

**JIMMA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL SCIENCES
DEPARTMENT OF CHEMISTRY**



M.Sc THESIS ON

**DETERMINATION OF ORGANOPHOSPHOROUS AND
ORGANOCHLORINE PESTICIDES IN WATER SAMPLES
OF GILGEL GIBE (I) HYDROELECTRIC DAM AND ITS TRIBUTARIES,
OROMIA, ETHIOPIA**

BY MEAZA DEJENE

**NOVEMBER, 2018
JIMMA, ETHIOPIA**

**DETERMINATION OF ORGANOPHOSPHOROUS AND
ORGANOCHLORINE PESTICIDES IN WATER SAMPLES
OF GILGEL GIBE (I) HYDROELECTRIC DAM AND ITS TRIBUTERIES,
OROMIA, ETHIOPIA**

BY

MEAZA DEJENE

ADVISOR: ABERA GURE (PhD)

CO ADVISOR: KASIM KEDIR (M.Sc)

SEBLEWORK MEKENNON (PhD)

**M.SC THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES COLLEGE OF
NATURAL SCIENCE JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY
(ANALYTICAL).**

M.Sc Thesis Submitted to School of Graduate Studies College of Natural Science Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry (Analytical)

APPROVAL SHEET

Advisors	Signature	Date
Abera Gure (PhD) Assoc. Prof. on Analytical Chemistry Department of Chemistry College of Natural Sciences, Jimma University	_____	_____
Kasim Kedir (M.Sc) Department of Chemistry College of Natural Sciences, Jimma University	_____	_____
Seblework Mekonnen (PhD) Department of Environmental Health Science and Technology Jimma University	-----	-----

TABLE OF CONTENTS

List of tables	iii
List of figures.....	iv
Abbreviations.....	v
Acknowledgments.....	vi
Abstract	vii
1. Introduction.....	1
1.1. Back ground of the study	1
1.2. Statement of the problem	4
1.3. Objective of the study	5
1.3.1. General objective.....	5
1.3.2. Specific objectives.....	5
1.4. Significance of the study.....	6
2. Review of related literatures.....	7
2.1. Enviromental pollution	7
2.1.1. Water pollution.....	7
2.2. Sources of water pollution.....	8
2.2.1. Point sources of pollution	8
2.3. History of pesticides	9
2.4. Use of pesticides.....	9
2.5. Pesticides and public health	9
2.6. Fate of pesticides	10
2.7. Distribution of pesticides in different environmental compartments	10
2.8. Chemical properties of pesticides.....	11
2.9. Classification of pesticide	12
2.9.1. Organophosphorous pesticides	12
2.9. 2. Organochlorine pesticides.....	14
2.10. Analytical determination of pesticides.....	16
2.11. Sample preparation methods	17
3. Methodology.....	19

3.1 Description of the area	19
3.2. Sample collection.....	19
3.3. Chemical and reagents	20
3.4. Instruments and equipments.....	21
3.5. Gas chromatography-electron capture detector condition	21
3.6. DLLME Extraction procedure.....	22
3.7. Method Validation	23
3.8. Statistical Analysis.....	23
4. Results and discussion.....	24
4.1. Calibration Curves and Analytical Performance study.....	24
4.2. Relative recovery studies	24
4.3. The concentration of pesticides residues in water samples.....	25
5. Conclusion and recommendations	30
5.1. Conclusion.....	30
5.2. Recommendations.....	31
6. References.....	32
Appendix.....	40

LIST OF TABLES

Table 1: List of chemical structure and common names with their P_{ka} and solubility in water values of the target pesticides.	14
Table 2: List of chemical structure and common names with their molecular weight and solubility in water values of the target pesticides.	16
Table 3: Analytical performance characteristics of the utilized method.....	24
Table 4: Relative recovery of the method	25
Table 5: Mean level ($\mu\text{g/L} \pm \text{SD}$) of OCPs in water samples ($n = 4$).	26

LIST OF FIGURES

Figure 1: Use of pesticides.	9
Figure 2: Fate of pesticides in the ecosystem.	10
Figure 3: Distribution of pesticides in different environmental compartments	11
Figure 4: Map of the study area and the specific sampling sites.	20
Figure 5: Chromatograms of the target OPPs and OCPs. Description of analytes (retention time, t_R , in min); (1) Methidathion (4.328); (2) Malathion (8.708); (3) Chlorphyrifos (8.978); (4) Chlorflurenol- Methyl (10.725); (5) DDT (12.095); (6) Endrin (12.957); (7) and Dieldrin (17.412).	22

ABBREVIATIONS

DLLME	Dispersive Liquid-Liquid Micro Extraction
GC-ECD	Gas Chromatography-Electron Capture Detector
OCP	Organochlorinepesticides
OPP	Organophosphorouspesticides
LLE	Liquid-Liquid Extraction
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
LPME	Liquid Phase Micro Extraction
GC- NPD	Gas Chromatography-Nitrogen Phosphorous Detector
GC-FPD	Gas Chromatography-Flame Photometric Detector
GC-MS	Gas Chromatography-Mass Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
RR	Relative Recovery
DDT	Dichlorodiphenyltrichloroethane
WHO	World Health Organization
EPA	Enviromental Protection Agency

ACKNOWLEDGMENTS

First and foremost, I would like to offer praise and glory to Almighty God for providing me good health, peace and strength in all my life. Next, I would like to express my heartfelt thanks and profound gratitude to my advisors Dr. Abera Gure, Mr Kasim Kedir and Dr. Seblework Mekonnen for their valuable and constructive guidance's as well as comments starting from the beginning up to the end of the work. I want to specially express my deep heartfelt appreciation to Dr Abera Gure for his suggestion, motivation and guidance throughout this work.

I would also like to acknowledge all my families, specially my father Dejene Tilahun, my mother Woyinitu Debebe, my sisters Selamawit and Mahilet Dejene for their moral and financial encouragement. My gratitude also goes to chemistry department stuffs for their unreserved contributions during my M.Sc study. My appreciation also goes to Environmental Health Science and technology department of Jimma University for their kind support to analyze my samples in their laboratory using GC-ECD instrument. Finally, I want to express my earnest appreciation for Jimma university community school for the financial support and permission they offered me to carry out this work.

ABSTRACT

In this study, the levels of some selected organophosphorous (OPPs) and organochlorine pesticides (OCPs) in water samples collected from Gilgile Gibe (I) hydroelectric dam and potential tributaries, Jimma zone, Oromia Regional State, Ethiopia were determined using gas chromatography electron capture detector (GC–ECD). Low density based dispersive liquid-liquid microextraction (LD-DLLME) using toluene (as extractant) and acetonitrile (as disperser) was used for extraction of the pesticides from water samples. Calibration curves were constructed at six concentration points by spiking and extracting distilled water a representative matrix. And the obtained calibration curves have good linearity with coefficient of determination (r^2), ranging from 0.995-0.999. The limits of detection (LOD) and quantification (LOQ) of the utilized method which were determined as 3 and 10 times the signal-to-noise ratio were ranging from 0.0001-2.5810 $\mu\text{g/L}$ and 0.0005-8.6050 $\mu\text{g/L}$, respectively. The efficiency of the methods was also evaluated through recovery studies by spiking the samples with known concentrations of the target analytes. The obtained recoveries were ranging from 67.2-105% with 0.79 - 12.5% relative standard deviations. The study results demonstrated that all water samples contain the target pesticides. But endrin was not detected in all the water samples. Methidathion was also not detected in the dam, as well as Nadi and Gibe river samples. The detected residual concentrations of the pesticides were all above the maximum residue limits, with the exception of DDT in acute toxic level. Results of the study indicated the studied water samples contain considerable amount of residues of pesticides that can influence the health of aquatic organisms and other consumers.

Keywords: *Organochlorine pesticides; Organophosphorous pesticides, Water sample; Gas chromatography electron capture detector.*

1. INTRODUCTION

1.1. Back ground of the study

Environmental pollution is the most concerned subject for many countries. Pollution has major effects on all aspects of life and threatens human health, animals, plants, and environment [1]. The environment is being increasingly altered by the presence and accumulation of waste and by products arising from both natural origin and man-made activities [2]. Pollution of the various compartment of the environment such as water, air and soil is increasing from day to day due to increase in population, industrialization and urbanization. Pollutions could originate from different sources including industrial wastes and agricultural chemicals such as pesticides [3].

Pesticides are chemical compounds that are used to kill pests including insects, rodents, fungi and unwanted plants (weeds) [4]. For instance, organochlorine pesticides (OCPs) and organophosphorus pesticides (OPPs) are widely used as pesticides.

OCPs own highly persistent properties and can exhibit a potential threat to human health. OPPs are often used in many countries as less toxic alternatives. Although, OPPs can disintegrate in the environment to some extent, they have been shown to frequently remain in surface water, fruit, vegetables and crops resulting from their excessive use [5].

The widespread uses of pesticides for agricultural and non-agricultural purposes have resulted in the presence of their residues in various environmental matrices, such as soil, environmental water (surface water and ground water) and air [6]. Most pesticides are characterized by pronounced persistence against chemical or biological degradation, high environmental mobility, strong tendency for bioaccumulation in human and animal tissues, and significant impacts on the health human being [5-6]. From the total applied pesticides, less than 0.1% could reach the target pests, whereas, the rest proportion could be potentially disseminated into other environmental compartments such as environmental soils, sediments and water [7]. Water pollution occurs when containments enter in to the water bodies such as lakes, rivers and oceans. Pesticides may reach the water ecosystem through various mechanisms such as leaching, agricultural or urban runoff, drift, and etc. [8]. Hence, they are considered as potential water pollutants and identified to cause

risks on crops, aquatic plants, and microorganisms and human being. Exposure to even low concentration of pesticides could cause series health effect [9]. For instance according to WHO estimation every year about three million people died because of pesticides poisoning [10]. This indicates that contamination of pesticides in different enviromental system including water ecosystems is a serious problem.

Gilgel Gibe I hydroelectric dam is one of the artificial lake formed by preparation of dam on Gibe river in Jimma zone, Oromia regional State, Ethiopia, to generate hydroelectric power. The lake also receives additional input from Nada gudda, Nada qalla, and Nadi Rivers. The dam surrounded by intensified farmlands, which utilize fertilizers and pesticides for various purposes [11]. This could have directly or indirectly pollution of the environment and cause a serious health effect [8-10]. Thus, regular monitoring of the levels of these agrochemicals in water ecosystem is crucial.

