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ANALYSIS OF ORGANOCHLORINE PESTICIDES IN WATER, SEDIMENT AND FISH OF GILGEL GIBE (I) HYDROELECTRIC DAM, OROMIA, ETHIOPIA

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Abbreviations

OCPs	Organochlorine pesticide
EPA	Environmental Protection Agency
IT'S	World Health Organization
EU	European Union
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
MS	Mass Spectroscopy
ECD	Electro Capture Detector
NPD	Nitrogen Phosphorous Detector
µECD	Micro Electro Capture Detector
FID	Flame Ionization Photometry
UV	Ultraviolet Visible
LLE	Liquid-Liquid Extraction
SPE	Solid Phase Extraction
HF-LPME	Hollow Fiber Based Liquid-Phase Micro Extraction
SPME	Solid Phase Micro Extraction
SE	Soxhlet Extraction
DLLME	Dispersive Liquid-Liquid Micro Extraction
DAD	Diode Array Detection

Acknowledgements

This work comes in to completion because of not only my effort, but also the contribution of from other individuals whom I would like to forward my appreciations and acknowledgments. First of all, I would like to thank my advisors, Dr. Abera Gure, Mr. Feyisa Wedajo and Dr. Seblework Mekonnen for their endless support from the beginning of the work to the end. I would also like to acknowledge all my families, specially my mother Shashitu Lakew, my daughter Yemariam Anbessa and my wife Sisay Solomon, for their financial and moral encouragement .My gratitude also goes to of Environmental Health Science and technology of Jimma University for their kind support to analyze my samples using their GC-ECD instrument. Finally, I want to express my earnest appreciation to Bulen Wereda Benishagul Gumuze Regional state of Ethiopia and particularly, Fikadu Kumsa for their financial support and permission they offered me to carry out this work.

Abstract

In this study, analysis of organochlorine pesticides (OCPs) has been carried out in water, sediment and fish samples collected from Gilgle Gibe (I) hydroelectric dam of Ethiopia, using gas chromatography with electron capture detector (GC-ECD). Low density based dispersive liquid-liquid microextraction (LD-DLLME) using toluene (as extractant) and acetone (disperser) was used for extraction OCPs from water samples and soxhlet extraction, using 150 mL acetone: n-hexane (20:80 v/v) was used for extraction of sediment and fish samples. After soxhlet procedure, sediment and fish extracts were cleaned with Florisil solid phase extraction (SPE). External calibration curves constructed at five concentration points were exhibited wide linear ranges, i.e., 0.2 – 100 µg/L, with excellent coefficient of determination, r2, ranging 0.999 -1.00. The limits of detection and quantification (LOD & LOQ) of the utilized method which were determined as 3 and 10 times the signal-to-noise ratio were ranging 0.06–0.72 µg/L and 0.30–2.40 µg/L, respectively. The efficiency of the methods was also evaluated by spiking the samples with known concentration of the target analytes and the obtained recoveries were ranging from 67 - 118%. Except gamma-chlordane, which was not detected in fish samples, all the studied samples, i.e., water, sediment and fish samples contain all target OCPs in the increasing order of their concentrations water < fish < sediment samples. Among the studied OCPs, DDE, endrin and dibuthyl chlorendate were observed at smallest concentration levels, in water, sediment and fish sample respectively and heptachlor epoxide was observed at highest concentration level in all sample type. The bioconcentration studies also demonstrated that the analytes are more concentrated in sediment than in fish samples. In general, the finding demonstrated that Gilgel Gibe I hydroelectric dam water and sediment as well as fishes grown in the dam water contain the studied pesticides residues by far above the WHO permissible limits. Thus, apart from hydroelectric power generation, use the dam water for agricultural purpose such as irrigation and/or fish production as well as recreation has health risk to the consumers.

Keywords: Organochlorine pesticides; Water; Sediment; Fish; Gas chromatography electron capture detector.

