main objectives of the present studies are:

i) To determine the concentration of DDT and its metabolites in the environmental impacts especially on soil, sediment, water and fish samples in the godown of the Chittagong Chemical Complex (CCC) area.

ii) To assess DDTs in human blood of the people residing at CCC area.

iii) To study the dissipation pattern of Cypermethrin and Fluxapyroxad in vegetable samples.

iv) To analyze pesticide residues in some vegetable samples grown in summer season in different places of Bangladesh.

EXPERIMENTAL

2.1 General

2.1.1 Materials and Methods

2.1.1.1 Reagents, Chemicals and Solvents

Analytical grade reagents and chemicals were used in all kind of experiments. Anhydrous sodium sulphate (Na_2SO_4), sodium chloride (NaCl) potassium hydroxide (KOH), potassium chloride (KCl), alumina, anhydrous magnesium sulphate ($MgSO_4$), sodium chloride (NaCl) and silica gel 60 (70-230 mesh) were purchased from Merck, Germany. Florisil from Acros organics, USA; Charcoal from Uni-Chem, China; PSA, C_{18} sorbent from Agilent, USA.

Analytical or pesticide residue grade solvents 2-propanol, *n*-hexane, ethyl acetate, ethanol, dichloromethane (DCM) acetone, acetonitrile (ACN), methanol and fuming hydrochloric acid (37%); were purchased from (Merck, Germany), HPLC grade water & *tert*-butyl methyl ether (MTBE; 99.9% Sigma-Aldrich, USA) and sulfuric acid (98%, w/w, RCI Labscan, Ltd.)

2.1.1.2 Standard Compounds

The standards of *2,4'*-DDT & *4,4´*-DDT (99% purity), *4,4´*-DDE (99% purity), *4,4´*-DDD (99% purity), CB-53 (99.5% purity), Diazinon (97.5% purity), Chlorpyrifos (99.5% purity), Cypermethrin (91% purity) and Fenvalerate (98.5% purity) were purchased from Dr. Ehrenstorfer, Germany. The standard Fluxapyroxad (99.7% purity) was obtained from Kyung Nong Co. Ltd., Seoul, Republic of Korea.

2.1.1.3 Glassware and Equipment

All required glass wares like volumetric flasks and pipettes were calibrated by BSTI (Bangladesh Standard Testing Institute). All glassware were cleaned by detergent, rinsed with distilled water followed by acetone, dried in oven at 105°C and then finally were rinsed with solvent. The analytical balance (Adventure, AR 1140 by Ohaus Corp, USA) was calibrated by BSTI. The other instruments used include centrifuge machine, rotary evaporator (Büchi) for reducing solvent, ultrasonic bath and oven for drying.

2.1.1.4 Methods

2.1.1.4.1 Activation of Chemicals

Sodium sulphate was activated by heating at 300 °C for 8 h in a furnace (GSM 11/8 Hope valley, S336RB, England) and was allowed to cool at room temperature before use.

Alumina and florisil were activated by heating for 12 h at 105 ºC in an oven (Eyela, Japan) and were allowed to cool at room temperature before use. Charcoal was activated by washing successively with distilled water, n-hexane, methanol and acetone then dried in an oven at 105 ºC for 1 hour.

2.1.1.4.2 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 shaken about five minutes and standing allowed to stand for 1 hr, the acid layer was collected and stored in an amber color bottle until non polar hexane layer was discarded.

2.1.1.4.3 Impregnation of Silica Gel

The silica gel 60 (70-230 mesh) was activated at 300 °C overnight and allowed to cool to room temperature. Sulphuric acid was added (sulphuric acid /silica gel ::1/2, w/w) and the gel was mixed until no lumps were left. The prepared silica gel was stored in an amber colored bottle and the bottle was kept in a desiccator until use.

2.1.2 Instruments

2.1.2.1 Homogenizer, Centrifuge Machine, Shaker & Vortex Machine

Moie (Butter bar) leaves were homogenized by Health Miller-Artlon homogenizer from Korea. Soil, fish and vegetable samples were also homogenized by normal kitchen blender.

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

Orbital shaker (Lab companion, Model- IS 971R) or by vertical shaker (Resipro shaker, Model- RS 1) and vortex machine (KEBO Lab REAX 2000) were used for the experiments.

2.1.2.2 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.

2.1.2.3 Gas Chromatograph

Gas chromatographs with electron capture detector (GC-ECD) Shimadzu-17A with manual injector (**Plate-1**) and Shimadzu-2010 with auto injector (**Plate-2**) were used for determination of DDTs and also pesticide residues in vegetable samples.

Plate-1: Gas Chromatograph Shimadzu GC-17 A

Plate-2: Gas Chromatograph Shimadzu GC-2010

2.1.2.4 Liquid Chromatograph

Liquid Chromatograph with mass spectrometry (LC-MS/MS); Water 2695: Separations Module; TQ detector; having auto injector and auto sampler was used for analysis of fluxapyroxad in moi leaves (**Plate-3)**.

Plate-3: Waters Acquity UHPLC- MS/MS

2.1.3 Preparation of Standard Solutions

2.1.3.1 Preparation of Primary, Middle and Working Standard Solutions

The primary standard solutions (100 µg L^{-1}) were prepared in 100 ml volumetric flask by separately dissolving standard reference sample (appropriate amount ≈ 10 mg mol^{-1}) of *4,4´*-DDE, *4,4´*-DDD, *2,4´*-DDT, *4,4´*-DDT (98.5~99.5% purity), diazinon, chlorpyrifos, cypermethrin, fenvalerate in n-hexane and fluxapyroxad in pure acetonitrile. These primary standard solutions were diluted to 20 µg L^{-1} and 5 µg L^{-1} as the middle and working standard solutions, respectively.

All prepared solutions were labelled indicating the concentration of standard 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.

2.1.3.2 Calibration Curves

The working standard solutions were serially diluted and prepare ten different concentrations in order to obtain standard calibration curves. The calibration curves were found by plotting each peak area *vs*. amount of concentrations of working standard solutions using GC-ECD. The calibration curves were linear over the range of the verified concentrations as shown by the detail that the correlation coefficients (R^2) for the linearity. The R^2 value was suggested by the Codex guideline (R^2 = 0.95). The calibration curves were prepared using Microsoft Excel-2010 software.

2.1.4 Selectivity, Sensitivity and Linearity

Selectivity (or specificity) means to analyze 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 is to determine limits of detection (LODs) and limits of quantification (LOQs) for each pesticide in each matrix.

To determine the LOD, working standard solutions were serially diluted to get preferred concentration. The solvent was injected first then the diluted standard solutions were injected soon lower concentration to higher concentration until the peak heights of the standards were equal to the noise level. The limit of detection (LOD) of the test compounds was determined by using a signal-to-noise ratio (S/N) 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 (S/N) of 10 (USP, 2009).

Linearity was evaluated by constructing calibration curves for each pesticide by injecting standard mixture to GC/LC at 5-8 different concentration levels covering the expected range of pesticides that might be present in the samples.

2.1.5 Identification and Quantification by GC/ LC-MS/MS

The reference standard solutions were injected into the respective instrument (GC-ECD/ LC-MS/MS) 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 pesticides present in the samples were identified.

For quantification, concentration of the corresponding analytes were found from standard calibration curve taking into consideration that the peak area was in the midpoint of the curves.

2.1.6 Control

For recovery experiments control or untreated samples were used, which were previously confirmed that they had no pesticide. Three control samples were spiked with known amount of pesticides 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.

2.1.7 Recovery

The recovery experiments of soil, water, fish and vegetable samples were conducted on control samples by spiking the sample at 3 replicates in two or three concentration levels. The spiked samples were permitted to equilibrate for 3-4 h before extraction, in order to allow the pesticide to penetrate 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:

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

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.

Recovery experiment of human sample was done by internal standard CB-53 to evaluate the reproducibility of the method. Recovery was calculated by the following formula.

$$\text{Recovery (Y)} = \frac{\text{Peak area of standard in sample internal}}{\text{Peak area of internal standard}} \text{ X } 100$$

2.1.8 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 the soil, water, fish and field (vegetable) samples. The recovery experiments were conducted in 3 replicates at 2-3 fortification levels. The extraction method was validated by using internal standard in human blood samples.

2.1.9 Matrix Effect

The matrix effect was evaluated by using a standard solution prepared in sample extract and pure solvent and calculated via the equation:

$$ME = \frac{(A_{ms} - A_{ss})}{A_{ss}} \times 100$$

Where *ME* is the matrix effect in %, A_{ms} is the peak area of the matrix standard, and A_{ss} is the peak area of the solvent standard (Kanrar *et al*., 2010).

The positive value obtained from the equation reflects the matrix-induced enhancement and negative value indicates suppression of signals.

2.1.10 Human Health Risk Assessment

Various international organizations have subsequently established a series of standards and instructions to estimate the risks to human health from environmental pollutants in fish (USEPA, 2013). A straight forward risk assessment is performed through comparison with the levels set by laws and guidelines. However, this comparison was made without the consideration of factors like different eating habits and consumption rates. Thus, in this study, we investigated the risk assessment by two approaches.

Part - A

DDTs in Environmental and Human Blood Samples of Chittagong Chemical Complex

2.2 Site Selection & Collection of Samples

Samples were collected from regions surrounding the Chittagong Chemical Complex area in the Southern, South Western and Eastern directions. These regions are situated in the vicinity of Barabkundu Bazar in Chittagong district, Southeastern part of Bangladesh (22.584000° N, 91.685005° E) (**Fig. 2.4**).

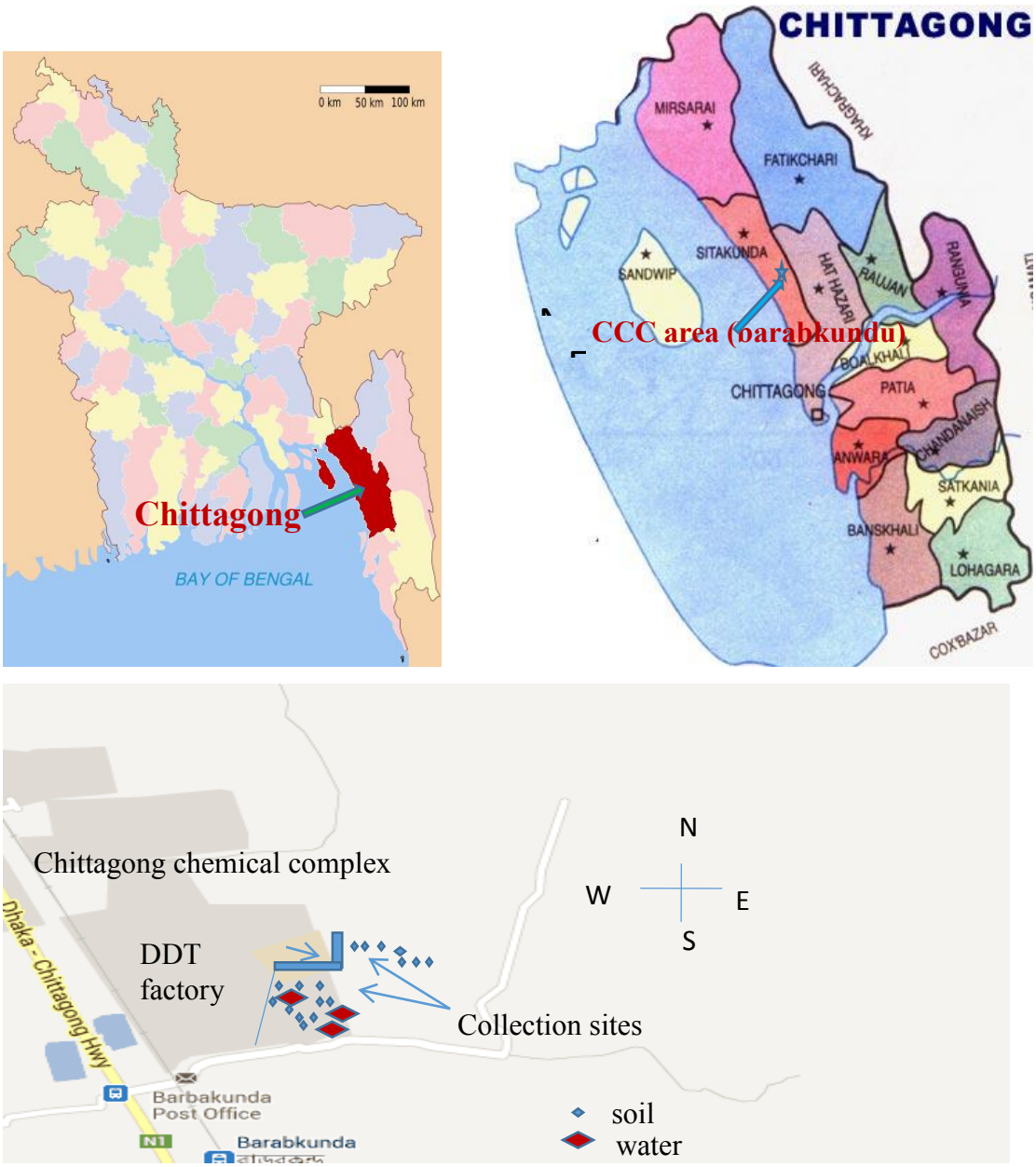


Fig-2.1: Sample collection sites, Chittagong Chemical Complex; Bangladesh.

2.2.1 Collection of Soil, Sediment and Water Samples

Soil and sediment were collected on 13 July 2011 at a depth of 0−20 cm by spade from 21 different locations. Three samples per location at the point of marked 1-ft triangular area were collected and mixed together and kept in one plastic zip-lock bag. Two sediment samples were collected by shovel from two ditches (three sides for each ditch and mixed together) located at a distance of 70 ft (21.336 m) southwest and 300 ft (91.440 m) and 350 ft (106.680 m) south from the storage area (**Fig-2.1 & Table-2.1)**.

Water samples (n=36) were collected from two ditches and from a pond (seven samples per water body), located at a distance of 70 ft southwest and 300 ft and 350 ft (106.680 m) south from the storage area. The water samples were collected in 1-L plastic bottles sterilized by adding a few drops of 70 % alcohol

Plate-4: Soil & Water samples collection from CCC; Chittagong, Bangladesh

Table-2.1: List of soil and water samples collected from Chittagong Chemical Complex area

Types of sample	Direction	Distance from the godown (ft)	Number of the Samples	Total samples	Period of samples
Soil	South/West	20	3	23	13.07.11
		30	3		
		50	3		
		60	3		
	East	20	1		
		30	1		
		40	1		
		50	1		
		70	1		
		200	1		
		250	1		
		300	1		
	South	20	3		
		30	3		
		50	3		
		60	3		
		70	3		
		100	3		
		150	3		
		200	3		
		250	3		
		300	3		
		330	3		
Sediment	South	70	11	22	
		300	11		
Water	South	70	16	36	
		300	16		
		Pond	16		

2.2.2 Collection of Fish Samples

Fifteen different fresh fish (n=15) samples (0.5-1.0 kg), were collected from a pond 350 ft (106.680 m) South from the storage area of CCC on June 2016. One shrimp fish and fourteen most common fish (0.5-1.0 kg) samples were included for this study (**Table-2.2**). The samples were some carnivores like climbing perch (koi), several varieties of catfish; fresh water shark (boal) and snake head fish (shol and taki),

herbivores and planktivores like mola, minnow (kachki) and common carp (rui) and omnivore's catfish (traditional variety of magur, shing) and tilapia. (**Table-2.2).**

(a) Katla **(b) Taki** **(c) Chitol**

Plate-5: Fish samples collected from CCC area

Table-2.2 : Names and nature of different fresh fish samples

Scientific name	Local name	English name	Family	Nature
Amblypharyngodon mola	Mola	Mola	Cyprinidae	Planktivore
Labeo rohita	Rui	Common carp	Cyprinidae	Herbivore
Corica soborna	Kachki	Minnow	Clupeidae	Omnivore
Clarias batrachus	Magur	Cat fish	Clariidae	Omnivore
Notopterus notopterus	Chitiol	Clown knifefish	Notopteridae	Carnivore
Heteropneustes fossilis	Shing	Cat fish	Heteropneustidae	Omnivore
Oreochromis nilotica	Telapia	Mozambique tilapia	Cichlidae	Omnivore
Gibelion catla	Katla	Catla	Cyprinidae	Herbivore
Wallago attu	Boal	Wallago	Siluridae	Carnivore
Anabas testudineus	Koi	Climbing perch	Anabantidae	Carnivore
Mystus vittatus	Tengra	Cat fish	Bagridae	Carnivore
Channa striatus	Shol	Snake head	Channidae	Carnivore
Channa punctatus (Bloch, 1794)	Taki	Snake head	Cyprinidae	Carnivore
Macrobrachium malcolmsonii	Chingri	Prawn	Dacapodae	Carnivore
Cyperinuscarpio	Karfo	Karp	Cyprinidae	Herbivore

2.2.3 Social Survey

Blood donors were motivated by Community leaders of the factory area through coordination and several visits by this study group. All donors were then psychologically self-motivated to donate blood for determination of toxicant present in their body. During collection of the blood samples a social survey was carried out according to a prepared questionnaire. People were asked the name, measured height and weight, per day quantity of fish eating, how many time/week they eat fish? How many times they eat dry fish?

2.2.4 Blood Collection

By taking consent of **Ethical Review Committee**, total thirty human blood samples were randomly collected from a group of men and women with different occupations (male donors n=20 and female donors n=10) from the closed down factory area during June 2014. It is to be mentioned that **local policy makers** were involved in blood collection programme and **consent from donors** were obtained with followed **proper safety measures**. Ages of men were within 40-65 years (n=12) and 25-39 years (n=8). Ages of women were within 40-50 years (n=4) and 24-39 years (n=6). Among them (n=5) and (n=8) people had been working in DDTs factory more than 15 years and less than 15 years respectively. Another (n=17) people were non workers (**Table 2.3**). Blood samples were drawn from the donors with the help of a technician from Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Dhaka.

Blood sample processing

All blood samples (5 ml in each) were collected in teflon tubes containing heparin as an anticoagulant and kept in a chilled-box. The blood samples were centrifuged immediately after collection at the sampling site and the serum isolated was stored in an ice box during transfer to the laboratory.

Plate-6: Survey from CCC, Chittagong, Bangladesh

Plate-7: Blood collection from CCC, Chittagong, Bangladesh

Table-2.3: Information of blood donors of CCC surroundings

Sample ID	Age Year	Height	Weight kg	Gender	Occupation	Duration of work in CCC	Education
sample -01	60	5'2"	48	M	Business	14 years (67-82)	Nil
sample -02	33	5'6"	70	M	Service	8 years (94-01)	S S C
sample -03	35	5'6"	69	M	CNG driver	5 years (93-97)	up to VIII
sample -04	48	5'4"	55	M	CNG driver	20-25 years	Nil
sample -05	56	5'4"	64	M	Carpenter	Nil	up to VII
sample -06	56	5'4"	63	M	Carpenter	20 years (77-97)	Nil
sample -07	55	5'2"	48	M	Farmer	8-9 years	up to IV
sample -08	49	5'4"	65	M	CNG driver	Nil	up to V
sample -09	38	5'2"	40	F	House wife	Nil	Nil
sample -10	35	5'2"	42	F	House wife	Nil	Nil
sample -11	45	5'3"	45	M	Driver	4-5 years	Nil
sample -12	65	5'4"	46	M	None	Whole life	up to III
sample -13	30	5'3"	48	F	House wife	Nil	Nil
sample -14	28	5'5"	56	M	Service	Nil	up to VIII
sample -15	55	5'4"	50	M	Farmer	Nil	Nil
sample -16	55	5'4"	50	M	Farmer	Nil	Nil
sample -17	33	5'4"	50	M	Carpenter	6 years	Nil
sample -18	24	5'6"	60	M	Mason	Nil	up to V
sample -19	26	5'8"	68	M	Mason	Nil	S S C
sample -20	22	5'4"	50	M	Mason	Nil	up to VIII
sample -21	60	5'4"	40	M	Farmer	2 years	Nil
sample -22	40	5'4"	60	M	Mason	18 years	up to VIII
sample -23	50	5'2"	55	F	House wife	Nil	Nil
sample -24	24	5'2"	45	F	Service	Nil	Nil
sample -25	40	5'2"	50	F	House wife	Nil	Nil
sample -26	34	5'4"	64	F	House wife	Nil	up to VIII
sample -27	40	5'2"	50	F	House wife	Nil	up to V
sample -28	25	5'4"	54	M	Carpenter	1 year	up to IV
sample -29	40	5'6"	78	F	House wife	Nil	Nil
sample -30	38	5,4"	76	F	House wife	Nil	Nil

2.2.5 Sample Storage

All samples were brought to Dhaka in a chilled box and immediately transferred to Organic Research Laboratory. Water, fish and blood samples were stored at −20 °C and soil/sediment samples were air-dried, mixed thoroughly, sieved (200 mesh), transferred to pre-sterilized amber-colored glass bottles and stored in a refrigerator pending further processing.

2.3 Moisture Content of Soil Samples

Moisture content of the soil samples were determined by heating the homogenized sample at 105 °C in an oven until constant weight was obtained. Moisture content was found to be 12.07–27.67% **(Table 3.5).**

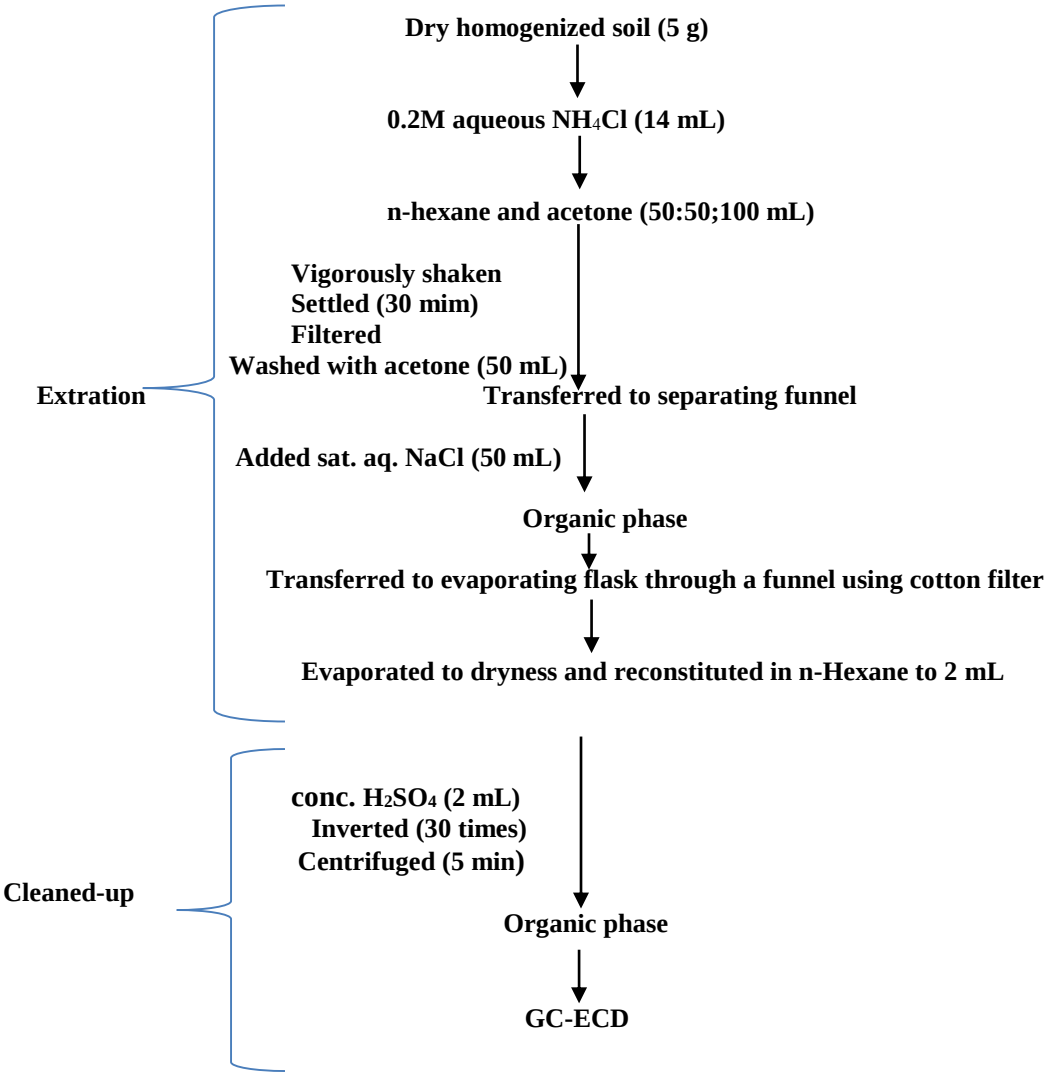
Before extraction the samples were taken out from the freezer and thaw well at room temperature. The samples were grinded with the help of kitchen blender. The grinded soil/sediment samples were then ready for extraction.

2.4 Extraction and Cleaned-up of Soil Samples

This extraction process was conducted following a SIDA guideline with modifications (Åkerblom, 1995). Homogenized soil samples (5 g) were taken in conical flask (250 mL), NH_4Cl solution (14 mL, 0.2 M) was added and shaken by hand for 1 min. A mixture of *n*-hexane- acetone (1:1, 100 mL) was added to it and also shaken in shaker for 1 h at 200 rpm. Then, the mixture was filtered through Whatman-41 filter paper into a separating funnel. Water (100 mL) and saturated aqueous NaCl (50 mL) were added to the filtrates and the mixture was manually agitated by for 1 min. The organic layer was collected in round-bottomed flask and evaporated to dryness, re-dissolved in *n*-hexane (2 mL).The extract (2 mL) was kept in a graduated test tube and then cleaned up.

The 2 mL extracted in a graduated test tube was treated with concentrated H_2SO_4 (saturated with n-hexane). The test tube was inverted for 30 times and then centrifuged for 5 minutes. Supernatant (1 mL) was taken by using pipette in clean GC

vial and analysed by GC-ECD (**Scheme-1**). The dilution factor was ranged from 1,600 − 1,200,000 times.

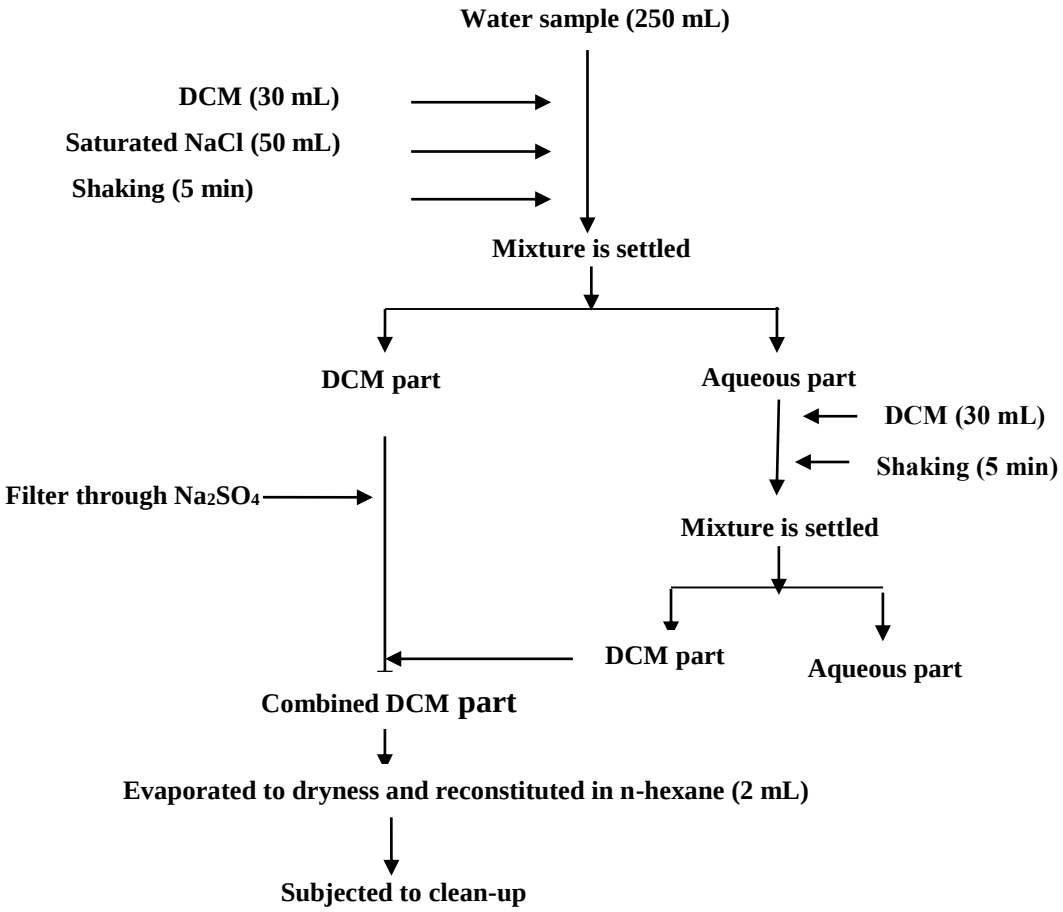


Scheme -1: Extraction and Cleaned-up procedure of soil samples

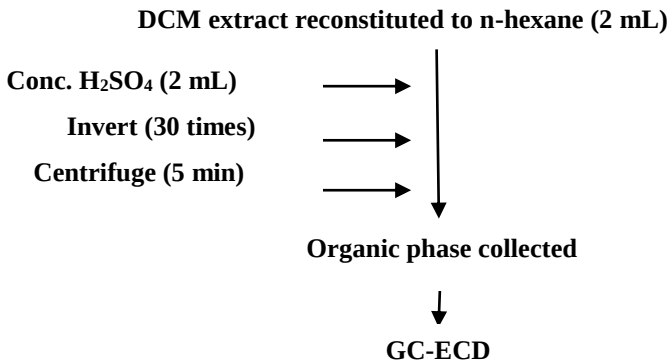
2.5 Extraction and Cleaned-up of Water Samples

Water samples (250 mL) were taken in 500 mL separating funnel. Then saturated aqueous NaCl (50 mL) solution and dichloromethane (30 mL) were added and shaken by hand for 1 min. The funnels were vigorously agitated in a mechanical shaker for 3 min and allowed to stand until the complete separation of two phases. Then, the organic phase was collected in a round-bottomed flask by filtration using cotton filter

toping of 20 g anhydrous sodium sulphate. The aqueous phase was further separated using dichloromethane (30 mL). The organic phase from this secondary separation was collected in the same flask. The extract was evaporated to dryness, re-dissolved in *n*-hexane (2 mL). The 2 mL extracted in a graduated test tube was cleaned up with H_2SO_4 treatment and analysed by GC-ECD (**Scheme-2, 3**)



Scheme-2: Extraction procedure of water samples



Scheme-3: Cleaned-up procedure of water samples

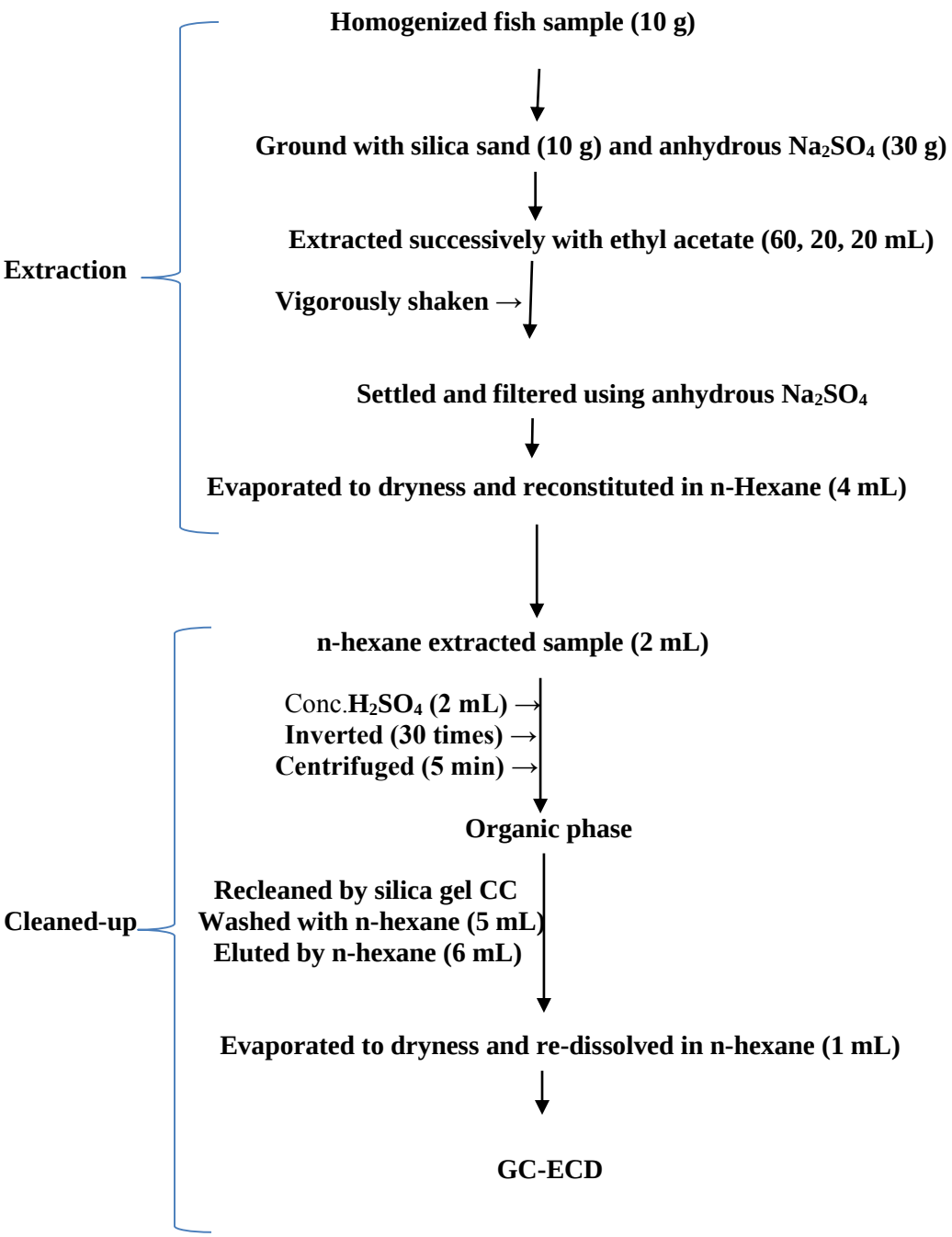
2.6 Sample Preparation for Fish Samples (Extraction)

Each of the collected fish samples was taken from the freezer and thaw well at room temperature. Then the scales and bones of the fish samples were removed except mola fish which was extracted with head and bone. The fish samples were chopped into small pieces and homogenized by a kitchen blender. The homogenized samples were divided into several portions of 10 g for replicate analysis.