But, to analyze residues of pesticides from water samples, sample preparation that involves isolation, cleanup and/or preconcentration steps is mandatory. In most cases, liquid-liquid extraction (LLE) and solid phase extraction (SPE) sample preparation methods are used for analysis of residue of pesticides from different matrixes [12-13].

However, these methods are time consuming, requires large amounts of toxic organic solvents and provide low enrichment of analytes [14] Therefore, in the last couple of decades, several simple, fast, cheap, sensitive and environmentally safe sample preparation methods have been proposed. Some the proposed methods include solid phase micro extraction (SPME) [15] and liquid phase micro extraction (LPME) [16]. Which have been extensively used for analysis of residues of pesticides from environmental water and from other matrices.

These days, dispersive liquid–liquid microextraction (DLLME), which was first reported in 2006 by Assadi and co-workers [17], has gained great attention for extraction and/or preconcentration of various organic and inorganic pollutants from different matrices. The method is characterized by its simplicity of operation, rapidity, low cost, high recoveries, high enrichment factor and being relatively environmentally benign [18].

After extracting and preconcentrating residues of pesticides, chromatographic methods are used for separation and determination [19]. These techniques includes with gas chromatography with

various detectors such as flame photometric detection (GC-FPD) [20], nitrogen phosphorus detection (GC-NPD) [21], electron capture detector (GC-ECD) [22], mass spectrometry (GC-MS) [23], as well as liquid chromatography was used for separation and determination of pesticides from different matrixes in coupled with mass spectrometry (LC-MS) [24] and so on. GC-ECD is the most common and chosen method because of its selectively and good sensitivity, for analysis of pesticides that contain halogen in their structure [22].

OC and OP pesticides have been used for long time in Ethiopia for controlling of agricultural pests, malaria and other organisms [25]. These pesticides could persist in the environment depending on its property and the amount of usage [26]. Pesticide residues which persist in the environment could have a direct or indirect impact on the health of living things including aquatic organisms. Thus, determination of pesticides residues in surface water and other environmental compartments is crucial. Surface water like Gilgel Gibe I hydroelectric dam water, which is surrounded by intensified farm lands and used for multipurpose such as fish production, irrigation, bathing, drinking and so on could adversely affect the health of the consumers, if they contain the residues pesticides. A study conducted on Gilgel Gibe I hydroelectric dam and its four potential tributaries Gibe, Nada gudda, Nada Qalla and Nadi rivers indicated that the water contain residues of OCPs including Aldrin, Dibutylchlorendate, 4,4-DDE, Gamma-chloridane, Edirne, Endosulfan sulfate, Dieldrin, Methoxychlor and Heptachlor epoxide [27]. This finding also indicated the possibility of the presence of the residues of the other commonly used pesticides like OPPs in the dam and its tributaries. Therefore, in the present study, the levels of selected OP and OC pesticides residues in the dam and the tributaries: Gibe, Nada gudda, Nada Qalla and Nadi rivers were investigated by using GC-ECD.

1.2. Statement of the problem

The environment contains countless complex pollutants, including pesticides, which are potentially damaging the environment and then, the health of human being and other animals [28]. These pollutants can be transferred from one particular area to another by natural phenomena (leaching, drifting and run-off) or by organisms including human beings [29]. The presence of these and several varieties of their transformation products at different concentration levels in waters could have direct or indirect impact on the health and safety of human beings [30]. As a result, continuous assessment and monitoring of the contamination level of different types of environmental water is important to identify their suitability for human consumptions.

In Ethiopia, for many years, OCP and OPP were used for controlling of insecticides on agricultural fields as well as for controlling of malaria at house hold level [25]. These pesticides have been also detected in staple foods including chat [31], teff and red pepper [32], which were collected from Jimma Zone, Ethiopia. These evidences clearly indicate that determination of these pesticides in different environmental and biological samples has of great importance to rescue the health of consumers in the area. In addition, some selected OC pesticides were also detected in Gilgel Gibe I hydroelectric dam and its four tributaries [27]. However, study has not been conducted on the residual levels of OPPs of the dam water and its four tributaries. Therefore, in this study, the levels of some selected OCPs and OPPs in Gilgel Gibe-I hydroelectric dam water and its four tributaries were determined using GC-ECD.

1.3. Objective of the study

1.3.1. General objective

- ◆ The main objective of this study is to analyze the concentrations of selected organophosphorus and organochlorine pesticides in water samples using gas chromatography with electron capture detector (GC–ECD).

1.3.2. Specific objectives

- ◆ To determine the concentrations of some selected organophosphorus pesticides such as methidathion, malathion and chlorpyrifos and organochlorine pesticides including chlorflurenol-methyl, DDT, endrin and dieldrin in water samples collected from Gilgel Gibe I hydroelectric dam and its four potential tributaries Gibe, Nada gudda, Nada Qalla and Nadi rivers.
- ◆ To identify the pollution status of the water samples based on EPA of ambient water quality guideline.
- ◆ To identify the contributions of the tributaries on pollution of the dam water in terms of the studied pesticides.

1.4. Significance of the study

It is well known that pesticides are one of the vital inputs in agriculture to prevent loss of production. However, an intensive and uncontrolled uses of these agrochemicals could result in the contamination of the environment, including waters. Therefore, the analysis of pesticide residues from water is crucial. In general, the findings of this study could have the following significances:

- ✓ It could be used as first hand information regarding the pollution status of the dam water and the tributaries.
- ✓ It could be used as the background information for the researchers who want to work on the dam or other related area.
- ✓ It might be used as supplementary information by the policy makers or other concerned bodies who closely follow up the dam water.

2. REVIEW OF RELATED LITERATURES

2.1. Environmental pollution

Environment pollution is a wide-reaching problem in whole over the world [33]. It is any substance whether liquid, solid or as which directly or indirectly alters the quality of any part of the receiving environment so as to affect its beneficial use adversely or produces toxic, substances, diseases, objectionable odor, radio activity noise, vibration, heat or any other phenomena on that is hazardous or potentially hazardous to human health or to other living things [34].

Pollutants may cause primary damage, with direct identifiable impact on the environment, or secondary damage in the form of minor perturbations in the delicate balance of the biological food web that are detectable only overlong time periods. Commonly pollution is classified according to the environment in which it occurs a water pollution, soil pollution and air pollution [35].

2.1.1. Water pollution

Water pollution is one of the most serious environmental problems. Water pollution is caused by a variety of human activities such as industrial, agricultural and domestic. Agricultural runoff laden with excess fertilizers and pesticides, industrial effluents with toxic substances and sewage water with human and animal wastes pollute our water thoroughly. Natural sources of pollution of water are soil erosion, leaching of minerals from rocks and decaying of organic matter [36].

Polluted water consists of Industrial discharged effluents, sewage water, and rain [37] and polluted by agriculture or households cause damage to human health or the environment. This water pollution affects the health and quality of soils and vegetation. Some water pollution effects are recognized immediately, whereas others don't show up for months or years [38]. Estimation indicates that more than fifty countries of the world with an area of twenty million hectares area are treated with polluted or partially treated polluted water including parts of all continents and this poor quality water causes health hazard and death of human being, aquatic life and also disturbs the production of different crops. In fact, the effects of water pollution are said to be the

leading cause of death for humans across the globe, moreover, water pollution affects our oceans, lakes, rivers, and drinking water, making it a widespread and global concern [39].

2.2. Sources of water pollution

Water pollution occurs when a body of water is adversely affected due to the addition of large amounts of materials to the water. The sources of water pollution are categorized as being a point sources or a non-source point of pollution [40].

2.2.1. Point sources of pollution

Occur when the polluting substance is emitted directly in to the way. A pipe spewing toxic chemicals directly in to the river are an example. The oil spill best illustrates point source water pollution [39, 40]. Direct sources include effluent outfalls from factories, refineries and waste treatment plants etc. that emit fluid of varying quality directly into urban water supplies. In the United States and other countries, these waters. The technology exists for point sources of pollution to be monitored and regulated [41].

2.2.2. Non-point source of pollution

Occurs when there is runoff of pollutants in to a waterway, an example of this type of water pollution is when fertilizers and pesticides from a field are carried in to a stream by rain, in the form of run-off which in turn affects aquatic life [42]. Non-point source of pollution delivers pollutants indirectly through enviromental changes. Pollution arising from non-point sources accounts for a majority of the contaminants in streams and lakes. Indirect sources include contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water. Soils and ground waters contain the residue of human agricultural practices (fertilizers, pesticides, etc.) and improperly disposed of industrial wastes. Atmospheric contaminants are also derived from human practices (such as gaseous emissions from automobiles, factories and even bakeries). Contaminants can be broadly classified into organic, inorganic, radioactive and acid/base [42].

2.3. History of pesticides

Humans have been using pesticides for a long time to control damaging of their agricultural yields. For instance; ancient Romans had control insect pests by burning sulfur and weeds by using salt [43]. Synthetic pesticide was reported first in 1939. The first reported pesticide was dichlorodiphenyl-trichloroethane (DDT). DDT was exceptionally effective and the most widely used insecticide in the world [44]. Since then, various synthetic pesticides were emerged and commercialized worldwide. According to the USA EPA, currently more than 20,000 pesticides are registered and commercialized, indicating the availability of varieties of pesticides utilized for either agricultural or non-agricultural purposes [43, 44].

2.4. Use of pesticides

The major advantage of pesticides is that they can save farmers. They are used to protect crops from insects and other pests. It can also be used for controlling pests and plant disease vectors, controlling of human/livestock disease vectors and nuisance organisms, controlling of organisms that harm other human activities and structures [45]. Figure 1 shows how pesticides are applied on agricultural field to control pests.



Figure 1: Use of pesticides [45].

2.5. Pesticides and public health

The extensive application of variety types of pesticides is a common practice to enhance the quality as well as quantity of agricultural products [46]. Although the production and use of some

persistent pesticides were banned, most of these products are continuously in use, without knowing fully the negative impact on ecosystems and public health. According to the World Health Organization (WHO) estimations, pesticide residues poison every year three million people. This indicates that pesticides pose a risk on the public health, so it is necessary to conduct more studies using different models and indicators to assess the potential risks to health and the ecosystem [10]. Moreover, improper utilization of such chemicals could also lead to reduced connectivity (tropic interaction between organisms), loss of biodiversity, ecological imbalance and decreased environmental stability.