1. Introduction

1.1. Back ground of the study

Environmental pollution is the most important subject for many countries. Pollution has major effects on all aspects of life and threatens human health, animals, plants and the environment [1]. Environmental, i.e., water, air and soil, pollution is increasing from day to day due to increase in population, industrialization, urbanization and agricultural activities. Pollutions could originate from different sources including industrial waste and agricultural chemicals such as the use of fertilizers and pesticides as means to increase agricultural production [2].

Pesticides are chemicals that have great impact on increasing the yield of productions [3]. Pesticide is any chemically synthesized substance or mixture of substances that are routinely utilized in agriculture to manage, destroy, attack or repel pests, pathogens and parasites [4]. Pesticides usually contain both organic and inorganic moieties and they can be classified into different groups based on their chemical composition. These classifications include organochlorines, organophosphates, carbamates, formamidines, thiocyanates, organotins, nitro phenols, synthetic pyrethroids and so on [5]. The widespread use of these pesticides for agricultural and nonagricultural purposes has resulted in the presence of their residues in various environmental compartments such as soil, water and air [6]. In contrast to their benefits, pesticides may have wide range of toxic effects to the environment and human health [7].

On the other hand the fate and transport of pesticides in the soil depend on the cumulative effects of the pesticide's characteristics (e.g., adsorptivity, solubility, volatility and degradation rate), as well as soils characteristics (e.g., texture and organic matter). The application methods used (e.g., aerial or ground) and the site conditions (e.g., topography, weather and irrigation [8]. Certain pesticides, which are more resistant to degradation by abiotic (physical, chemical and other factors) and biotic (micro and macro organisms of the soil food web) could leach into the lower strata of the soil and then they can be absorbed by plant roots, accumulate in the food chain and are ultimately biomagnified in the food web [9,10]

Organochlorine pesticides (OCPs) are persistent in the environment. In environmental waters they disappear via secondary mechanisms: absorption on sediment, biological breakdown by micro

flora and fauna, and absorption by fish through gills, skin and feeding [11]. They also persist in food chains and are readily accumulated in animal tissues

Pesticides can be transported from the sprayed area to non-target areas, and thus potentially affect non-target and endogenous species [12].The transport and destination of pesticides involve complex mechanisms that are influenced by many processes including volatilization, leaching, adsorption and chemical and biological decomposition .Pesticides may be introduced into the aquatic systems by diffuse surface or subsurface hydrological pathways. A major pathway for pesticide transport into surface waters includes field spray drift during application, surface runoff and leaching with subsequent transport through drainage channels during rain events and farm and farmyard improper operations [13].

Different analytical methods including gas chromatography-mass spectrometry (GC-MS) [14, 15], gas chromatography with electron capture detector (GC-ECD) [16, 17, 18, 19], with gas chromatography flame photometric detection (GC-FID) [20], gas chromatography with nitrogen phosphorus detection (GC-NPD) [21]. In addition, high performance liquid chromatography (HPLC) with MS [22], HPLC with fluorescence detector (FLD) [23], HPLC with ultraviolet (UV) [24] and HPLC with diode array detector (DAD) [25] are commonly used for pesticide s residues from different matrices.

Particularly, GC-ECD and GC-MS are widely used for separation and quantification of residues of OCPs [14-18]. But, prior to their chromatographic analysis separation and pre-concentration of the target analytes from the matrix or from other coexisting components are needed. Thus, the two most traditional methods: liquid–liquid extraction (LLE) and solid-phase extraction (SPE) have been widely used for extraction and pre-concentration of pesticides from liquid matrices [26, 27]. Nevertheless, they are time-consuming, often requiring large amounts of expensive and hazardous organic solvents and the use of multistep procedures associated with the high risk of analyte losses, tedious and environmentally unsafe [27].

In the last couple of decades, other efficient, economic and environmental friendly miniaturized sample preparation method like solid-phase microextraction [28], hollow fiber-based liquid-phase microextraction (HF-LPME) [29], stir bar sportive extraction [30], single-drop microextraction [31] and dispersive liquid- liquid micro extraction (DLLME) [32] have been proposed as

alternative methods of LLE and SPE. These methods have advantages such as simplicity of operation, rapidity, low cost, high recovery high enrichment factors and environmental safe.