Homogenized sample (10 g) was taken in a mortar. Then 10 g silica sand and 30 g anhydrous sodium sulfate were added to the samples and the mixture was ground until the sample floated freely (additional sodium sulfate was added wherever needed). The powdery sample was taken in a conical flask (quick fit 250 mL) and extracted successively with 60, 20, 20 mL of ethyl acetate by shaking 3 minutes each time. The extracts were combined and filtered using filter paper with few gram of anhydrous sodium sulfate. The extract (filtrate) was evaporated about to dryness. (Akerblom, 1995). Weight of fat was collected, then re-dissolved in *n*-hexane (4 mL).

2.6.1 Cleaned-up of Fish Samples

2 mL of n-hexane extract was partitioned with 2 mL of concentrated H_2SO_4 acid by inverting the tube 30 times by an inverter and then centrifuged it for approximately 5 min. The n-hexane part was transferred to a new test tube for additional clean-up. A pasture pipette column was packed with 1 g of silica gel impregnated with concentrated sulphuric acid (silica gel-sulphuric acid; 2:1, w/w). The column was first washed with 5 mL of n-hexane and then (1 mL) extract n-hexane part was applied to the column and was eluted with 6 mL of n-hexane, then concentrated to 1 mL through air blow before injection in GC-ECD. Extraction and clean-up procedure was given in **Scheme-4.**



Scheme-4: Extraction & cleaned-up procedure of fish samples

2.6.2 Lipid Determination for Fish Samples

Amount of lipid in the fish samples (n=15) were determined. The n-hexane extract was evaporated to dryness with gentle flow of nitrogen until constant weight was

obtained.The dry materials determined the amount of lipid present in the fish samples **(Table-3.8)**.

2.7 Sample Preparation for Human Blood

The extraction procedure followed in this study was identical to the method described by Hovander and co-workers (2006) for some minor adjustments related to these particular samples. Human blood serum (approx. 5 g) was spiked with 10 ng and 20 ng of CB-53. Prior to extraction, sample which were less than 5 g were adjusted to 5 g with aqueous 1% of KCl solution.

Denaturation and extraction

HCl (6 M, 1.0 mL) was added in to each of the sample, vortexed for 30 seconds then 6 mL of 2-propanol was added to the sample and vortexed again. The extract was denatured with n-hexane/MTBE (1:1, v/v) two times (6 mL & 3 mL) by inverting with a mechanical inverter for 5 minutes and centrifuged for five minutes (3000 rpm). The organic phase was transferred to a new test tube and washed with 4 mL of 1% aqueous KCl solution (w/w). The washed organic phase was transferred to a new pre-weighed test tube (14 mL) and the aqueous phase was re-extracted with 3 mL of n-hexane/MTBE (1:1, v/v) and combined the extract (Hovander *et al*.,2006).

2.7.1 Lipid Determination for Human blood samples

Amount of lipid in the blood samples (30) were determined. The combined n-hexane/MTBE extract was evaporated to dryness with gentle flow of nitrogen until constant weight was obtained. The dry materials determined the amount of lipid present in the human blood **(Table-3.11)**.

2.7.2 Separation of Neutral and Phenol-type of Organochlorine Compounds

The dry lipid samples were redissolved in 4 mL of n-hexane and partitioned with 2 mL 0.5M KOH solution in 50% ethanol by inverting 30 times. The materials were centrifuged for 3 minutes (3000 rpm) and the hexane soluble part was transferred to a new test tube (10 mL). The lower part in alcoholic alkaline solution was re-extracted with 3 mL of n-hexane. The n-hexane part was combined and evaporated to 4 mL with gentle flow of nitrogen which constitutes the neutral fraction of OC compounds.

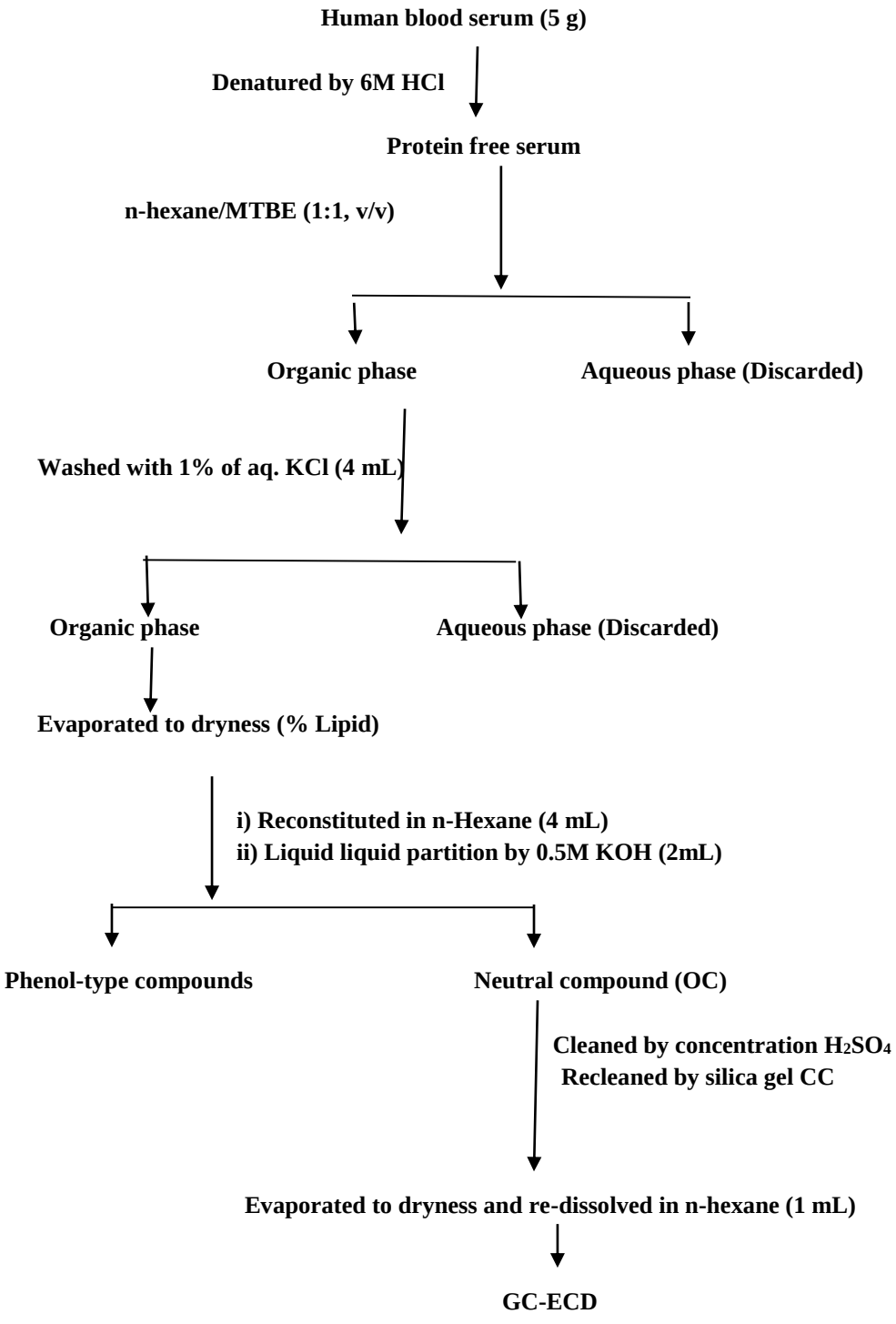
2.7.3 Cleaned-up

2.7.3.1 Removal of Lipid from Neutral OC Compounds

Each 4 mL of n-hexane extract was partitioned with 2 mL of concentrated H_2SO_4 acid by inverting the tube 20 times by an inverter and then centrifuged it for approximately 5 min. The n-hexane part was transferred to a new test tube and the H_2SO_4 phase was re-extracted with 3 mL of n-hexane. The combined n-hexane part was reduced to 0.5 mL (lipid free) with gentle flow of nitrogen at 30°C.

2.7.3.2 Additional Cleaned-up of Neutral OC compounds

Neutral fraction: A pasture pipette column was packed with 1g of silica gel impregnated with concentrated sulphuric acid (silica gel-sulphuric acid; 2 : 1, w/w). The column was first washed with 8 mL of n-hexane and then the lipid free neutral fraction (0.5 mL) was applied to the column and was eluted with 10 mL of n-hexane. It was then concentrated to 1 mL through air blow before injection in GC-ECD. Extraction and clean-up procedure was given in **Scheme-5.**



Scheme-5: Extraction and Cleaned-up procedure for human blood samples

2.8 Analytical Conditions of GC−ECD (17A, Shimadzu, Japan)

A Gas Chromatograph (GC 17A, Shimadzu, Japan) having Electron Captured Detector was used for identification and quantification of organochlorine compounds in soil and water samples. A quartz capillary column (HP-5 MS) of 30 meter length and 0.25 mm inner diameter (ID) was used for the analysis. Nitrogen was used as a carrier and as well as a make-up gas (flow rate, 2 mL min^{-1}). The injector and detector temperatures were set at 290 °C and 310 °C respectively. All samples (1 µL volume) were injected in a splitless − split mode.

Column oven temperature program: for soil, sediment and water

Initial Temperature: 120 °C

Rate, °C/minute	Temperature, °C	Hold time, minute
0.0	120.0	1.0
20.0	300.0	3.0

Total program time: 13.00 minutes

2.9 Analytical Conditions of GC−ECD (Shimadzu-2010 Japan)

GC condition for separation: A Gas Chromatograph having Electron Captured Detector, (GC−-ECD) (Shimadzu−2010 Japan) was used for identification and quantification of organochlorine compounds. A quartz capillary column (HP−5) of 30 meter length, 0.25 mm inner diameter (ID) and 0.25 µm film thickness was used for analysis. Nitrogen was used as a carrier and as well as a make-up gas (flow rate, 1 mL min^{-1}). The injector and detector temperatures were set at 220 °C and 290 °C respectively. All samples (1 µL volume) were injected in a splitless − split mode.

Column oven temperature program: for fish samples

Initial Temperature: 120 °C

Rate, °C/minute	Temperature, °C	Hold time, minute
0.0	120.0	1.0
10.0	280.0	4.0

Total program time: 21.00 minutes

Column oven temperature program: **for human blood**

Initial Temperature: 120 °C

Rate, °C/minute	Temperature, °C	Hold time, minute
0.0	120.0	1.0
20.0	260.0	0.0
5.0	280.0	4.0

Total program time: 16.00 minutes

2.10 Risk Based Consumption Limit for Contaminated Fish

To assess public health risk posed through fish consumption, the cancer risk estimates and hazard ratios (HRs) were measured on the basis of the guidelines of the United States Environmental Protection Agency (USEPA). An area of concern is present between 10^6 and 10^4 (USEPA, 2005).

HR for cancer risks was assessed by comparing the EDI with the benchmark concentration (BMC) (Solomon *et al*., 2000; Jiang *et al*., 2005) using the following equation:

$$HR = EDI / BMC$$

Estimated daily intake (EDI)

Estimated dietary intakes of OCPs were calculated as follows:

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

Where C is the measured concentration of OCPs (ng g^{-1}, w/w), DR is average daily consumption rate of fish (52.88 g/day) (Fisheries' resource, Bangladesh 2013-14) and BW is body weight (60 kg) (WHO, 2010). The average daily consumption rate was derived from FAO (2011).

Benchmark concentration (BMC)

The BMC for carcinogenic effects was derived from the cancer slope factor (CSF), which was obtained from the USEPA (USEPA, 2012). The BMC for carcinogenic effects represents the exposure concentration at which lifetime cancer risk is one million for lifetime exposure. A hazard ratio that is greater than one indicates that there is potential risk to human health (Dougherty *et al*., 2000).

2.11 Statistical Methods for Human Blood

Statistical analysis was performed by ANOVA for human blood. Basic descriptive statistics, ANOVA, LSD and Correlations test were performed on SPSS, to identify the relationship between lipid, DDTs residues with gender, age and duration of work. A p-value of <0.05 was denoted statistically significant**.**

Part - B

Pesticide Residues in Vegetable Samples

2.12 Site Selection

2.12.1 Dissipation Pattern of Cypermethrin

Vegetables are grown extensively in different part of Bangladesh. Five different vegetable samples (tomato, bitter gourd, pumpkin, eggplant, & green chili) were collected from farmer's field of Nurundi, one of the largest vegetable growing area of Jamalpur district on 4th February 2016 (**Fig-2.2).**

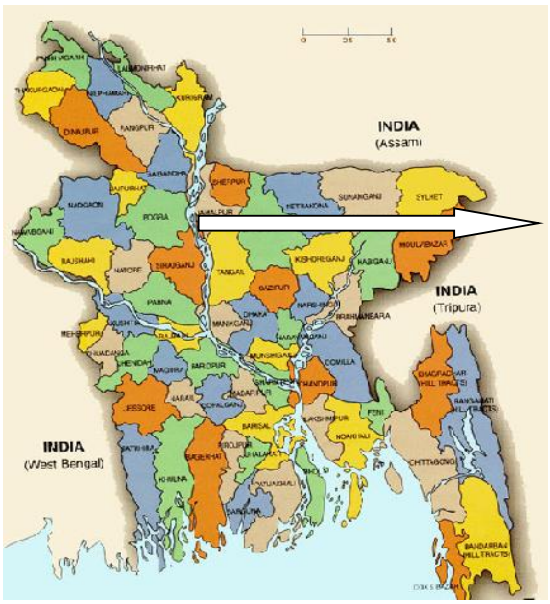


Fig-2.2: Area of sample collection **Plate-8: Vegetables field**

2.12.2 Dissipation Pattern of Fluxapyroxad

The moie leaves were grown in greenhouse from the last week of March until the first week of June, 2015. Experiments were conducted in a greenhouse in the experimental fields of Naengcheon-ri, Masan-myeon, Gurye-gun, Jeollanam-do, and Republic of Korea. The experimental area contained into two plots, in which a random block scheme was established with three replicates (plot A for double spraying and plot B

for triple sprayings). In addition, control samples were cultivated in a separate plot without receiving any treatment (**Fig.-2.3**)

Plate-9: Moie control field

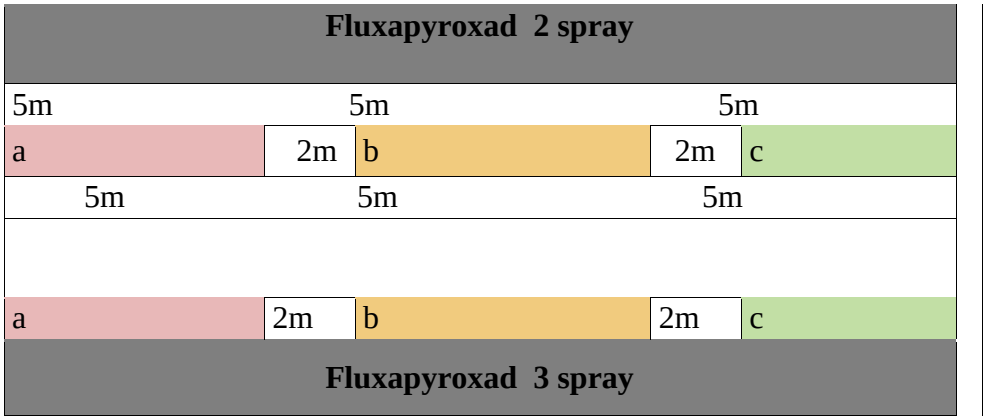


Fig-2.3: Green house experimental moie field design

2.12.3 Pesticide Residues

Four varieties of summer vegetables (patal, chichinga, jhinga and dhundol) were collected from different farmer's cultivation fields from Kaliganj in Jhenidah and local bazaar of Nandail & Gaforgau from Mymensingh district and Gobindapur & Burichang from Comilla district of Bangladesh.

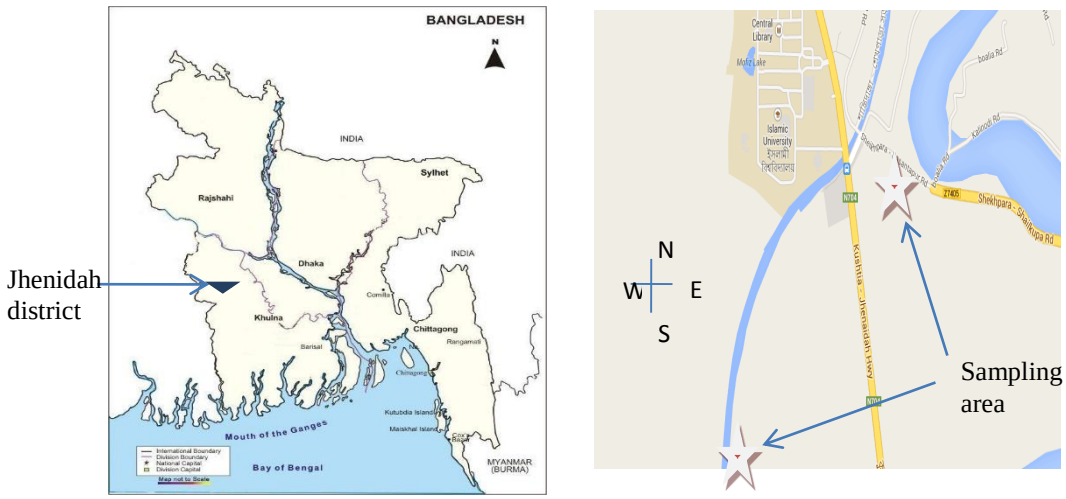


Fig-2.4: Sampling location of vegetables in Jhenidah district

2.13 Social Survey

During the collection of vegetable samples in Nurundi at Jamalpur and in Kaligonj at Jhenaidah district a social survey was carried out according to a prepared questionnaire. We had discussion with local farmers and also examined their health condition. They do not know about the proper uses & doses of pesticides.

Plate-10: Survey with farmers in Jamalpur district

Plate-11: Survey with farmers in Kaligonj in Jhinaida district

2.14 Application of Pesticides

Cypermethrin

Cypermethrin under the trade name Shobicron/Ektara, was sprayed at recommended dose (10% EC, 200 mL ha^{-1} *i.e.* 20 g a.i. ha^{-1}) on tomato, bitter gourd, pumpkin, egg plant and green chili by the farmers in their fields in Norundi.

Plate-12: Treated pesticides Shobikron/Ektara & application of pesticides

Fluxapyroxad

Fluxapyroxad (15% active ingredient, flowable) concentrate from Kyung Nong, Seoul, and Republic of Korea, was diluted and sprayed using a portable hand sprayer (Apollo Corp., Gimje, Republic of Korea). The solution was dissolved in water (fluxapyroxad 5 g in 20 L water) at the recommended doses by the manufacturer in the greenhouses. In plot B, fluxapyroxad was sprayed first on 18 April 2015. Then on 25 April 2015 in plot A and B and finaly on 2 May 2015 in plot A and B of the greenhouse. Every spray was done after 7 days interval. Climatic conditions were monitored using a thermo-hygrometer (model DK-102, Daekwang, Inc. Seoul, Republic of Korea), and temperatures ranged from 20 to 35 ºC. Relative humidity ranged from 15 to 55% throughout the experimental period.

Plate-13: Pesticide application on moie field

Plate-14: Greenhouse design for the pesticide application on moie leaves

2.15 Collection of Vegetable Samples

i) For dissipation pattern of cypermethrin, all vegetable samples were collected before the application of pesticides and termed as untreated (control). Later cypermethrin was applied to these vegetable samples and collected 2 hours after application and termed as pesticide treated samples. Before pesticide spraying control samples (~ 1 kg) were collected in jip-locked bag from the respective fields and kept in chilled-box. After two hours of pesticides application samples (~ 10 kg) were collected and immediately stored in a chilled-box at 4°C (0 day sample) and then all the samples were transferred to the laboratory as soon as possible.

Table-2.4: Names and nature of different fresh vegetable samples

Common name	**English name**	**Scientific name**	**Family**	**Dose Rate/ha** (mL/L Water)
Begoon	Eggplant/ Brinjal	*Solanum melongina*	Solanaceae	2
Misti kumda	Pumpkin/ Sweet gourd	*Cucurbita maxima*	Cucurbitaceae	
Karolla	Bitter gourd	*Momordica charantia*	Cucurbitaceae	
Tomato	Tomato	*Lycopersicon esculentum*	Solanaceae	
Kacha morich	Green chili	*Capsicum species*	Solanaceae	

(a) Eggplant) (b) Tomato (c) Korolla

Plate-15: Vegetable samples collected from Jamalpur district

ii) For the analysis of pesticide residues in some vegetable samples grown in summer season (like - snake gourd, ribbed gourd, sponse gourd & pointed gourd) were collected from three different areas of Bangladesh. One control vegetable samples were collected before pesticides spraying, from Kaligonj. Samples were kept in a chilled-box (samples in jip-locked bag) and then stored in freezer at -20 ^{0}C.

Table-2.5: List of the selected vegetable samples

Local name	English name	Scientific name	Family
Chichinga	Snake gourd	*Trichosanthes anguina*	Cucurbitaceeae
Jhinga	Ribbed gourd	*Luffa acutangula*	Cucurbitaceeae
Dhundul	Sponse gourd	*Luffa cylindrica*	Cucurbitaceeae
Patol	Palwal / Pointed gourd	*Trichosanthes dioica*	Cucurbitaceeae

(a) Pesticide spray **(b) Jhinga** **(c) Patol** **(d) Chichinga**

Plate-16: Vegetable samples collection from different places of Bangladesh

Table-2.6: Vegetables sampling date from farmer's field and market

Name of vegetables	Collection place	Date of sampling
Chichinga and Jhinga	Kaligonj, Jhenidah	26-06-2013
	Nandail, Mymensingh	06-07-2013
	Gaforgau, Mymensingh	05-07-2013
Dhondol and Patol	Kaligonj, Jhenidah	26-06-2013
	Gaforgau, Mymensingh	05-07-2013
	Gobindopur& Burichong, Comilla	12-07-2013

iii) For dissipation pattern of fluxapayroxd, moie leaves were collected on 2 May 2015.The leaves were collected from each replicated plot at 0 (after 3h), 3, 7, 10 and 14 days after the application in plot A and at the same periods after a second application in plot B. All collected samples (about 500 g) were immediately brought to the laboratory.

Moie is Korean leafy vegetables. Its English name is Butterbur. It is similar to Pumpkin leaf in our country. This leaf is popular in Korea. Korean people usually have these leaves as salad & Bangladeshi people have this as cooked leafy vegetables.

Plate-17: Moie leaves collection

Table-2.7: Spraying of fluxapyroxad and harvesting schedule of moie leaves

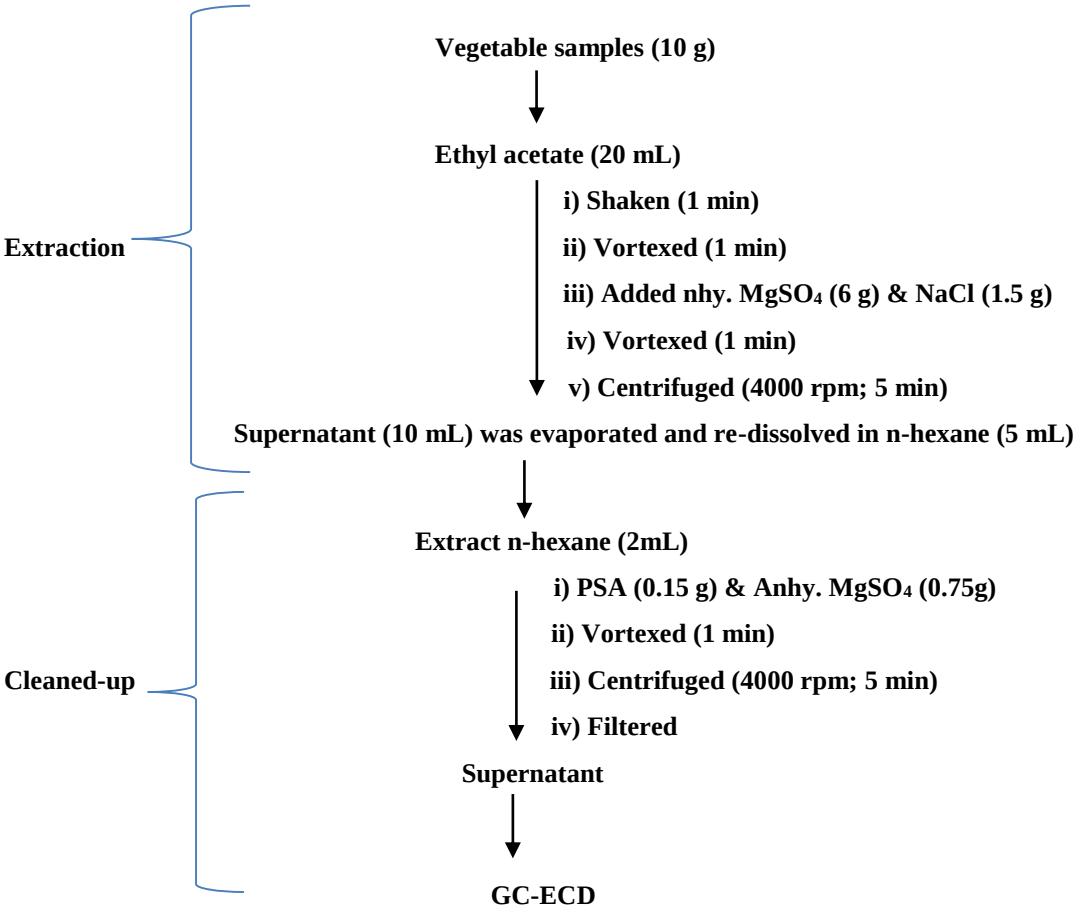
Spraying date	Interval prior to harvest (days) Treatment		Harvest date
	frequency =2	frequency = 3	
1st spray-18 April' 2015 2nd spray-25 April' 2015 3rd spray- 2 May' 2015	0	0	2015. 05. 02
	1	1	2015. 05. 03
	3	3	2015. 05. 05
	5	5	2015. 05. 07
	7	7	2015. 05. 09
	10	10	2015. 05. 12
	14	14	2015. 05. 16
Harvest day of the untreated sample (control) – 4 April, 2015			

2.16 Sample Preparation

Vegetable samples were chopped into small pieces and about 200 g of each was homogenized by kitchen blender. Before analysis, all vegetable samples were kept in freezer at -20° C. Except for analysis of dissipation pattern of cypermethrin, control and 0 day (after 2 hours) samples were kept in the freezer and rest of the samples were kept in ambient temperature. The samples were then extracted and cleaned-up after 1, 3, 5, 8, 10 & 12 days.

2.16.1 Extraction and Cleaned-up Methodology of Vegetable Samples

10 g of homogenized vegetable samples was taken in a 50 mL screw cup teflon tube and ethyl acetate (20 mL) was added. The content was shaken for 1 minute in hand and vortexed for 1 min. Anhydrous $MgSO_4$ (6 g) and NaCl (1.5 g) were added and vortexed for another 1 min and then centrifuged for 5 minutes at 4000 rpm. The supernatant (10 mL) was transferred to a round bottom flask (100 mL), evaporated to dryness and reconstituted in n-hexane (5 mL). The extracted sample (2 mL) was taken in a screw test tube (10 mL) then PSA (0.15 g) and anhydrous MgSO4 (0.75g) were added to it. The content was vortexed for another 1 min and then centrifuged for 5 minutes at 4000 rpm. The supernatant was passed through cotton filter and transferred to a clean GC vial and finally analysed by GC-ECD (**Scheme-6).**



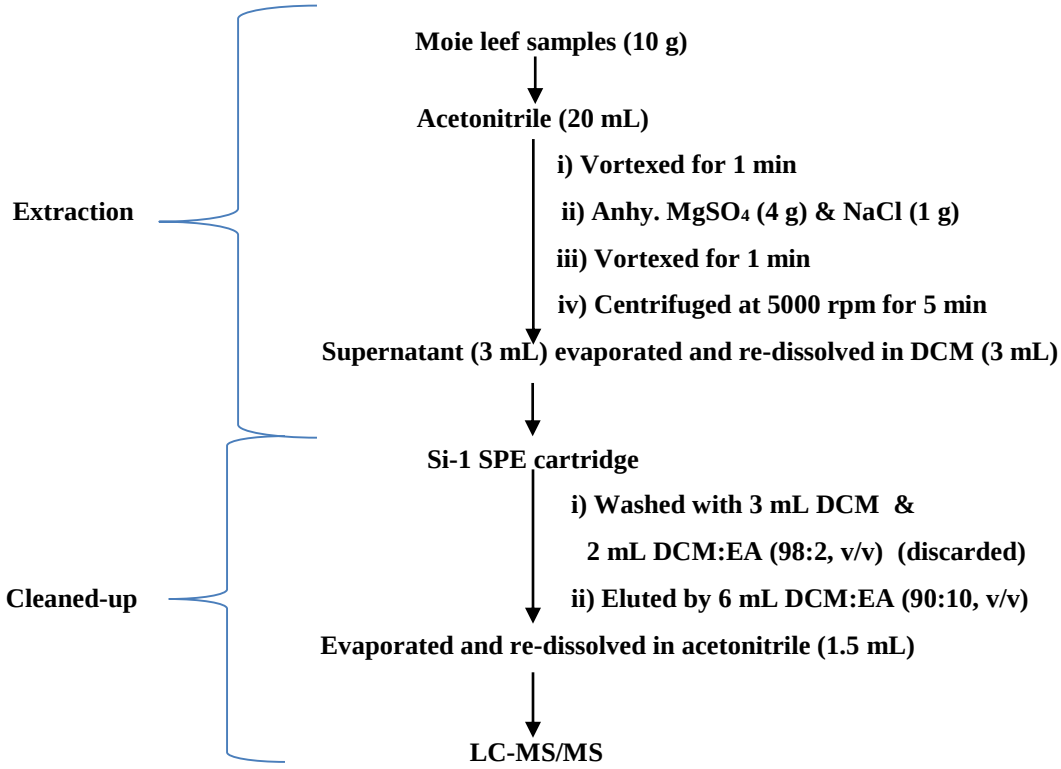
Scheme-6: Extraction and cleaned-up method of vegetable samples

Moie leaves were chilled in chill-box (samples in jip-locked bag) and then stored in freezer under -24 °C for analysis of dissipation pattern of fluxapyroxad. Before storing in freezer the samples were chopped and homogenized in kitchen blender. Subsamples weighing approximately 100 g each stored at -24 °C prior to analysis.

Plate-18: Chopped moie leaves

2.16.2 Extraction and Cleaned-up Methodology of Moie Leaves

Approximately 10 g of homogenized moie leaves were extracted by QuEChERS method with some modification. The extract was evaporated under N_2 gas and purified with solid phase extraction (SPE) cartridge (Si-1 Silica). 10 g homogenized (by Health Miller- Artlon, Republic of Korea) moie samples were taken in 50 mL teflon centrifuge tube. 20 mL aliquot of acetonitrile was added to the sample and then vigorously shaken for 1 min. 4 grams of anhydrous magnesium sulphate and 1 g NaCl were added and the sample was again shaken for 1 min. Then the extract was centrifuged for 5 min at 5000 rpm, 20 °C. 3 mL of the upper layer was transferred to a 10 mL Teflon centrifuge tube, evaporated by N_2 gas and reconstituted in DCM (3 mL). A Silica SPE cartridge (1g, 6 mL) was used for cleaning the extract. The cartridge (Si-1 Silica) was equilibrated with 5 mL DCM and then the sample extract was applied into the cartridge. The cartridge was washed with 3mL DCM and 2 mL DCM: EA (98:2, v/v) and then discarded. The pesticide was eluted with 6 mL DCM: EA (90:10, v/v) at a rate of 1 mL min^{-1}. The eluent was evaporated to dryness, re-dissolved in with acetonitrile (1.5 mL) and analyzed by LC-MS/MS. (**Scheme-7**).



Scheme-7: Extraction and cleaned-up method of moie leaves

2.17 Analytical Condition of GC-ECD

The quantitative analysis pesticide residues were conducted by gas chromatograph (GC-2010 Shimadzu) equipped with ^{63}Ni Electron Capture, (EC) detector. A non-polar (Rtx-5 MS) or (HP-5 MS) Quartz capillary column (30 m long × 250 µm i.d. x 0.25 µm film thicknesses) from Agilent, USA was used to carry out the separation. Nitrogen was used as both carrier (column flow 1.92 mL/min.) and make up gas. The injector and detector temperatures were 220 °C and 295 °C, respectively. All injections were made in split-less-split mode and injection volume was 1µL.

Chromatograph separation condition

Column oven temperature program: For Cypermethrin

Initial Temperature: 150 °C

Rate, °C/minute	Temperature, °C	Hold time, minute
0.0	150.0	0.0
30.0	285.0	0.0
2.0	295.0	1.0

Total program time: 10.00 minutes

Column oven temperature program: For pesticide residues

Initial Temperature: 120 °C

Rate, °C/minute	Temperature, °C	Hold time, minute
0.0	120.0	2.0
10.0	270.0	1.0
2.0	290.0	3.0

Total program time: 31.00 minutes

Identification of residues was achieved by running samples and external reference standards in GC and then comparing the corresponding retention times.

2.18 Analytical Conditions of LC−MS/MS

The analysis of fluxapyroxad was conducted on a waters ACQUITY UHPLC system (Milford, MA) equipped with a Waters Acquity UHPLC. Kinetex C_{18} column (2.1 mm × 100 mm, 2.6 µm particle size) the temperature of which was kept at 35 ^{0}C and

auto injector. The analysis of the analytes was conducted in the multiple reaction monitoring (MRM) positive electro spray ionization (ESI) mode with high resolution. Mobile phase A contained 0.1% formic acid in acetonitrile and B contained 0.1% formic acid in H_2O. Injection volume was 5 µL. Isocratic condition and the column flow was 350 µL min^{-1}. The analysis was carried out in triple quadruple (TQ) mode in which three characteristic ions (m/z = 131, 274, 380) for fluxapyroxad were monitored.

Chromatographic separation condition

- Column: Kinetex C18 (100 × 2.1 mm i.d., 2.6 µm particle size)
- Mobile phase: A : 0.1% Formic acid in acetonitrile; B : 0.1% Formic acid in H_2O
- Flow rate: 350 µL/min
- Column temp.: 35°C
- Injection volume: 5µL

Isocratic condition

Time (min)	A	B
5	75	25

MRM condition

M.W.	Precursor ion (*m/z*)	Product ion (*m/z*)	Cone (V)	CE[b] (V)
381	380	131[a] 274	46	23 19

a) Quantification ion

b) Collision Energy

2.19 Storage Stability

Storage stability of the cypermethrin in freezer (-20 °C) was done by spiking the pesticide on control samples at the concentration level 1 mg kg^{-1} in triplicate analysis and the samples were stored in a freezer from 4 February 2016 to 20 March 2016 (about 40 days). Then these were extracted, cleaned-up and analyzed with all samples following same procedure on 20 March 2016 to know the stability of the analytes in vegetables (tomato, eggplant, bitter gourd, pumpkin & green chili) at freezing condition.