2.6. Fate of pesticides

Once applied in the agricultural field or product or other environment pesticides could be disseminated to the surrounding compartments such as, water, air, soil, and living organisms, via various mechanisms including leaching, runoff, drifts, and so on [47] Figure 2 shows that fate of pesticides in the ecosystem [47].

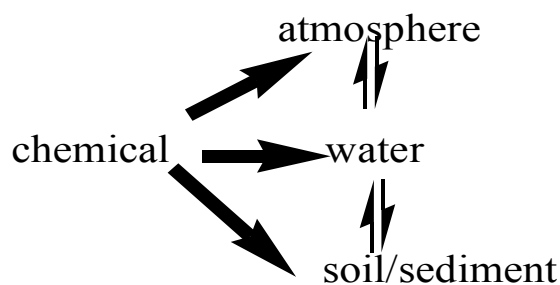


Figure 2: Fate of pesticides in the ecosystem [47].

2.7. Distribution of pesticides in different environmental compartments

The environment is a major source of exposure to pesticides from farming. Approximately 47% of the applied product is deposited at or adjacent soil and water is dispersed in the atmosphere. The environment can be divided into four major compartments, namely; air, water, soil and biota (Figure 2). The widespread use and disposal of pesticides by farmers, institutions and the general public provide many possible sources of pesticides in the environment. Pesticides once released into the environment may have many different fates. Pesticides that are sprayed can move through

the air and may eventually end up in other parts of the environment, such as in soil or water. Pesticides that are applied directly to the soil may be washed off into the nearby bodies of surface water or may percolate (leach) through the soil to lower soil layers and groundwater [48].

Pesticides can also get into water via drift during pesticide spraying. In some cases pesticides can be applied directly onto water surface e.g. for control of mosquitoes. Water contamination depends mainly on nature of pesticides (water solubility, hydrophobicity), soil properties, weather conditions, landscape and also on the distance from an application site to a water source. Rapid transport to groundwater may be caused by heavy rainfall shortly after application of the pesticide to wet soils [49].

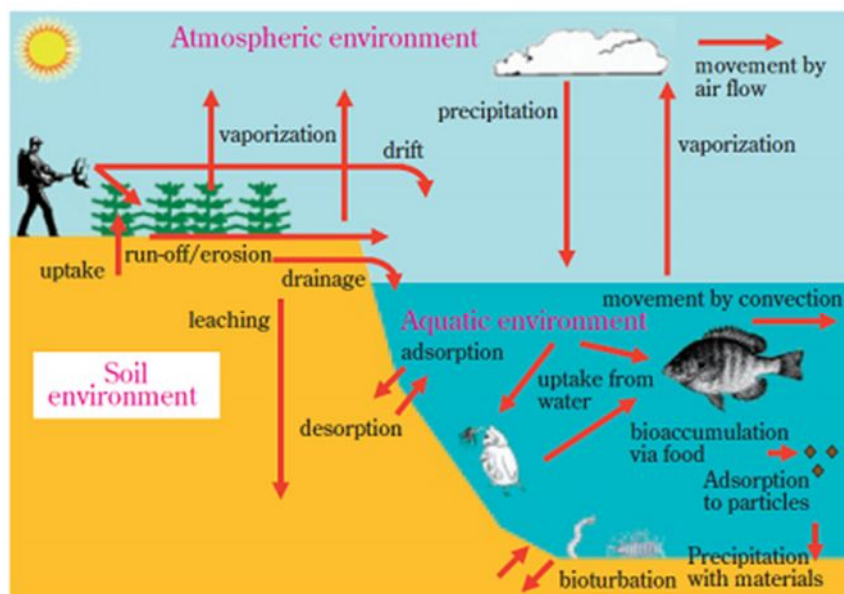


Figure 3: Distribution of pesticides in different environmental compartments [49].

2.8. Chemical properties of pesticides

Following the release of pesticides in the environment, they undergo a complex series of interdependent processes that are collectively called chemodynamics of pesticides. The chemodynamic processes that a pesticide undergoes is essentially determined by its inherent physico-chemical properties and partly by environmental parameters such as pH, temperature, moisture, precipitation, salinity, light intensity and topography. The major chemodynamic

processes that determine the pesticides persistence, distribution and the ultimate fate in the environment include transportation, retention, degradation and biota uptake. Among all these chemodynamic processes, degradation is of much relevance as it entails the chemical transformations of pesticides in the environment, hence chemical properties of pesticides [50]. Degradation of pesticides is the breakdown or chemical transformation of pesticide molecules into other forms that are not necessarily simpler and less toxic compared to the parent molecule. In some cases the degradation products are also toxic and have some pesticidal effects as well. A good example of this is the degradation of DDT to DDD, which is itself a pesticide [51]. The rate of degradation of pesticides is usually measured in terms of half-life ($t_{1/2}$), which is the time required for the depletion of half (or 50%) of the amount of pesticide present initially. The degradation processes that bring about pesticides transformation can be categorized into two major groups; chemical degradation and biological degradation. Chemical degradation generally occur in water or atmosphere and it follows one of four reactions namely; oxidation, reduction, hydrolysis and photolysis. Biological degradation generally occurs in soil and in living organisms and it utilizes one of four reaction; oxidation, reduction, hydrolysis and conjugation. The type of the reaction in which a pesticide undergoes is largely determined by the pesticide inherent phyco-chemical properties and the environmental compartment (water, soil, air, biota) in which it is hosted [52].

2.9. Classification of pesticide

Pesticides can be classified in different ways. For instance, they could be classified based on their functions and the type of the intended target pests: insecticides; herbicides; rodenticides; fungicides, etc and based on their chemical compositions: organochlorine, organophosphorus, carbamates, triazines, pyrethroids etc [53].

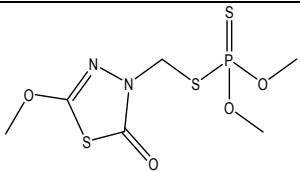
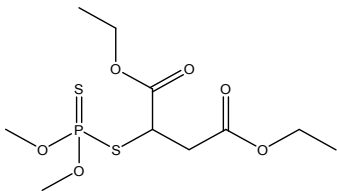
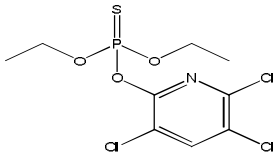
2.9.1. Organophosphorous pesticides

OPPs are the general name of organic derivatives of phosphorus. They are the most commonly used insecticides in the world because of their rapid hydrolysis and little long-term accumulation in the environment [54]. They have been widely used as an alternative to OCPs for the control of insecticide in a wide range of fruit, vegetables, and grain all over the world. But, due to their

extensive use and moderate persistence, OP pesticide residues could be found on commodities at the time of sale and natural waters. They also exhibit toxicity, bioaccumulation, and mobility between different compartments [55].

The toxicity of some of these pesticides is due to the action of their metabolites. For example, metabolites of malathion; malaoxon and isomalathion are 40 times more toxic than their parent compound [54, 55]. Malaoxon can induce DNA breaks in places where on cogenes or tumor suppressor genes are located, so that this metabolite may be considered as a carcinogenic and a mutagenic agent. In mammals, OPPs degradation occurs through hydrolytic phosphatases and carboxyl esterases. It has low toxicity to mammals and was widely used in public health. It is used as a systemic pesticide that acts up on contact and ingestion to combat insects in foliage and soil. When absorbed through the airways, digestive tract and skin, it can cause death from respiratory failure in humans since the elevated concentration of acetylcholine blocks neuromuscular transmission. OPPs are generally more toxic to vertebrates and invertebrates as cholinesterase inhibitors leading to a permanent overlay of acetylcholine neurotransmitter across a synapse. As a result, nervous impulses fail to move across the synapse causing a rapid twitching of voluntary muscles and hence can cause paralysis and death. Unlike OCPs, OPPs are easily decomposed in the environment by various chemical and biological reactions, thus they are not persistent in the environment [56].

Table1: List of chemical structure and common names with their P_{ka} and solubility in water values of the target pesticides.

Pesticide name	Structures	Molecular weight (g/mol)	Class	Solubility
Methidathion		302.318	OPP	240 mg/L at 20 °C
Malathion		330.358	OPP	145 mg/L at 20 °C
Chlorphyrifos		350.57	OPP	1 mg/L at 20 °C

2.9. 2. Organochlorine pesticides

Organochlorines are the compounds which contain a minimum of one covalently bonded chlorine atom. Organochlorines exhibit a large variety of structures with much diverse chemical properties. Due to high atomic weight of chlorine, these compounds are found to be denser than water [57]. They were the first synthetic organic pesticides to be used in agriculture and in public. Most of them were widely used as insecticides for the control of a wide range of insects; these chlorinated pesticides persist in the environment for very long periods, undergo bioaccumulation and biomagnifications and therefore impart toxicity to non-target organisms including human beings. Once released in the environment OCPs, break down very slowly in air, water, soil and in living organisms and thus, they are considered as persistent pollutants [57].

Most of them have shown to be formidable to biodegradation due to the strength of C-Cl bond. OCPs can biomagnified through the food chain and thus, consumers of food of animal origin such

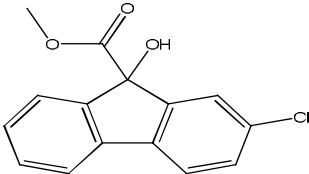
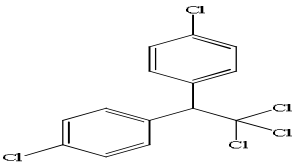
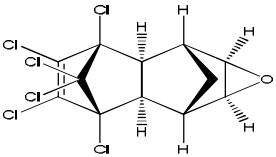
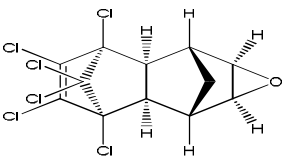
as fish, meat, milk and dairy products end up with high levels of exposure. This is happened since OCPs accumulate in the body, resulting from their slow bio-degradation. Insecticides such as OPPs, pyrethroids and carbamates have become attractive alternatives to OCPs because they do not persist in the environment [58].