Such simple alternative methods are of great importance for the determination of OCPs in different environmental and biological samples, as their presences have a negative impact on the life of human beings and other life. Perhaps, pesticides have been used for long time in Ethiopia for controlling of agricultural pests, malaria and other organisms, negligible studies have been done on their residual analyses in different matrices. Particularly, environmental waters which are commonly used for multipurpose including fish production, recreation, and irrigation so on are adversely affecting the health of consumers, if they contain residues of these chemicals. Therefore, in this study, concentrations of OCPs in Gilgel Gibe I hydroelectric dam water, sediment and fish were investigated for the first time using GC-ECD.

1.2. Statements of problem.

Agrochemicals like pesticides and fertilizers are used as a vehicle to improve crop production. But, because of their toxicity, particularly, pesticides could potentially damage the environment and/or non-target living things including human beings. From their application point, pesticides are usually transported to different environmental compartments such as environmental waters, mainly via water runoff, leaching, atmospheric depositions and drainage water and thus, cause acute and chronic effects on aquatic species and humans who are directly or indirectly using [33]. Although, some pesticides, like OCPs, have been worldwide banned not to be used, their residues are still detected in environmental and biological samples, resulting from their persistence nature.

In Ethiopia, for many years, OCPs were used for controlling of insecticides on agricultural fields as well as for controlling of malaria at house hold level [34]. These pesticides have been also detected in various samples including milk [35], cereals [35], Khat [36], and coffee [35], which were collected 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 relation to this fact, although Gilgel Gibe-I hydroelectric dam water is currently used as the main source of fishes for the surrounding community, including Jimma Town, and as well, recreation center for the people of the area no study has been carried out on the level of OCPs of the dam water, sediment and fish. Therefore, in this study, the

concentration OCPs of Gilgel Gibe-I hydroelectric dam water, sediment and fish were determined using GC-ECD.

1.3. Objectives

1.3.1. General objective.

The main objective of this study is to analyze the concentration of various OCPs in Gilgel Gibe (I) hydroelectric Dam water, sediments and fish samples using gas chromatography with electron capture detector (GC–ECD).

1.3.2. Specific objectives

- To determine the concentration of OCPs such as P, DDT, 4, 4, DDE, endosulfansulfate, dieldrin, methoxychlor, Endrin, γ-chlordane. Aldrin, dibuthylchlordate and heptachlor epoxide in water and sediment of Gibe (I) hydroelectric dam.
- To analyze variation of the concentrations of OCPs in fish samples based on their body mass or size.
- To investigate bio concentrations of OCPs in sediment and fish samples.
- To compare concentration of pesticide among water, sediment and fish samples.

1.4. Significance of the study.

Findings of the study could have the following significances:

- It could be used as firsthand information regarding the concentrations of OCPs of the dam water, sediment and fish samples.
- It could be used as the background information for the researchers who want to work on the dam or related area.
- It might be used as supplementary information to policy makers or other concerned bodies who closely follow up the dam water.

2. Review Literature

2.1. Sources of pesticide

2.1.1. Point-source

The contamination can be traced to specific points of discharge from water treatment plants industry and factories or from combined sewers [38].

2.1.2. Nonpoint-source

Non-point source of pesticide comes from many diffuses sources including agricultural and residential lands starts with precipitation falling on the ground. As the resulting runoff moves over and through the soil, it picks up and carries away natural and human made contaminants, finally transporting them to streams, rivers, wetlands, lakes and even ground water. These are the leading and most widespread cause of water-quality degradation, and it can have harmful effects on drinking-water supplies, recreation, fisheries, and wild life. It is more difficult to develop solutions for non-point sources, which are vastly more widespread and difficult to identify and quantify than point sources [39].

2.2. Historical developments of pesticide

Pesticides have been used since the beginning of human history. The first known pesticide was elemental sulfur dusting used in Sumeria approximately 4500 years ago. Homer (1000 BC) mentions that Odysseus burned sulfur to purge the hall, the house and the court. Chinese by 900 AD used arsenic sulfides to control garden insects. In the seventeenth century, nicotine sulfate extracted from tobacco leaves was used as insecticide. In the nineteenth century, two more natural pesticides were introduced, pyrethrum and rotenone that are derived from chrysanthemum and from the roots of tropical plants, respectively. Until the 1930s, pesticides were mainly inorganic compounds or of natural origin [40].