For the determination of storage stability of fluxapyroxad, 10 g control samples were spiked with standard fluxapyroxad at the spiking level (0.5 mg kg^{-1}) in 50 mL centrifuge tube and were frozen (-24 °C) as three replicates on 2 May 2015. This experiment was carried out at the final stage of field sample analysis on 20 June 2015.

2.20 Health Risk Index (HRI)

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

$$HRI = \frac{EDI}{ADI}$$

Estimated Daily Intake (EDI)

EDI of pesticide residues was calculated by multiplying the residual pesticide concentration (mg/kg) by the food consumption rate (0.345 kg/person/day) and dividing by a body weight of 60 kg for an adult people (Wang *et al*., 2005).

Acceptable Daily Intake (ADI)

Acceptable daily intake represents the daily concentration below which there is a high probability of no adverse health effect. Acceptable Daily Intake (ADI) for human is 0.05 mg/kg body weight (Codex, 2013).

HRI of the residues was intended using the results and other statistics equation which was modified after EFSA (EFSA, 2013). HRI value more than 1 is considered as not safe for human health (Darko *et al*., 2008).

A. Results and Discussion

3.1 DDTs in Environmental Samples of CCC area

3.1.1 Soil, Sediment and Water Samples

3.1.1.1 Recovery Experiments of Soil and Water Samples

The recovery experiments for soil were conducted at two spiking levels (0.025 mg kg^{-1} & 0.1 mg kg^{-1}) in three replicate analysis.

The recovery for water samples were carried out in triplicates at two spiking levels (4 and 20 µg L^{-1}) using Milli-Q water (LC grade). The spiked samples (soil and water) were settled for 4 h and subsequently extracted and analyzed **(Scheme 1−3 & Table 3.1)**.

Table-3.1: Recovery of DDTs in soil and water samples

Pesticide	% Recovery ± RSD %			
	Soil		Water	
	Spiking level			
	0.025 mg kg^{-1}	0.1 mg kg^{-1}	4.0 µg L^{-1}	20.0 µg L^{-1}
4,4'-DDE	88±1.95	84±1.62	83±2.55	98±6.15
4,4'-DDD	76±10.23	80±4.28	89±2.71	105±6.14
2,4'-DDT	79±4.11	88±1.78	86±2.29	102±6.3
4,4'-DDT	73±3.98	94±10.29	87±3.68	110±9.31

3.1.1.2 Calibration Curve

In order to obtain standard calibration curves, stock solution of standard certified samples were serially diluted to get ten different concentrations (0.005 − 0.5 mg L^{-1}). The calibration curves were linear over the range of different concentrations as shown by detail that the correlation coefficients (R^2) for the linearity range were 0.9975−0.9996 **(Fig-3.1).**

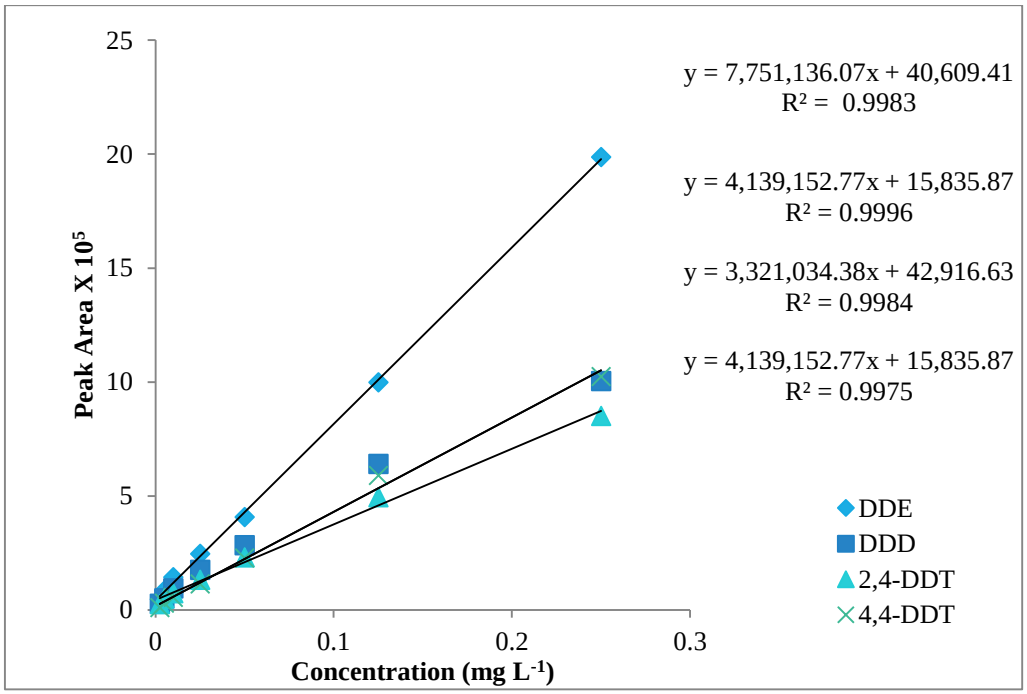


Figure-3.1: Calibration curves of DDTs for soil, sediment and water

3.1.1.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The residual level of DDTs in soil and water samples were quantified by GC–ECD (Shimadzu-17A Japan). The Linearity of the ECD controlled by running series of dilution standards. LOD and LOQ of each of the standard sample were found to be 0.005 and 0.017 mg kg^{-1} for soil; 0.04 and 0.132 µg L^{-1} for water respectively (**Table-3.2)**.

Table- 3.2: Linear range, retention times (RT), correlation coefficients (R^2), LOD and LOQ of DDTs in soil and water samples

Pesticide	Linear range (mg L^{-1})	RT (min)	Linearity (R^2)	LOD	LOQ
4,4'-DDE	0.005− 0.5	9.29	0.9983	0.005 mg kg^{-1} (soil)	0.017 mg kg^{-1} (soil)
4,4'-DDD		9.79	0.9996		
2,4'-DDT		10.14	0.9984	0.04 µg L^{-1} (water)	0.132 µg L^{-1} (water)
4,4'-DDT		10.82	0.9975		

3.1.1.4 Moisture Content in Soil Samples

The moisture content of the soil samples was 19–30 % (Table-3.3) as well as Bar diagram (Fig- 3.2).

Table-3.3: Moisture contents (%) of soil samples

Sample ID	% Moisture content
20 ft S	25.24
30 ft S	27.09
50 ft S	24.55
60 ft S	24.74
70 ft S	23.98
100 ft S	27.67
150 ft S	26.54
200 ft S	22.97
250 ft S	23.78
300 ft S	21.96
330 ft S	19.27
20f S/W	22.07
30f S/W	19.31
50f S/W	28.59
60f S/W	26.59
20 ft E	23.60
30 ft E	19.51
40 ft E	20.42
50 ft E	21.64
70 ft E	20.57
200 ft E	12.07
250 ft E	21.79
300 ft E	22.22

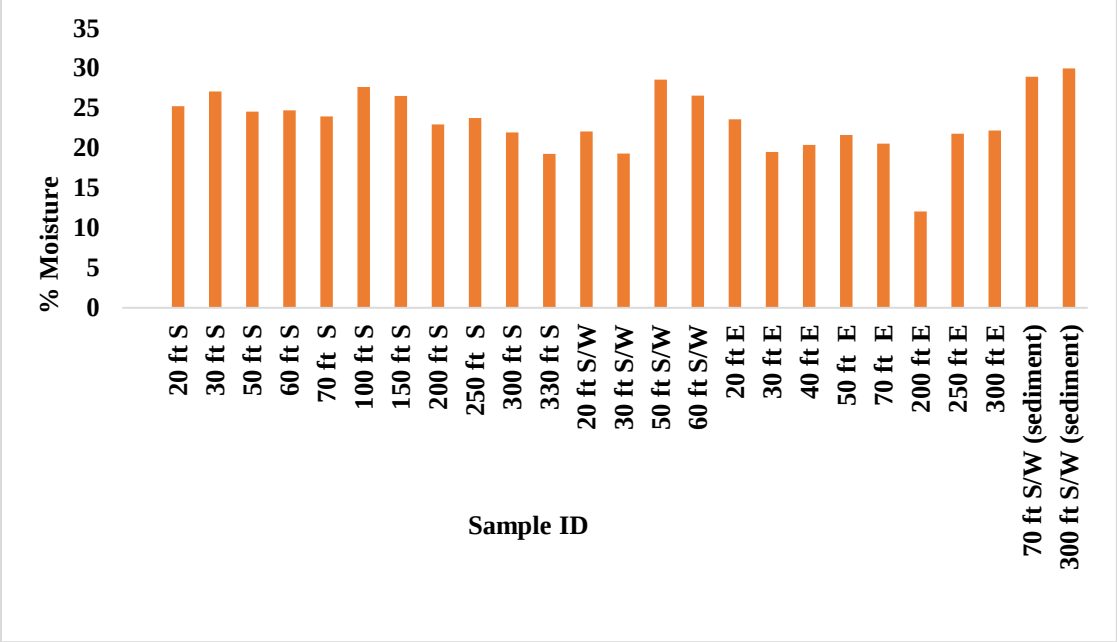


Fig-3.2: Bar diagram of moisture contents in soil samples

3.1.1.5 Total DDTs Residues in Soil, Sediment and Water Samples

The amount of DDTs in CCC area were detected as 1 x 10^2–1635 x 10^2 mg kg^{-1} and 16 x 10^2–7 x 10^2 mg kg^{-1} for soil and sediment samples respectively **(Table-3.4** & **Bar diagram Fig.-3.3)**.

Table-3.4: Residues of DDTs (Average ± SD, mg kg^{-1}) in soil and sediment samples

Sample ID (n=3)	*4,4'*-DDE (mg kg^{-1}) (x10^2)	*4,4'*-DDD (mg kg^{-1}) (x10^2)	*2,4'*-DDT (mg kg^{-1}) (x10^2)	*4,4'*-DDT (mg kg^{-1}) (x10^2)	∑DDT (mg kg^{-1}) (x10^2)	*4,4'*DDT/ ∑DDT
20 ft S	30 ± 2.37	288 ± 26.64	0.16 ± 0.07	617 ± 150.92	935	0.66
30 ft S	15 ± 2.05	71 ± 10.37	0.15 ± 0.04	194 ± 17.54	280	0.69
50 ft S	103 ± 18.67	61 ± 4.86	2 ± 1.38	191 ± 41.13	354	0.54
60 ft S	9 ± 0.81	31 ± 3.25	0.5 ± 0.06	98 ± 14.57	139	0.71
70 ft S	5 ± 0.55	21 ± 3.87	0.27 ± 0.02	118 ± 17.45	144	0.82
100 ft S	3 ± 0.35	14 ± 2.52	1 ± 0.64	67 ± 4.20	85	0.79
150 ft S	5 ± 0.88	4 ± 0.52	0.02 ± 0.01	6 ± 0.16	14	0.41
200 ft S	0.32 ± 0.02	1 ± 0.20	0.22 ± 0.25	4 ± 0.30	6	0.75
250 ft S	0.23 ± 0.03	0.41 ± 0.08	0.19 ± 0.77	1 ± 0.20	2	0.68
300 ft S	0.88 ± 0.18	0.80 ± 0.14	0.16 ± 0.02	5 ± 0.96	7	0.75
330 ft S	0.32 ± 0.56	0.21 ± 0.02	0.21 ± 0.32	4 ± 0.74	5	0.89
20 ft S/W	9 ± 1.12	159 ± 16.88	0.22±0.51	1468 ± 92.80	1635	0.90
30 ft S/W	61 ± 4.75	459 ± 31.27	0.06±4.13	563 ± 26.21	1084	0.52
50 ft S/W	29 ± 2.37	23 ± 2.13	0.22± 0.55	29 ± 5.91	82	0.36
60 ft S/W	5 ± 1.08	4 ± 42.38	0.24±0.50	6±1.22	16	0.40
20 ft E	2 ± 0.39	1 ± 0.21	0.24±0.23	19 ± 2.63	22	0.85
30 ft E	3 ± 0.62	2 ± 0.17	0.23±0.38	32 ± 6.06	38	0.85
40 ft E	0.77 ± 0.1	4 ± 0.35	0.20±0.34	40 ± 6.24	44	0.90
50 ft E	70 ± 2.01	351 ± 7.19	0.13±2.91	565 ± 21.84	984	0.57
70 ft E	0.73 ± 0.92	4 ± 0.31	019±0.87	40 ± 8.10	45	0.89
200 ft E	4 ± 0.68	0.4 3± 0.65	0.7 ± 0.06	0.73 ± 0.14	1	0.61
250 ft E	7 ± 1.40	1 ± 0.12	1.7 ± 0.1	3 ± 0.24	5	0.75
300 ft E	6 ± 0.56	0.23 ± 0.04	4.8 ± 0.2	0.74 ± 0.09	1	0.72
70 ft S/W (sediment)	5.0±1.1	4.4±0.4	0.3±0.02	6.4±1.1	16.2	1.48
300 ft S/W (sediment)	0.9±0.1	0.8±0.1	0.1±0.003	5.06±0.9	6.9	0.33

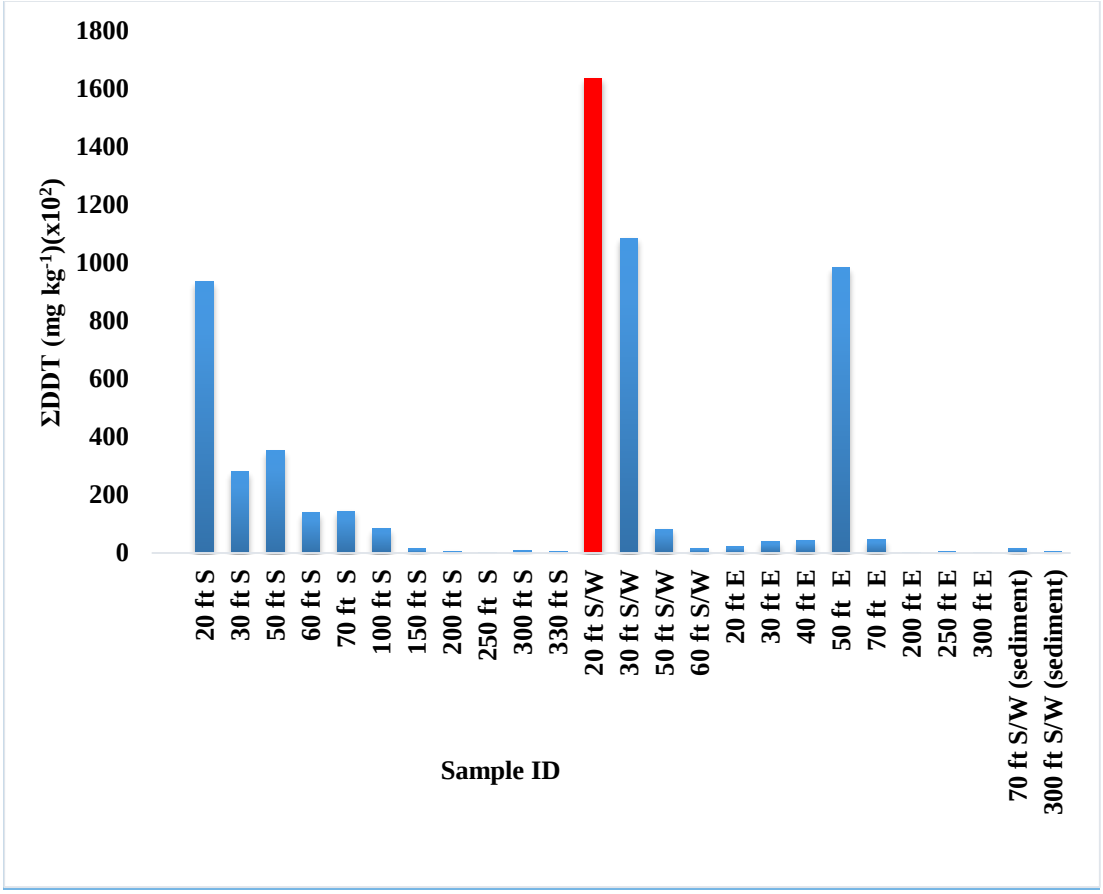


Fig -3.3: Bar diagram of ∑DDTs in soil

Liquid-liquid extraction process was conducted for water sample as per standard procedure provided by US EPA (Methods 500 Series). The amount of DDTs in CCC area were detected as 0.4 – 18 µg L^{-1} for water samples **(Table-3.5 & Bar diagram; Fig-3.4).**

Table-3.5: Residues of DDTs (µg L^{-1}) in water samples

Sample ID (n=7)	*4,4′*-DDE (µg L^{-1})	*4,4′*-DDD (µg L^{-1})	*2,4′*-DDT (µg L^{-1})	*4,4′*-DDT (µg L^{-1})	∑DDT (µg L^{-1})	*4,4'*DDT/∑DDT
300 ft – 1	1.0719	1.1727	BQL	10.7343	12.9789	0.8271
300 ft – 2	0.3139	0.8289	BQL	4.9943	6.1371	0.8138
300 ft – 3	0.3663	0.9181	BQL	4.0875	5.3719	0.7609
300 ft -4	0.4138	0.2548	BQL	0.7079	1.3764	0.5143
300 ft – 5	0.3653	0.8928	BQL	3.8940	4.2593	0.9142
300 ft – 6	0.1789	0.3596	BQL	1.1285	1.3074	0.8632
300 ft - 7	0.2837	0.1279	BQL	0.1041	0.5158	0.2019
Pond water-1	0.3699	0.7790	BQL	2.1040	3.2528	0.6468
Pond water-2	0.1027	0.4403	BQL	1.4475	1.9905	0.7272
Pond water-3	0.2409	0.2289	BQL	0.2219	0.4966	0.4468
Pond water-4	0.1594	0.1853	BQL	0.1699	0.3844	0.4418
Pond water-5	0.1212	0.2291	BQL	1.0519	1.4022	0.7502
Pond water-6	0.2655	0.2029	BQL	0.1232	0.5917	0.2083
Pond water-7	0.0501	0.0610	BQL	0.2292	0.3403	0.6734
70ft – 1	0.2823	0.4697	BQL	0.2818	1.0339	0.2726
70ft – 2	0.1207	0.2408	BQL	0.8229	1.1844	0.6948
70ft – 3	0.2883	0.5803	BQL	1.5934	2.4620	0.6472
70ft – 4	0.1349	0.2715	BQL	0.9132	1.3195	0.6920
70ft – 5	0.2795	0.3749	BQL	1.3396	1.9939	0.6718
70ft – 6	0.7935	1.8143	BQL	15.2317	17.8395	0.8538
70ft – 7	0.4448	0.6941	BQL	3.7898	4.9286	0.7689

*BQL = Below Quantification Level

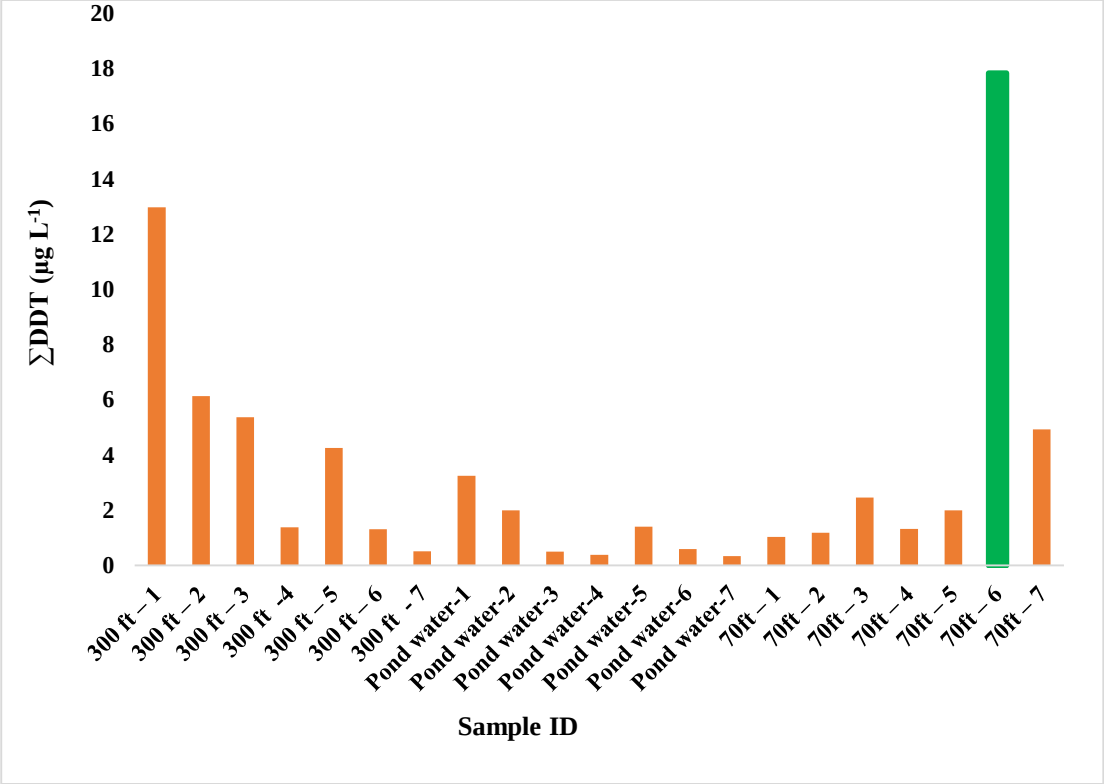


Fig-3.4: Bar diagram of ∑DDTs in water sample

3.1.2 DDTs in Fish Samples

3.1.2.1 Recovery Experiment for Fish

The recovery for fish samples were carried out in triplicates at three spiking levels (0.05, 0.1 and 0.2 mg kg^{-1}). The spiked samples (fish) were settled for 4 h and subsequently extracted and analyzed **(Scheme 3−4 & Table-3.6)**.

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 (**Table-3.6**), which were in the range 70-120% and acceptable for fish samples according to standard methodology (Codex, 2003).

Table-3.6: Recovery of DDTs in fish samples

Pesticide	% Recovery ± RSD %		
	Spiking level		
	0.05 mg kg^{-1}	**0.1 mg kg^{-1}**	**0.2 mg kg^{-1}**
4,4'-DDE	92±2.76	93±7.13	87±3.02
4,4'-DDD	114±1.54	101±6.17	102±16.34
2,4'-DDT	76±2.05	88±8.30	105±9.29
4,4'-DDT	72±3.64	70±0.78	91±15.50

3.1.2.2 Calibration Curves

In order to construct standard calibration curves, stock solution of standard references certified samples were serially diluted (0.00025-1 mg L^{-1}) to obtain different concentrations. The calibration curves were linear over the range of concentrations as shown by the fact that the correlation coefficients (R^2) for the linearity range were 0.9980−0.9992 (**Fig-3.5 & Table-3.7)**.

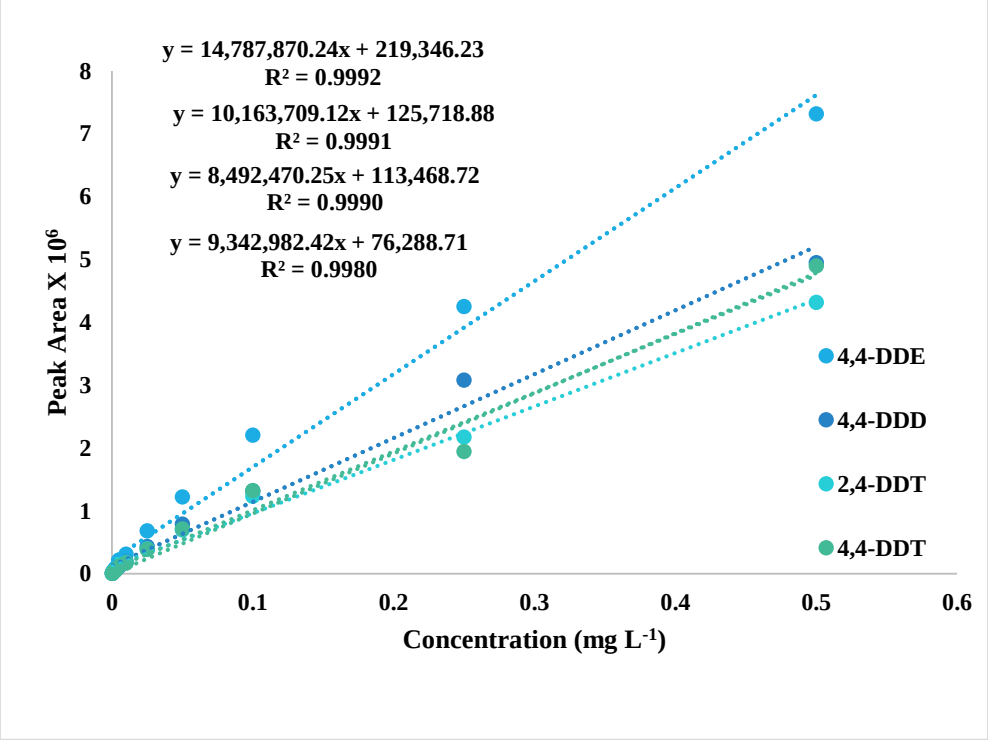


Fig.-3.5: Calibration curves for fish samples

3.1.2.3 Quantification

The concentration levels of DDTs in fish samples were quantified by GC–ECD (Shimadzu-2010 Japan). The Linearity of the ECD was controlled by running series of standard dilution mixtures.

3.1.2.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

To elucidate the sensitivity of the experiment of method, limit of detection (LODs) and limit of quantification (LOQs) were determined (**Table-3.7**).

Table 3.7: Retention times (RT), correlation coefficients (R^2), LOD and LOQ of fish

Pesticides	**Linear range (mg L^{-1})**	**RT (min)**	**Linearity (R^2)**	**LOD (µg kg^{-1})**	**LOQ (µg kg^{-1})**
4,4'-DDE	0.00025− 1	10.39	0.9992	0.0625	0.2063
4,4'-DDD		11.04	0.9991		
2,4'-DDT		11.14	0.9990		
4,4'-DDT		11. 68	0.9980		

3.1.2.5 Lipid Contents in Fish Samples

Amount of lipid contents in the fish samples (n=15) were determined. The extract was kept evaporated to dryness with gentle flow of nitrogen until constant weight was obtained. The dry materials gave amounts of lipid present in the fish samples. The lipid content was determined gravimetrically **(Table-3.8 & Bar diagram** in **Fig-3.6).**

Table-3.8 : Lipid contents (%) in fish samples of CCC area

Local name	Sample ID	Fresh wt (g)	% of Lipid
Mola	MoF	4.4	0.91
Rui	RF	5.02	0.4
Kachki	KaF	5.28	0.76
Magur	MgF	4.22	0
Chitiol	ChF	5.06	0.2
Shing	ShiF	0.36	2.7
Telapia	TpF	4.67	0.43
Katla	KatF	4.79	0.21
Boal	BF	5.03	0.87
Koi	KoF	2.66	1.13
Tengra	TeF	4.31	0.46
Shol	ShF	4.09	0.24
Taki	TaF	5.13	0.19
Chingri	ChF	4.66	0.43
Karfo	KarF	5.05	0.92

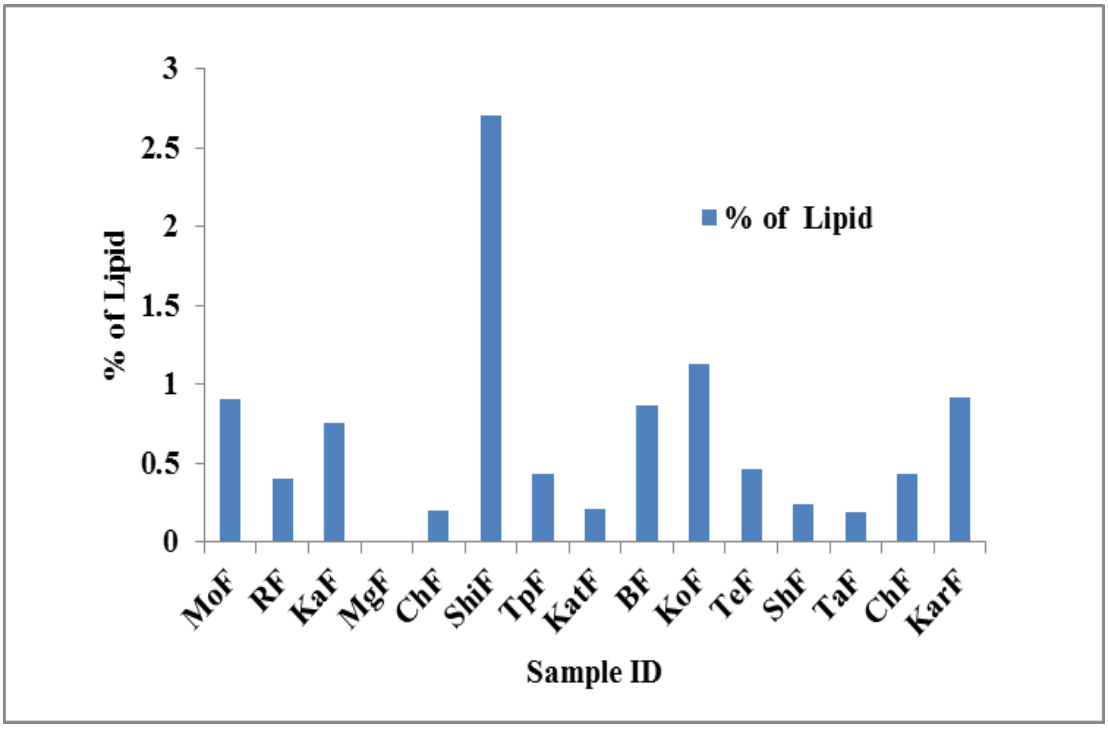


Fig – 3.6: Bar diagram of percentage (%) of lipid of fish samples

3.1.2.6 Total DDTs Residues in Fish Samples

All fish samples were of medium sizes which are available in all common fish markets. One of the good quality freshwater shrimp was also included in this analysis.

The residual amounts of DDTs were found in different fish samples (expressed µg kg^{-1}) were presented in **Table 3.9** as well as in **Bar diagram (Fig-3.7)**.

Table-3.9: Residues of DDTs (Average ± SD, µg kg^{-1}) in fish samples

Sample ID (n=3)	Local name	*4,4'*-DDE (µg kg^{-1})	*4,4'*-DDD (µg kg^{-1})	*2,4'*-DDT (µg kg^{-1})	*4,4'*-DDT (µg kg^{-1})	∑DDT (µg kg^{-1})	*4,4'*-DDT /∑DDT
MoF	Mola	3.63±0.43	0.43±0.05	0.37±0.05	0.12±0.02	4.55	0.03
RF	Rui	0.38±0.03	0.36±0.01	0.09±0.36	0.31±0.04	1.14	0.27
KaF	Kachki	2.71±1.68	0.80±0.12	0.02±0.01	3.02±0.93	6.54	0.46
MgF	Magur	0.22±0.03	0.06±0.01	0.42±0.07	0.38±0.07	1.08	0.35
ChF	Chitiol	0.54±0.08	0.76±0.03	0.08±0.01	0.08±3.02	1.46	0.05
ShiF	Shing	4.75±0.60	2.68±0.34	0.82±0.13	0.65±1.08	8.9	0.07
TpF	Telapia	0.10±0.01	0.39±0.01	0.45±0.05	0.15±0.05	1.09	0.14
KatF	Katla	0.37±0.01	0.26±0.04	0.14±0.02	0.08±2.05	0.85	0.09
BF	Boal	0.27±0.01	0.21±0.02	0.09±0.01	0.09±0.03	0.66	0.14
KoF	Koi	1.09±0.05	1.14±0.14	0.65±0.04	1.42±0.07	2.9	0.01
TeF	Tengra	1.05±0.04	1.32±0.03	0.05±0.02	0.06±.12	2.48	0.02
ShF	Shol	1.78±0.31	0.17±0.01	0.16±0.01	0.07±0.65	2.18	0.03
TaF	Taki	0.56±0.04	0.22±0.02	0.36±0.04	0.05±0.67	1.19	0.04
ChF	Chingri	0.37±0.04	0.63±0.09	0.16±0.04	0.26±0.05	1.42	0.18
KarF	Karfo	0.94±0.09	1.14±0.03	0.18±0.6	0.29±0.9	2.55	0.11

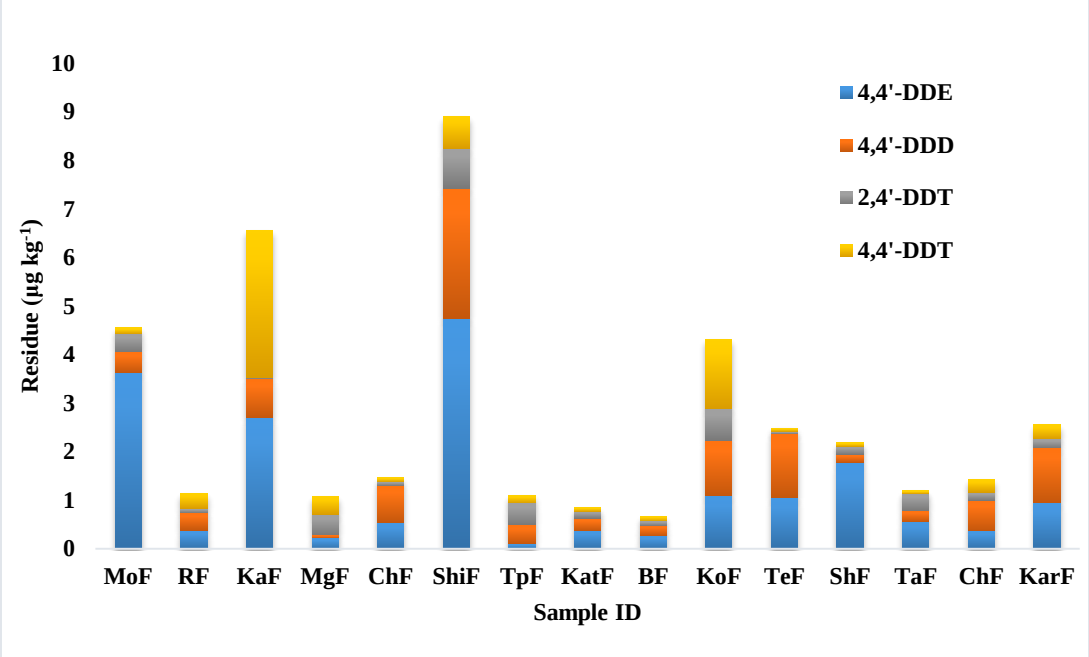


Fig-3.7: Bar diagram of DDTs in fish samples

3.2 Human Blood Samples

3.2.1 Recovery Experiment in Human Blood Samples

Recovery calculation was done by internal standards to evaluate the reproducibility of the method. The method was optimized and validated by doing recovery experiments using internal standard CB-53 in human blood samples. Blood samples were spiked in 0.025 µg L^{-1} (sample ID 1−15) and 0.05 µg L^{-1} levels (sample ID 16−30) before extraction. The recoveries of the internal standards were 93% with RSD 12% (0.05 µg L^{-1}) (n=15) and 85% with RSD 6% (0.025 µg L^{-1}) (n=15) for CB- 53. The procedures outlined for DDTs assessment in this study were adjudged reliable and efficient. The percentage of recovery was within the range, 70-120% (Codex Alimentarius, 2003).