The majority of the chemicals that were developed were organochlorine pesticides, such as aldrin, benzene hexachlorocyclohexanes (HCHs), chlordanes, dichlorodiphenyltrichloroethanes (DDTs), dimethyl tetrachloroterephthalate (DCPA), diazinon, dieldrin, endosulfans, endrin, hexachlorobenzene (HCB), methoxychlor, and mirex. Most of these chemicals persisted in the environment long after they were used, accumulated in lipid tissues of a variety of biota, and increase in concentration at the higher levels of food webs [59].

OCPs act as nervous system disruptors leading to convulsions and paralysis of the insect and its eventual death. Some of the commonly used representative examples of OCPs are DDT, lindane, endosulfan, aldrin, endrin, dieldrin, chlordane, Chlorflurenol-methyl and so on [60].

Human can be exposed to pesticides through inhalation, ingestion, or dermal contact, which might result in many toxicological health effects, including endocrine disruptions, reproduction and birth defects, immune system dysfunction, and cancer. OCPs are efficiently transported into the aquatic systems by infiltration, runoff, and atmospheric deposition as a result of their volatilization. Organochlorine compounds have been found to have toxic effects on aquatic organisms [61]. OCPs have been banned in many countries since the 1970s. So because of their slow chemical and biological degradation rate, OCPs are still detected in the environment. Therefore, determination of residual levels OCPs is important [61].

Table2: List of chemical structure and common names with their molecular weight and solubility in water values of the target pesticides.

Name of pesticides	Structures	Molecular weight (g/mol)	Class	Solubility in water
Chlorflurenol-methyl		215.589	OCPs	21.8 mg/L
DDT		354.49	OCPs	250 mg/L
Endrin		380.907	OCPs	125 mg/L
Dieldrin		380.91	OCPs	-

2.10. Analytical determination of pesticides

After extracting and preconcentrating residues of pesticides, chromatographic methods are used for separation and determination [19]. These techniques includes with gas chromatography with various detectors such as flame photometric detection (GC-FPD) [20], nitrogen phosphorus detection (GC-NPD) [21], electron capture detector (GC-ECD) [22], mass spectrometry (GC-MS) [23], as well as liquid chromatography was used for separation and determination of pesticides from different matrixesin coupled with mass spectrometry (LC-MS) [24] and so on. GC-ECD is

the most common and chosen method because of its selectivity and good sensitivity, for analysis of pesticides that contain halogen in their structure [22].

2.11. Sample preparation methods

In spite of substantial technological advances in analytical field, most instruments cannot directly handle complex sample matrixes yet. As a result, a sample-preparation step is commonly involved before instrumental analysis. The main aim of sample preparation is to clean up and concentrate the analytes of interest, while rendering them in a form that is compatible with the analytical system [62]. Various sample preparation methodologies including traditional methods such as; LLE and SPE were used. Liquid-liquid extraction (LLE), based on the transfer of analyte from the aqueous sample to a water-immiscible solvent, is widely employed for sample preparation. Despite its enormous applications for quantitative extraction of multiresidue pesticides from different matrices, LLE has a number of drawbacks: it is laborious, tedious and time-consuming procedures, it requires relatively large amount of sample and toxic organic solvents and multistage operations [63].

Another popular sample-preparation approach is solid-phase extraction (SPE). It was developed in the mid of 1970s as an alternative approach to LLE for isolation, preconcentration and clean-up of the target analytes in a single step [64]. Although it uses much less solvent than LLE, the usage can still be considered significant, and normally an extra step of concentrating the extract down to a small volume was needed. SPE can be automated but this entails complexity and additional cost. Compared to LLE, SPE is simple and safe to use. Moreover, the benefits of SPE include high recoveries of analytes, purified extracts, ease to automation, compatibility with chromatographic analysis and reduction in consumption of organic solvents. As a result, SPE has been applied in preparation of environmental, biological, food and pharmaceutical samples. It has long been accepted as an alternative standard sample preparation method to LLE in many of US Environmental Protection Agency (USEPA) methods for the determination of pollutant organic compounds in drinking water and wastewater [65].

In the past couple of decades, with the increasing interest in miniaturization and resultant solvent and sample saving, some new and novel sample preparation techniques, which are collectively

called liquid phase microextraction (LPME) techniques, have been introduced and applied with great success for analyses of different classes of organic and inorganic substances in various matrices [66].

In LPME procedures, the analytes of interest are selectively transferred from the aqueous sample solution (donor phase) to several micro liters of water-immiscible organic solvent (extractant /acceptor phase). These methods, generally, comprises extraction, clean-up and preconcentration processes in a single step. Based on the introduction mechanisms of an extracting solvent into the sample solution, LPME may be broadly categorized into three main variations: single-drop microextraction (SDME), hollow-fiber liquid-phase microextraction (HF-LPME) and dispersive liquid-liquid microextraction (DLLME) [67].

In 2006, Assadi and co-workers [17] developed a novel liquid-phase microextraction technique, named dispersive liquid-liquid microextraction (DLLME). Since after its introduction, DLLME has gained great attention for extraction and/or preconcentration of pesticide residues from various matrices [68, 69]. The method employs ternary solvent system comprising: aqueous sample, extraction solvent (water immiscible solvent) and disperser solvent (water miscible solvent). In the procedure, the mixture of extraction solvent and dispersive solvent is rapidly injected into aqueous solution and thus, an emulsion (cloudy solution) containing fine droplets of the extraction solvent is dispersed in aqueous sample solution. Then, analytes are rapidly transferred into the dispersed fine droplets of the extraction solvent (organic phase). The dispersed fine droplets are then separated after centrifuging. The extraction solvent, which is either accumulated at the bottom or top of centrifugation tube based up on the mode of DLLME procedure, is collected for further analysis [70]. In the last decade, DLLME has received great attentions as one of the best sample preparation method because of its low cost, rapidity, easy operation as well as its pronounced high enrichment factors. The advantages of DLLME method are simplicity of operation, rapidity, low cost, high-recovery, high enrichment factor, and environmental benignity [71], with wide application prospects in trace analysis.

3. METHODOLOGY

3.1 Description of the area

The Gilgel Gibe I hydroelectric dam is geographically located at latitude 7°49'52.45"N and longitude 37°19'18.79"E, in Jimma zone, Oromia Regional state at about 260 km in south west of Addis Ababa and about 70 km northeast of Jimma town. The dam occupies about 4225 km² area. The area is largely surrounded by cultivated farm land and villages [72].

3.2. Sample collection

The water samples were collected (one sample from each) from Gilgel Gibe I hydroelectric dam (D) and the four potential tributaries rivers: Nada qalla(NQ), Nada gudda(NG), Nadi(NR) and Gibe (GR) using grab sampling method. Water samples were taken in 1 L amber glass bottles, which were previously washed with 10% of nitric acid, oven dried, thoroughly rinsed with ultrapure water and then dried in an oven at 75 °C. Before sampling, the bottles were flushed three times with the sampled water. The collected water samples were transported to Jimma university analytical chemistry research laboratory using ice box and kept below 4 °C in refrigerator until analysis, without any pretreatment. Figure 4 shows map of the study area and the specific sampling sites.

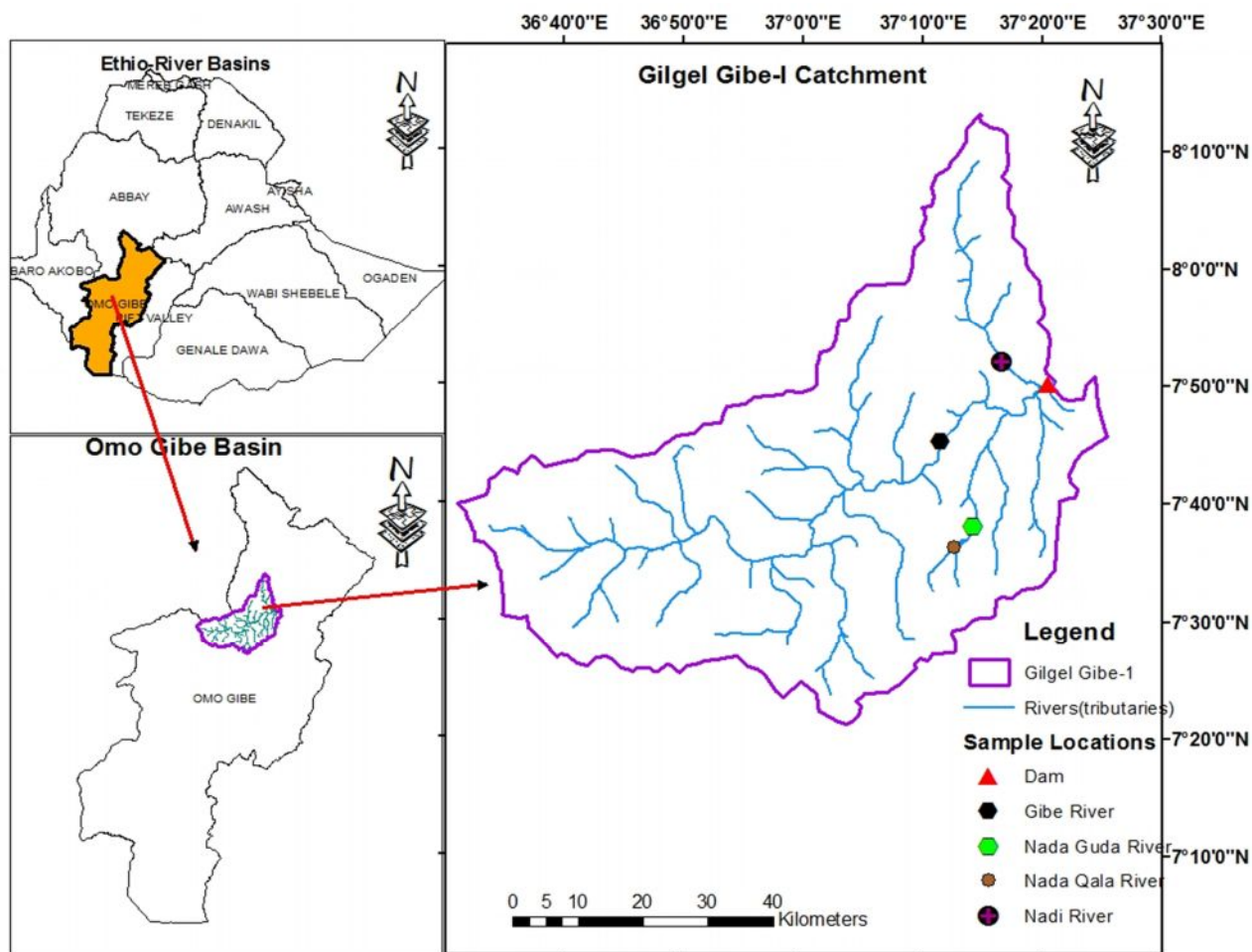


Figure 4: Map of the study area and the specific sampling sites.