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The first synthetic pesticide was 1, 1, 1-trichloro-2, 2-bis [4-chlorophenyl] ethane (DDT) in 1874, but not used until 1939, when Muller and coworkers discovered its insecticidal properties. DDT was successfully used in the malaria eradication programs since the 1950s. Together with DDT,

other chlorinated hydrocarbon insecticides were developed in the same years in agriculture for protection of cotton, deciduous fruits, cereals, and potatoes. In the 1960s, Rachel Carson demonstrated the bioaccumulation potential of DDT representing a serious threat to biodiversity. DDT is now banned in the large majority of countries, but it is still used in some developing nations to prevent malaria and other tropical diseases, following risk/benefit criteria. Since the removal of organochlorine insecticides, organophosphates became the most widely used class of insecticides. Shortly afterwards, insecticide carbamates were introduced in the market. In the 1970s, an important class of synthetic insecticides was discovered, the pyrethroids, characterized by a low mammalian toxicity and low environmental persistence. Pesticides are grouped according to the type of pest they control or by chemical classes. Since then the various synthetic pesticides were emerged and commercialized worldwide. According to the USA Environmental Protection Agency (EPA), currently more than 20,000 pesticides are registered and commercialized, indicating the availability of the varieties of pesticides utilized for either agricultural or non-agricultural purposes [41].

2.3. Pesticide and human health

An estimated 2.2 million people are at risk to exposure from agricultural pesticide with the majority of this population being locating in developing nation [42]. Pesticide can enter the human body through inhalation ingestion or by dermal penetration through the skin, those who work with agricultural pesticide are the most at the risk if they were not properly dressed or by broken and leaching equipment's. The majority of average citizens who are affected by pesticide through using water that was been contaminated with pesticide.

In 2004, carbofurne pesticide residues found in several batch of noodle manufactured in Nigeria may have results 23 reported cases of vomiting and one death [43]. Pesticide cause headaches, blurred vision, vomiting abdominal pain, suppress the immune system, lead to blood and liver diseases, depression, asthma and nerve damage with this adverse effects pesticide bioaccumulation and its concentration increase further up to food chain [44].Studies have shown that long-term low-dose exposure to pesticides leads to the development of respiratory diseases such as asthma [43]. Such exposure also leads to reduced sperm quality and sperm count, causing sterility [45].Pesticide poisoning is more significant in developing countries compared to developed

countries. Pesticide residues affect environmental quality. Farmers serve as the main unit of agricultural production; their life and their daily production are closely related to the natural environment. Consequently, their irrational economic activities and unscientific ecological behavior directly and inevitably worsens the ecological environment [46]. According to data released by the World Health Organization (WHO) [47] suicide by pesticides is common in many Asian and Latin American countries. Pesticides are often poorly controlled and widely available, particularly in countries of low and middle income [48].

2.4. Fate of pesticide

The widespread use and disposal of pesticide by farmers, institution and peoples provide many possible source of pesticide in the environment. The pesticide sprayed can move through the air and may eventually end up in other parts of the environment such as in soil or water [8]. The pesticide, which applied directly to the soil, may be washed off the soil in to nearby bodies of surface water. or may percolate through the soil to lower soil layer and ground water and indirectly results leaching from boat paints runoff from soil may also contribute to air level through evaporation and can may break down or degraded by action of sun light, water or other chemical or microorganism such as bacteria [49].