3.2.2 Calibration Curve

The concentration of DDT and its metabolites were quantitatively determined by external standard method using peak area. Linear calibration curves for DDTs were done by GC-ECD over eight calibration levels (0.5, 0.25, 0.125, 0.05, 0.025, 0.01, 0.005 and 0.001 µg L^{-1}).The calibration curves were linear over the range of the tested concentrations as shown by the fact that the correlation coefficients (R^2) for the linearity range were 0.9981−0.9992 (**Fig-3.9)**.

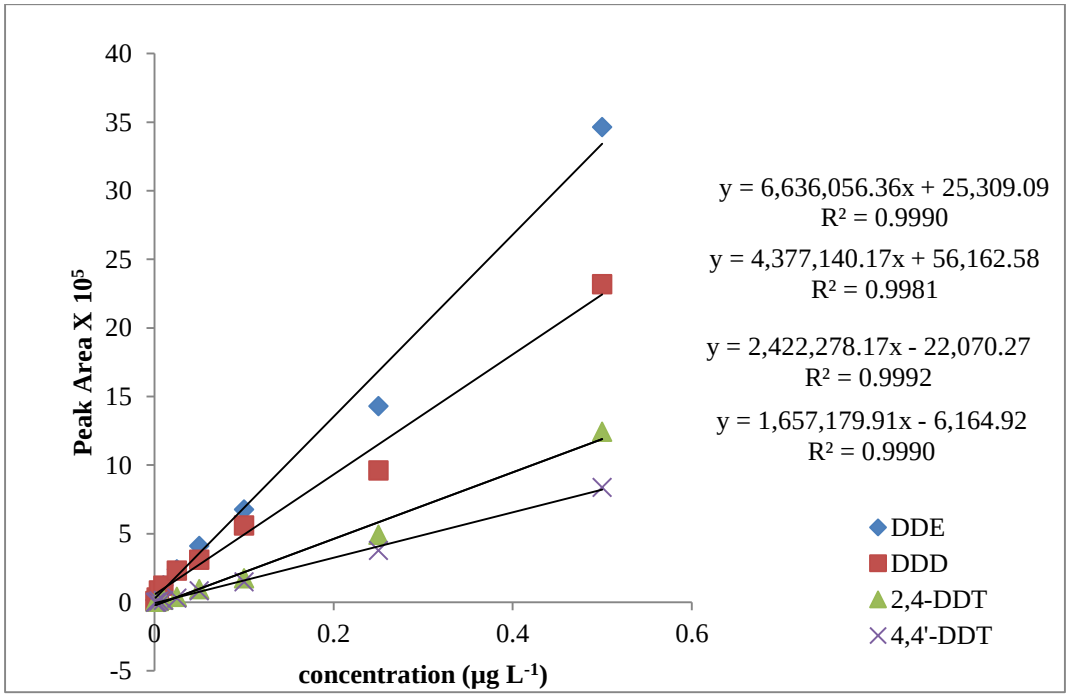


Figure-3.8: Calibration curves of DDTs for human blood

3.2.3 Quantification

The concentrations of DDTs in human blood plasma were quantified by GC-ECD (Shimadzu-2010 Japan). The Linearity of the ECD controlled by running series of

standard dilution mixtures. LOD and LOQ of each of the standard sample were also determined (**Table-3.10**).

Table-3.10: Retention times (RT), correlation coefficients (R^2), LOD and LOQ of human blood

Pesticide	Linear range (µg L^{-1})	RT (min)	Linearity (R^2)	LOD (µg kg^{-1})	LOQ (µg kg^{-1})
4,4'-DDE	0.001− 0.5	10.55	0.9990	0.025	0.0825
4,4'-DDD		11.22	0.9981		
2,4'-DDT		11.31	0.9992		
4,4'-DDT		11.87	0.9990		

3.2.4 Lipid Contents in Human Blood Samples

Amount of lipid contents in the blood samples (n=30) were determined. The combined n-hexane/MTBE extract was kept evaporated to dryness with gentle flow of nitrogen until constant weight was obtained. The dry materials gave amounts of lipid present in the human blood. The lipid contents were determined by gravimetrically (**Table-3.11**) and **Bar diagram** (**Fig-3.10**).

Table-3.11: Lipid contents (%) in blood samples of local people of CCC area

Sample ID	Age (year)	Gender	Fresh wt (g)	% of Lipid
sample -01	60	M	4.4	0.91
sample -02	33	M	5.02	0.4
sample -03	35	M	5.28	0.76
sample -04	48	M	4.22	0
sample -05	56	M	5.06	0.2
sample -06	56	M	0.36	2.7
sample -07	55	M	4.67	0.43
sample -08	49	M	4.79	0.21
sample -09	38	F	5.03	0.87
sample -10	35	F	2.66	1.13
sample -11	45	M	4.31	0.46
sample -12	65	M	4.09	0.24
sample -13	30	F	5.13	0.19
sample -14	28	M	4.66	0.43
sample -15	55	M	5.05	0.92
sample -16	55	M	0.8	1.25
sample -17	33	M	3.72	0.27
sample -18	24	M	4.74	1.69
sample -19	26	M	4.33	1.15
sample -20	22	M	4.49	2.45
sample -21	60	M	4.98	1.8
sample -22	40	M	3.73	1.6
sample -23	50	F	4.3	0.47
sample -24	24	F	3.8	0.26
sample -25	40	F	4.7	0.64
sample -26	34	F	4.19	0.95
sample -27	40	F	3.32	0.9
sample -28	25	M	0.66	1.5
sample -29	40	F	5	1
sample -30	38	F	5	1

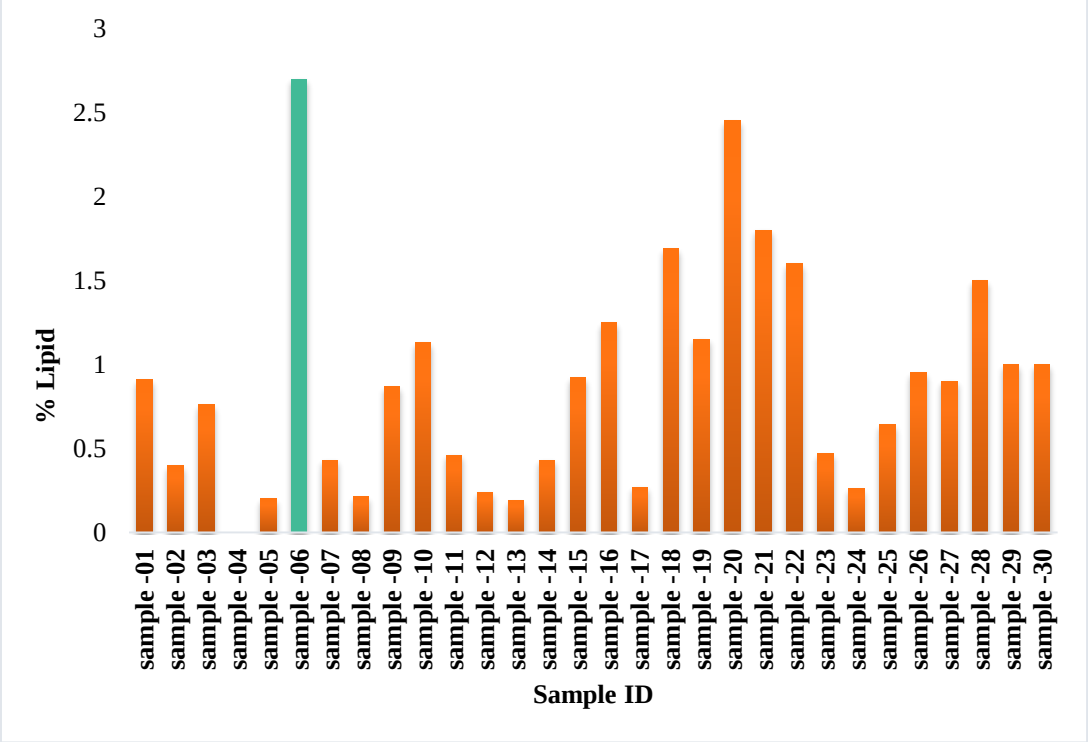


Fig-3.9: Bar diagram of lipid contents (%) in human blood samples

3.2.5 Statistical Analysis

Statistical analysis was performed by ANOVA. Basic descriptive statistics, ANOVA, LSD and Correlations test were performed on SPSS, to identify the relationship between lipid, DDTs residues with gender, age and duration of work. A p-value of <0.05 was denoted statistically significant (**Table 3.12**).

Table-3.12: Comparison total DDT, ratio of DDT and lipid contents (%) according to gender, age and duration of work

		∑DDT	Ratio of DDT	Lipid contents
Gender	M	261.86±503	0.14±0.10	0.97±0.78
	F	326.87±471	0.20±0.23	0.74±0.33
p – value		NS	NS	NS
Age	< 30	121.44±185	0.18±0.10	1.10±0.85
	31 – 50	210.06±408	0.20±0.19	0.71±0.41
	>50	606.14±714	0.07±0.07	1.06±0.86
p – value		NS	NS	NS
Duration of work	Non worker	270.34±469	0.18±0.18	0.87±0.58
	<10 yrs	130.66±184	0.9±0.07	0.80±0.60
	10 – 20 yrs	852.66±841	0.16±0.13	1.74±0.90
	>20 yrs	18.0±7	0.20±0.08	0.12±0.17
p – value		NS	NS	0.04

*NS = None Significant

3.2.6 Total DDTs Residues in Human Blood Samples

The amount of DDTs in CCC area were detected as 0–1686 µg kg^{-1} for human blood samples which was given in (**Table-3.13)**.

Table-3.13: Residues of DDTs in blood samples of local people of CCC surroundings

Sample ID	*4,4'*-DDE µg kg^{-1}	*4,4'*-DDD µg kg^{-1}	*2,4'*-DDT µg kg^{-1}	*4,4'*-DDT µg kg^{-1}	∑DDT µg kg^{-1}	*4,4'*DDT/∑DDT
sample -01	34	810	13	11	868	0.01
sample -02	0.61	13	4.6	2.8	21	0.13
sample -03	2.6	36	6.4	6.4	51	0.12
sample -04	2.3	7.0	8.1	5.7	23	0.25
sample -05	2.0	27	4.3	2.3	36	0.06
sample -06	583	331	435	337	1686	0.20
sample -07	3.0	214	BDL	BDL	217	0
sample -08	1.6	40	9.2	7.7	59	0.13
sample -09	1.5	1.5	7.9	3.5	14	0.24
sample -10	1.9	4.1	4.4	6.5	17	0.39
sample -11	0.70	2.4	2.9	BDL	5.95	0
sample -12	4.3	3.6	3.1	1.8	13	0.14
sample -13	0.82	6.9	BDL	0.97	8.7	0.11
sample -14	0.77	2.6	4.3	3.1	11	0.29
sample -15	3.1	3.3	7.8	1.0	15	0.07
sample -16	20	1295	59	34	1408	0.02
sample -17	0.39	9.7	5.2	3.9	19	0.20
sample -18	10	7.20	9.33	9.32	36	0.26
sample -19	2.60	0.47	3.11	2.20	8.37	0.28
sample -20	6.05	10	3.42	4.82	24	0.20
sample -21	BDL	BDL	BDL	BDL	BDL	BDL
sample -22	0.02	0.02	2.92	1.08	3.99	0.27
sample -23	71	137	BDL	BDL	207	0
sample -24	3.9	266	13	10	292	0.03
sample -25	31	1376	6.6	41	1455	0.03
sample -26	19	845	22	9.4	869	0.01
sample -27	0.51	51	4.32	2.4	59	0.04
sample -28	69	313	36	52	470	0.11
sample -29	8.7	39	23	102	173	0.59
sample -30	10	45	25	94	174	0.54

*BDL = Below Detection Limit

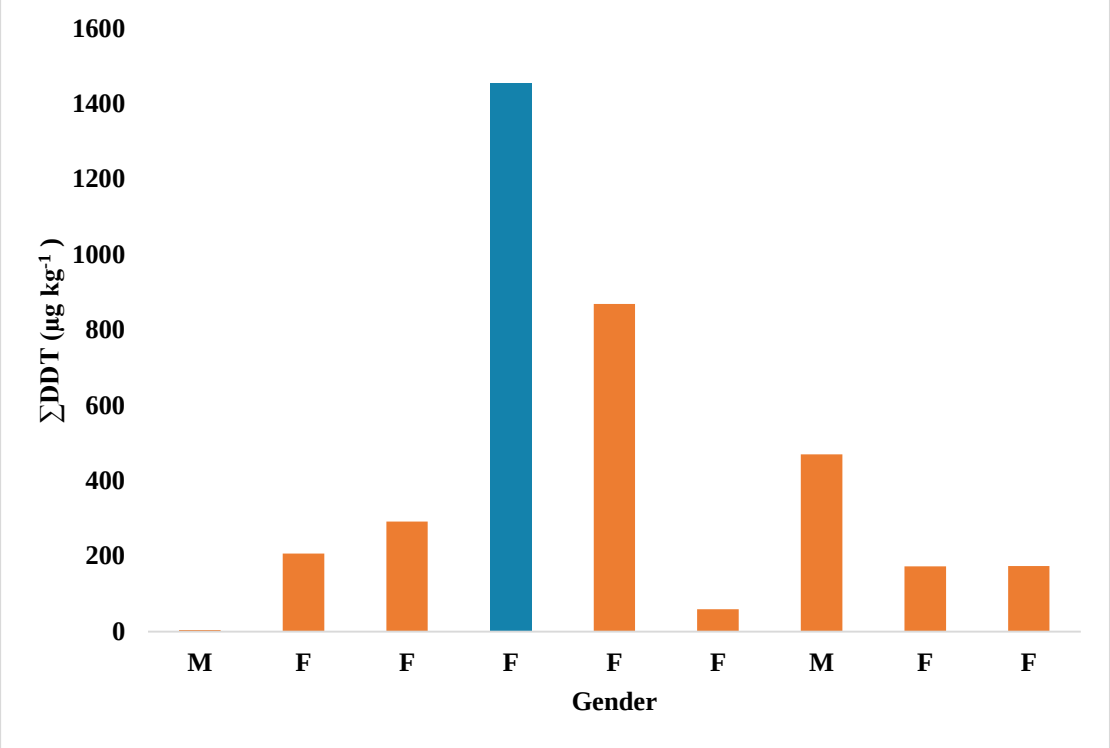


Fig-3.10: Bar diagram of ΣDDTs in human blood samples with respect of gender

3.3 Discussion

3.3.1. Method Validation

The analytical method was confirmed in terms of selectivity, linearity, sensitivity and recovery.

Selectivity

The **selectivity** (or specificity) was examined by the autogenously compounds interfering with the analytes were investigated by comparing the chromatograms of the standard,

blank soil/water/fish control sample and spiked samples **(Fig.-3.11)**. No interfering peaks were detected at the retention time of DDTs.

Linearity

The **linearity** of the GC machine was tested for DDTs by constructing calibration curves. Calibration curves were established for *4,4′*-DDE, *4,4′*-DDD, *2,4′*-DDT, and *4,4′*-DDT by plotting the peak area against the concentration of each of the standards. The calibration curves were linear to the correlation coefficients (R^2) ≥ 0.9950. The residual DDTs in the spiked and treated samples (soil, sediment, water, fish & blood) were also resolute using the calibration curves.

Sensitivity

The **sensitivity** of the instruments were evaluated by (S/N) limits of detection (LODs) and limits of quantifications (LOQs). The LODs and LOQs were found to be 0.005 and 0.0165 mg kg^{-1} for *4,4′*-DDE, *4,4′*-DDD, *2,4′*-DDT, and *4,4′*-DDT for all tested procedures in soil/sediment samples. On the other hand, the values were 0.04 and 0.132 µg L^{-1} respectively in water **(Table 3.2)**. At alteration to our results, Rissato *et al*. (2006) conveyed a lower LOQ (0.00001 mg kg^{-1}) and Kihampa and Mato (2009) found a lower LOD (0.0002 mg kg^{-1}) for *4,4′*-DDE, *4,4′*-DDD, *2,4′*-DDT, and *4,4′*-DDT in soil, the results, were different due to the differences in the used methods and/or machine.
The LODs and LOQs were found to be 0.0625 µg kg^{-1} and 0.2063 µg kg^{-1} respectively in fish samples (**Table-3.7**).

The LODs and LOQs were found to be 0.025 µg kg^{-1} and 0.082 µg kg^{-1} respectively in the blood sample (**Table 3.10**).

Recovery

Accuracy and **precision** (for soil, water, fish and human blood recoveries) were evaluated. The **accuracy**, expressed as a percentage of recovery. **Precision** was articulated as the relative standard deviation (RSD). The analytical precision of DDTs were extracted from spiked soil, water and fish matrices. The accuracy (72 – 120%) and precision (≤ 15%) were in concurrence with the series listed in the Codex guidelines (Codex Alimentarius, 2003).

The recoveries of the internal standards in human blood samples were 93% with RSD 12% (0.05 µg L^{-1}) (n=15) and 85% with RSD 6% (0.025 µg L^{-1}) (n=15) for CB-53. The procedures outlined for DDTs assessment in this study were adjudged to be reliable and efficient.

Table -3.14: Accuracy & Precision of DDTs in different matrix

Name of samples	Accuracy (%Recovery)	Precision (% RSD)
Soil	73 – 94	10.29
Water	83 – 110	9.5
Fish	73 – 113	16.34
Human blood	85 – 93	12

3.3.2 DDTs in Soil, Sediment and Water Samples

The soil/sediment nearby the factory was exposed to be extremely contaminated with DDT and its metabolites (**Table-3.4 & Fig-3.3**). The DDT concentrations were reduced with increasing distantness from the factory in the southern direction. However, this trend was not detected in the eastern direction. DDTs in CCC soil were calculated (Al Mahmmud *et al*. 2015). The amounts of separate DDTs were $0.04\times10^2 - 81.3\times10^2$ mg kg^{-1} (for *4,4′*-DDE), $0.03\times10^2 - 585\times10^2$ mg kg^{-1} (for *4,4′*-DDD), $0.001\times10^2 - 510\times10^2$ mg kg^{-1} (for *2,4′*-DDT), and $0.7\times10^2 - 2308\times10^2$ mg kg^{-1} (for *4,4′*-DDT). DDTs were originate 0.59 –3.01 μg L^{-1} in water samples, (**Table-3.5 & Fig- 3.4**). The concentrations of *4,4′*-DDT were established to be greater than those of *2,4′*-DDT in soil, sediment, and water (**Tables-3.4 and 3.5**).

These clearly notice that the contamination was conceded by technical-grade DDT, which mainly consists of 65 – 80% *4,4′*-DDT and 15 – 21% *2,4′*-DDT (Tomlin 2000). The maximum contamination of soil by DDTs was initiated up to 290 mg kg^{-1} (29 %) (**Table-3.4**) in 50-ft east samples. In line, Younas *et al*. (2013) described more concentrations of DDTs up to 65 % in surface soil samples at a former DDT producing factory in Amman Garh near Nowshera, Khyber Pakhtunkhwa, Pakistan. They also found more *4,4′*-DDT than those of *2,4′*-DDT and their metabolites (*4,4′*-DDE and *4,4′*-DDD). In addition, Elfvendahl *et al*. (2004) conveyed similar concentrations up to 28 % total DDTs in surface soil samples at a former pesticide 99 stock site in Tanzania.

If the ratio exceeds 0.5, the new basis exist *i.e.* current DDT input in the environment. (Covaci and Hurab, 2001, Zamir *et al*. 2008). In our study, the ratio was >0.5 in all samples (**Table-3.4 and 3.5**), which meant the contamination by DDT happened recently in all of the samples. But the factory was closed in 1993 and after that doubtlessly the huge stockpile was predisposed around the factory randomly. No information was obtained about the discarding time of the huge stockpile from the factory warehouse.

Enormous amounts of DDT were found in the soil, and in some places DDT had been vigorously discarded. Because of the conditions in such dumpsites, there was often no aerobic degradation of DDT.

No other information, excluding a survey and research by our research group in association with Department of Environment (DoE), exposed that soil in the CCC area was contaminated with DDTs (Nahar, 2006), though the factory was locked in 1993. In slightly associated reports by Zhang *et al*. (2009) who inspected organochlorine pesticides (OCPs) contamination in surface soils from two pesticide factories in Southeast China. In general, the total OCPs (largest concentration of DDTs) concentrations of surface soils from new and old factories were 0.84 and 166 mg kg^{-1}, respectively. The total OCPs concentration of old was approximately 200 times as much as that of new factory, since it has more than 30 years history than new factory. As the soils of the factories were highly contaminated with OCPs, the authors suggested on-site remediation technologies and the best available techniques/best environmental practices (BAT/BEP) should be carried out on these factories with the national implementation of the Stockholm Convention.

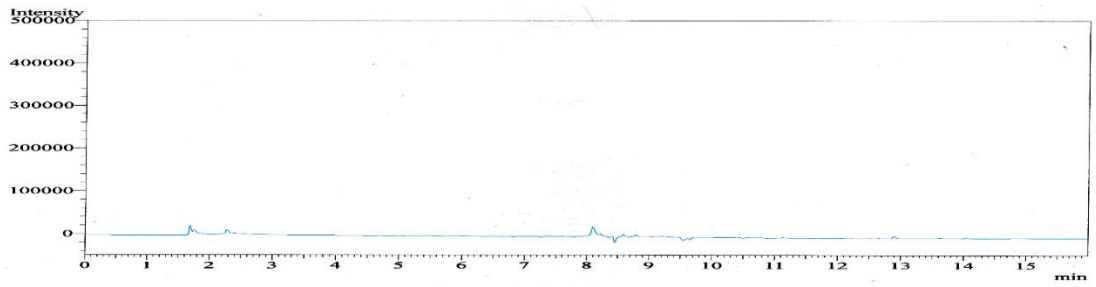
The effects of pesticide contaminations on aquatic ecosystems have been well studied in North America, Japan and many parts of Europe (Yamaguchi *et al*., 2003). In contrast, there is very little data on the levels of residues in developing countries (Albert, 1996). Therefore, monitoring of DDTs residues in developing countries is essential to determine its impacts on the environment. Moreover, human health would be seriously threatened by drinking water contaminated with these pollutants.

In another connected works by Dalla Villa *et al*. (2006) who considered the contamination level of DDT in soil of Mato Grosso, Brazil and dissipation of *4,4'*-DDT and *4,4'*-DDE of the area. After the embargo of organochlorine-pesticide use in Brazil, the beyond DDT was collected in Mato Grosso state and was deposited irregularly in an

open air area. They described that *4,4'*-DDT were 3800 – 7300 mg kg^{-1} in surface soil, 0.036 – 440 mg kg^{-1} in 30-40 cm deep soil, 0.069 -180 mg kg^{-1} in 90 – 100 cm deep soil in the nearby area of the contaminated zone. The authors decided that the *4,4'*-DDT is moving slowly downhill in the soil profile, however, the levels of this contaminant are high enough to present risk to underground water.

A survey of the soil was carried out to observe DDT levels of the contaminated soil by Khwaja (2008) in and around a destroyed DDT factory at Nowshera, NWFP of Pakistan. 81 soil samples were composed within 500 m of the old gate of the factory and at different depths in eight different directions. It was conveyed that 90.91% of the soil samples studied were contaminated with DDT, with 66.6% of the samples indicating residual DDT levels higher than DDT minimum risk level (MRL) in soil (0.05 mg kg^{-1}) and highest level of DDT found in the soil was 7.16 ± 1.70 mg kg^{-1}. But the contamination levels are much inferior to the soil of our CCC factory**).**

a)



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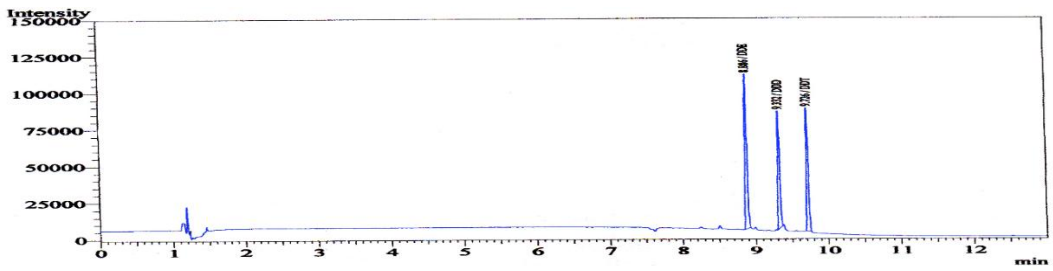

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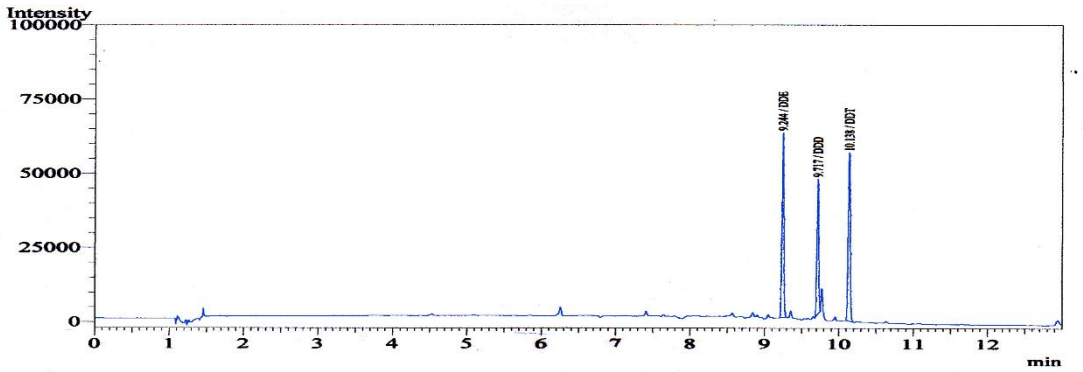

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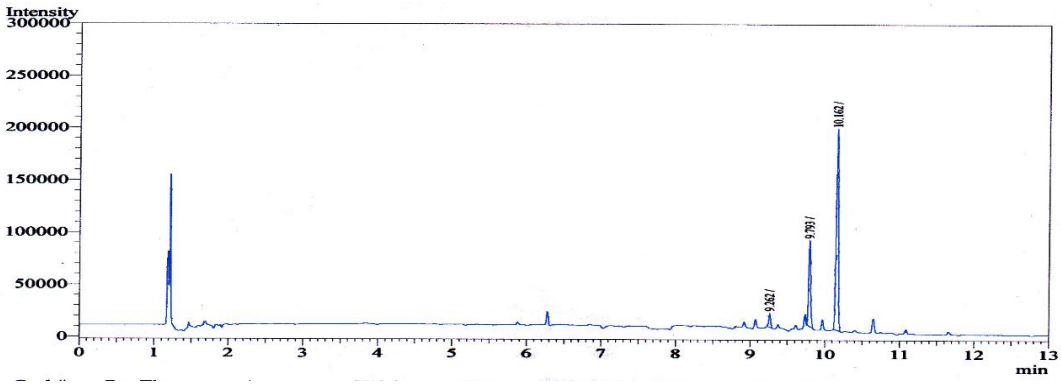

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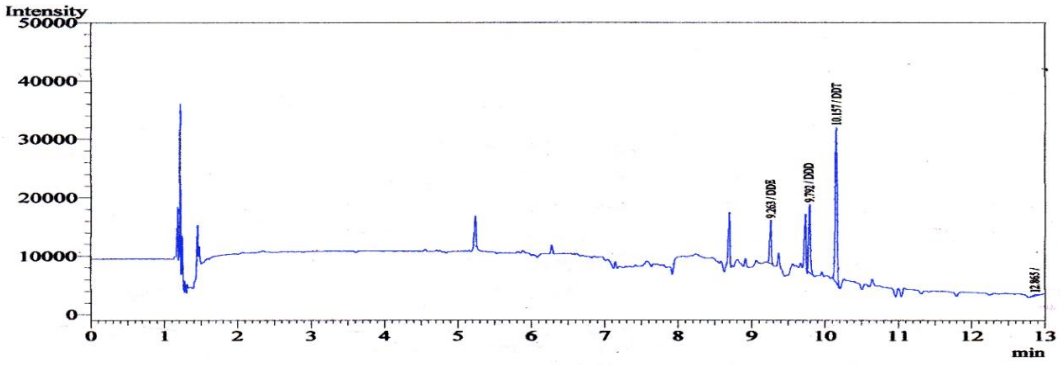


Fig. 3.11 : Gas chromatography electron capture detection of a) blank soil sample, b) DDT standards at 0.25 mg L^{-1}, c) fortified samples at 0.1 mg kg^{-1}, and d) soil samples obtained 20 ft south/west of the factory storage area e) water sample (sequence of peak: DDE, 9.26; DDD, 9.79; *2,4*-DDT, 10.14; and *4,4'*-DDT, 10.82)

3.3.3 Lipid Contents

The lipid contents of (n=15) fish samples were determined by evaporating to dryness with gentle flow of nitrogen. The lipid content was determined gravimetrically and found 0 − 6 % **(Table-3.8)** and **Bar diagram (Fig-3.6)**. Among them the highest amount of lipid content was found in shing fish.

The lipid content of (n=30) human blood samples were determined from the n-hexane/MTBE extract by evaporating to dryness with gentle flow of nitrogen. The lipid content was determined gravimetrically and found 0 − 2.45 % (**Table 3.11 & Fig-3.9)**.

3.3.4 DDTs in Fish Samples

Fish is an essential and irreplaceable food in the rural Bangladeshi diet. Contamination with toxic residual pesticides is at least partly responsible for fish mortality during hatching of eggs and growing of the post larvae (Hirose, 1975; Park *et al*., 2004; Singh and Singh, 2006).

Food is the primary route of exposure to DDTs. In animals, DDT causes adverse health effects such as reproductive and developmental failure and may cause immune system defects. DDT causes widespread contamination of water and soil resources, resulting in serious health effects in humans and animals (ATSDR, 2002). Anthropogenic activities and biochemical degradation of any original compound results the OCPs and its residues become contaminant to the environment. OCPs are known to be capable of long lasting resistance (McAloon and Mason 2003) to biodegradation and as a result food chains can be affected through their concentration and can be a reason of a momentous intensification at the end of the food chain (Shankararamakrishnan *et al*.2004; Darko and Acquaah 2007). As with many other OCPs, the nervous system is the major target of acute DDT exposure (RFI, 2001). Chronic exposure leads to formation of cancer cells

(Settimi *et al*. 2003), neurological and immunological effects (Donkin *et al*. 1996: Galloway and Handy 2003; Kamel and Hoppin 2004). On the other hand, there are abnormalities in reproductive and fetal development (Garcia *et al*. 1999; Yucra *et al.* 2006), enzyme inhibitor (Pesticide News 2000: Manirakiza *et al.* 2002) and endocrine disruption (Pesticide Trust 1995: Barlow 2005).

For the purpose of boosting food production, the Government of Bangladesh supplied organochlorine pesticides including POPs free of cost to the farmers till 1970 and at reduced price up to 1980. But due to the worldwide understanding of serious adverse effects of POPs and other organochlorine pesticides these were banned in Bangladesh in phases and the industry producing DDT was closed down in 1993.

DDT and its metabolites are not soluble in water but can be present as suspended materials associated with the phytoplankton, algae or through adsorption on soil or sediment. Fish and other aquatic organism can easily be contaminated by taking these suspended materials as their food. Being persistent organic pollutants, DDT and its metabolites can accumulate in the fatty tissues of living organism for long time and continuous consumption of contaminated fish by human may result into bio magnifications and cause various health problem. World Health Organization's Acceptable Daily Intake (ADI) allowance (0.02 mg kg^{-1}bw) (IPCS and IARC 2009) and the MRL value is 0.05 mg kg^{-1} (EPA, 2009). It is to be believed that estimated lethal dose for human 500 mg kg^{-1} (ICPS, 1976). DDT has half-life of 15 years (PULSE, 2009). The potential route of DDT contamination of aquatic ecosystems is atmospheric deposition (Bouwman *et al*. 2008). In addition the result of surface runoff is a possible illegal use of the pesticides in surrounding areas could also be possible sources of contamination (Bornman *et al*. 2007).

In this study, 15 fish samples were randomly collected from the closed down factory area to determine the presence of DDTs and to see bioaccumulation. Therefore, the wide

detection of DDTs in analyzed fish and prawn species may be related to the extensive applications of DDT in the surrounding environments. Food grains, vegetables, banana trees are cultivated on the bank of the pond of CCC area. The soil/sediment surrounding the factory was discovered to be highly contaminated with DDT and its metabolites (Al Mahmmud *et al*.2015).

There may be a relation between previous use of DDT as pesticide in crop production on the bank of the pond which could wash out into the adjacent water body and higher levels of DDT residues in the analyzed fishes. Reports from fish of other rivers and common fish markets also indicates the level of DDTs residues (Nahar *et al*. 2008, Amzad *et al*. 2016).

In present study the highest amount of DDT and its metabolites (8.9 μg kg^{-1}) were found in the Shing fish which might be due to its lipid content (Mustafa, 2006). Boal showed small amount of DDT and its metabolites (**Table-3.8** & **Fig- 3.6**).

Although, organochlorine pesticides were progressively banned in Bangladesh more than a decade ago and the DDT factory was closed in 1995, the present study show that organochlorine compounds have persisted in the environment. Findings of DDT and its metabolites in fish samples indicated that it can be found in other fresh water fish also. However, none of the samples were found to contain residual level exceeding the value (5.0 mg kg^{-1} for total DDT in fish) of Maximum Residue Limit (MRL) suggested by FAO/WHO (Codex, 1993).