3.3. Chemical and reagents

All chemicals and reagents used were analytical grade and solvents are HPLC grade. The organic solvents toluene was obtained from Blulux international PLtd (Stockholm, Malmo, Malmohus), n-hexane from Lobachemiepv. Ltd., (Jehangir villa, Mumbai, India), methanol and acetonitrile was obtained from Carlo erba reagents S.A.S (Mumbai, India) and (Bologna, Italy) respectively. Sodium chloride (NaCl) was from Tecnopharmchem, (Bahadurgarh, India). Whatman filter paper (grade 1 and size 8.5 cm) was used for filtration of the water samples.

Analytical standards of OPPs such as methidathion, malathion, chlorpyrifos, and OCPs such as; DDT, chlorflourenol methyl, endrin and Dieldrin were obtained from Sigma Aldrich (St. Louis,

MO, USA). Stock solutions containing 1000 mg/L of each pesticide were separately prepared in methanol and stored in refrigerator below 4°C. Intermediate working standard solution containing a mixture of 100 mg/L of methidathion, malathion, chlorpyrifos, and chlorflurenol-methyl as well as 10 mg/L of DDT, endrin and dieldrin analyte was then prepared by diluting appropriate volume of each standard in methanol and then, the solution was stored in the refrigerator at 4 °C. Working standard solutions was then prepared from this intermediate standard solution by diluting in n-hexane.

3.4. Instruments and equipments

Separation and quantification of the target analytes were performed using Agilent Gas chromatography equipped with an electro capture detector (GC-ECD) auto sample, pump, column compartment model 7980A (Agilent technologies, Singapore). An HP-5 capillary column (30 m, 0.25 mm inner diameter; 0.25-mm film thickness) coated with 5% phenyl methyl siloxane model was also obtained from Agilant. Vortex mixer obtained from Fisher scientific model FB15024 (Belgium). Other equipment such as 15 mL of centrifuge tube, centrifuge of model plc-02 Germany industrial colp (Taiwan), and Analytical balance from Kern and Sohn GMBH D-72336 (Balingen) were also used during the study.

3.5. Gas chromatography-electron capture detector condition

All pesticide residue analyses were performed using gas-liquid chromatography with an electron capture detector having an ALS auto-sampler. An HP-5 capillary column (30 m × 0.25 mm inner diameter; 0.25-mm film thickness) coated with 5% phenyl methyl siloxane (model 7890A, Agilent Technology) was used in combination with the following oven temperature program: initial temperature of 80 °C, ramped at 30 °C min⁻¹ to 180 °C, ramped at 3 °C min⁻¹ to 205 °C, held for 4 min, ramped at 20 °C min⁻¹ to 290 °C, held for 8 min, ramped at 50 °C min⁻¹ to 325 °C. The total GC run time was 27.92 min. Nitrogen (99.99% purity) was used as a carrier gas at a flow rate of 20 mL min⁻¹ and as a makeup gas at a flow rate of 60 mL/min. An aliquot of 1 µL was injected in split mode at a split ratio of 50:1 and injection temperature of 280 °C. The pesticide residues were detected with µ-ECD operating at a temperature of 300 °C. The

chromatogram of the target analytes using the above mentioned GC-ECD operating condition is shown in Figure 5.

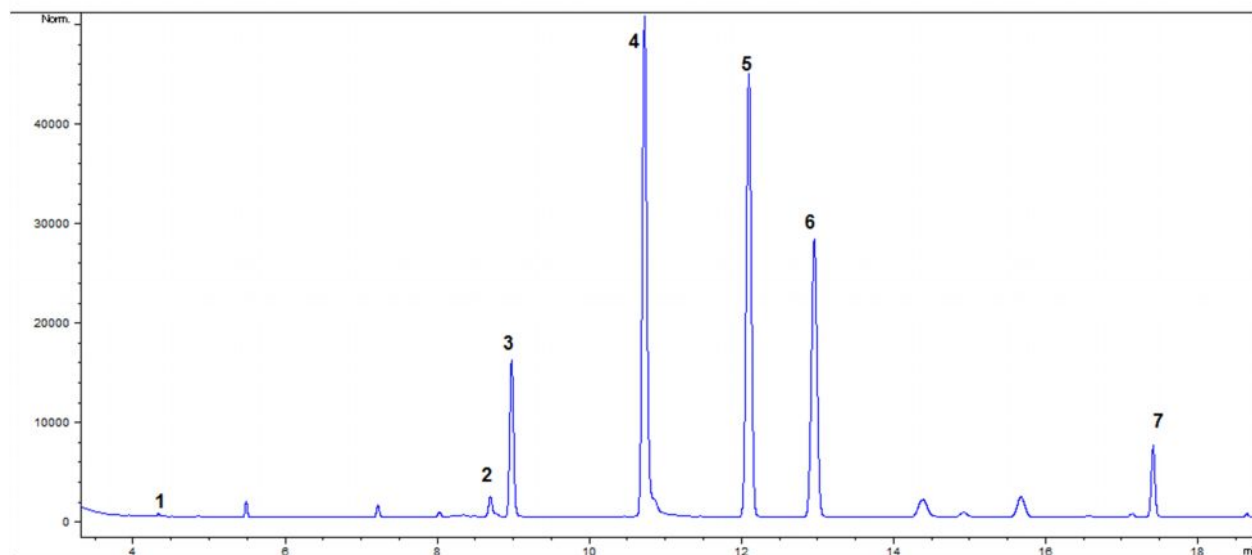


Figure 5: Chromatograms of the target OPPs and OCPs. Description of analytes (retention time, t_R , in min); (1) Methidathion (4.328); (2) Malathion (8.708); (3) Chlorpyrifos (8.978); (4) Chlorflurenol- Methyl (10.725); (5) DDT (12.095); (6) Endrin (12.957); (7) and Dieldrin (17.412).

3.6. DLLME Extraction procedure

LD-DLLME method, which was earlier, reported by Shen and coworkers [22] was used for extraction of the residues of the OC and OP pesticides from the water samples. Accordingly 5 mL water sample was taken in to 15 mL centrifuge tube and then, a mixture of 100 μ L toluene and 500 μ L acetonitrile as extraction and disperser solvents, respectively, was rapidly injected using a 5 mL medical syringe. Subsequently, after adding 0.5 g NaCl (i.e., 10% *m/v*) the content was manual shaken until the salt was completely dissolved. Then, the sample solution was vortexed for 30 s to enhance the homogeneous distribution of cloudy suspension into the sample solution and hence to accelerate the transfer of analyte from aqueous phase to the extraction phase. The content was then centrifuged for 3 min at 3000 rpm to facilitate phase separation. Finally, 50 μ L of the floating organic phase was then carefully with withdrawn using a micro pipette and

transferred into 100 μL insert vial which was housed in 1.5 mL autosampler vial to inject 1 μL into GC-ECD instrument.

3.7. Method Validation

Applicability of the utilized method to the target pesticides in the matrix under the study was evaluated by constructing calibration curves using distilled water as representative sample. Then, the analytical performances of the method was evaluated in terms of linear dynamic range, determination of limits of detection (LOD) and quantification (LOQ), repeatability and relative recovery studies.

Calibration curves were constructed using six concentration points corresponding to 10, 100, 250, 500, 750 and 1000 $\mu\text{g/L}$ for methidathion, malathion, chlorpyrifos and chlorflurenol-methyl and 0.1, 1, 2.5, 5, 7.5, and 10 $\mu\text{g/L}$ for DDT, endrin and dieldrin. Each concentration level was spiked in distilled water extracted LD-DLLME. Each concentration level was extracted in duplicate and each extract was then injected in duplicate. Then, calibration curves were obtained by considering the peak areas as the instrumental response versus the analytes concentrations. LOD and LOQ were determined as 3 and 10 times the signal to noise ratio, respectively. Precision (repeatability) of the method was investigated in terms of relative standard deviation (RSD) of replicate determinations. The accuracy of the method was assessed by performing relative recovery studies by spiking pesticide standards on to NQ river water sample as representative matrix 2.5 and 5 $\mu\text{g/L}$ concentrations levels.

3.8. Statistical Analysis

Statistical analytical software (SAS) version 9.0 with design of complete block design (CBD) was used for one-way ANOVA ($p \leq 0.05$) to investigate whether concentrations of OPPs and OCPs in the studied water samples are significantly different or not.

4. RESULTS AND DISCUSSION

4.1. Calibration Curves and Analytical Performance study

Calibration curves were constructed by spiking standard solutions of the target pesticides at six concentration levels in distilled water as a representative matrix and then extracting with considered LD-DLLME method. Then, calibration curves were obtained by considering the peak areas as the instrumental response versus the analytes concentrations. . The obtained calibration curves were exhibited wide linear ranges and good coefficient of determinations (r^2) ranging 0.995–0.999. LOD and LOQ of the method were ranging from 0.0001-2.5810 $\mu\text{g/L}$ and 0.0005-8.6050 $\mu\text{g/L}$, respectively. The performance characteristics of the utilized method are shown in Table 3.

Table 3: Analytical performance characteristics of the utilized method.