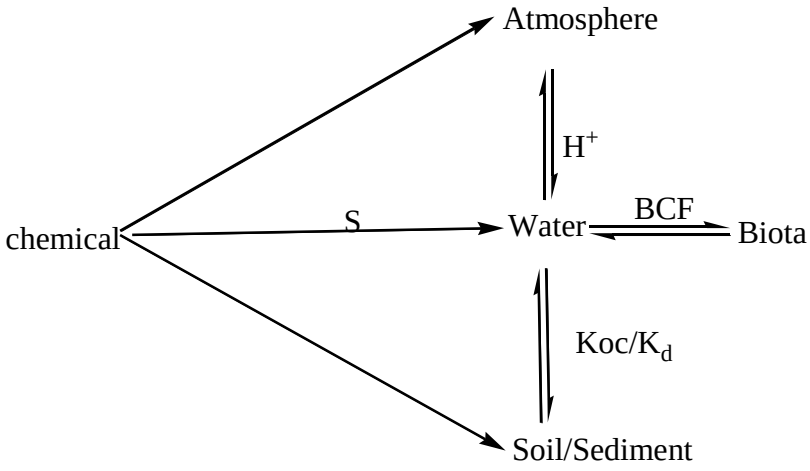

Figure 1. Fate of pesticides in the ecosystem [50].

2.5. Classification of pesticides

Pesticides can be classified in different ways either by target pest (insecticide that target insects and herbicide that target plant) and chemical identity (organophosphate, organochlorine, carbamate and pyrethroid and so on [51].These research only targeted to organochlorine pesticides

2.6. Organochlorine pesticide

Organochlorine pesticide (OCPS) is chlorinated hydrocarbon insecticide, solvents widely used around the world. This class of chemicals comprises variety of compounds containing carbon, hydrogen and chlorine [52]. And were the first synthetic chemicals used for agricultural and industrial purposes and are among the most serious environmental contaminates because of their persistence, bioaccumulative properties and potential toxic effect on wildlife and human [53,54]. A number of previous study pointed out that organochlorine pesticide may affects normal function of the endocrine system to induce such toxic side effects as humane breast or liver cancer, testicular tumors and even low sperm count in humane [55, 56]. Whereas without considering the above mentioned problems organochlorine insecticide were successfully use to control of malaria and typhus, yet in India, China, Asia and Africa. The statics on the use of different pesticide shows that 40% all pesticide used belongs to organochlorine class of chemical [57, 58]. Due to their low coast and they needs against various pests, such pesticides are DDT,HCH, aldirin and dieldrin were most widely used pesticide in developing countries were listed above [58].

Exposure to organochlorine occurs via ingestion of contaminated food or water, inhalation of vapor, and absorption through the skin. Occupational and other domiciliary exposures are also possible. Dietary exposure results in bioaccumulation of these chemicals in the human body [59]. Organochlorine has similar structure they all contain a cyclodiene ring. The lungs, gastrointestinal tract, and skin can absorb all these compounds. In addition, although the organism absorbs approximately 10% of the applied dose, lipid solvents increase dermal penetration [60]. The accumulation of organochlorine compounds is a result of their chemical structure and their physical properties such as polarity and solubility. These fat-soluble compounds persist in both the body and the environment. Consequently, researchers, most of advanced countries and regulatory agencies have banned several organochlorine [61, 62].

2.7. Analytical determination of pesticide

Numerous analytical methods have been used for the determination of organochlorine pesticide in the environment. Nowadays, the most important and common methods used for organochlorine determination are GC and LC. GC is a chromatographic technique that is used for the detection of polar, volatile compounds because of its sensitivity.

The analytical determination of natural and synthetic pesticide in water sample has been dominated by the use of GCMS [14, 15] GC-μECD [19], GC-FID [23], and GC-NPD [21]. And high-performance liquid chromatography like HPLC-FID, [23], HPLC-MS [22], HPLC, UV [24] although, various chromatographic techniques have been utilized, proper sample preparation technique is always needed, prior to their final chromatographic separation and quantitative analysis [63, 64].

2.8. Sample pretreatment and clean-up methods

Sample pre-treatment and clean-up is usually a difficult step and time consuming segment in the analysis of pesticide in the environment. Due to the low level concentrations in which they exist, as well as the complexity of the matrices in which they are detected. However, sample preparation is necessary to convert the sample into a form that is suitable for the analysis to be performed without the loss of the secondary sample [65]. The analysis of organic compounds in highly complex matrixes such as food, biological and environmental solid sample was highly difficult. Due the interference of matrixes components as result the extracts may not be compatible with DLLME procedures so to overcame this problem usually two kind of procedure were takes place in complex samples, one the sample was homogenized and centrifuged or the filtrate juice was taken for DLLME processes. Second, the sample pre extracted and the extract was used in DLLME processes [66].