If the ratio exceeds 0.5, the new basis exist *i.e*. current DDT input in the environment. (Covaci and Hurab, 2001, Zamir *et al*. 2008). The ratios of the *4,4'*-DDT/∑DDT were in the range of 0.01-0.46 which indicated that exposure to DDT is not due to recent uses.
DoE, Government Bangladesh (Hashmi, 2005) in collaboration with our group analyzed fresh and dry fish samples using less hazardous methods Malin, (1995) and reported the

presence of DDTs in dry fish samples. No detectable amount of DDTs were found in fresh fish samples of sea areas.

Pesticides reach water bodies or aquatic environment either by direct application or indirectly. The indirect sources include run off from agricultural fields, spray drifts, rainwater, sewage and effluent from the industries, manufacturing pesticides or using them in their process. The flood and rainwater carry a part of the agrochemical residues to the river or to different water body systems and the distribution may be scattered. So availability of pesticides in different water bodies are different.

3.3.5 Health Risk Estimates

The estimated daily intake (EDI) and health risk index (HI) were calculated for the contaminant of each fish. Here the HI of all fishes were found less than 1 (HI<1). From the obtained concentrations of DDTs residues, dietary exposure and health risks were calculated for adult.

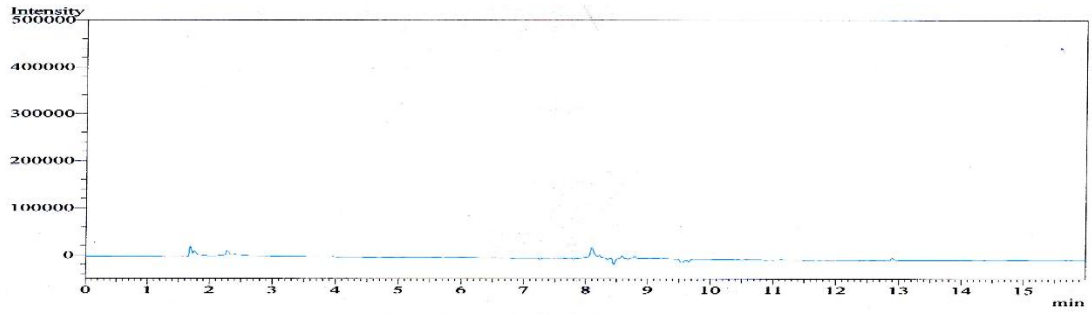
From a statistic analysis, the daily food intake of an adult person in our country exposes that fish is in fourth position after rice, cereal and vegetables, which give about 4% of the total daily food consumption in weight (Report of the household income and expenditure survey, 2011). Although detail study was absent 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.* 2009). Therefore it can be believed that although the HI of the studied fishes are less than 1 but together with other food items of daily meal, total HI would be greater than 1. This shows that there may be health risks related with lifetime consumption of the studied fish, tougher 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 associated with health hazards.

Table-3.15: Health risk assessment based on BMC

Sample ID	∑DDT µg kg^{-1}	EDI µg kg^{-1}/day	HR
MoF	4.55	4.01	0.0004
RF	1.14	1.00	0.0001
KaF	6.54	5.76	0.0006
MgF	8.9	7.84	0.0009
ChF	1.46	1.29	0.0001
ShiF	1.08	0.952	0.95×10^{-6}
TpF	1.09	0.961	0.96×10^{-6}
KatF	0.85	0.749	0.75×10^{-6}
BF	0.66	0.582	0.00006
KoF	2.9	2.56	0.0003
TeF	2.48	2.19	0.0002
ShF	2.18	1.92	0.0002
TaF	1.19	1.05	0.0001
ChF	1.42	1.25	0.0001
KarF	2.55	2.25	0.0002

a)



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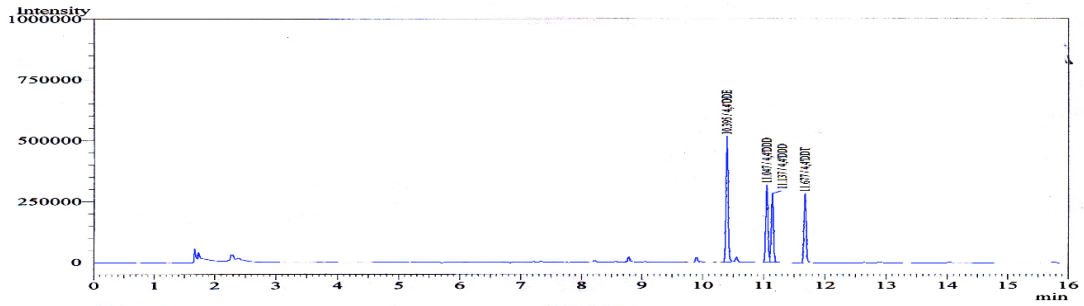

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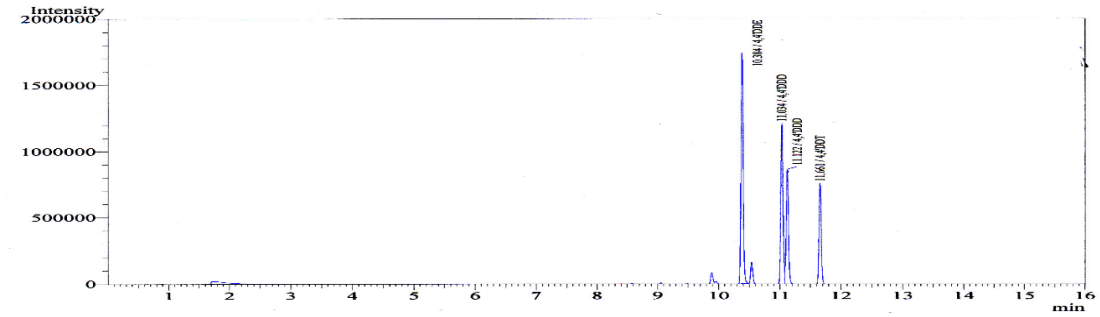

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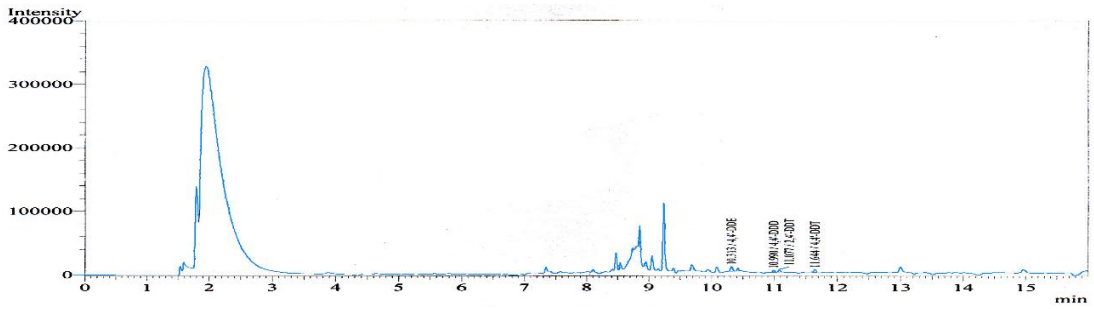


Fig-3.12: Gas chromatography electron capture detection of a) blank sample, b) DDTs standards at 0.05 mg L^{-1}, c) fortified samples at 0.25 mg kg^{-1}, and d) fish sample (sequence of peak: DDE, 10.39; DDD, 11.04; *2,4*-DDT, 11.14; and *4,4'*-DDT, 11.68)

3.3.6 DDTs in Human Blood Samples

In this study, human blood samples (n=30) were randomly collected from groups of men (n=20) and women (n=10) with different occupations in the closed down factory area to determine the presence of DDTs and to see bioaccumulation in different age groups.

Thirty human blood samples were analyzed for the presence of DDTs (**Table 3.13 & Fig-3.10**). The concentration were found 0 – 583 µg kg^{-1} for *4,4'*-DDE, 0 – 1376 µg kg^{-1} for *4,4'*-DDD, 0 – 435 µg kg^{-1} for *2,4'*-DDT and 0 – 337 µg kg^{-1} for *4,4'*-DDT.

The highest concentration of *4,4'*-DDE, *2,4'*-DDT & *4,4'*-DDT was detected in a male's blood sample whose age is 56 years and he worked in CCC for 20 years (583 µg kg^{-1}, 435 µg kg^{-1}& 337 µg kg^{-1}), respectively. And the highest concentration of *4,4'*-DDD was detected in female blood sample whose age is 50 years and she is a housewife (1376 µg kg^{-1}). This may be attributed to her higher lipid content (1.13%) and long life spend. DDT is lipophilic that accumulated in fat body. DDTs accumulation increases with age.

The highest ∑DDT was detected in male blood sample 1686 µg $kg^{-1}$and in female blood sample 1455 µg kg^{-1}. The range of *4,4'*-DDT/∑DDT ratio from 0.01 to 0.59.

Among the 30 human blood samples DDT and its metabolites were detected except one. That sample was a male's blood sample whose age is 60 and he worked in CCC for only two years. (MRL value of DDTs in human blood is 0.0005 mg/kg/day).

Since Bangladesh has started developing as an agricultural country it has encountered health problems due to contamination by OCPs. From this study it is revealed that the highest concentration of organochlorine compounds were detected from *4,4'*-DDD and interestingly it comes from its parent compound *4,4'*-DDT. My data has clearly shown

that it was early exposure to fresh commercial DDT as the *4,4'*-DDT/∑DDT ratio was low. In 100% blood samples the *4,4'*-DDT/∑DDT ratio was <0.5.

In early study September 2005 twenty four human blood samples were collected from children, teenagers and adults (male donors n=24) from Dhaka city and analyzed. The highest concentrations of *4,4'*-DDE (3600 µg kg^{-1}) and *4,4'*-DDT (380 µg kg^{-1}) were found in that study (Mamun *et al.*, 2007). Fish and dry fish are popular items in the daily diet of most Bangladeshi people. Background information revealed that all the donors ate fish almost every day and dry fish 2-3 days in a week. Accordingly a large dose of DDT has been found in dry fish.

In March–June 2006 a study was carried out by collecting ninety eight human blood samples from five professions of various parts of Bangladesh and analyzed. The highest concentrations of *4,4'*-DDE (2900 µg kg^{-1}) and *4,4'*-DDT (2300 µg kg^{-1}) were found in that study (R. Zamir *et al.*, 2008). Fish and dry fish are popular items in the daily diet of most Bangladeshi people specially low income group people like garment workers, fisher men, PDB workers, students of Dhaka university. Background information revealed that almost all fishermen apply indiscriminately high intensity of DDT while making fish into dry fish. So, the presences of DDT in blood samples are directly coming from DDT used by the professionals (fishermen) or indirectly from dry fish.

Another study in 2008 about seventy three blood samples were collected from children, living in five different areas of Dhaka, Bangladesh and analyzed. Showed that Bangladeshi children have rather high *4,4'*-DDT/*4,4'*-DDE ratio (mean 0.2; range 0.09−0.5) (L. Linderholm *et al.* 2011)**.**

Table -3.16: Comparison with other studies (only the highest level)

Place	Year	Month	No. of samples	*4,4′*-DDE µg kg^{-1}	*4,4′*-DDD µg kg^{-1}	*4,4′*-DDT µg kg^{-1}	∑*DDT* µg kg^{-1}	DDT/ ∑*DDT*
Dhaka	2005	September	24	3600	13	380	3900	0.24
Dhaka	2006	March	78	2900	130	2300	5260	0.56
Barisal	2006	June	20	480	10	1300	1460	0.89
CCC	2014	June	28	583	1376	337	1686	0.39

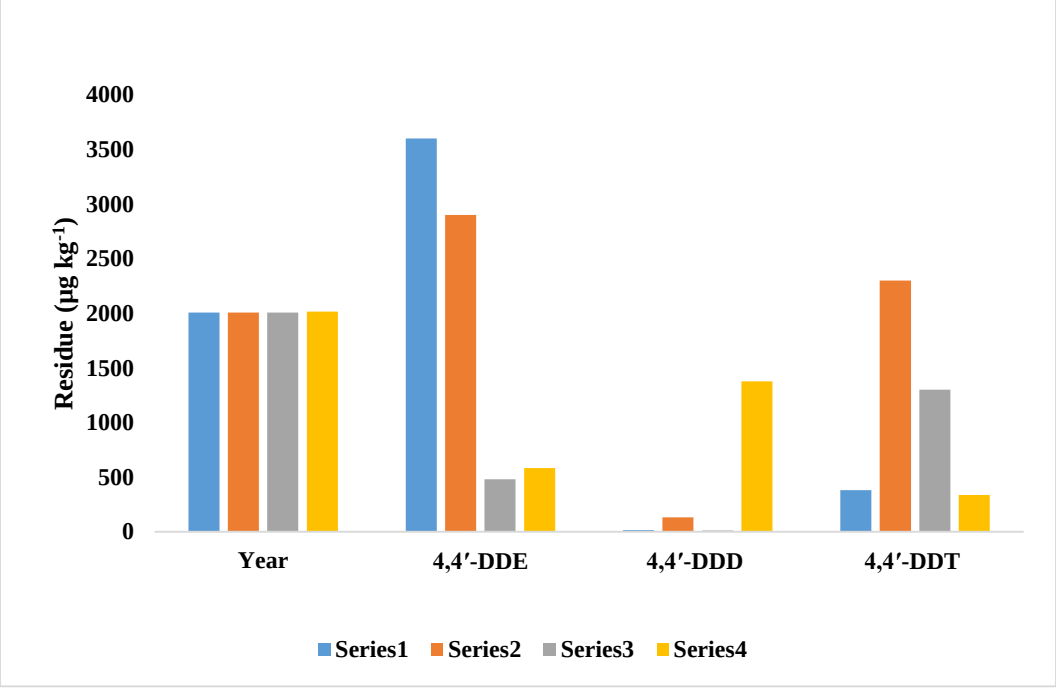


Fig-3.13: Bar diagram of comparison with other studies of DDTs in human blood samples

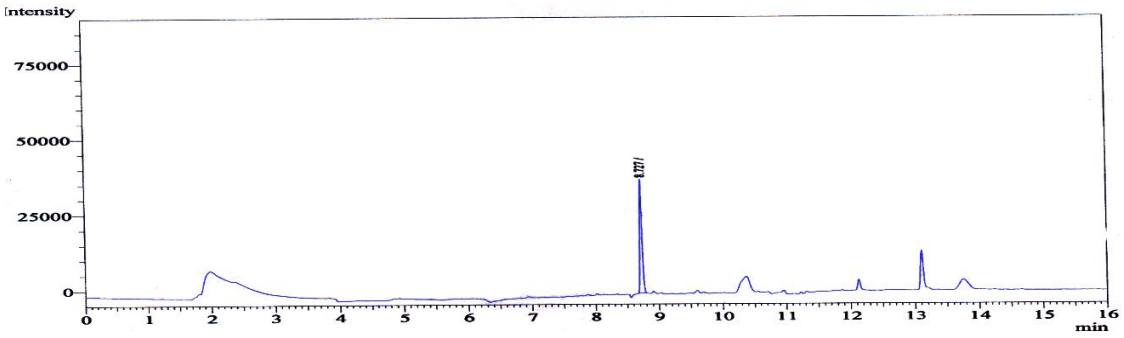
The levels of *4,4′*-DDE among the subjects from Bangladesh is however matched by humans from several countries, i.e., Mexico (Waliszewski *et al*., 1999), Nicaragua (Cuadra *et al*., 2006), Slovakia 322 (Petrik *et al*., 2006), Romania (Dirtu *et al*., 2006) and even Greenland (Bjerregaard *et al*., 2001).

From this studies the most abundant contaminant, in all groups studied, **4,4′**-DDD is dominating, and comes from its parent compound **4,4′**-DDT. With **4,4′**-DDT/∑DDT ratios indicating recent and ongoing DDT exposure.

Table -3.17: Values of DDTs & Ratio in different Sex, Age, Duration of Work in CCC & % Lipid

Values of DDTs	Sex		Age				Duration of work			% Lipid	
	Male (20)	Female (8)	Male		Female		>15 yr	<15 yr	Non worker	>0.5	<0.5
			>40 yr	<40 yr	>30 yr	<30 yr					
∑DDT (µg kg^{-1})	0-1686	9-1455	13-1686	11-470	9-1455	9-292	4-1686	0-868	9-1455	0-1686	11-292
Range of ratio	≤ 0.29	≤ 0.39	≤ 0.25	≤ 0.39	≤ 0.39	≤0.03	≤0.25	≤0.2	≤0.39	≤0.39	≤0.29

a)



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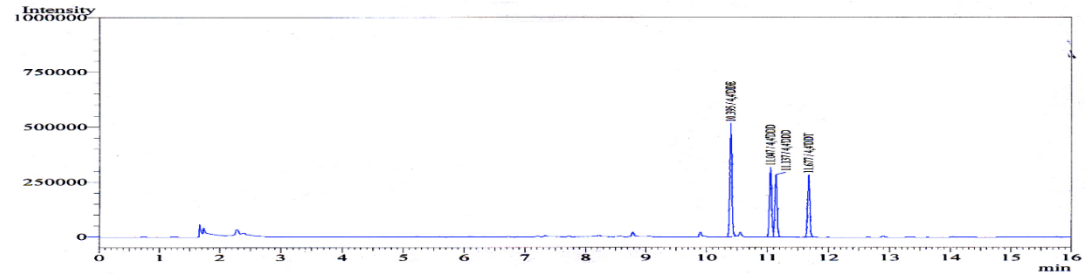

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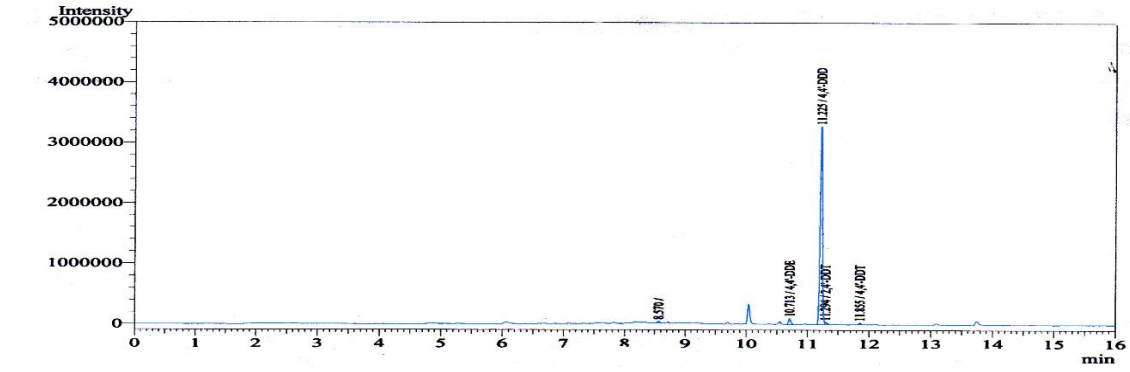


Fig-3.14: Gas chromatography electron capture detection of a) CB-53 standards at 0.025 mg L^{-1} , b) DDT standards at 0.25 mg $L^{-1}$and c) blood sample (sequence of peak: CB-53;8.73, DDE, 10.55; DDD, 11.22; *2,4*-DDT, 11.31; and *4,4'*-DDT, 11.87)

Table -3.18: Comparison with fish and human blood sample

Fish							Human blood						
Sample ID	**4,4'-DDE (µg kg^{-1})**	**4,4'-DDD (µg kg^{-1})**	**2,4'-DDT (µg kg^{-1})**	**4,4'-DDT (µg kg^{-1})**	**∑DDT (µg kg^{-1})**	**4,4'DDT/ ∑DDT**	**Sample ID**	**4,4'-DDE µg kg^{-1}**	**4,4'-DDD µg kg^{-1}**	**2,4'-DDT µg kg^{-1}**	**4,4'-DDT µg kg^{-1}**	**∑DDT µg kg^{-1}**	**4,4'DDT/ ∑DDT**
MoF	3.63±0.43	0.43±0.05	0.37±0.05	0.12±0.02	4.55	0.03	sample -01	34	810	13	11	868	0.01
RF	0.38±0.03	0.36±0.01	0.09±0.36	0.31±0.04	1.14	0.27	sample -02	0.61	13	4.6	2.8	21	0.13
KaF	2.71±1.68	0.80±0.12	0.02±0.01	3.02±0.93	6.54	0.46	sample -03	2.6	36	6.4	6.4	51	0.12
MgF	0.22±0.03	0.06±0.01	0.42±0.07	0.38±0.07	1.08	0.35	sample -04	2.3	7.0	8.1	5.7	23	0.25
ChF	0.54±0.08	0.76±0.03	0.08±0.01	0.08±3.02	1.46	0.05	sample -05	2.0	27	4.3	2.3	36	0.06
ShiF	4.75±0.60	2.68±0.34	0.82±0.13	0.65±1.08	8.9	0.07	sample -06	583	331	435	337	1686	0.20
TpF	0.10±0.01	0.39±0.01	0.45±0.05	0.15±0.05	1.09	0.14	sample -07	3.0	214	BDL	BDL	217	0
KatF	0.37±0.01	0.26±0.04	0.14±0.02	0.08±2.05	0.85	0.09	sample -08	1.6	40	9.2	7.7	59	0.13
BF	0.27±0.01	0.21±0.02	0.09±0.01	0.09±0.03	0.66	0.14	sample -09	1.5	1.5	7.9	3.5	14	0.24
KoF	1.09±0.05	1.14±0.14	0.65±0.04	1.42±0.07	2.9	0.01	sample -10	1.9	4.1	4.4	6.5	17	0.39
TeF	1.05±0.04	1.32±0.03	0.05±0.02	0.06±.12	2.48	0.02	sample -11	0.70	2.4	2.9	BDL	5.95	0
ShF	1.78±0.31	0.17±0.01	0.16±0.01	0.07±0.65	2.18	0.03	sample -12	4.3	3.6	3.1	1.8	13	0.14
TaF	0.56±0.04	0.22±0.02	0.36±0.04	0.05±0.67	1.19	0.04	sample -13	0.82	6.9	BDL	0.97	8.7	0.11
ChF	0.37±0.04	0.63±0.09	0.16±0.04	0.26±0.05	1.42	0.18	sample -14	0.77	2.6	4.3	3.1	11	0.29
KarF	0.94±0.09	1.14±0.03	0.18±0.6	0.29±0.9	2.55	0.11	sample -15	3.1	3.3	7.8	1.0	15	0.07

Fish							Human blood						
Sample ID	**4,4'-DDE (µg kg^{-1})**	**4,4'-DDD (µg kg^{-1})**	**2,4'-DDT (µg kg^{-1})**	**4,4'-DDT (µg kg^{-1})**	**∑DDT (µg kg^{-1})**	**4,4'DDT/ ∑DDT**	**Sample ID**	**4,4'-DDE µg kg^{-1}**	**4,4'-DDD µg kg^{-1}**	**2,4'-DDT µg kg^{-1}**	**4,4'-DDT µg kg^{-1}**	**∑DDT µg kg^{-1}**	**4,4'DDT/ ∑DDT**
MoF	3.63±0.43	0.43±0.05	0.37±0.05	0.12±0.02	4.55	0.03	sample -16	20	1295	59	34	1408	0.02
RF	0.38±0.03	0.36±0.01	0.09±0.36	0.31±0.04	1.14	0.27	sample -17	0.39	9.7	5.2	3.9	19	0.20
KaF	2.71±1.68	0.80±0.12	0.02±0.01	3.02±0.93	6.54	0.46	sample -18	10	7.20	9.33	9.32	36	0.26
MgF	0.22±0.03	0.06±0.01	0.42±0.07	0.38±0.07	1.08	0.35	sample -19	2.60	0.47	3.11	2.20	8.37	0.28
ChF	0.54±0.08	0.76±0.03	0.08±0.01	0.08±3.02	1.46	0.05	sample -20	6.05	10	3.42	4.82	24	0.20
ShiF	4.75±0.60	2.68±0.34	0.82±0.13	0.65±1.08	8.9	0.07	sample -21	BDL	BDL	BDL	BDL	BDL	BDL
TpF	0.10±0.01	0.39±0.01	0.45±0.05	0.15±0.05	1.09	0.14	sample -22	0.02	0.02	2.92	1.08	3.99	0.27
KatF	0.37±0.01	0.26±0.04	0.14±0.02	0.08±2.05	0.85	0.09	sample -23	71	137	BDL	BDL	207	0
BF	0.27±0.01	0.21±0.02	0.09±0.01	0.09±0.03	0.66	0.14	sample -24	3.9	266	13	10	292	0.03
KoF	1.09±0.05	1.14±0.14	0.65±0.04	1.42±0.07	2.9	0.01	sample -25	31	1376	6.6	41	1455	0.03
TeF	1.05±0.04	1.32±0.03	0.05±0.02	0.06±.12	2.48	0.02	sample -26	19	845	22	9.4	869	0.01
ShF	1.78±0.31	0.17±0.01	0.16±0.01	0.07±0.65	2.18	0.03	sample -27	0.51	51	4.32	2.4	59	0.04
TaF	0.56±0.04	0.22±0.02	0.36±0.04	0.05±0.67	1.19	0.04	sample -28	69	313	36	52	470	0.11
ChF	0.37±0.04	0.63±0.09	0.16±0.04	0.26±0.05	1.42	0.18	sample -29	8.7	39	23	102	173	0.59
KarF	0.94±0.09	1.14±0.03	0.18±0.6	0.29±0.9	2.55	0.11	sample -30	10	45	25	94	174	0.54

B. Results and Discussion

3.4 Pesticide Residues in Vegetable Samples

3.4.1 Dissipation Pattern of Cypermethrin in Five Different Vegetable Samples

3.4.1.1 Recovery Experiment of Cypermethrin

Vegetable samples were collected from the farmer's field before pesticides were sprayed which samples were used as the control samples. These were found to contain no targeted pesticides by doing blank experiments. No peaks were found in the retention time of pesticide in matrix (vegetables) control extract. The recovery experiments were conducted at two spiking levels (0.25 mg kg^{-1} & 1 mg kg^{-1}) in three replicate analysis**.**

Table- 3.19: Recovery experiment of cypermethrin in vegetable samples

Vegetables	**% Recovery ± RSD % (n=3)**	
	Spiking level	
	0.25 mg kg^{-1}	**1 mg kg^{-1}**
Eggplant	95±5.35	87±6.01
Pumpkin	81±6.97	79±7.37
Tomato	102±7.65	83±8.48
Bitter gourd	98±11.45	77±6.78
Green Chili	101±9.86	95±3.76

3.4.1.2 Calibration Curve

The prepared standard cypermethrin solutions were analyzed with GC-ECD. The calibration curves were prepared by two levels. Lower level (0.001−0.02 mg L^{-1}) and higher level (0.02−1 mg L^{-1}).

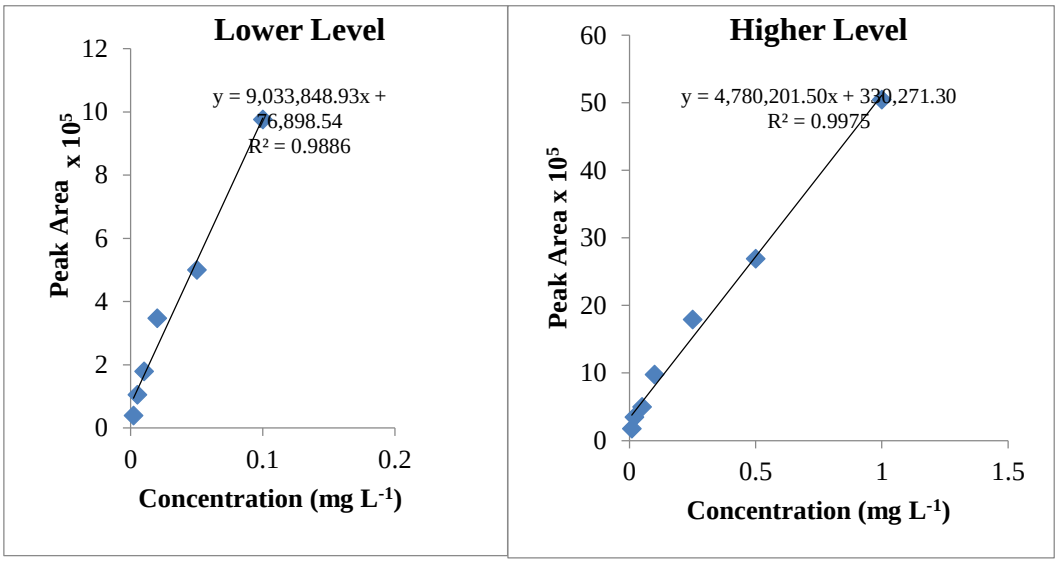


Fig-3.15: Calibration curves for vegetable samples (Lower & Higher level)

3.4.1.3 Matrix Matched Calibration

Matrix matched calibration curves of cypermethrin were prepared in matrices that was extracted from control samples *i.e.* eggplant, pumpkin, tomato, bitter gourd and green chilli. The matrix matched calibration curves of cypermethrin in eggplant, pumpkin, tomato, bitter gourd and green chilli were constructed in the concentration range of 0.025 – 2 mg L^{-1}. The linearities were excellent with a correlation coefficient of R^2 > 0.9990 (**Table- 3.20**).

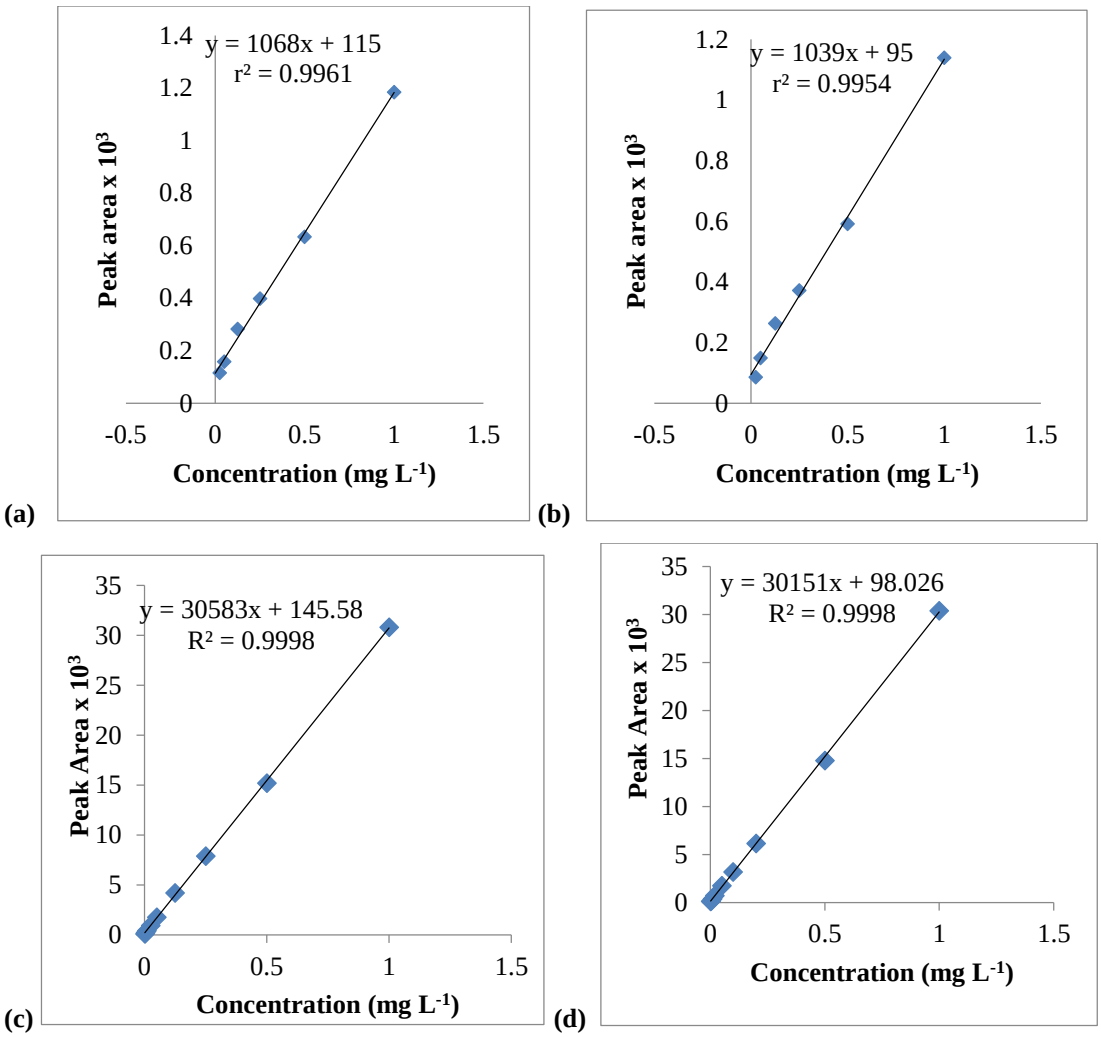


Fig-3.16: Matrix matched calibration curves (a) eggplant (b) tomato (c) Bitter gourd (d) Green chilli

3.4.1.4 Quantification

The residual level of cypermethrin in eggplant, pumpkin, tomato, bitter gourd and green chilli were quantified by GC-ECD (Shimadzu−2010 Japan). The Linearity of the ECD was controlled by running series of standard dilution mixtures. LOD and LOQ of each of the standard sample were given in (**Table-3.20**).

Table-3.20: Retention times (RT), correlation coefficients (R^2), LODs and LOQs of cypermethrin in vegetable samples

Vegetables	Linear range (mg L^{-1})	Linear range for Matrix Matched	RT (min)	Linearity (R^2)	LOD (µg kg^{-1})	LOQ (µg kg^{-1})
Egg plant	0.001– 0.5	0.025 – 2	9.56	0.9961	0.01	0.033
Pumpkin				0.9984		
Tomato				0.9954		
Bitter gourd				0.9998		
Green chili				0.9998		

3.4.1.5 Cypermethrin Residues in Vegetable Samples

Use of pesticide is necessary to increase crop production. However, unselective use of insecticides and without maintaining the proper pre-harvest interval can make the food unsafe, especially when tomatoes are used as salad (N. Nahah *et al*. 2012). Cypermethrin is a modern pesticide that undergoes degradation quickly. During the present study, selected five vegetables are usually cultivated everywhere in Bangladesh. Cypermethrin was applied to the vegetables in the farmers' fields at the recommended dose. The residue levels were determined of the five vegetables.

Samples were extracted by QuEChERS method following on (Mastovska *et al*., 2010) with some modification. The extract was evaporated under reduced pressure and cleaned up by dispersive solid phase method and then analyzed by GC-ECD (**Table 3.21).**

Statistical analysis were done *i.e*. mean, standard deviations, correlation coefficients (R^2), LODs, LOQs were also calculated.