Analyte	LDR ($\mu\text{g/L}$)	r^2	LOD ($\mu\text{g/L}$)	LOQ($\mu\text{g/L}$)
Methidathion	10 – 1000	0.996	2.5810	8.6050
Malathion	10 – 1000	0.995	1.3460	4.4850
Chlorphyrifos	10 – 750	0.996	0.0490	0.1640
Chlorflurenol-methyl	10 – 800	0.999	0.0060	0.0200
DDT	0.1 – 8	0.996	0.0001	0.0005
Endrin	0.1 – 10	0.999	0.0002	0.0010
Dieldrin	0.1 – 10	0.997	0.0020	0.0070

LDR: Linear dynamic range, LOD: Limit of detection, LOQ: Limit of quantifications, r^2 coefficient of determinations

4.2. Relative recovery studies

Relative recovery (%RR) experiments were performed by spiking NQ river sample (as a representative sample) and distilled water at two concentrations levels (2.5 and 5 $\mu\text{g/L}$). The %RR of the analytes were determined by dividing the peak areas obtained from the differences of non spiked and the spiked NQ water sample to the peak area obtained for the spiked distilled water times 100, as indicated in the formula below. Table 4 presents relative recovery studies.

$$\%RR = \frac{A_{S(NQ)} - A_{us(NQ)}}{A_{S(DW)}} \times 100$$

Where: $A_{S(NQ)}$, $A_{us(NQ)}$, and $A_{S(DW)}$ are peak areas of spiked NQ, unspiked NQ and spiked distilled water samples.

Table 4: Relative recovery of the method

Analyte	Level-1	Level- 2
Methidathion	97.5 (7.4)	82.5(7.58)
Malathion	75.9 (4.2)	69.3 (7.2)
Chlorphyrifos	93.3 (9.6)	86.5 (12.5)
Chlorflurenol-methyl	71.6 (8.6)	104 (3.65)
DDT	83 (9.3)	88.7 (6.3)
Endrin	95.26 (1.1)	105 (0.79)
Dieldrin	67.2 (6.9)	81.8 (1.08)

The values in bracket are relative standard deviation (RSD %)

4.3. The concentration of pesticides residues in water samples

The obtained concentrations of the target pesticides in the dam and river water samples are presented in Table 5.

Table 5: Mean level ($\mu\text{g/L} \pm \text{SD}$) of OCPs in water samples ($n = 4$).

Pesticides	Sample sites							MRL [73].	
	NQ	NG	NR	GR	D	LSD	CV	Acute	Chronic
Methidathion	142.66 ± 0.79^A	110.73 ± 3.24^B	ND	ND	ND	14.5	20.5	NA	NA
Malathion	30.82 ± 0.97^A	29.92 ± 0.32^B	29.67 ± 0.40^{CB}	29.06 ± 0.31^{CD}	28.54 ± 0.29^D	0.83	1.82	NA	0.1
Chlorpyrifos	3.17 ± 0.57^C	10.94 ± 0.29^A	1.67 ± 0.10^D	3.45 ± 0.42^C	5.71 ± 0.59^B	0.69	8.97	2.400	0.0043
Chlorflurenol-methyl	8.89 ± 0.05^B	8.84 ± 0.04^B	9.62 ± 0.47^{BA}	11.19 ± 2.60^{BA}	12.05 ± 1.99^A	2.55	16.3	NA	NA
DDT	0.61 ± 0.22^{BC}	0.60 ± 0.20^C	0.62 ± 0.01^{BAC}	0.70 ± 0.12^A	0.67 ± 0.50^{BA}	0.08	8.53	1.100	0.001
Endrin	ND	ND	ND	ND	ND	ND	ND	0.190	0.061
Dieldrin	0.93 ± 0.25^B	0.92 ± 0.05^B	0.94 ± 0.40^B	0.97 ± 0.04^A	0.95 ± 0.02^{BA}	0.03	2.19	0.360	0.061

ND: not detected, NA: not available, SD: Standerd devation, LSD: least significance difference, CV: coefficient of variance, NQ: nada qalla, NG: nada gudda, NR: nadi river, GR: gibe river, D: dam; and MRL: Maximum residue level

The obtained results revealed the presence of OPPs and OCPs in the studied all water samples. But, endrin was not detected in all samples and Methidathion was also not detected in NR, GR and D water samples. Results of One way ANOVA ($p \leq 0.05$) indicated the presence differences in the concentrations the studied water samples collected from the dam and the four potential tributaries.

Methidathion

Methidathion was detected at high concentration levels NQ and NG, $142.66 \pm 0.79 \mu\text{g/L}$ and $110.73 \pm 3.24\mu\text{g/L}$, respectively. But, in other water samples it was not detected. This may indicate that the pesticide is excessively used in the study area and thus, can easily mix to the water system via run off, drift or other mechanisms. On the other hand, the compound is low persistent has short life time, moderately soluble in water and relatively highly volatile [74]. These properties may contribute for the absence of the target analytes in the other samples. Methidathion is highly acutely toxic to aquatic organisms [75]. But to compare with the standard guideline, there is no information is available of MRL guideline for these pesticides.

Malathion

Malathion was detected in all water samples. The lowest and highest malathion concentrations were observed in D ($28.54 \pm 0.29\mu\text{g/L}$) and NQ ($30.82 \pm 0.97 \mu\text{g/L}$). One-way ANOVA ($p \leq 0.05$) demonstrated that the absence of significant differences in the concentration of the pesticides between NG and NR; NR and GR; as well as between GR & D water samples (Table 5). However, NQ, which contains the highest concentration of the pesticides which is significantly different from the other water samples. The lowest concentration of the pesticide in the dam water may attribute to dilution effect.

Malathion is harmful even in small concentration level, for aquatic life such as fish and other organisms [75]. It has relatively high water solubility, (i.e., 145 mg/L) and thus, it has high potential to transport in surface water and also ground water. In water it undergoes chemical and microbial degradation and converted to malaoxon and isomalathion [76]. The rate and extent of its degradation is dependent on the chemical and physical properties of the water system,

particularly temperature and the solution pH, in addition to the composition of the microbial population present in the system. Its degradation rate is fast in water at pH > 7.0. Biodegradation also plays a role when pH < 7.0 and the rate of hydrolysis is slower relative to the rate of biodegradation. It's less toxic than its metabolites, malaoxon and isomalathion [75]. The concentrations of malathion in all samples were above the EPA MRL set by EPA- for ambient water quality criteria for aquatic organisms for river water [73]. The studied water samples were not suitable for aquatic organism such as fish and also other consumption.

Chlorpyrifos

In the studied water samples the concentrations of Chlorpyrifos were ranging from 1.67 ± 0.1 $\mu\text{g/L}$ (in NR) to 10.94 ± 0.29 $\mu\text{g/L}$ (in NG). One-way ANOVA ($p \leq 0.05$) indicated the concentration of chlorpyrifos in the studied water samples was significantly different from each other, with the exception of in NQ and GR samples (Table 5). Chlorpyrifos is one of the highly toxic pesticides to fish and to aquatic invertebrate animals [77]. It is characterized by its low water solubility (1.0 mg/L) or moderate hydrophobicity and moderate volatility [75].

The present study demonstrated that, except NR sample, all the studied water samples have very high amount of chlorpyrifos residue, which were higher than acute and chronic MRL of the pesticide set by EPA for ambient water quality criteria for aquatic organisms [73]. In NR the observed concentration was below the acute MRL but, its value was above the chronic MRL of EPA. This may indicate that the pesticide was intensively used by the farmers in those areas. Generally, the water samples were highly contaminated by chlorpyrifos pesticide at the level that it can cause acute and chronic health effect on aquatic organisms and other consumers of the water.

Chlorflurenol-methyl

The concentrations of chlorflurenol-methyl in the studied water were ranging from 8.84 ± 0.04 $\mu\text{g/L}$ in NG to 12.05 ± 1.99 $\mu\text{g/L}$ in the dam sample, while the lowest was observed. One-way ANOVA results demonstrated that the absence of significance differences in the concentration of the chlorflurenol-methyl, except in the dam water, which was significantly different from the others (Table 5).

Chlorflurenol-methyl is an obsolete herbicide that is not approved for use in the EU or EPA [75]. It has a low mammalian oral toxicity, but it has not well evaluated for chronic health impacts. It is moderately toxic to fish and aquatic invertebrates [75]. Generally, the studied water samples contain high concentrations of the pesticide, indicating that the surrounding communities are using the pesticide on their farmlands to control weeds.

DDT

DDT was also observed in the water samples ranging from 0.60 ± 0.20 $\mu\text{g/L}$ in NQ to 0.70 ± 0.12 $\mu\text{g/L}$ in GR. Although the observed concentration of DDT in all water samples are below MRL set by EPA, in all samples and it was detected above its chronic toxic effect or aquatic organisms lives in fresh water [73]. One way ANOVA ($p \leq 0.05$) indicated the presence of significance differences in concentrations of DDT in NG and GR water samples. The difference among the other samples was not significant. The order of the water samples in terms of the concentrations of DDT was $\text{NG} \approx \text{NQ} \approx \text{NR} < \text{D} < \text{GR}$. This indicated that the study areas are still contaminated by DDT, and thus, use of the water may have long term impact on the health of the consumers. According to [27], the metabolite of DDT i.e. DDE concentration result was higher than the present study. This indicates that there is still usage of these pesticides in the study areas.

Dieldrin

The observed concentrations of dieldrin were ranging from 0.92 ± 0.05 $\mu\text{g/L}$ in NG to 0.97 ± 0.04 $\mu\text{g/L}$ in GR, which were above EPA to the acute and chronic MRL for fresh water for aquatic organisms [73]. Dieldrin is highly acute toxic to fish. It is persistence in the environment. One way ANOVA ($p \leq 0.05$) indicated that except in GR, there was no significant difference in the concentrations of dieldrin in the water samples. Generally, the observed results showed that the compound is still intensively used in the study areas. According to [27], the concentration of dieldrin still higher than the present studies. This is also indicates that the usage of this pesticide and have impact to the environment.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

In this study, the determination of some selected OP and OC pesticides including malathion, chlorpyrifos, methidathion, chlorflurenol-methyl, Endrin, DDT and Dieldrin in Gilgle Gibe I hydroelectric dam and its four potential tributaries water samples were carried out using GC-ECD. LD-DLLME was used for extraction and preconcentration of the target pesticides. To perform quantitative determinations, calibration curves were constructed by extracting the spiked distilled samples at six concentration levels.

The results of study demonstrated that all water samples contain the target pesticides. However, endrin was not detected in all samples, as well as methidathion was also detected only in NQ and NG. The obtained results indicated that the concentrations of the detected pesticides were higher than the EPA acute (except DDT) and chronic toxicity MRL set for the ambient water quality criteria for aquatic organisms. Generally, obtained finding indicated that the studied river water contains high concentrations of the studied pesticide residues and thus, consumption of the dam and its tributaries waters have great effect on the consumers.