Sample preparation steps, such as homogenization filtration, extraction and purification are employed before the final determination [67]. Various sample preparation methodologies including traditional methods (e.g., LLE and SPE) [68] and modern method such as DLLME methods have been proposed for extraction and/or preconcentration of pesticide residues from various matrices [26, 27, 69]. Since its introduction in 2006, DLLME has gained great attention for extraction and/or preconcentration of pesticide residues from various matrices. 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. In the procedure, the analytes are rapidly transferred into the dispersed fine droplets of the extraction solvent (organic phase). The dispersed fine droplets are then separated after centrifuging. Either the extraction solvent, which is 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 [20]. It has been successfully applied for extraction and preconcentration of sulfonylurea and organophosphorus pesticides from water samples [71] and organochlorine pesticides from water [16], Fish sample [72].

3. Materials and Methods

3.1 Descriptions of study area

The Gilgel Gibe-I hydroelectric dam project is located at latitude 7º49`52.45``N and longitude 37º19`18.79´´E, in Jimma zone, Oromia Regional state, Ethiopia; at 7º49`52.45``N latitude and 37º19`18.79´´E longitude, Figure 2. The dam occupies about 4225 km^2 area. The area is largely comprised of cultivated land and surrounded by man village [73].

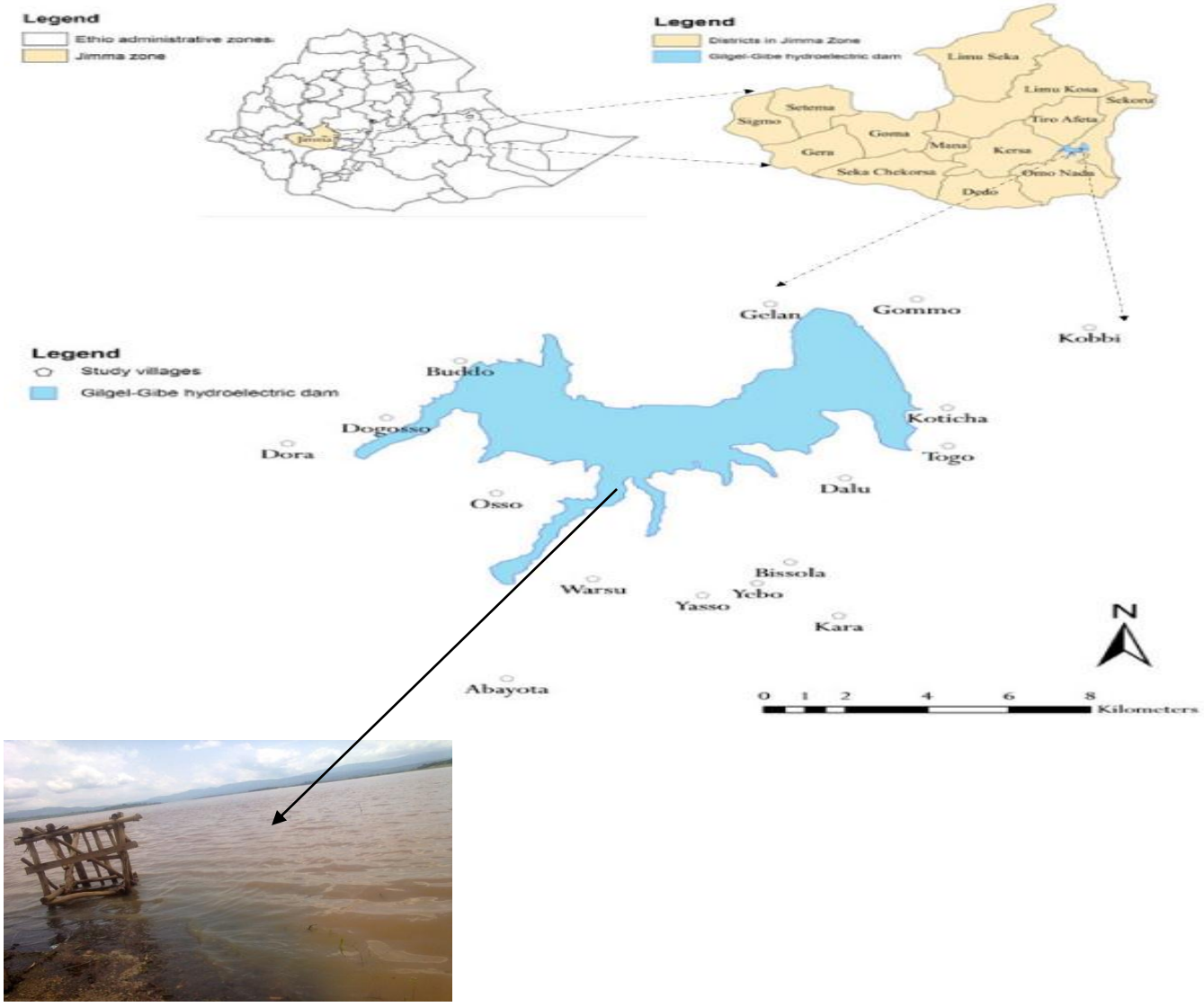