Table-3.21: Cypermethrin residues (Average ± SD, µg kg^{-1}) in vegetable samples

Samples (n=3)	Day after spraying	Cypermethrin in Tomato (µg kg^{-1})	Cypermethrin in Egg plant (µg kg^{-1})	Cypermethrin in Bitter gourd (µg kg^{-1})	Cypermethrin in Green chili (µg kg^{-1})	Cypermethrin in Pumpkin (µg kg^{-1})
Control	-	ND	ND	ND	ND	ND
Samples after pesticides spraying	0	157.67±4.65	113.51±4.99	133.67±3.42	88.13±5.26	54.53±5.23
	1	143.14 ±14.33	106±7.54	125.25±7.98	74.50±3.10	43.68±1.53
	3	82.21±6.19	83.42±4.92	89.39±7.67	49.32±2.36	34.55±5.66
	5	74.99±2.87	44.23±0.15	48.42±4.71	36.38±1.57	17.03±1.87
	8	40.19±2.14	28.57±0.77	35.02±4.60	26.75±1.14	8.17±1.01
	10	28.64±5.51	22.66±3.37	26.84±2.48	19.00±1.33	–
	12	18.54±2.67	16.19±2.87	–	–	–
	MRL	0.2 mg kg^{-1} (Codex 2013) 0.5 mg kg^{-1} (EU 2009)	0.03 mg kg^{-1} (Codex 2009) 0.5 mg kg^{-1} (EU 2009)	2 mg kg^{-1} (Codex 2009)		

*ND – Not Detected

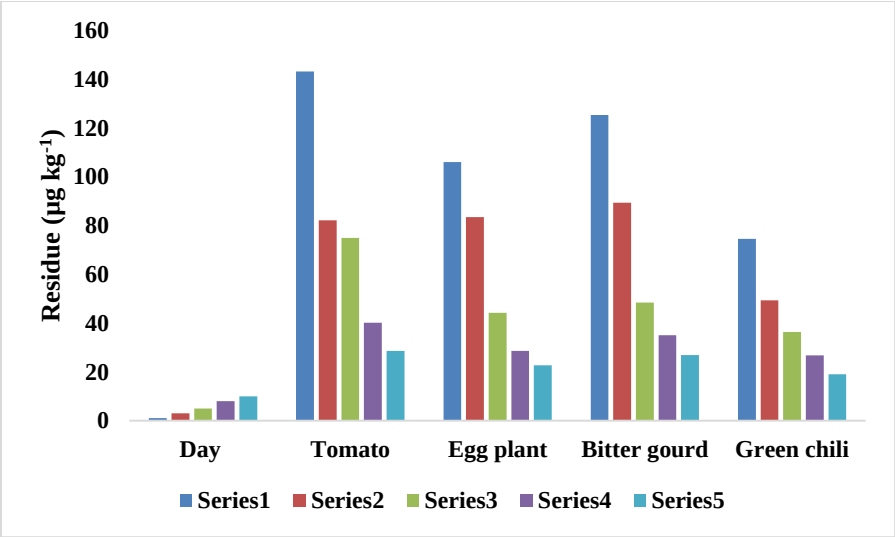


Fig- 3.17: Bar diagram of Cypermethrin in four Vegetables

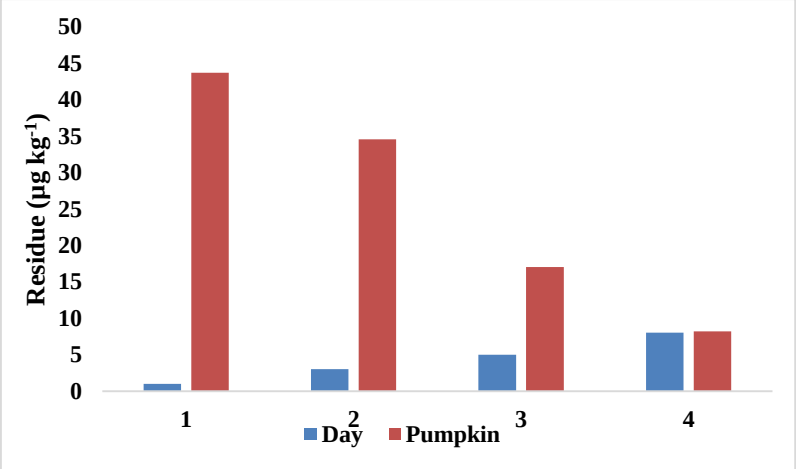


Fig- 3.18: Bar diagram of Cypermethrin in Pumpkin

3.4.1.6 Dissipation Studies of Cypermethrin in Vegetable Samples

The analytical method was applied to field samples to determine the dissipation pattern of total cypermethrin in five different vegetables. The dissipation kinetics of total cypermethrin in different vegetables were determined by plotting the residue concentration against time. Mean concentrations of each sample (three replicates) were obtained and plotted against day after pesticide spraying for all samples in recommended dose separately to prepare dissipation curves (**Fig.-3.19).**

The residual concentration and half-life of cypermethrin were calculated by first-order kinetics equations $A=A_0e^{-kt}$ and $t_{1/2} = \ln 2/k$ respectively, where t is time (days) after pesticide application, A_0 is an initial pesticide concentration after application (at t=0), k is a dissipation coefficient and $t_{1/2}$ is defined as the time required for pesticide residue level to fall to half the initial residue level after application (Park 2011),.

Table-3.22: Dissipation pattern (%) of Cypermethrin in vegetable samples

Day after spraying	Cypermethrin in Tomato	Cypermethrin in Egg plant	Cypermethrin in Bitter gourd	Cypermethrin in Green chili	Cypermethrin in Pumpkin
0	-	–	–	–	–
1	9.22	6.12	6.05	15.91	18.52
3	42.57	21.70	28.80	33.78	20.90
5	47.61	58.49	61.60	51.35	61.03
8	71.92	72.64	72.00	63.51	81.30
10	79.99	78.30	78.40	74.32	–
12	87.05	84.91	–	–	–
Equation	$Y=163.29e^{-0.18x}$	$Y=127.52e^{-0.18x}$	$Y=139.46e^{-0.17x}$	$Y=97.45e^{-0.14x}$	$Y=62.06e^{-0.25x}$
Regrassion	$R^2=0.99$	$R^2=0.98$	$R^2=0.97$	$R^2=0.98$	$R^2=0.98$
Half life	$t_{1/2} = 3.85$	$t_{1/2} = 3.85$	$t_{1/2} = 4.08$	$t_{1/2} = 4.95$	$t_{1/2} = 2.77$

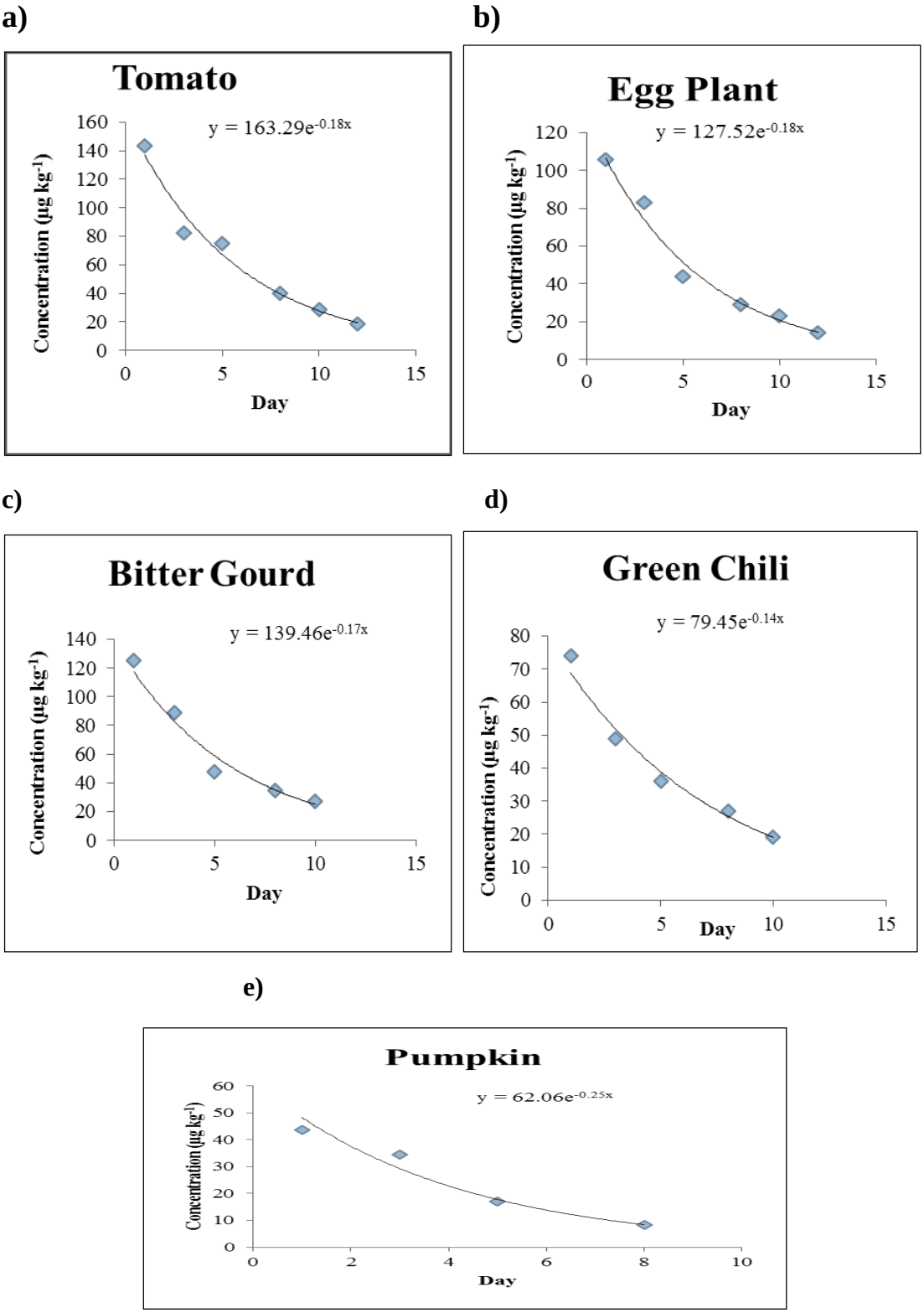


Fig-3.19: Dissipation curves of (a) Tomato, (b) Eggplant, (c) Bitter gourd, (d) Green chili & (e) Pumpkin.

3.4.1.7 Storage Stability of Cypermethrin

Average recovery of cypermethrin was found to be 83% (RSD 9) which showed that the pesticide was quite stable at -20 °C storage condition for 40 days because these values were also consistent with the ranges listed in the Codex guidelines (Codex, 2003). The stability results revealed that the cypermethrin was not degraded in vegetables matrix during the period of analysis.

3.4.2 Dissipation Pattern of Fluxapyroxad in Moie Leaves

3.4.2.1 Recovery Experiment

The extraction efficiency of the analytical procedure was evaluated via recovery experiments. A QuEChERS method was developed base on a method described by Anastassiades *et al* (2003) for the analysis of fluxapyroxad in moie leaves after some modification. The recovery experiments were conducted at two spiking levels (0.1 mg kg^{-1} & 0.5 mg kg^{-1}) in three replicate analysis.

Table-3.23: Recovery experiment of fluxapyroxad in moie leaves

Pesticide	**% Recovery ± RSD %** **(n=3)**	
	Spiking level	
	0.1 mg kg^{-1}	**0.5 mg kg^{-1}**
Fluxapyroxad	88±8.79	93±1.69

3.4.2.2 Calibration Curve

The prepared standard fluxapyroxad solutions were analyzed by LC–MS/MS. The calibration curve was linear over the range of the concentrations and the correlation coefficients (R^2) for the linearity range was 0.9985.

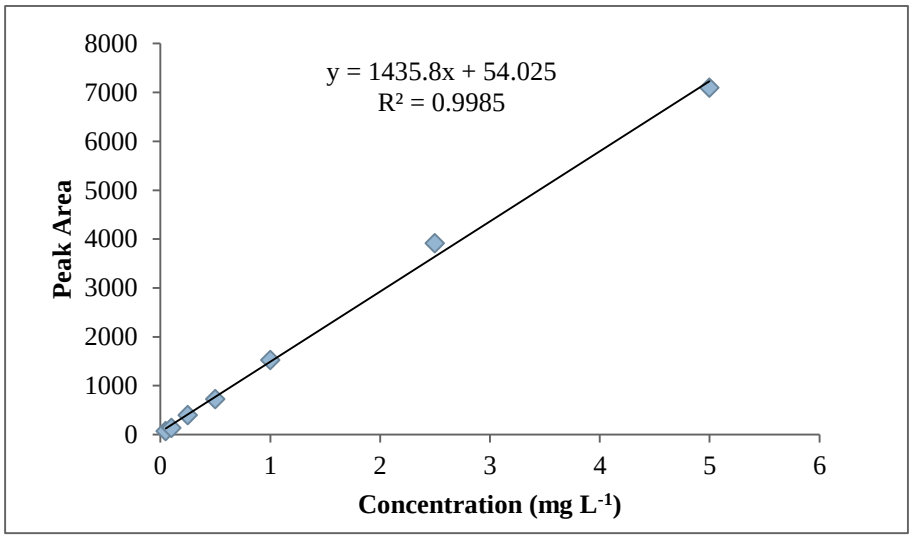


Fig-3.20: Calibration curve of Fluxapyroxad

3.4.2.3 Matrix Matched Calibration

Control samples were extracted and cleaned-up. 1 mL cleaned-up solution was evaporated upto dryness. Then added 1 mL different diluted standard solutions and vortexed. Matrix matched calibration standards were prepared and then injected in LC–MS/MS.

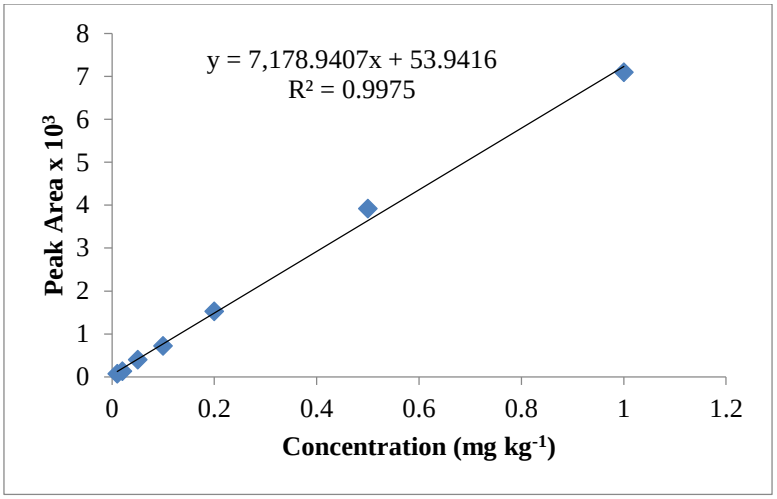


Fig-3.21: Matrix matched calibration curve of fluxapyroxad in moie leaves

3.4.2.4 Quantification

The concentrations of fluxapyroxad in moie leaf was quantified by LC–MS/MS (Water 2695). The Linearity of the LC–MS/MS was prepared by running series of dilution mixtures standards. LOD & LOQ were also measured (**Table -3.24**).

Table-3.24: Retention times (RT), correlation coefficients (R^2), LOD, LOQ and main ions in mass spectra of fluxapyroxad in moie leaf

Pesticide	Linear range (mg L^{-1})	Linearity (R^2)	Linear range for MM (mg L^{-1})	RT (min)	Linearity (R^2)	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Main ions (m/z)
Fluxapyroxad	0.01–1	0.9975	0.05 – 5	3.66	0.9989	0.01	0.033	380, 131

3.4.2.5 Fluxapyroxad Residues in Moie Leaves

Leafy vegetable moie grow everywhere in South Korea. Pesticides are used extensively by the farmers in the production of vegetables to fulfill the need of food. During the present study, Fluxapyroxad was applied to moie leaves in the experimental fields at the recommended dose. Moie leaves were harvested at 0, 1, 3, 5, 7, 10 and 15 days after application of the pesticide following standard protocol of WHO guideline. The residue level of fluxapyroxad was determined in moie leaves. Samples were extracted by QuEChERS method and purified with solid phase extraction (SPE) cartridge (Si – 1 and analyzed by LC–MS/MS (**Table 3.24)**.
Statistical analysis were done *i.e.* mean standard deviations, correlation coefficients (R^2), LODs, LOQs were also calculated.

Table-3.25.: Fluxapyroxad residues (with half-life & % dissipation) in moie leaves at various time intervals following different application rates (T2 & T3)

Day after application	Treated twice (T2) Plot A		Treated thrice (T3) Plot B		Untreated Plot
	Residue (Average±SD) (µg kg^{-1})	Dissipation (%)	Residue (Average±SD) (µg kg^{-1})	Dissipation (%)	
0	10.87±0.48	–	19.58±1.81	–	ND
1	8.38±0.29	22.91	14.77±1.58	24.57	
3	5.29±0.68	51.33	13.54±0.13	30.85	
5	3.81±0.97	64.95	5.64±0.27	71.20	
7	2.34±0.09	76.82	3.47±0.52	82.28	
10	0.92±0.56	91.54	2.01±0.18	89.73	
14	0.47±0.10	95.68	0.36±0.17	98.16	
$Y = 10.913e^{-0.228x}$ $R^2 = 0.9916$ Half life, $t_{1/2} = 3.04$			$Y = 19.58e^{-0.259x}$ $R^2 = 0.9654$ Half life, $t_{1/2} = 2.68$		

*ND – Not Detected

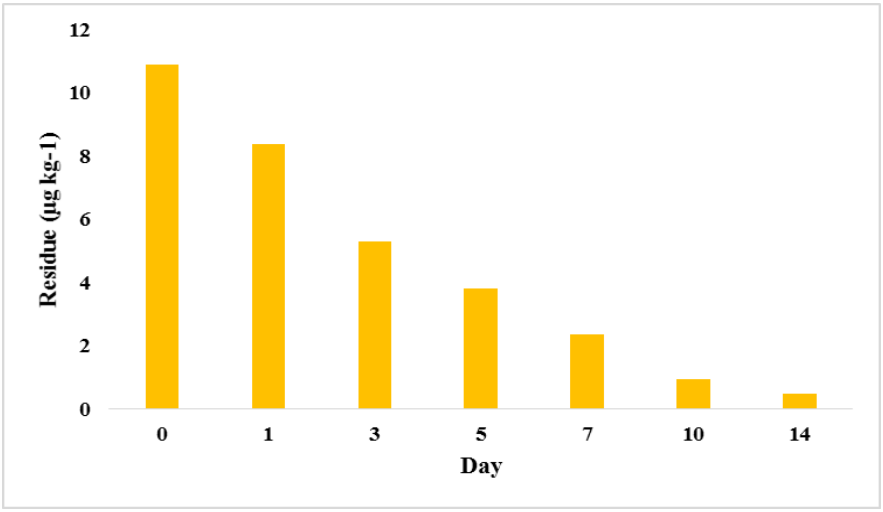


Fig- 3.22: Bar diagram of fluxapyroxad in moie leaves at T2

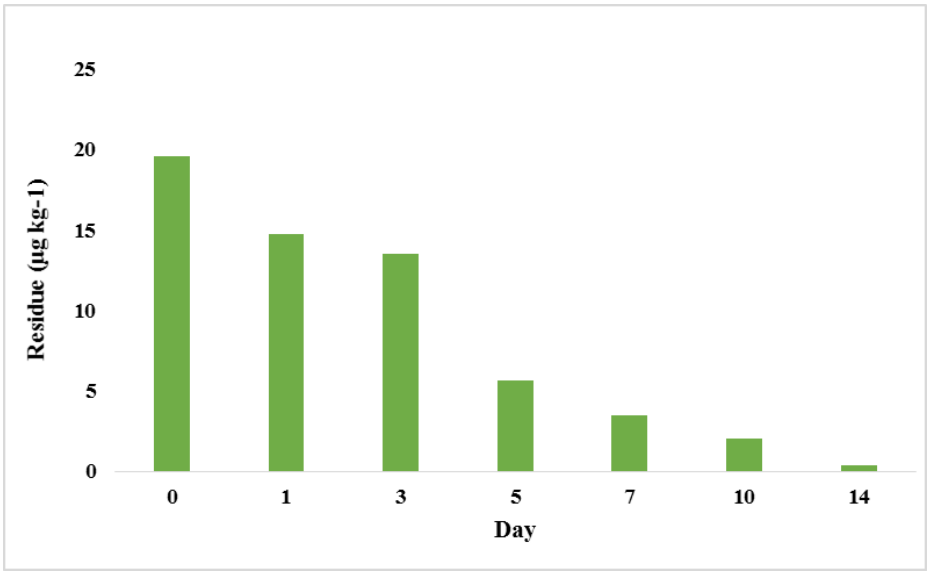


Fig- 3.23: Bar diagram of fluxapyroxad in moie leaves at T3

3.4.2.6 Dissipation Studies of Fluxapyroxad Residues in Moie Leaves

The investigative method was applied to field samples to determine the dissipation pattern of total fluxapyroxad in moie leaves. The half-life ($t_{1/2}$) of the residues was established, which provides information about the persistence of pesticides in crops and calculated as ln0.5/k (Putnam *et al*. 2003). The initial deposit of total

fluxapyroxad in moie leaves were 11 and 20 µg kg^{-1} for double and triple doses, respectively (**Table-3.24**).

The dissipation regressive equation were:

$Y = 10.913e^{-0.228x}$ ($R^2 = 0.9916$) (**Fig-3.25**) for double doses and

$Y = 19.58e^{-0.259x}$ ($R^2 = 0.9654$) (**Fig-3.26**) for triple doses of application.

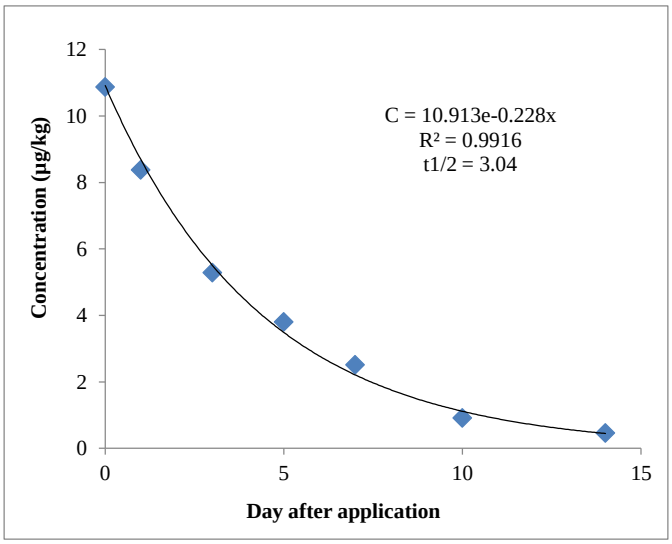


Fig-3.224: Dissipation curves of fluxapyroxad in moie leaves for treated twice (T2)

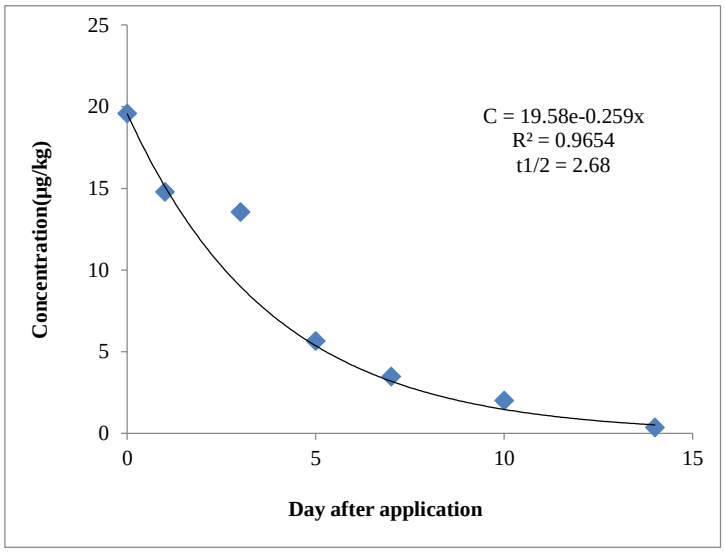


Fig-3.25: Dissipation curves of fluxapyroxad in moie leaves for treated thrice (T3)

3.4.2.7 Storage Stability of Fluxapyroxad

Average recovery of fluxapyroxad was found to be 95% (RSD 7), which showed that fluxapyroxad was quite stable at -24 °C storage condition for 40 days because these values were also consistent with the ranges listed in the Codex guidelines (Codex, 2003). The stability results revealed that the fluxapyroxad was not degraded in moie leaves matrix during period of analysis.

3.4.3 Analysis of Pesticide Residues in Some Vegetable Samples Grown in Bangladesh during Summer Season

3.4.3.1 Recovery Experiment

Vegetable samples were collected from the farmer's field before pesticides spray were used as the control samples. These were found to contain no targeted pesticides by doing blank experiments. The recoveries were calculated (n=3) at two spiking concentrations (0.25 and 0.5 mg kg^{-1}) with RSD below 7% (**Table-3.26**).

Table-3.26: Pesticides Recovery experiment in vegetable sample

Vegetables	Pesticides	% Recovery ± RSD % (n=3)	
		Spiking level	
		(0.25 mg kg^{-1})	(0.5 mg kg^{-1})
Chichinga	Diazinon	93±3.55	104±3.26
	Chlropyrifos	86±1.23	95±2.88
	Cypermethrin	83±2.76	85±2.69
	Fenvelarate	81±3.39	94±1.29
Jhinga	Diazinon	85±1.76	97±1.76
	Chlropyrifos	87±3.60	98±1.32
	Cypermethrin	115±4.51	97±1.57
	Fenvelarate	84±4.84	96±2.33
Dhundol	Diazinon	95±1.12	102±4.04
	Chlropyrifos	79±3.79	80±6.28
	Cypermethrin	92±4.11	88±1.78
	Fenvelarate	73±3.98	82±7.92
Patol	Diazinon	84±1.48	93±1.54
	Chlropyrifos	93±1.77	90±2.85
	Cypermethrin	89±4.17	98±1.58
	Fenvelarate	96±0.94	94±1.81

3.4.3.2 Calibration Curve

Calibration curves were constructed in the concentration range of 0.025 – 2 mg L^{-1}. The linearities were excellent with a correlation coefficient of $R^2 > 0.999$ (**Table-3.27 & Fig.-3.26**).

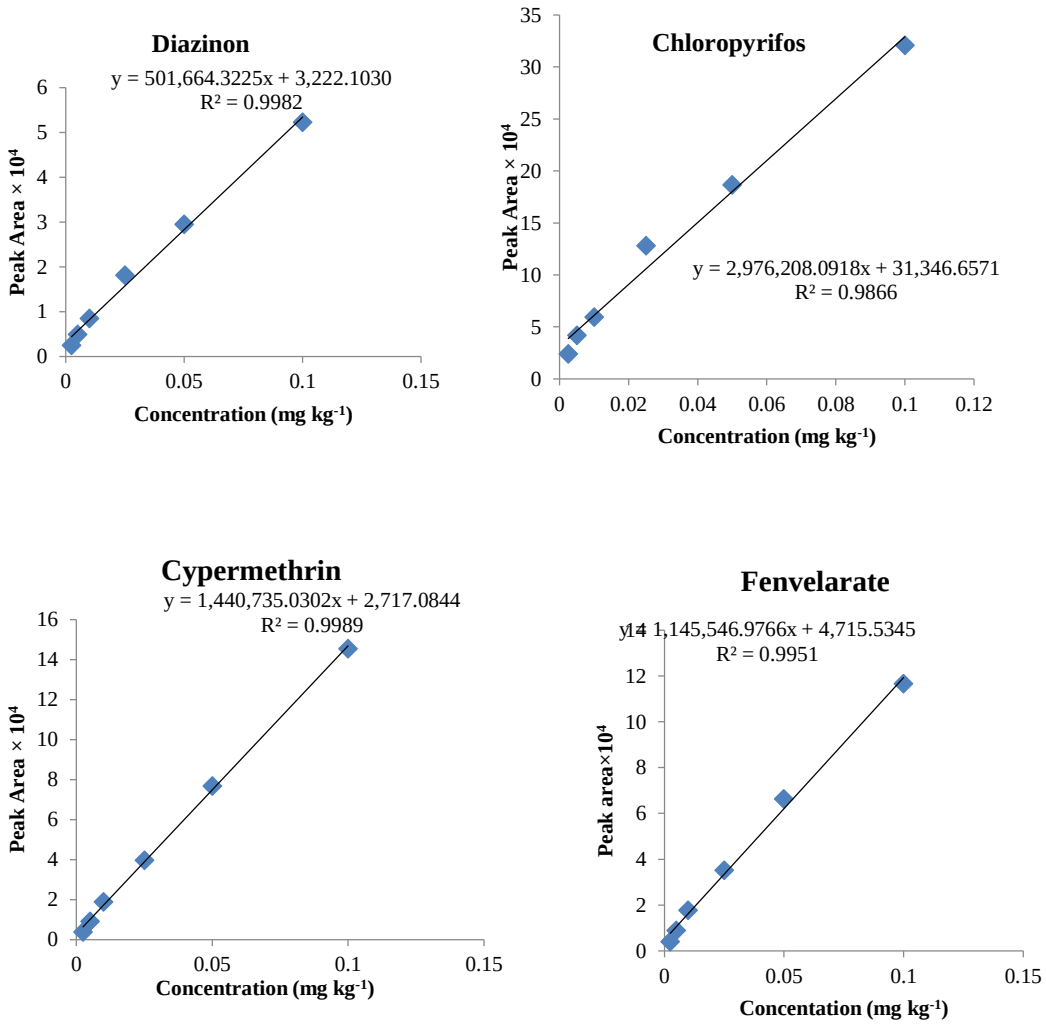


Fig.-3.26: Calibration curves for vegetable samples

3.4.3.3 Matrix Matched Calibration

Calibration curves were prepared in matrices that extracted from control samples *i.e.* chichinga and patol. The linearities were excellent with a correlation coefficient of R^2 > 0.9999 (**Table-3.27**).

3.4.3.4 Quantification

The limits of detection (LODs) and limits of quantification (LOQs) were calculated (**Table- 3.27**). The LODs and LOQs were 0.8 and 2.64 µg kg^{-1} for diazinon; 0.01 and 0.033 µg kg^{-1} for cypermethrin; 0.002 and 0.0066 µg kg^{-1} for fenvalerate; 0.002 and 0.0066 µg kg^{-1} for chlorpyrifos, respectively (**Table-3.27**).

Table-3.27: Retention times (RT), correlation coefficients (R^2), LOD and LOQ of summer vegetables

Pesticide	Linear range (mg L^{-1})	RT (min)	Linearity (R^2)	Vegetables	LOD (µg kg^{-1})	LOQ (µg kg^{-1})
Diazinon	0.0025 − 0.1	12.46	0.9982	Chichinga	0.8	2.64
Chloropyrifos		14.64	0.9866	Jhinga	0.002	0.0066
Cypermethrin		24.83	0.9989	Dhundol	0.01	0.033
Fenvalarate		27.27	0.9951	Patol	0.002	0.0066

3.4.3.5 Pesticide Residues in Vegetable Samples

The field samples were collected from Jhenidah district were analyzed following the same extraction and cleaned-up method. Some samples were also collected from three local markets of three different districts on different dates. Though almost every samples were found with chlorpyrifos, cypermethrin, diazinon and fenvalerate residues but cypermethrin residues and fenvalerate was not found in patol and chichinga, jhinga samples respectively (**Table-3.28**).

Table-3.28: Pesticide residues (Average ± SD, µg kg^{-1}) in vegetable samples

Vegetables	Location	Chlorpyrifos (µg kg^{-1})	Cypermethrin (µg kg^{-1})	Diazinon (µg kg^{-1})	Fenvalerate (µg kg^{-1})
Chichinga	Gaforgau,Mymensingh	ND	7.00±2.75	2.18±0.18	ND
	Kaligang,Jhinaidha	ND	ND	ND	ND
Jhinga	Nandail, Mymensingh	18.50±0.58	2.04±0.32	ND	ND
	Gaforgau,Mymensingh	ND	ND	ND	ND
	Kaligang,Jhinaidha	ND	ND	ND	ND
Dhundol	Kaligang,Jhinaidha	15.29±0.58	ND	ND	ND
	Gabindopur,Comilla	19.82±0.68	41.29±1.78	20.25±4.18	6.27±0.48
	Burichong,Comilla	19.15±0.70	21.38±3.54	9.10±0.53	ND
	Comilla,local market	19.60±0.34	ND	16.70±2.13	ND
Patol	Gaforgau,Mymensingh	19.65±1.03	ND	5.82±3.41	ND
	Kaligang,Jhinaidha	ND	ND	6.53±1.22	7.83±3.23
	Nandail,Mymensingh	ND	ND	ND	ND
MRL		0.5 mg kg^{-1}	0.5 mg kg^{-1}	0.5 mg kg^{-1}	1.0 mg kg^{-1}

*ND = Not Detection

3.5 Discussion

3.5.1 Method Validation

Selectivity

The selectivity was examined by comparing the chromatograms of the standard, blank sample and spiked sample. There were no interference peaks at the retention time of cypermethrin (**Fig-3.28**), fluxapyroxad (**Fig-3.30**) and diazinon, chlorpyrifos, cypermethrin & fenvalerate (**Fig.-3.35**).

Linearity

The linearity of the GC machine was evaluated by constructing calibration curves. Matrix matches calibration curves (cypermethrin in eggplant, tomato, bitter gourd & green chili) were constructed in the concentration range of 0.025–2 mg L^{-1} (**Table-3.20**). Matrix matched calibration curves (diazinon in patol, chlorpyrifos in dhundol, fenvalerate in jhinga & cypermethrin in chicinga) were constructed in the concentration range of 0.0025–1 mg L^{-1} (**Table-3.27**). The linearity of LC–MS/MS machine was evaluated by constructing calibration curves. Matrix matched calibration curve of fluxapyroxad in moie was constructed in the concentration range of 0.025 – 2 mg L^{-1} (**Table-3.24**). The linearity was excellent with correlation coefficients of $R^2 \geq$ 0.999 (Codex, 2003).

Sensitivity

The limit of detection (LOD) is the smallest concentration from which it is possible to assume the presence of an analyte, and the limit of quantification (LOQ) is the smallest quantity required to quantify the analyte with a reasonable degree of statistical certainty. The LOD and LOQ were found 0.01 and 0.033 µg kg^{-1} for the cypermethrin in eggplant, tomato, bitter gourd, pumpkin and green chili respectively (**Table-3.20**).

The LOD and LOQ were 0.01 & 0.033 µg kg^{-1} for fluxapyroxad respectively (**Table - 3.24**).

The LODs and LOQs were 0.8 and 2.64 µg kg^{-1} for diazinon; 0.01 and 0.033 µg kg^{-1} for cypermethrin; 0.002 and 0.0066 µg kg^{-1} for fenvalerate; 0.002 and 0.0066 µg kg^{-1} for chlorpyrifos (**Table-3.27**).