5.2. Recommendations

The following recommendations could be forwarded based on the results of this study

- ✓ The researcher recommends that additional study should be necessary for the determination of OC and OP pesticide residues in the dam and its tributaries of water, sediment and fish samples to obtain the pollution profile of the water.
- ✓ Researcher recommends that monitoring of OP and OC pesticides at different environmental compartments should be done at regular interval to determine the environment of the release of this compound to environment.

6. REFERENCES

1. Alrumman, S.A.; Kott, A.F.; Kehsk, M.A. Water pollution: source and treatment. *American Journal of Environmental Engineering*. **2016**, 6, 88-98.
2. Briggs, D. Environmental pollution and the global burden of disease. *British medical bulletin*. **2003**, 68, 1-24.
3. Srinivasan, R. Advances in application of natural clay and its composites in removal of biological, organic, and inorganic contaminants from water. *Advances in Material Science and Engineering*. **2011**, 56, 1-18.
4. Ming, Y.B.; Jonatan, W.; Ambikapakan, S. Occupational pesticide exposures and respiratory health. *International Journal of Environmental pollution and Public Health*. **2013**, 10, 5-45.
5. Bempah, K.; Buah, K.A.; Denutsui, D.; Asomaning, J.; Tutu, A. Monitoring of pesticide Residues in Fruits and vegetables and related Health risk assessment in Kumasi Metropolis, Ghana. *Research Journal of Environmental and Earth Sciences*. **2011**, 3, 761-771.
6. Zhou, R.; Zhu, L.; Yang, K.; Chen, Y. Distribution of organochlorine pesticides in surface water and sediments from Qiantang River, East China. *Journal of Hazard Mater*. **2006**, 137, 68-75.
7. Owa, F.D. Water pollution: sources, effects, control and management. *Mediterranean Journal of social science*. **2013**, 4, 65-8.
8. Desai, N.; Vanitaben, S. A study on the water pollution based on the environmental problem. *Indian Journal of Research*. **2014**, 3, 95-96.
9. Halder, J.N.; Islam, M.N. Water pollution and its impact on the human health. *Journal of Environment and Human*. **2015**, 2(1), 36-46.
10. WHO. Organophosphorous pesticides in the environment - Integrated Risk Assessment, Geneva: **2001**.
11. Girma, B. Environmental Impact Monitoring For Gilgel Gibe I Dam Using An Integrated Methods Of Remote Sensing and Geographic Information System (GIS). AAU School of Graduate Studies department of Earth Science. **2010**.
12. Berrueta, L.A.; Gallo, B.; Vicente, F.A. Review of solid phase extraction, basic principles and new developments. *Chromatographia*. **1995**, 40, 474-483.

13. Archivio, A.D.; Fanelli, M.; Mazzeo, F. P. Comparison of different sorbents for multiresidue solid-phase extraction of 16 pesticides from groundwater coupled with high-performance liquid chromatography. *Talanta*. **2007**, *71*, 25–30.
14. Wang, S.; Xiang, B.; Tang, Q. Trace Determination of dichlorvos in Environmental Samples by Room Temperature Ionic Liquid-Based Dispersive Liquid-Phase Microextraction Combined with HPLC. *Journal of Chromatographic Science*. **2012**, *00*, 1–7.
15. Gonçalves, C.; Alpendurada, M.F. Solid-phase micro-extraction–gas chromatography–(tandem) mass spectrometry as a tool for pesticide residue analysis in water samples at high sensitivity and selectivity with confirmation capabilities. *Journal of Chromatography A*. **2004**, *1026*, 239–250.
16. Han, D.; Row, K.H. Trends in liquid-phase microextraction and its application to environmental and biological samples. *Microchimica Acta*. **2012**, *176*, 1–22.
17. Rezaee, M.; Assadi, Y.; Hosseini, M.R.M.; Aghaee, E.; Ahmadi, F.; Berijani, S. Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A*. **2006**, *1116*, 1–9.
18. Farajzadeh, M.A.; Seyedi, S.E.; Shalamzari, M.S.; Bamorowa, T.M. Dispersive liquid–liquid Micro extraction using extraction solvent lighter than water. *Journal of Separation Science*. **2009**, *32*, 3191–3200.
19. Bedassa, T.; Gure, A.; Megersa, N. Low density solvent based dispersive liquid –liquid microextraction and preconcentration of multiresidue pesticides in environmental waters for liquid chromatographic analysis. *Journal of Analytical Chemistry*. **2015**, *70*, 1199–1206.
20. Zhao, W.; Sun, X.; Deng, X.; Ahmad, K.; Bahruddin, S. Cloud point extraction coupled with ultrasonic-assisted back-extraction for the determination of organophosphorus pesticides in concentrated fruit juice by gas chromatography with flame photometric detection. *Food Chemistry*. **2011**, *127*, 683–688
21. Qian, L.; Hongguo, Z.; Chongtian, Y. Determination of pesticide residues in drinking water using automated solid-phase extraction and gas chromatography with nitrogen phosphorus detection. *Journal of Fish Science*. **2006**, *9*, 1-2.
22. Shen, Z.; He, Z.; Wang, P.; Zhou, Z.; Sun, M.; Li, J.; and Liu, D. Low-density magneto fluid dispersive liquid–liquid microextraction for the fast determination of organochlorine pesticides in water samples by GC-ECD. *Analytica Chimica Acta*. **2013**, *793*, 37– 43.

23. Zacharis, C.; Tzanavaras, P.; Roubos, K. Solvent-based emulsification dispersive liquid–liquid microextraction combined with gas chromatography–mass spectrometry for determination of trace organochlorine pesticides in environmental water samples. *Journal of Chromatography A*, **2010**, *1217*, 5896–5900.
24. Benno, A.I.; Ruud, C.J.; Dam, v.; Rob, J.; Hans, G.J.; Odile, M. Determination of polar organophosphorus pesticides in aqueous samples by direct injection using liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*. **2001**, *918*, 67-78.
25. Inglezakis, V.J.; Moustakas, K. Household hazardous waste management: A review. *Journal of Environmental Management*. **2015**, *150*, 310-321.
26. Gavrilescu, M. Fate of pesticides in the environment and its bioremediation. *Engineering in Life Sciences*. **2005**, *5*, 497-526.
27. Teshale, A. Analysis of organochlorine pesticides in water, sediment and fish of Gilgel Gibe (I) hydroelectric dam, Oromia, Ethiopia (M.Sc thesis from Jimma university chemistry department, **2017**).
28. Kazemi, M.; Tahmasbi, A.M.; Valizadeh, R.; Naserian, A.A.; Soni, A.; Moheghi, M.M. Importance and toxicological effects of organophosphorus pesticides: a comprehensive review. *Journal of Agricultural Science*. **2012**, *1*, 43–57.
29. Andreu, V.; Pico, Y. Determination of currently used pesticides in biota. *Analytical and Bioanalytical Chemistry*. **2012**, *404*, 2659–2681.
30. Omar, E.N.; Carlos P.L.; Eduardo, B.O. Organophosphorouspesticides: their effects on biosentinel species and humans. control and application in Chile. *International Journal of Morphology*. **2017**, *35*(3), 1069-1074.
31. Mekonen, S.; Ambelu, A.; Negassa, B.; Spanoghe, P.; Exposure to DDT and its metabolites from khat (*Catha edulis*) chewing, consumers risk assessment from southwestern Ethiopia. *Regulatory Toxicology and Pharmacology*. **2017**, *87*, 64-70.
32. Mekonen, S.; Ambelu, A.Z.; Spanoghe, P.; Pesticides residue evaluation in major staple food items of Ethiopia using the Quenchers method: A case study from Jimma zone. *Environmental Toxicology and Chemistry*. **2014**, *33*, 1294-1302.

33. Donald, D.B.; Cessna, A.J.; Sverko, E.; Glozier, N.E. Pesticides in surface drinking water supplies of the northern Great Plains. *Environmental Health Perspective*. **2007**, 115, 1183–1191.
34. Halder, J.N.; Islam, M.N. Water pollution and its impact on the human health. *Journal of Environment and Human*. **2015**, 2(1), 36-46.
35. Yonglong, L.; Song, S.; Wang, R.; Impacts of soil and water pollution on food safety and health risks in China. *Environment International*. **2015**, 77, 5-15.
36. Pruss, A.; Kay, D.; Fetrell, L.; Bartram, J.; Estimating the burden of disease from water, sanitation and hygiene at a global level. *Environmental Health Perspectives*. **2002**, 110, 537–42.
37. Sexton, K.; Adgate, J.L. Looking at environmental justice from an environmental health perspective. *Journal of Exposure Analysis and Environmental Epidemiology*. **2000**, 9, 3–8.
38. Perry, M.J.; Marbella, A.; Layde, P.M. Association of pesticide safety knowledge with beliefs and intentions among farm pesticide applicators. *Journal of Occupational and Environmental Medicine*. **2000**, 42, 187-193.
39. Landrigan, P.J.; Fuller, R. Environmental pollution: an enormous and invisible burden on health systems in low and middle income countries. *World Hospital and Health Service*. **2015**, 50, 35–41.
40. Ashraf, M.A.; Maah, M.J.; Yusoff, I. and Mehmood, K. Effects of Polluted Water Irrigation on Environment and Health of People in Jamber, District Kasur, Pakistan, *International Journal of Basic and Applied Sciences*. **2010**. 10, 37-57.
41. Schell, L.M.; Gallo, M.V.; Denham, M.; & Croft, R.J. Effects of pollution on human growth and development: an introduction, *Journal of Physiological Anthropology*, **2006**, 25(1), 103-112.
42. Ganguly, S.; Paul, I.M.; Kumar, S. Increasing water pollution from various sources affecting the inhabitant fishes adversely: A matter of great concern. *Fishing Chimes*, **2011**, 34, 44–48.
43. Huppert, H. E.; Sparks, R. S. J. Extreme natural hazards: population growth, globalization and environmental change. *Philosophical Transactions of the Royal Society*, **2006**, 364(1845), 1875-1888.
44. Berg, H.V.; Manuweera, G.; Konradsen, F. Global trends in the production and use of DDT for control of malaria and other vector-borne diseases. *Malaria Journal*. **2017**, 16(401), 1-8.