Figure 2. Location map of Gilgel Gibe basin in Ethiopia [73].

3.2 Chemical and Reagents

All chemicals and reagents used were analytical grade and solvents were HPLC grade. The organic solvents; toluene, acetone, hexane and methanol obtained from BDH Chemicals Ltd (Poole, England). Sodium chloride (NaCl), hydrous sodium sulfate (Na_2SO_4) was also from BDH Chemicals Ltd. Dual layer Florisil/Na_2SO_4, SPE 2g/2g) purchased from Sigma Aldrich (St. Louis, MO, USA). Ultrapure water obtained after purification utilizing Mill-Q water purification system, (Millipore, Bedford, France) was used throughout the work. Whatman filter paper (grade 1 and size 8.5 cm) and 3 µm nylon filters was obtained from Whatman International Ltd (Maidstone, England) and was used for filtration of the water, sediment and fish samples.

Analytical standards of p, p'-DDT, 4, 4, DDE, endosulfansulfate, dieldrin, methoxychlor, endrin, γ-chlordane. Aldrin, dibuthylchlordate and heptachlor epoxide Sigma Aldrich (St. Louis, MO, USA) products were used. Stock solutions containing 1000 mg/L of each pesticide were separately prepared by dissolving accurately weighed amount in methanol and stored in refrigerator below 4^0C. Intermediate working standard solution containing a mixture of 20 mg/L of each analyte was then prepared by diluting appropriate volume of each standard in methanol and then, the solution was stored in the refrigerator at 4 ^{0}C. Working standard solutions was then prepared from this intermediate standard solution. The chemical structures and common names of the target pesticides are given in Figure 3