Matrix effect

The matrix effect is regarded as a signal suppression or enhancement of the analyte due to the co-elution of matrix components (Jansson *et al*., 2004; Kruve *et al*., 2008). It can vary considerably from matrix to matrix and differs significantly in pure solvent and in matrix. Therefore, it is necessary to use the matrix-matched calibration standards in order to minimize quantitative errors in pesticide residues analysis. Positive values obtained from the equation reflect matrix-induced enhancement, and negative values indicates suppression of signals. No matrix effect is observed when ME% is equal to 0%. In this study, the matrix effects were calculated 28% (cypermethrin in tomato), 23% (cypermethrin in eggplant), 17% (cypermethrin in bitter gourd) 15% (cypermethrin in green chili) and 12% (cypermethrin in pumpkin).
The peak areas of the fluxapyroxad at a level of 0.5 mg kg^{-1} prepared in untreated moie leaves extract or solvent were compared. The matrix effect was 20%. These effects were rather mild and not so much significant (Kmellar *et al*., 2008).

Recovery

Accuracy and precision

Accuracy was expressed as a percentage of recovery and **precision** as a relative standard deviation (RSD). The extraction efficiency was assessed by doing recovery experiment carried out with control samples collected from field before pesticide spray. The recovery experiments were performed in three replicates at two spiking concentration levels.

The recoveries were 87 – 95% for cypermethrin in eggplant, 79 – 81% in pumpkin, 77 – 98% in bitter gourd, 83 – 102% in tomato , 95 – 101% in green chili with **precision** (RSD) below 10% (**Table-3.19**). The average recoveries of cypermethrin in the five vegetables (77 – 102%) with RSD ≤10%.

The recoveries were 88% (RSD 9) and 93% (RSD 2) for fluxapyroxad at 0.1 mg kg^{-1} and 0.5 mg kg^{-1} concentration levels respectively (**Table-3.23**). These accuracy and precision values were consistent with the ranges listed in the Codex guidelines (Codex, 2003), and thus the method described herein can be considered as a reliable, reproducible, and accurate routine analytical method.

The highest recoveries were 104% for diazinon in chichinga, 115% for cypermethrin in jhinga, 95% for chlorpyrifos in chichinga and 96% for fenvelarate in patol with **precision** (RSD) below 8% (**Table-3.26**). The average recoveries of the four pesticides in the four vegetables (73 – 115%) with RSD ≤8% indicated good performance of extraction, cleaned-up and chromatographic parameters for the analysis of targeted pesticide residues in vegetable samples.

The variations in the recoveries of different pesticides in vegetables might be due to several factors including the interaction with the matrices. Higher recoveries were found for cypermethrin in tomato and cypermethrin in jhinga indicated that matrix may have played a role affecting the recovery (Ajay *et al.* 2004). The results are comparable with earlier other research that report recoveries of pesticides in fruits and vegetables; 63–129% (Pang *et al*., 1995), 70–116% (Patel *et al*., 2004), 70–120% (Gebara *et al*., 2005), 88-108% (Bai *et al*., 2006).

Optimisation of SPE Conditions.

The SPE condition was optimised to obtain good recoveries for fluxapyroxad. Among the parameters studied were flow rate and solvent polarity. Development of an optimum solvent system for fluxapyroxad was based on several criteria.

3.5.2 Dissipation Studies of Cypermethrin

A number of vegetables are grown in Bangladesh every year. Throughout the year people usually eat fresh vegetables like: tomato, eggplant, pumpkin, bitter gourd, bottle gourd, green chili etc. The consumption of vegetables are high. For this reason, vegetables are growing in commercial level at different corner of Bangladesh. Farmers usually use different kinds of pesticides to protect vegetables from pests and diseases to increase the product's quality and quantity. But most of the farmers are illiterate and do not maintain the safe pre-harvest interval.

Farmers in Bangladesh apply pesticides in their fields almost every day sometimes even more than one time in a day or just before harvesting. The crops come directly to the markets without any PHI of degradation. Concerning the level of food safety, the study was designed to reproduce the way of using pesticides in the real fields and the samples available in the market for the consumers. After discussion with the farmers, vegetables fields were selected where pesticides were not applied within last 10 days. Blank samples were collected from those fields before applying the pesticides and no residue of any of the targeted pesticides was detected (**Fig-3.28**).

0-day sample (2 h after spray) and the control sample were kept immediately into the chill box to avoid any degradation or contamination during transport. Rest of the samples was transported at ambient temperature in the similar way they are transported to the market commercially. These samples were kept also at ambient temperature after they have reached the laboratory to see the dissipation at this temperature.

The maximum residue levels of cypermethrin were detected in 0-day samples (2 h after spray) of all vegetables (**Table-3.22**). The residue levels went down gradually with days and 74–88% dissipations was observed in all matrices within 10 days.

The decreasing of concentration of the pesticides followed a first-order kinetics pattern (**Fig-3.19**). The biological half-life ($t_{1/2}$) was calculated by the formula $t_{1/2} = \ln 2/k$ (Park 2011), in which the constant k is the slope of the linear regression ($A=A_0e^{-kt}$). The half-life of cypermethrin for five vegetables were calculated as 3.85,

3.85, 4.08, 4.95 and 2.77 days in tomato, eggplant, bitter gourd, green chili and pumpkin respectively. Similar results were reported by Awasthi (1994) who determined persistence and degradation of different pyrethroids in green chilli. The half-life of permethrin was 2.2–3.2 days, that of cypermethrin was 2.6–2.8 days and of deltamethrin was 2.8–3.0 days. Dissipation of cypermethrin in tomato samples after pesticide application followed first order kinetics at recommended and double the recommended doses, a residue level of 0.29 ppm at zero day followed by 0.24 ppm at 1 day and gradually declined to 0.07 ppm at 10 days (N. Nahar *et al*. 2012).

Pesticide residues of the present study were compared with MRL value established by European Union (EU, 2012) and Codex Alimentarius (FAO/WHO, 2004). It was found that cypermethrin residues went below the MRL value after 1 day of spraying in tomato (143.14 µg kg^{-1}) and in eggplant (106 µg kg^{-1}) and at 0 day *i.e*. two hours after spray in bitter gourd (133.67 µg kg^{-1}) (Codex, 2013, 2009). The results were in covenant with the judgments of Chai *et al*. (2009) where dissipation of cypermethrin was studied in green mustard and soil in a humid-tropical vegetable production system. The results were also similar with the results reported by Awasthi (1994) and Ahmed *et al*. (1993) where the waiting periods of pyrethroids were mentioned as 0–5 days. For cypermethrin residues in vegetable soybean, the application dose was safe for the consumption after three days of application in dry season where it fell below MRL (Md. Abdullah *et al*., 2001). Another study observed that the persistence of cypermethrin for 11 days which was below prescribed MRL within 8 days on cauliflower which was close to the safe period for wet season (Rai *et al.,* 1986).

World Health Organization has settled MRL for pesticide residues in different vegetables. In line with that very recently Bangladesh Food Safety Authority has enacted a regulation titled “Chemical Contaminates, Toxins and Harmful Residues, 2017” (Bangladesh gazette No: SRO No 183-Law/2017).

3.5.3 Health Risk Assessment

ADI, EDI and HRI of cypermethrin residues were given in **Table-3.29**. EDI of pesticide residues was calculated by multiplying the residual pesticide concentration (mg/kg) by the food consumption rate (0.345 kg/person/day) and dividing by a body weight of 60 kg for an adult people (Wang *et al*., 2005).

The Maximum Residue Limit (MRL) for cypermethrin in tomto is 0.2 mg kg^{-1}, eggplant is 0.03 mg kg^{-1}, bitter gourd 2 mg kg^{-1} according to Codex Alimentarius Commission (Codex, 2013) and the Acceptable Daily Intake (ADI) for human is 0.05 mg/kg body weight.

The estimated daily intake and health risk index (HI) were calculated. The HI showed that cypermethrin residues in all vegetables were found less than 1 (HI<1). From the obtained concentrations of cypermethrin residues, dietary exposure and health risks were calculated for adult. The statistic of daily food intake of an adult person of our country exposes that vegetables are in third position after rice, cereal and fish which give the daily food consumption in weight (Report of the household income and expenditure survey, 2011). When HI is less than 1, the food concerned is considered as acceptable. If the value is greater than 1 the food is considered a risk to the consumer (Darko and Akoto 2008, Akoto *et al*. 2015).

Table-3.29: Health risk assessment based on ADI of cypermethrin in vegetables

Pesticide	Vegetables	ADI (mg/kg /day)	Residues (mg/kg) 0-day	EDI (mg/kg/ day)	HRI	Health Risk
Cypermethrin	Tomato	0.05	0.158	0.00091	0.018	No
	Eggplant		0.114	0.00065	0.013	No
	Bitter gourd		0.134	0.00077	0.015	No
	Green chili		0.088	0.00051	-	-
	Pumpkin		0.055	0.00032	-	-

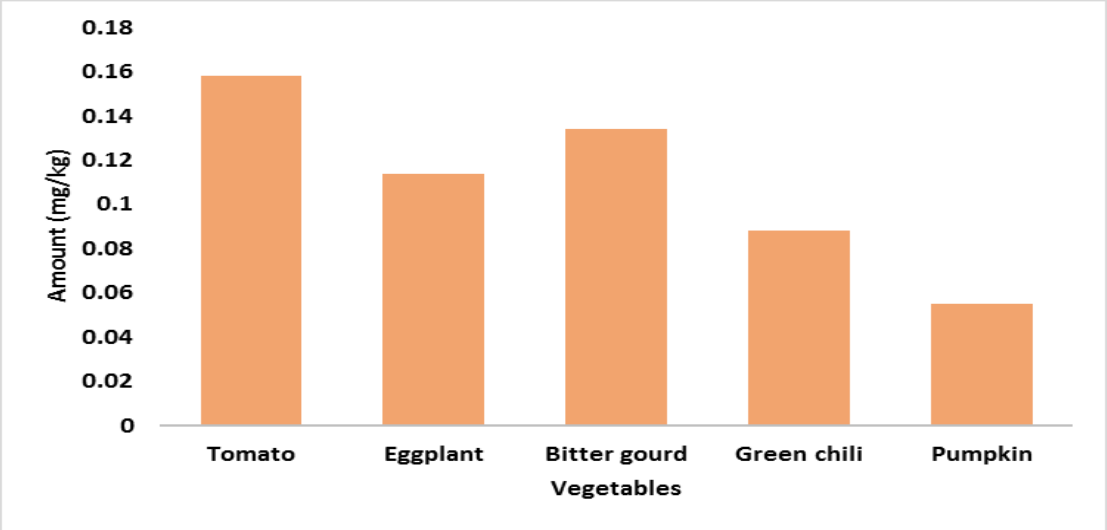


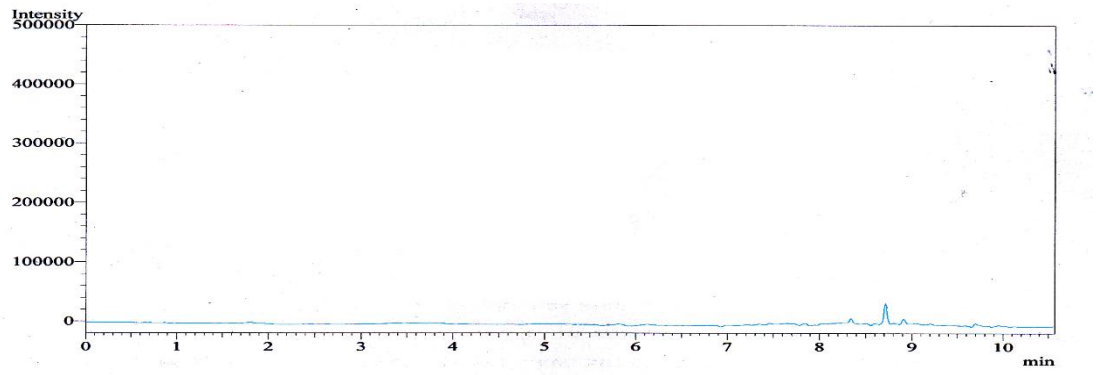
Fig- 3.27: Bar diagram residues of cypermethrin (0 day) in vegetable samples

This research was carried out to know the presence of cypermethrin residues in five different vegetables. As a result, all samples were below the MRL of cypermethrin so the most of the vegetables samples were not able to cause serious health hazard. The

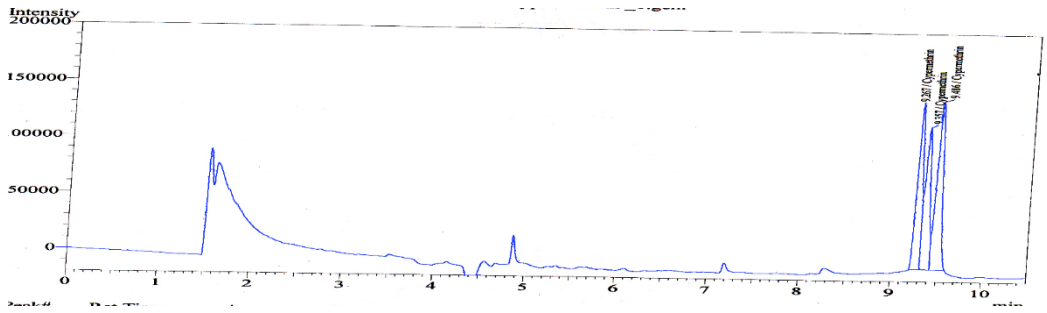
results of this study were significant to a previous investigation carried out in Lahore (Tahir *et al*., 2009).

Cypermethrin is used widely for vegetables in Bangladesh. Sometimes vegetables and fruits growers use a pesticide very frequently, even twice a day and harvest crop immediately after pesticide application. This is very dangerous in two ways. First, the pesticide residue limit is likely to be higher than MRL and hence harmful for human and environment. Secondly, pests can make some genetic changes to be resistant against the pesticide. So, farmer should know about these facts and control the use of pesticide. However, the persistent nature of the pesticides is of great concern due to their bio accumulative behaviour and toxic biological effects on human (Tanabe *et al*., 2000). For cypermethrin it was observed that, the pre- harvest interval of 3 days for safe use by consumers where residue felt below the MRL (Md. Abdullah *et al*., 2001) in dry season.

a)



b)



c)

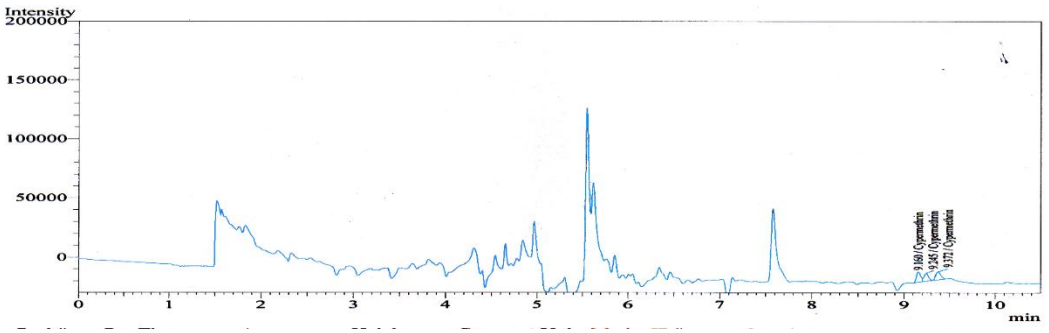
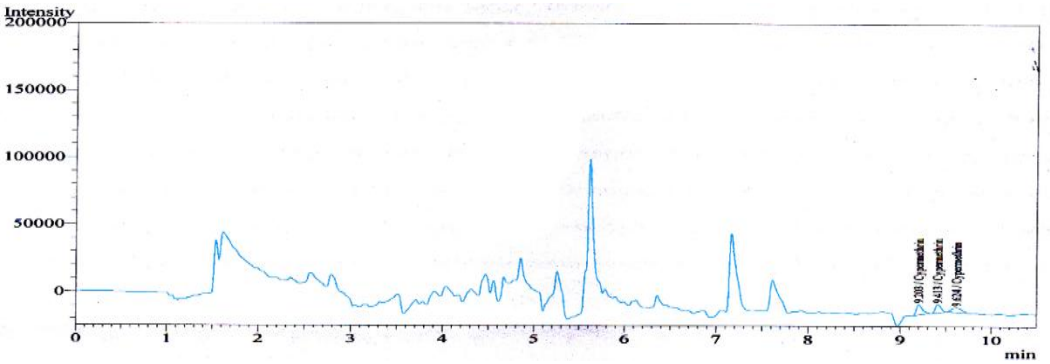
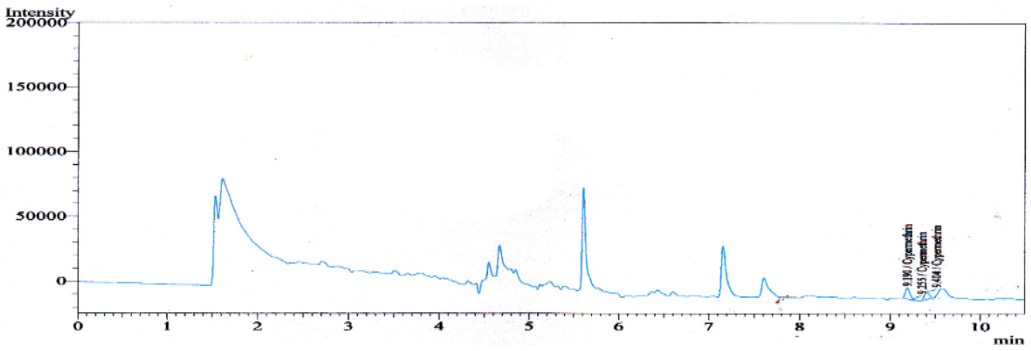


Fig-3.28: CG-ECD chromatogram of a) blank sample b) standard 1 mg L^{-1} of cypermethrin c) fortified in green chili sample at 0.25 mg kg^{-1} (sequence peak : cypermethrin; 9.27, 9.36 & 9.49).

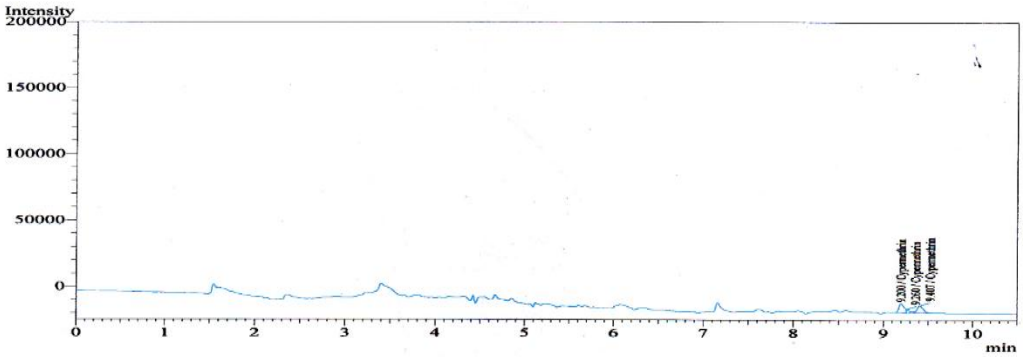
a) Green chili in 1 day



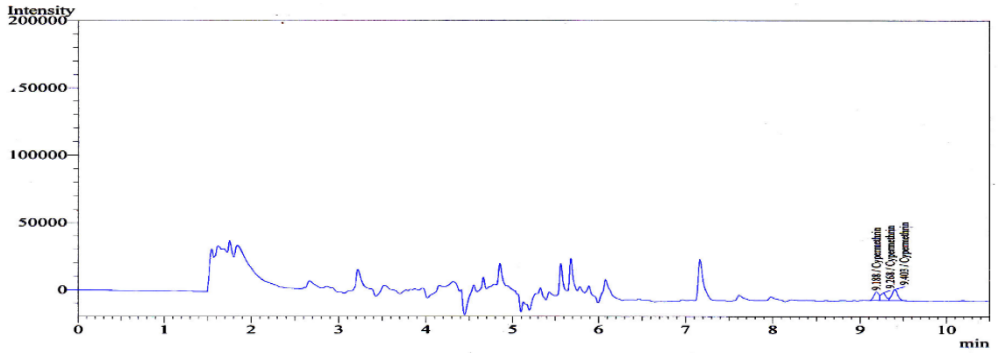
b) Tomato in 10 days



c) Bitter gourd in 3 days



d) Eggplant in 1 day



e) Pumpkin in 1 day

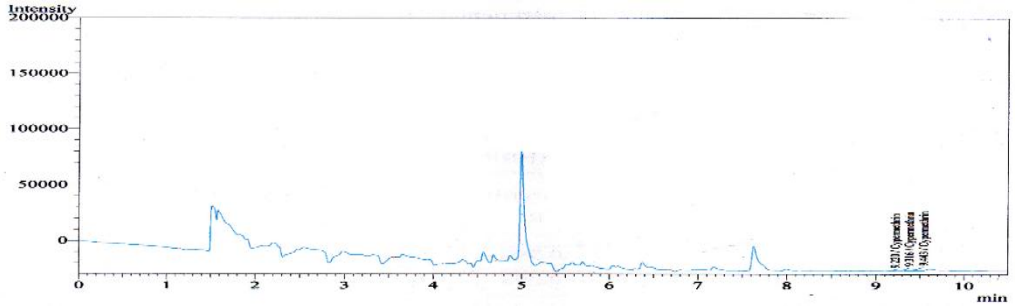


Fig-3.29: CG-ECD chromatogram of cypermethrin in a) green chili b) tomato c) bitter gourd d) eggplant & e) pumpkin

3.5.4 Dissipation Studies of Fluxapyroxad

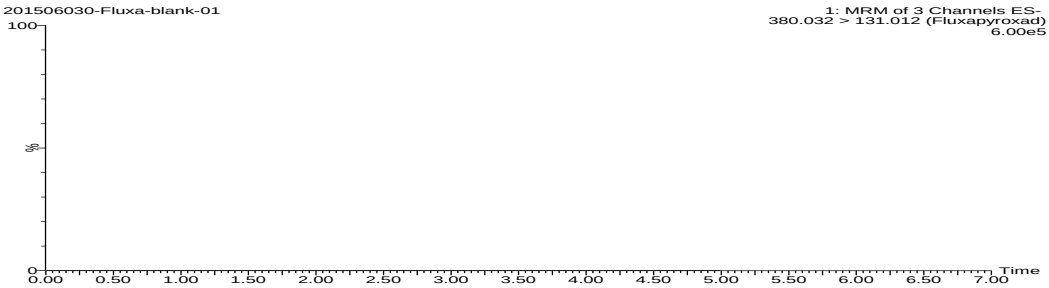
There were three plots in every greenhouse for pesticides B (treated twice), C (treated thrice) and A (untreated). No residue was detected on blank moie leaves collected from the untreated (A) plot (**Fig-3.30**). The maximum residue levels were detected on day 0 (for treated thrice). The residue concentrations of fluxapyroxad were found 11 and 20 µg kg^{-1} sample collected from the plot B (treated twice) and from plot C (treated thrice), respectively, on zero (0) day. Fluxapyroxad dissipated 97% and 98% after 14 days for the sample treated twice and thrice treated, respectively (**Table-3.25**). These levels were lower than the estimated MRL (0.1 mg kg^{-1}), and also lower than the MRLs in different fruits and vegetables (5.0 mg kg^{-1}: KFDA 2009). MRL value for fluxapyroxad in moie leaves as 1.0 mg kg^{-1} could be proposed. Therefore, a safety pre-harvest interval of 14 days (or 2 days more) is suggested for moie leaves at the recommended dosage application of fluxapyroxad.

In another study fluxapyroxad exhibits medium to very high persistence in soil of China. Rates of degradation were dependent on fluxapyroxad concentration, with half-lives of 158 and 385 days for different treatments (WU Xiao-hu *et al*. 2014). The half-life values at 0.75 mg kg^{-1} and 7.5 mg kg^{-1} DW (dry weight) are in accordance with those reported by EFSA (2012).

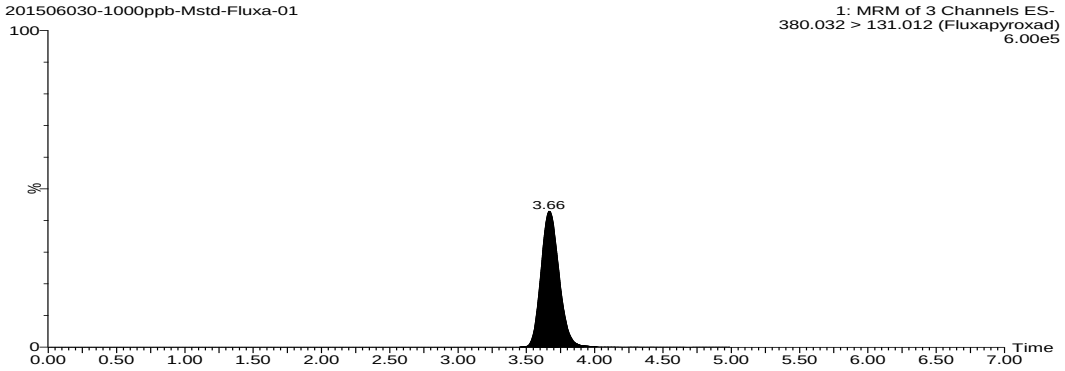
In addition, fluxapyroxad is stable against hydrolysis at pH values of 5, 7 and 9 and aqueous photolysis. Therefore, microbial degradation may be as the main factor responsible for fluxapyroxad dissipation in soils, but the degradation of fluxapyroxad in soils was very slow (WU Xiao-hu *et al*. 2014).

The decreasing of concentration of fluxapyroxad followed a first-order kinetics pattern (**Fig-3.24 & 3.25**). $t_{1/2}$ was calculated by the formula $t_{1/2} = \ln 2/k$, in which the constant k is the slope of the linear regression. The half-lives of fluxapyroxad were calculated as 3.04 & 2.68 days for the sample collected from plot B (treated twice) and plot C (treated thrice), respectively.

a) Control



b) Matrix-matched Standard-1mg kg^{-1}



c) High recovery

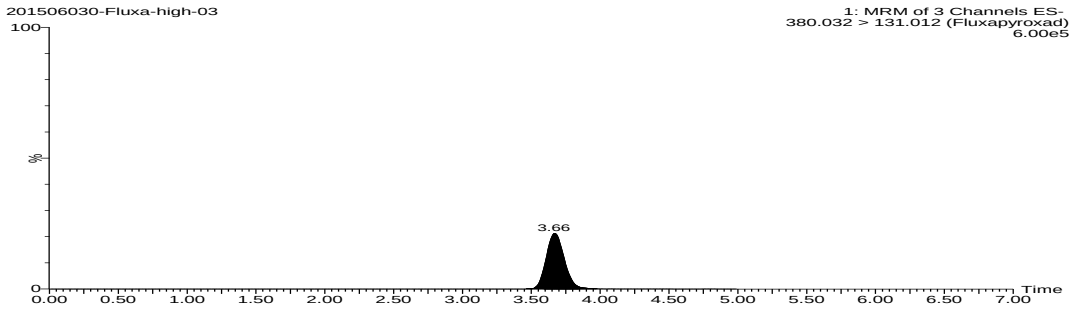


Fig-3.30: LC-MS/MS Chromatogram of fluxapyroxad (a) Blank sample, (b) Standard 1 mg kg^{-1} in moie leaves matrix and (c) Recovery equivalent to 0.5 mg kg^{-1} (peak : 3.7)

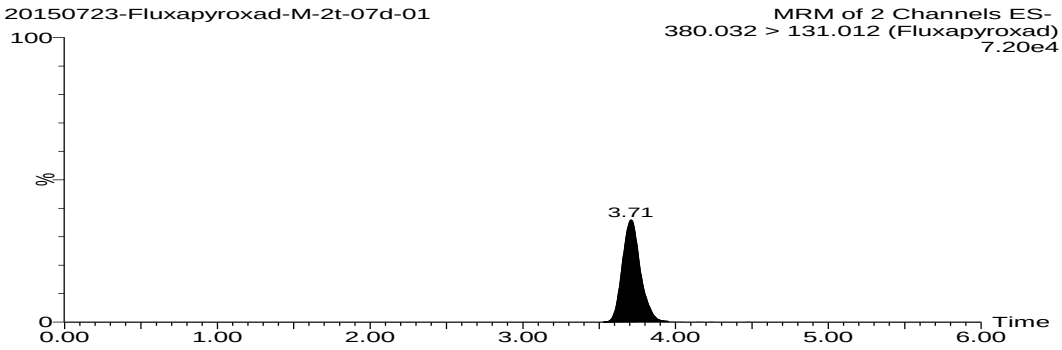


Fig-3.31: LS-MS/MS Chromatogram of fluxapyroxad in real sample (treated twice) on 7 day

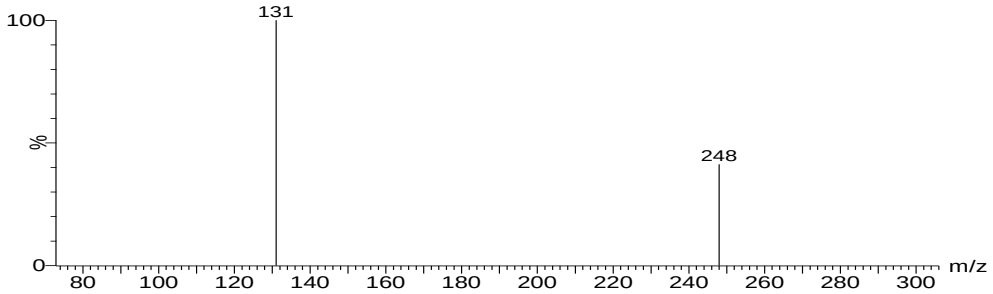


Fig-3.32: LS-MS/MS Chromatogram of fluxapyroxad in real sample (treated twice) on 7 day in selected ion mode

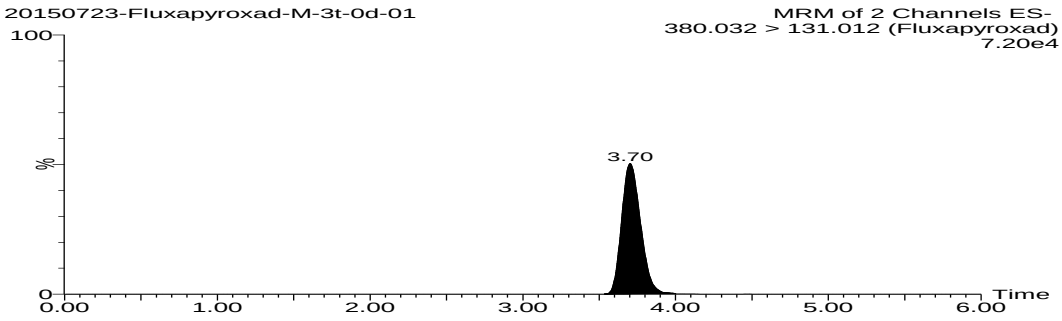


Fig-3.33: LC-MS/MS Chromatogram of fluxapyroxad in real sample (treated thrice) on 1 day

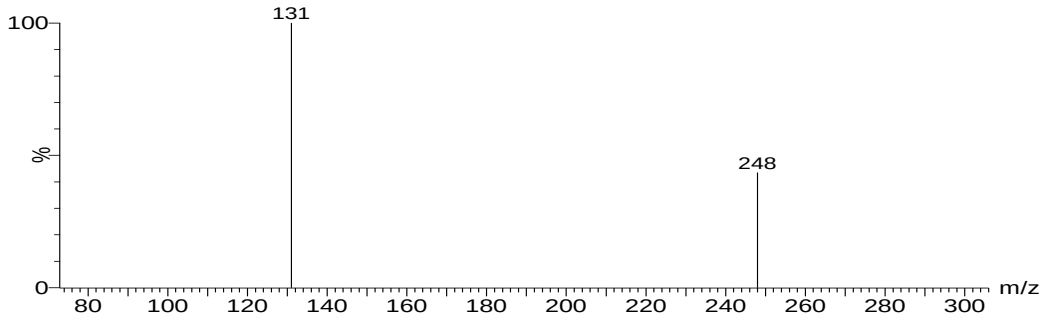


Fig-3.34: LS-MS/MS Chromatogram of fluxapyroxad in real sample (treated thrice) on 1 day in selected ion mode

3.5.5 Residue Analysis of Market Samples

Chichinga, jhinga, dhundol and patal samples of the same cultivar's variety were purchased from two local markets of Comilla and Mymensing district at two different days (**Table-2.5**). Cypermethrin & diazinon were detected in the chichinga samples, Chlorpyrifos & cypermethrin in jhinga samples. Chlorpyrifos, Diazinon, cypermethrin & fenvelarate were found in dhunol samples, diazinon & fenvelarate were in patol. Pesticide residues were detected in 40% of the market samples but all were below the MRL values. Similar results were reported in different survey reports conducted in Bangladesh such as:

Fardous *et al.* (2007) surveyed to determine the presence of some selected organophosphorus (chlorpyrifos and diazinon) and carbamates (carbofuran, carbaryl) in tomato collected from different locations of Bangladesh during 2005-2006. Out of 18 sampling locations, tomato from 7 locations was found to contain pesticide residues but below the MRL values.

Islam *et al*. (2009a) studied the pesticides residues (diazinon, malathion, chlorpyrifos and cypermethrin) in cauliflower. Pesticides were sprayed at recommended and double of the recommended doses and samples were collected after 6 hours. Diazinon

and chlorpyrifos were found above the MRL values in both the doses but malathion and cypermethrin were below the detection limit. (Chowdhury *et al*. 2013) collected 14 tomato samples randomly from different shops of local markets of Savar Upazilla and analyzed to identify the level of widely applied cypermethrin, chlorpyrifos and diazinon residues. Five samples were found to be contaminated with cypermethrin and one with chlorpyrifos but the concentrations were below the corresponding MRL values.

On the other hand, many survey or monitoring reports outside Bangladesh, especially in the subcontinent or region reported, slightly with different results, for example:

Sanghi and Tewari (2001) monitored 12 pesticide residues in summer fruits (12 samples) and vegetables (23 samples) from different parts of India. Malathion was the most abundantly present insecticide both in vegetables and fruits and few samples also contained DDT and its metabolite, DDE.

Kumari *et al*. (2003) monitored pesticide residues in 80 winter vegetable samples (cabbage, cauliflower, pea grains, brinjal, tomato, potato, and green chilly) from Hisar, Haryana, India during 1997-1998**.** Among the four major chemical groups, residue levels of organophosphorus insecticides were highest followed by carbamates, synthetic pyrethroids and organochlorines. About 32% of the samples showed contamination with organophosphorus and carbamate insecticides above their respective MRL values.