45. Aktar, M.W.; Sengupta, D.; Chowdhury, A. impacts of pesticide use in agriculture their benefits and hazards. *Interdisciplinary Toxicology*. **2009**, 2, 1-12.
46. Kazemi, M.; Tahmasbi, A.M.; Valizadeh, R.; Naserian, A.A.; Soni, A.; Moheghi, M.M. Importance and toxicological effects of organophosphorus pesticides: a comprehensive review. *Journal of Agricultural Science*. **2012**, 1, 43–57.
47. Gavrilesco, M. Fate of pesticides in the environment and its bioremediation. *Engineering Life Science*. **2005**, 5, 497-526.
48. Blair, A.; Thomas, K.; Coble, J.; Sandler, D.P.; Hines, C.J.; Lynch, C.F. Impact of pesticide exposure misclassification on estimates of relative risks in the agricultural health study. *Occupational and Enviromental Medicine*. **2011**, 68, 537–41.
49. Farshid, K. Assessment of organochlorine pesticide residues in water, sediments and fish from Lake Tashk. *Journal of Achievement in Life Science*. **2015**, 9, 107-111.
50. Zacharia, J.T. Identity, physical and chemical properties of pesticides, Tanzania. *Pesticides in the Modern World*. **2011**, 307-437.
51. Mekonen, S.; Lachat, C.; Ambelu, A.; Steurbaut, W.; Kolsteren, P.; Jacxsens, L.; Wondafrash, M.; Houbraken, M.; Spanoghe, P. Risk of DDT residue in maize consumed by infants as complementary diet in southwest Ethiopia. *Science of the Total Environment*. **2015**, 511, 454-460.
52. Zorawar, S.; Jasinder, K.; Ravneet, K.; Swarndeeep, S, H. Toxic effects of organochlorine pesticides, *American Journal of Bioscience*. **2016**, 4(3-1), 11-18.
53. Prieto, P.; Sandra, G.; Ascencio, C.; John, C.; Oyarzun,G.; Ceruelo, A.; Vazquez, P. Pesticides, Classification, uses and toxicity and Measures of exposure and genotoxicity risks. *Research journal of Enviromental Science and Toxicology*. **2012**, 42-76.
54. Tankiewicz, M.; Fenik,J.; Biziuk,M. Determination of organophosphorus and organonitrogen pesticides in water samples. *Trends in Analytical Chemistry*. **2010**, 29, 1050–1063.
55. De, A.; Pinheiro,S.; Jailson, B.; Andrade, D. Development, validation and application of a SDME/GC-FID methodology for the multiresidue determination of organophosphate and pyrethroid pesticides in water. *Talanta*. **2009**, 79, 1354–1359.
56. Hreljac, I.; Filipic, M. Organophosphorus pesticides enhance the genotoxicity of benzopyrene by modulating its metabolism. *Mutation Research*. **2009**, 671, 84-92.

57. Tryaki, O.; Temur, C. The fate pesticide in the environment. *Journal of Biological and Enviromental Science*. **2010**, 4, 29-38.
58. Schenck, F.J.; Howard, K.; V. Determination of organochlorine and organophosphorus pesticide residues in low moisture, non fatty products using a solid phase extraction cleanup and chromatography. *Journal of Enviromental Science and Health*. **2000**, 35, 1–12.
59. Arbeli, Z.; Fuentes, C. L. Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. *Crop Protection*. **2007**, 26, 1733-1746.
60. Li, Y.; Chen, P.S.; Huang, S.D. Water with low concentration of surfactant in dispersed solvent-assisted emulsion dispersive liquid–liquid microextraction for the determination of organochlorine pesticides in aqueous samples. *Journal of Chromatography. A*. **2013**, 1300, 51– 57.
61. Farahani, H.; Yamini, Y.; Shariati, S.; Reza, M.; Zanjani, K.; Baghahi, S. Development of liquid phase microextraction method based on solidification of floated organic drop for extraction and preconcentration of organochlorine pesticides in water samples. *Analytica Chimica Acta*. **2008**, 626, 166-173.
62. Namieśnik, J.; Szefer, P. Preparing Samples For Analysis, The Key To Analytical Success. *Ecological Chemistry And Engineering Science*. **2008**, 15(2), 181-189.
63. Kouzayha, A. Rabaa, A.R.; Mohamad, A.L.; Beh, D.; Budzinski, H.; Jaber, F. Multi-residue method for determination of 67 pesticides in water samples using solid-phase extraction with centrifugation and gas chromatography-mass spectrometry. *American Journal of Analytical Chemistry*. **2012**, 3, 257-265.
64. Gonçalves, C.; Alpendurada, M.F. Solid-phase micro-extraction–gas chromatography–(tandem) mass spectrometry as a tool for pesticide residue analysis in water samples at high sensitivity and selectivity with confirmation capabilities. *Journal of Chromatography A*. **2004**, 1026, 239-250.
65. Cserhati, T.; Forgacs, E.; Deyl, Z.; Miksik, I.; Esckhardt. A. Chromatographic determination of herbicide residues in various matrices. *Biomedical Chromatography*. **2004**, 18, 350-359.
66. Lambropoulou, D.A.; Albanis, T.A. Liquid phase micro extraction techniques in pesticide residue analysis. *Journal of Biochemical and Biophysical Methods*. **2007**, 70, 195-228.

67. Yazdi, S.A.; Amiri, A.; Liquid-phase microextraction. *Trends Analytical Chemistry*. **2010**, 29, 1-14.
68. Farajzadeh, M.A.; Djozan, D.; Khorram, P. Development of a new dispersive liquid–liquid microextraction method in a narrow-bore tube for preconcentration of triazole pesticides from aqueous samples. *Analytical Chimica Acta*. **2012**, 713, 70-78.
69. Zhou, Q.; Pang, L.; Xie, G.; Xiao, J.; Ba, H.; Determination of atrazine and simazine in environmental water samples by dispersive liquid–liquid microextraction with high performance liquid chromatography. *Analytical Science*. **2009**, 25, 73-76.
70. Cabuk, H.; Akyuz, M.; Ata, S.; A simple solvent collection technique for a dispersive liquid-liquid microextraction of parabens from aqueous samples using low-density organic solvent. *Journal of Separating Science*. **2012**, 35, 2645-2652.
71. Leong, M.I.; Huang, S.D; Dispersive liquid-liquid microextraction method based on solidification of floating organic drop for extraction of organochlorine pesticides in water samples. *Journal of Chromatography A*. **2009**, 1216, 7645-7650.
72. Demissie, T.A.; Saathoff, F.; Sileshi, Y.; Gebissa, A. Climate change impacts on the stream flow and simulated sediment flux to Gilgel Gibe 1 hydropower reservoir Ethiopia. *European International Journal of Science and Technology*. **2013**, 2, 64-65.
73. Hamilton, D.J.; Ambrus, A.; Dieterle, R.M.; Felsot, A.S.; Harris, C.A.; Holland, P.T.; Katayama, A.; Kurihara, N.; Linders, J.; Unsworth, J.; Wong, S.S. Regulatory limits for pesticide residues in water (IUPAC Technical report). *Pure Applied Chemistry*. **2003**, 75, 1123–1155,
74. Washburn, A.D. The Environmental Fate of Methidathion. *Journal of Environmental Monitoring*. **2003**, 4, 75-81.
75. Wang, D.F.; Sun, J.P.; Du, D.H.; Sun, L.P.; Chen, Z.D.; Xue, C.H. Degradation of extraction from seaweed and its complex with rare earths for organophosphorous pesticides. *Journal of Rare Earths*. **2007**, 25, 93–99.
76. Alamgir, M.D.; Banik, S.; Uddin, B.; Moniruzzaman, M.; Karim, N.; Gan, S.H. Organophosphorus and carbamate pesticide residues detected in water samples collected from paddy and vegetable fields of the Savar and Dhamrai Upazilas in Bangladesh. *International Journal of Environmental Research and Public Health*. **2012**, 9, 3318-3329.

77. Lee, I.; Eriksson, P.; Fredriksson, A.; Buratovic, S.; Viberg, H. Developmental neurotoxic effects of two pesticides: Behavior and bimolecular studies on chlorpyrifos and carbaryl. *Toxicology and Applied Pharmacology*. **2015**,*288*, 429–438.

APPENDIX

Statistical test data result from Statistical analytical software (SAS)

Table 1: Analysis of variance for Methidathion pesticides

Source of variation	Df	SS	MS	F-value	P-value
Treatment	4	90284.25655	22571.06414	256.55	<.0001
Replication	3	117.38737	39.12912	0.44	0.7254
Error	12	1055.75447	87.97954		
Corrected total	19	91457.39839			

Df-Degree of freedom, SS-Sum Square, Ms-Mean square, P-value-Probability

Table 2: Analysis of variance for Malathion

Source of variation	Df	SS	MS	F-value	P-value
Treatment	4	12.09595730	3.02398933	10.43	0.0007
Replication	3	0.64003940	0.21334647	0.74	0.5505
Error	12	3.47871710	0.28989309		
Corrected total	19	16.21471380			

Table 3: Analysis of Chlorphyrifos

Source of variation	Df	SS	MS	F-value	P-value
Treatment	4	210.3649727	52.5912432	262.28	<.0001
Replication	3	0.1719932	0.0573311	0.29	0.8347
Error	12	2.4061733	30.0767094		
Corrected total	19	212.9431392			

Table 4: Analysis of variance for Chlorflurenol-methyl

Source of variation	Df	SS	MS	F- value	P-value
Treatment	4	33.08696370	8.27174093	3.03	0.0610
Replication	3	0.08300695	0.02766898	0.01	0.9985
Error	12	32.78772430	2.73231036		
Corrected total	19	65.95769495			

Table 5: Analysis of variance for DDT

Source of variation	Df	SS	MS	F-value	P-value
Treatment	4	0.03870920	0.00967730	3.28	0.0491
Replication	3	0.00741855	0.00247285	0.84	0.4986
Error	12	0.03540320	0.00295027		
Corrected total	19	0.08153095			

Table 6: Analysis of variance for dieldrin

Source of variation	Df	SS	MS	F-value	P-value
Treatment	4	0.00502380	0.00125595	2.96	0.0646
Replication	3	0.00032800	0.00010933	0.26	0.8544
Error	12	0.00508900	0.00042408		
Corrected total	19	0.01044080			