In another study, 84 seasonal vegetable samples, viz., brinjal, okra, cauliflower, cabbage, knolknol, summer squash, smooth gourd, cucumber, pea and potato from Hisar were analyzed for monocrotophos, endosulfan, cypermethrin, malathion, methyl parathion, chlorpyrifos, aldicarb and quinalphos by Kumari *et al*. (2004).

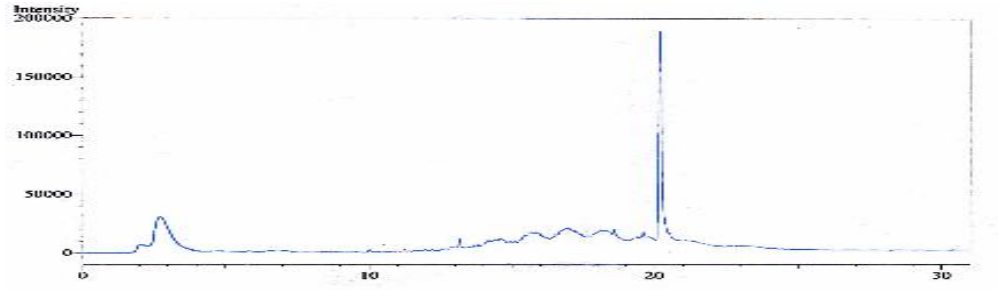
Parveen *et al.* (2005) monitored 24 pesticides residues, belonging to different pesticide classes like OPPs, OCPs, carbamates and pyrethroids in 206 samples of 27 different vegetables collected from the retail markets of Karachi, Pakistan during 2000-2003. 63% samples were contaminated with one or another pesticide and 46% samples had pesticide residues more than MRL values as given by FAO/WHO.

El-Saeid and Selim (2013) monitored 86 pesticide residues (OPPs, OCPs, pyrithroids and carbamates) in 1057 samples of fresh vegetables from import and domestic production (cold pepper, eggplant, carrot, cucumber, potato, hot pepper, cultivation tomato, squash, beans, okra, onions, cauliflower, and green house tomato) in market of Riyadh, Saudi Arabia. Pesticide residues above the MRL values were detected in 16% of the total samples (168 from 1057 samples), but 84% of the total samples (887 from 1057 samples) had no residues those were below MRL.

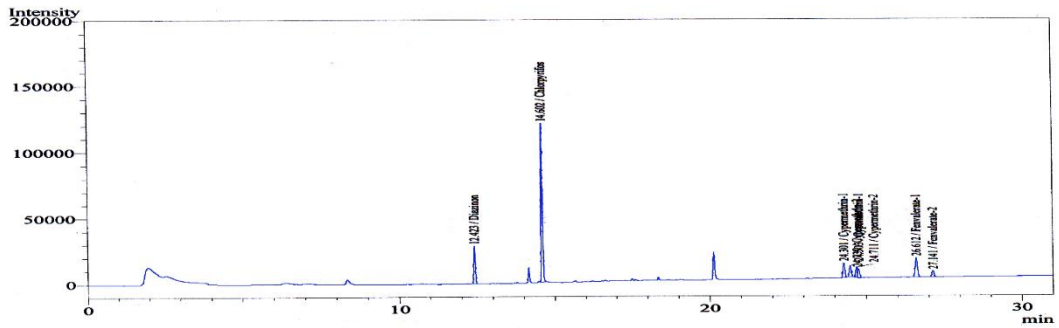
Eight organophosphorus pesticide residues (dichlorvos, demeton, diazinon, dimethoate, parathion methyl, pirimiphosmethyl, sumithion and parathion) in market food samples (cereals, vegetables and fruits) in Shaanxi area of China were investigated by Bai *et al*. (2006). Out of 200 samples, 18 samples contained five OPPs, viz., dichlorvos, dimethoate, parathion methyl, pirimiphos-methyl and parathion found in concentrations ranging from 0.004 mg/kg to 0.257 mg/kg. The most common OPPs residues found in vegetable and fruit samples of Shaanxi area were dichlorvos, dimethoate and parathion. The mean levels of dimethoate in fruits and parathion in vegetables exceeded the MRL values given by the Ministry of Health of China.

Comparing the above reports, our study wants to give a positive signal to Bangladesh that farmers can start following the good agriculture practice. The survey reports from regional countries revealed a significant percent of fruits and vegetables were contaminated with pesticides above the corresponding MRL values. The concept of Good Agricultural Practices may serve as a reference tool for deciding, at each step in the production process, on practices and/or outcomes that are environmentally sustainable and socially acceptable. The implementation of GAP should therefore contribute to Sustainable Agriculture and Rural Development (SARD). We need to make our farmers, retail suppliers and consumers that Good Agricultural Practices addresses environmental, economic and social sustainability for on-farm processes, and result in safe and quality food.

a) Blank sample



b) Std. DCCF 1 mg L^{-1}



c) Recovery (0.5 mg kg^{-1})

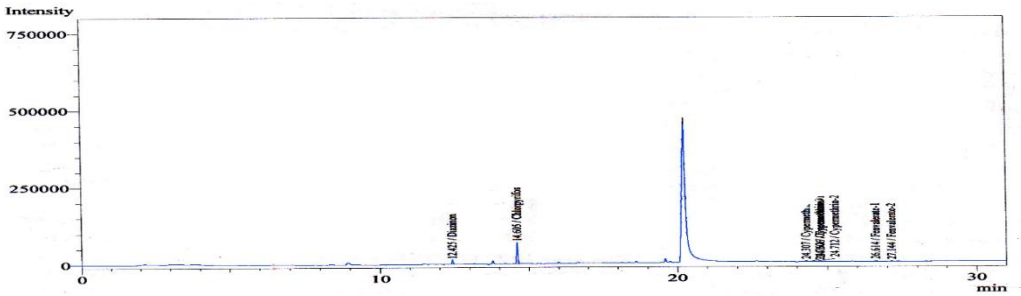
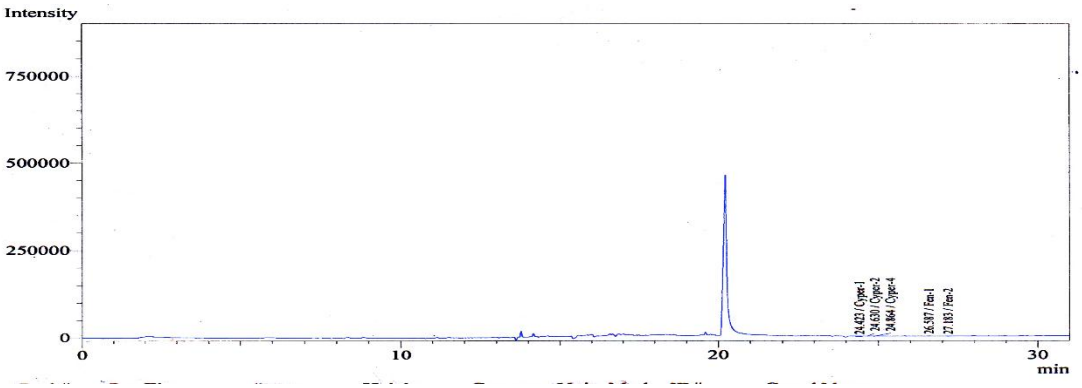
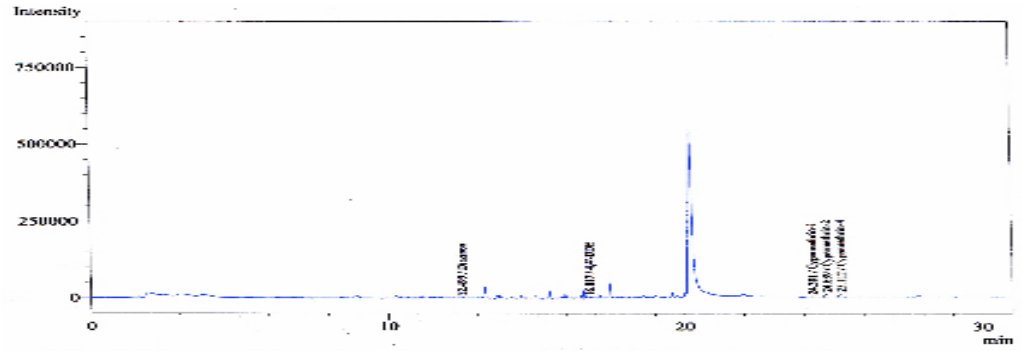


Fig-3.35.: GC-ECD Chromatogram of (a) Blank sample, (b) Standard DCCF 1 mg L^{-1} in and (c) Recovery equivalent to 0.5 mg kg^{-1} in (sequence of peak: Diazinon, 12.43; Chlorophyrifos, 14.61; Cypermethrin; 24.33, 24.73 & 24.77 and Fenvalarate, 26.64 & 27.17).

a) In patol



a) Jhinga



b) Dhodol

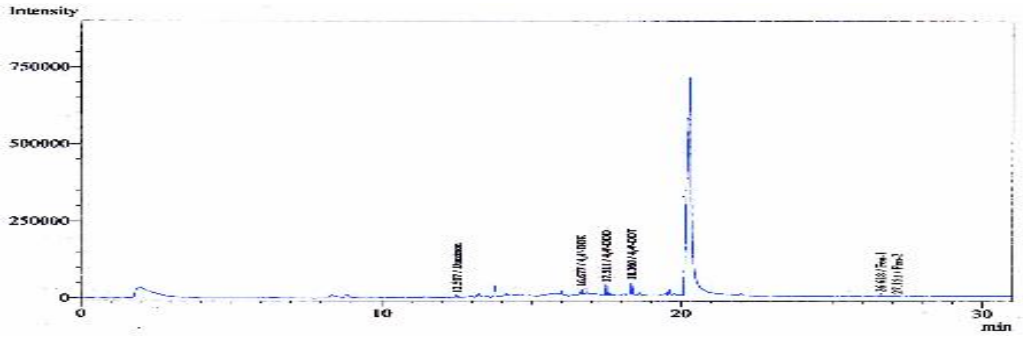


Fig-3.36: GC-ECD Chromatogram of (a) Patol, (b) Jhinga and (c) Dhodol (sequence of peak: Diazinon, 12.43; Chlorophyrifos, 14.61; Cypermethrin; 24.33, 24.73 & 24.77 and Fenvalarate, 26.64 & 27.17)

SUMMARY

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 depend 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).

The only DDT production factory within Chittagong Chemical Complex (CCC) area started in 1966 and soon supply started in the local area. However, due to long persistence in the environment, bioaccumulation, bio magnifications and accumulation to the fatty tissues of human over food chain, the consumption and manufacture of OCPs became restricted worldwide from nineties. The Stockholm Convention identified 12 persistent organic pollutants (POPs) and recently included 13 more, including DDT which are harmful for wildlife and human health and formulated a treaty in 2001 to stop production, usage and elimination of OCs pesticides where Bangladesh is a part of it and has been paying the fees regularly to the secretariat and actively participating in biannual conference (COP). DDT had been emitted in Bangladesh and the factory at CCC area was shut down as a signatory of Stockholm convention. Bangladesh closed down the DDT factory in 1995 without deciding what should happen to the stored DDT in the factory of the CCC area.

4.1 DDTs in Environmental and Human Blood Samples of CCC Area

This study was shown to monitor the range of dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDE & DDD) in environmental samples (soil, sediment, water and fish) and human blood from areas nearby a closed DDT factory in Bangladesh. Soil, sediment and water samples were collected on 13 July, 2011 from the CCC area in the southern, south western and eastern directions. Fifteen different fish (n=15) samples were collected from a pond in the factory area during June 2016. Thirty human blood (n=30) samples were randomly collected from people (men and women) living inside and near the factory on June 2014 to determine the level of exposure.

The samples were immediately kept in 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.

DDTs (DDT and its metabolites) from soil and sediment samples were extracted using solvent extraction (SE), water samples by liquid-liquid partitioning, fish samples by solid dispersion method and finally human blood samples by Hovander and coworkers method with slight modification with a mixture of n-hexane: MTBE (1:1) followed by cleaned up using silica gel impregnated with conc. sulphuric acid (2:1 w/w, 1 g). All samples were analyzed by Gas Chromatograph equipped with an Electron Capture Detector (GC-ECD). Linearity's expressed as coefficients (R^2) were ≥0.995. The recoveries were 72-120% and 83-110%, with <15% RSD in soil and water, respectively at two concentration levels. The limit of quantification (LOQ) was 0.0165 mg kg^{-1} in soil and 0.132 µg L^{-1} in water.

The recovery for fish samples were conducted at three concentration levels (0.05, 0.1 & 0.2 mg kg^{-1}) in three replicate analysis. The recoveries were 70–105 %, with <16 % RSD. LOD & LOQ was found 0.063 µg kg^{-1} & 0.206 µg kg^{-1} respectively in fish samples.

By using internal standard, the recoveries of human blood were 73−108 % (0.05 µg L^{-1}) and 75−98 % (0.025 µg L^{-1}) for CB-53. LOD & LOQ was found 0.025 µg kg^{-1} & 0.083 µg kg^{-1} respectively, in blood sample.

4.1.1 Moisture Content

The moisture content of the soil samples was 19–30 %.

4.1.2 Lipid Contents of Fish and Human Blood Samples

The lipid contents (%) of different fish species were determined gravimetrically and ranged from 0 − 6 %. Among them the highest amount of lipid content was found in shing fish. The lipid content of 30 human blood samples were 0 − 2.45 %.

4.1.3 Statistical Analysis

Statistical analysis was performed by ANOVA. Basic descriptive statistics, ANOVA, LSD and Correlations test were performed on SPSS, to identify the relationship between lipid, DDTs residues with gender, age and duration of work. A p-value of <0.05 was denoted statistically significant.

4.1.4 Total DDTs Residues of Soil, Sediment, Water, Fish and Human Blood Samples

The amount of DDTs (*4,4´*-DDE + *4,4´*-DDD + *2,4*-DDT + *4,4´*-DDT) in CCC area were detected as 1 x 10^2–1635 x 10^2 mg kg^{-1} and 16 x 10^2 –7 x 10^2 mg kg^{-1} for soil and sediment samples respectively Higher amounts of DDTs were revealed in the southern (2.2–936 × 10^2 mg kg^{-1}) or southwestern (86.3– 2067 × 10^2 mg kg^{-1}) track from the factory than in the eastern track (1.0– 48.6 × 10^2 mg kg^{-1}). An exception was the soil sample collected 50 ft (15.24 m) east (2904 × 10^2 mg kg^{-1}) of the factory. The range of DDTs in the water bodies (0.59–3.01 µg L^{-1}) was approximately equal in all directions.

In present study, the highest amount of DDT and its metabolites (8.9 µg kg^{-1}) were found in the Shing fish which might be due to its lipid content (Mustafa, 2006). Boal showed small amount of DDT and its metabolites. 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, lower DDTs residues may be related to their lower position in food chain that feed on plant materials while the lower residue levels may be related to their lower lipid content and positioned in lower trophic levels that mainly feed on zooplankton and plant materials. Therefore, it is clear that lipid contents, trophic positions and feeding habit all together influence the accumulation of pesticide residues in fishes.

In this study 30 human blood samples were randomly collected from groups of men (n=20) and women (n=10) with different occupations in the closed down factory area to determine the presence of DDTs and to see bioaccumulation in different age groups. The concentration were found 0 – 583 µg kg^{-1} for *4,4′*-DDE, 0 – 1376 µg kg^{-1} for *4,4′*-DDD, 0 –435 µg kg^{-1} for *2,4′*-DDT and 0 – 337 µg kg^{-1} for *4,4′*-DDT.

The highest concentration of *4,4′*-DDE, *2,4′*-DDT & *4,4′*-DDT was detected in a male's blood sample whose age is 56 years and he worked in CCC for 20 years (583 µg kg^{-1}, 435 µg kg^{-1}& 337 µg kg^{-1}), respectively. And the highest concentration of *4,4′*-DDD was detected in female blood sample whose age is 50 years and she is a housewife (1376 µg kg^{-1}). This may be attributed to her higher lipid content (1.13%) and long life spend. DDT is lipophilic that accumulated in fat body. DDTs accumulation increases with age.

The highest ∑DDT was detected in male blood sample 1686 µg $kg^{-1}$and in female blood sample 1455 µg kg^{-1}. The range of *4,4′*-DDT/∑DDT ratio from 0.01 to 0.59. Among the 30 human blood samples DDT and its metabolites were detected except one. That sample was a male's blood sample whose age is 60 and he worked in CCC for only two years. (MRL value of DDTs in human blood is 0.0005 mg/kg/day).

We established that DDTs might have been discarded randomly around the warehouse after the closing of the factory.

4.1.5 Meteorological Parameters and DDTs Concentrations

In the present study together with biological factors the meteorological parameters (temperature, humidity and rainfall) are also considered to assess the accumulation of DDTs in fish body. From the analysis of distribution pattern of contaminants in fish, 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 individually as persist together in the environment and influence together to accumulation DDTs.

4.1.6 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 might be associated with reproductive toxicity in several species of fish.

4.1.7 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 neighboring environments of Chittagong Chemical Complex area. Therefore, it can be said that DDT is still exposed to the environments in the country.

4.1.8 Human Health Risk

As the residue levels exceeded some tolerance limits together with their continuous exposure make these contaminants as major concern 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 species were <1. Though the HIs are less than 1 but together with other food items of daily meal, total HI would be greater than 1. This shows that there is health risk associated with lifetime consumption of the studied fishes, together with other contaminated foods.

4.2 Pesticide Residues in Vegetable Samples

Vegetables are being consumed by the local people of Bangladesh almost every day. Pesticides are being used to protect the crops and there is no guide line about the safe harvesting period of the crops and MRL values for any pesticides in Bangladesh. Studies of dissipation pattern of pesticides in growing crops is necessary which will give a safe harvesting period as well as MRL value after final application.

4.2.1 Dissipation Studies

Dissipation pattern of cypermethrin in five different vegetables (tomato, bitter gourd, pumpkin, eggplant & green chili) were collected February 2016 from the farmer's fields Norundi near Jamalpur district of Bangladesh. For these studies the samples were kept at ambient temperature. The samples were extracted by QuEChERS method, cleaned-up by adsorption chromatography technique and analyzed by GC-ECD. Linearity's (R^2) $\geq$ 0.995 for matrix-matched standard, LOD and LOQ was 0.01

µg kg^{-1} and 0.033 µg kg^{-1}. The recoveries were 82−106 % (RSD ≤ 17 %) at two fortified concentrations (0.25 & 1 mg kg^{-1}) and storage stability was 83% (RSD ≤ 9 %).

The maximum residue levels (MRL) of cypermetrin were identified in 0 day samples (2 h after spray) of all vegetables. The residue levels went down progressively with days and 74-88% dissipations were observed in all matrices within 10 days. It was established that cypermethrin residues went lower the MRL value after 1 day of spraying in tomato (143 µg kg^{-1}) and in eggplant (106 µg kg^{-1}) and at 0 day (134 µg kg^{-1}) in bitter gourd (Codex, 2013, 2009). The half-life of cypermethrin for five vegetables was calculated.

Fluxapyroxad is a second-generation carboxamide fungicide that inhibits succinate dehydrogenase of mitochondrial respiratory chain. This study was carried out to assure the safety of fluxapyroxad residues in butter bar (moie) by developing a method and the dissipation pattern was observed under greenhouse conditions from two different treatments (T2 and T3). This experiment was carried out in the laboratory in Republic of Korea. The leaves which were grown in greenhouse at Naengcheon-ri, Masan-myeon, Gurye-gun, Jeollanam-do, Republic of Korea, from the last week of February until the first week of April, 2015. The method was developed and validated using high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The extraction was carried out by the QuEChERS, and then purified with silica solid phase extraction (SPE) cartridge. Correlation coefficient (R^2) of matrix-matched standard was 0.998, LOQ was 0.01 mg kg^{-1} and recoveries were 88% and 93% at both fortified concentration 0.5 and 2.5 mg kg^{-1}, with RSD ≤ 10% and storage stability 95±7.04. The method was successfully applied to the experimental field samples, which were collected randomly at 0 to 14 days' post application. In this study, fluxapyroxad was dissipated below the MRL value after 10 days at triple of recommended dose. The rate of disappearance was described to 1^{st} order kinetics with half-life of 2.6 days. The initial residues after application were 11 and 20 µg kg^{-1} on the zero day for T2 and T3 respectively. After 14 days the residues declined to 0.42 and 0.36 µg kg^{-1} for T2 and T3 respectively.

4.2.2 Health Risk Assessment

EDI of pesticide residues was calculated by multiplying the residual pesticide concentration (mg/kg) by the food consumption rate (0.345 kg/person/day) and dividing by a body weight of 60 kg for an adult people (Wang *et al*., 2005).

The Maximum Residue Limit (MRL) for cypermethrin in tomto is 0.2 mg kg^{-1}, eggplant is 0.03 mg kg^{-1}, bitter gourd 2 mg kg^{-1} according to Codex Alimentarius Commission (Codex, 2013) and the Acceptable Daily Intake (ADI) for human is 0.05 mg/kg body weight.

The estimated daily intake and health risk index (HI) were calculated. The HI showed that cypermethrin residues in all vegetables were found less than 1 (HI<1). From the obtained concentrations of cypermethrin residues, dietary exposure and health risks were calculated for adult. The statistic of daily food intake of an adult person of our country exposes that vegetables are in third position after rice, cereal and fish which give the daily food consumption in weight (Report of the household income and expenditure survey, 2011). When HI is less than 1, the food concerned is considered as acceptable. If the value is greater than 1 the food is considered a risk to the consumer (Darko and Akoto 2008, Akoto *et al*. 2015). The most of the vegetable samples were not harmful to health as all samples had lower the MRL of cypermethrin.

4.3 Residue Analysis of Market Samples

For the analysis of pesticide residues in some vegetables (snake gourd, ridge gourd, wild ridge gourd & pointed gourd) twenty four vegetables samples were collected from different locations of Bangladesh and analysed for the presence of chloropyrifos, cypermethrin, diazinon and fenvelarate pesticides residues. Vegetables samples were extracted by QuEChERS method, cleaned up by dispersive solid phase method and analyzed by GC-ECD. The linearities were excellent with $R^2 \geq 0.99$. The LOD and LOQ were found 0.002 mg kg^{-1} & 0.007 mg kg^{-1} for chlorpyrifos, 0.01 mg kg^{-1} &

0.033 mg kg^{-1} for the cypermethrin, 0.004 mg kg^{-1} & 0.012 mg kg^{-1} and 0.002 mg kg^{-1} & 0.007 mg kg^{-1} for fenvalerate, respectively. The recovery experiments were conducted at two concentration levels (0.25 and 0.5 mg kg^{-1}) in three replicate analysis**.** The average recoveries of the four pesticides in the four vegetables (73 – 115%) with RSD ≤8%. Pesticides were detected in 40% of the market samples but all were below the corresponding MRL values.

CONCLUSION

We exposed that the adjacent soil or sediment was tremendously contaminated with DDTs. These results will be discussed for the consideration of the Bangladesh Chemical Industrial Corporation and Department of Environment, The Government of Bangladesh (Al Mahmmud *et al*.2015). Contamination of DDTs residue poses a significant health risk to the people living beside the CCC area that are consuming drinking water and fish. This study was continued to provide more information on DDTs contamination in the CCC area.

We expect that, after few decades DDTs will not be found in the environment. The concentrations of total DDTs in all the samples were within the permissible MRL level recommended by FAO/IAEA/WHO. As DDT is a long persistent and bio accumulative substance in the environment, intake of significant amount of this can slow poison our diet which is a matter of health concern. Significant relationships were observed between lipid contents of fish samples and DDTs residues. The ratios of the *4,4'*-DDT/∑DDT (0.01-0.46) which indicated that exposure to DDT is not due to recent uses. All the detected HIs of DDTs residues for studied fish species were <1. Though the HIs are less than 1 but together 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 fish samples, together with other contaminated foods.

The highest concentration of *4,4'*-DDD was detected in a female blood sample whose age is 50 years and she is a housewife (1376 µg kg^{-1}). This may be attributed to her higher lipid content (1.13%) and long life spend. DDT is lipophilic that are easily accumulated in fat body. DDTs accumulation increases with age. Presence of DDT in human blood can be from direct contamination or it can come from food indirectly. Surroundings (soil, sediment & water samples) contained very high amount of DDT and its metabolites. Former employees of CCC are at high risk of accumulation of DDTs as they bath, drink and culture fish in pond of that area. DDTs can easily spread

by air and water far away from the CCC factory areas day by day. Previous production, storage and concentration of huge DDT (2904×10^2 mg kg^{-1}) in soil samples, still keeps the human & environment near the DDT factory at high risk. Our previous results (soil, sediment, water & fish) were submitted to the Chairman of BCIC.

From the study, it can be concluded that though DDT was exposed in CCC area long before but because of their long life span; this contaminant persist in the soil, water and fish in that area. It also enter to the human body through food chain. The surroundings soil was extremely contaminated with DDTs and this report should be directed to the Bangladesh Chemical Industrial Corporation and Department of Environment, Bangladesh so that necessary action can be taken to quarantine the godown area of CCC DDT factory.

Different kinds of pesticides; organophosphorous (mainly diazinon and chlorpyrifos) and pyrethroids (cypermethrin, fenvalerate etc.) are being used vegetable cultivation to increase the food production in Bangladesh. The dissipation and residual analysis of different pesticides in vegetable samples were studied to assess the safety of food products in the country. A field trial was carried out to assess the dissipation patterns of cypermethrin in farmer's field.

All the samples were found not to exceed the MRL of cypermethrin. The food with such concentration of cypermethrin residues cause toxicity in living organism. However, pesticide residue in vegetables over MRL value can cause serious health hazard for both wild life and human. So, nonstop monitoring of pesticide residue would be needed in a large scale. Pesticide traders/sellers and vegetables cultivators should be given training on the safe use and handling of pesticides in order to protect crops from pest infestation and reduce health hazards of end user.

Proper use of pesticides in agriculture needs to be vocal in Bangladesh and other countries. This study may be helpful for public consciousness, a guideline to government for pest control management in case of fruits and vegetables production and preservation of nutritional necessities of population. For cypermethrin in vegetables they should apply it as recommended doses and harvest after 2-3 days for

consumer safety. They should not apply an overdose but if applied, the harvest period should be more than two weeks. And, they must never apply cypermethrin daily.

The maximum residue levels (MRL) of cypermetrin were identified in 0 day samples (2 h after spray) of all vegetables. The residue levels went down progressively with days and 74-88% dissipations were observed in all matrices within 10 days. It was established that cypermethrin residues went lower the MRL value after 1 day of spraying in tomato (143 µg kg^{-1}) and in eggplant (106 µg kg^{-1}) and at 0 day (134 µg kg^{-1}) in bitter gourd (Codex, 2013, 2009). The half-life of cypermethrin for five vegetables was calculated. The most of the vegetable samples were not detrimental to health as all samples had lower the MRL of cypermethrin.

In a collaborative research programme (between Chonnam National University, South Korea and Dhaka university, Bangladesh), a QuEChERS method was modified and validated for the residual analysis of fluxapyroxad in moie leaves grown at green house condition in South Korea. The samples were analyzed by LC-MS/MS. This is the first study focusing on the impacts of fluxapyroxad on moie leaves. A modified method was successfully optimized for analysis of fluxapyroxad in these green leafy moie leaves using both QuEChERS method and LC-MS/MS technique. Good recoveries were found and ranged between 88% and 93% with RSD less than 10%. The optimized method was successfully applied to the field sample. These data confirmed the usefulness of this method in detecting environmental exposure to pesticides through food. The dissipation rates of fluxapyroxad at two treatments were different. The half-life was found to be 3.04 days for double dose and 2.68 days for triple dose. In this study, fluxapyroxad was dissipated below the MRL value after 10 days at triple of recommended dose. European Food Safety Authority (EFSA) referred MRL for leafy vegetables and fresh herbs (0.1 mg/kg). The rate of disappearance was described to 1^{st} order kinetics with half-life of 2.6 days. The initial residues after application were 11 and 20 mg kg^{-1} on the zero day for T2 and T3 respectively. After 14 days the residues declined to 0.42 and 0.36 mg kg^{-1} for T2 and T3 respectively.

Pesticides are extensively used for manufacture of crop, protection of food, public health purpose and to control the insect infestation of plants. By nature pesticides are poisonous material which acts on insect and pest. Twenty four different vegetables

samples were collected from different locations of Bangladesh and analysed for the presence of chloropyrifos, cypermethrin, diazinon and fenvelarate pesticides residuies.

The estimated daily intake and health risk index (HI) were calculated. The HI showed that cypermethrin residues in all vegetables were found less than 1 (HI<1). The most serious alarm about the pesticide use is its hazardous effects on different components of the environment. In Bangladesh context, the vegetables growers have been using the pesticides frequently to have advanced and pest free harvest. But the overdose of pesticides make the residue problematic, which might contaminate our food and environment.

World Health Organization has settled MRL for pesticide residues in different vegetables. In line with that very recently Bangladesh Food Safety Authority has enacted a regulation titled "Chemical Contaminates, Toxins and Harmful Residues, 2017" (Bangladesh gazette No: SRO No 183-Law/2017).

There is always a time gap between the harvest and availability of vegetables in the local market. It is not enough simply to deliver manufacturers with a manual of food safety guidelines and skilful implementation and documentation. From these studies it worthwhile for the consumers to keep vegetables at ambient (2-3days) temperature rather than store in a refrigerator.

Recently a multiplicity of Good Agricultural Practices (GAP) codes, standards and regulations have been developed by various food industry and producers organizations in first world countries. Developed countries' governments and NGOs also aiming to codify agricultural practices at farm level for a range of commodities. We in our country we need to make a culture of Good Agricultural Practice to ensure safety and quality of produce in the food chain, capture new market advantages by modifying supply chain governance, improve natural resources use, workers health and working conditions, and/or create new market opportunities for farmers and exporters in developing countries. We need to make our farmers, retail suppliers and consumers that Good Agricultural Practices addresses environmental, economic and social sustainability for on-farm processes, and result in safe and quality food.

Comparing the above reports, our study wants to give a positive signal to Bangladesh that farmers can start following the good agriculture practice. The survey reports from regional countries revealed a significant percent of fruits and vegetables were contaminated with pesticides above the corresponding MRL values.

QUALITY ASSURANCE (QA)

To maintain the quality of this study was followed by ISO 17025. All the steps were performed with maximum efficiency and in an environmentally friendly manner. While collecting of samples, they were transported to the laboratory immediately and stored in a proper way. Samples which were about to store in freezer, were stored at -20 ^{0}C to reduce degradation and which were needed to be stored at ambient temperature, were stored at fume hood cupboard to allow the samples to be in a contact with air, heat and light.

Certified reference standards were used with highest purity (91-99%) and were stored at -20 ^{0}C in freezer. Working standard solutions were prepared in a very sensitive manner and were labeled indicating name, date of preparation and the meniscus layer was marked with permanent ink.

All required glass wares like volumetric flasks, pipettes, and the four digit analytical balance were calibrated by BSTI (Bangladesh Standard Testing Institute). Micro pipettes were certified by the company (country of origin) according to ISO 17025. All the glassware were cleaned with detergent, rinsed thoroughly with distilled water and finally rinsed with acetone and the solvent to be used. These were baked at 300 ^{0}C overnight, cooled and wrapped with aluminum foil. Water and the organic solvents were checked for possible interference with other contaminants by the analysis of reagent blank. All the instruments and chromatographic instruments were checked and calibrated regularly.

All the reported methods were validated with the parameters of validations; linearity, specificity, selectivity and by recovery experiments by spiking the control samples. The spiked samples were kept apart to allow perfect adsorption on the samples. Excellent recoveries (in a range of 70 - 120%) and precisions (less than 20%) were found.

Gas chromatograph coupled with electron capture detector (Shimadzu-17A & Shimadzu-2010) and high performance liquid chromatograph coupled with Tandem

Mass Spectrometer (Water 2695; LC-MS/MS, TQ detector) were used in this analysis for identification and quantification of analytes in the samples. All the sample extracts had been cleaned up before injecting into chromatography machines, by respective cleaned up procedure because the identification was based on retention time of analytes, matched with certified standards and this may cause some problems with co-elution. Quantification was performed within dynamic range of the respective detector. The control standards were analyzed every day during analysis to check performance of the analytical systems.

All electrical equipment, GCs and LC-MS/MS were connected with a power generator (50 kVA) with online UPS. All the equipment rooms' has air cooler facilities. Log books were maintained for each of the equipment. Solvents were stored separately. Hazardous solvents and chemicals were handled carefully. All the solvents waste were disposed safely.

To evaluate instrumental limits, the limit of detection (LOD) and the limit of quantification (LOQ) were measure as 3 and 10 times of the signal to noise ratio (S/N) in the chromatogram, respectively.

Separate laboratories were designated for sample storage, extraction and residual analysis by GC and LC-MS/MS. All rooms were neat and clean and separate shoes were available for each room. Separate IPS was used for instruments to avail continuous power supply and freezers and refrigerators were available for sample storage. Sufficient light and ventilation were available in the laboratory. Personal safety measures *i.e.* wearing apron, taking hand gloves and face protection (goggles and masks) were taken during the laboratory work.

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List of papers out of the work

1. **Farzana Khalil**, M. N. U. Al Mahmud, Md. Musfiqur Rahman, M. I. R. Mamun, Mohammad Shoeb, A. M. Abd El-Aty, Jong-Hyouk Park, Ho-Chul Shin Nilufar Nahar and Jae-Han Shim. Determination of DDTs in soil and water of a closed-down DDT factory in Bangladesh: Inter-method and inter-laboratory validation studies of soil samples. (2015. Environment Monitoring and Assessment, 187: 743).

2. **Farzana Khalil**, Nilufar Nahar, Mohammad Shoeb and M. I. R. Mamun. DDTs in fish samples of Chittagong Chemical Complex area in Bangladesh (Manuscript).

3. **Farzana Khalil**, Nilufar Nahar, Mohammad Shoeb and M. I. R. Mamun. DDTs in human blood of former DDT factory in Bangladesh: Chittagong Chemical Complex area. (Manuscript).

4. **Farzana Khalil**, Nilufar Nahar, Mohammad Shoeb and M. I. R. Mamun. Dissipation pattern of cypermethrin in five different vegetable samples of Bangladesh. (Manuscript).

5. **Farzana Khalil**, Nilufar Nahar, Mohammad Shoeb M. I. R. Mamun and Jae-Han Shim. Study on distribution and dissipation of fluxapyroxad in moie leaves (Manuscript).

6. **Farzana Khalil**, Mohammad Shoeb, Nilufar Nahar, M. I. R. Mamun, Analysis of pesticide residues in summer vegetables of Bangladesh. (Manuscript).