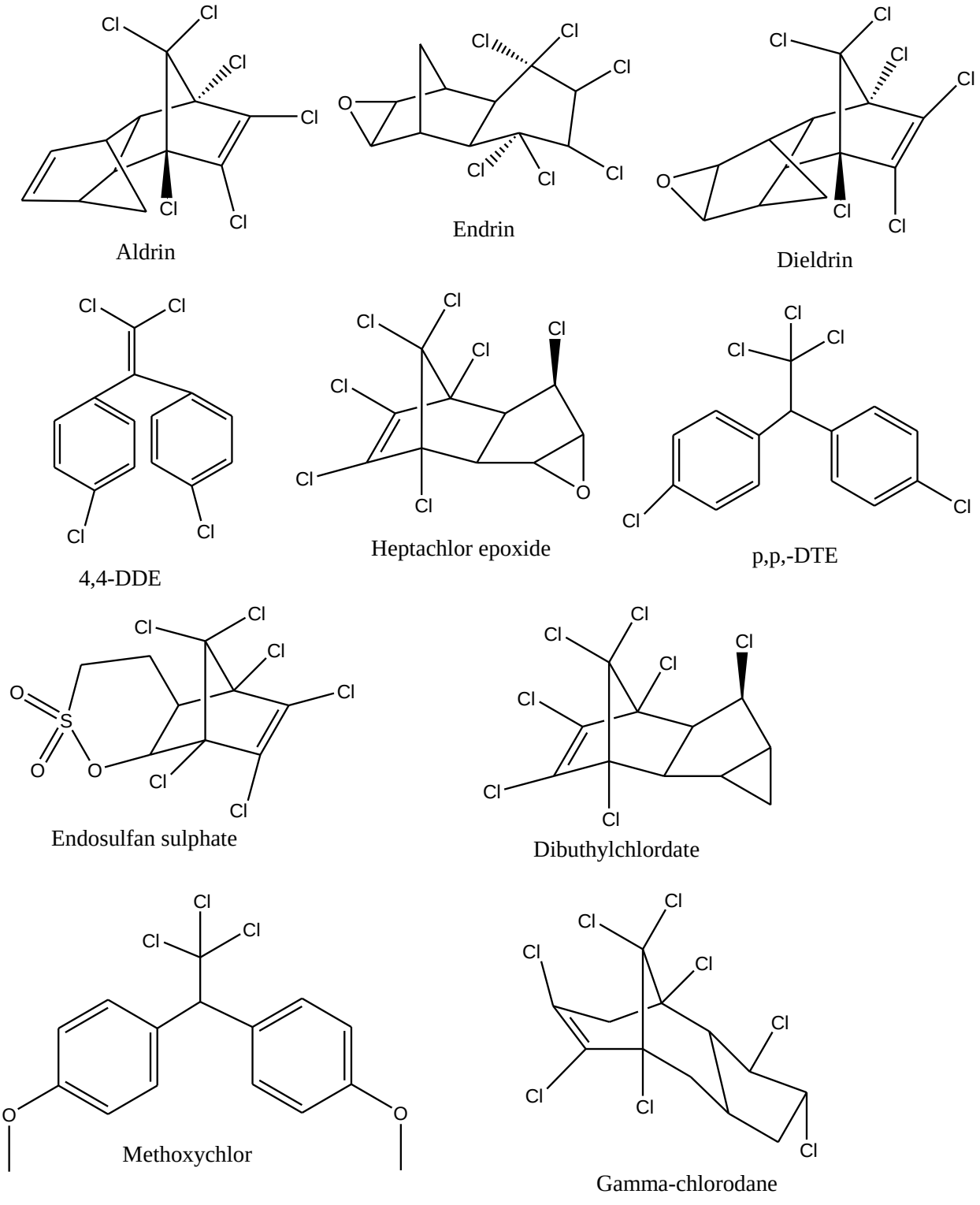

Figure 3. Chemical structure and common names of the target pesticides**.**

3.3. Instruments and equipment

Separation and quantification were performed using Agilent Gas chromatography equipped with an electro capture detector (GC-ECD) auto sample, pump, column compartment, and electro capture detector (ECD) with the model of 7980A, Vortex mixer obtained from Fisher scientific model FB15024 (Belgium) and cup vial and ultrasonic water bath Elma obtained from (Germany). Other equipment such as centrifuge tube, soxhlet thimble, centrifuge and 5 mL medical syringes were used for sample preparation.

3.4. Gas Chromatography Electro Capture Condition

All pesticides were determined by gas-liquid chromatography with electron capture detector (GC-ECD, Agilent Technologies 7890A)

Table .1. Gas Chromatography Electro Capture Condition

Parameters	**Requirements**
Detectors:	µECD
Column	250°C
Auto-sampler	ALS
Injection	Split less
Split less period	Mode
Inlet temperature program	250
Carrier gas	N_2
Injection volume	1µl
Column temperature	30m, 0.25µm and
Detector temperature	300°C
Inlet pressure	10.04Psi
Oven program	Initial 80°C, 180°C ramp at 15°C to 205°C, hold for 4min then, ramp at 30°C to 290°C hold for 8 min
Post run time	27.9
Post run temperature	290°C
Post run pressure	18.04

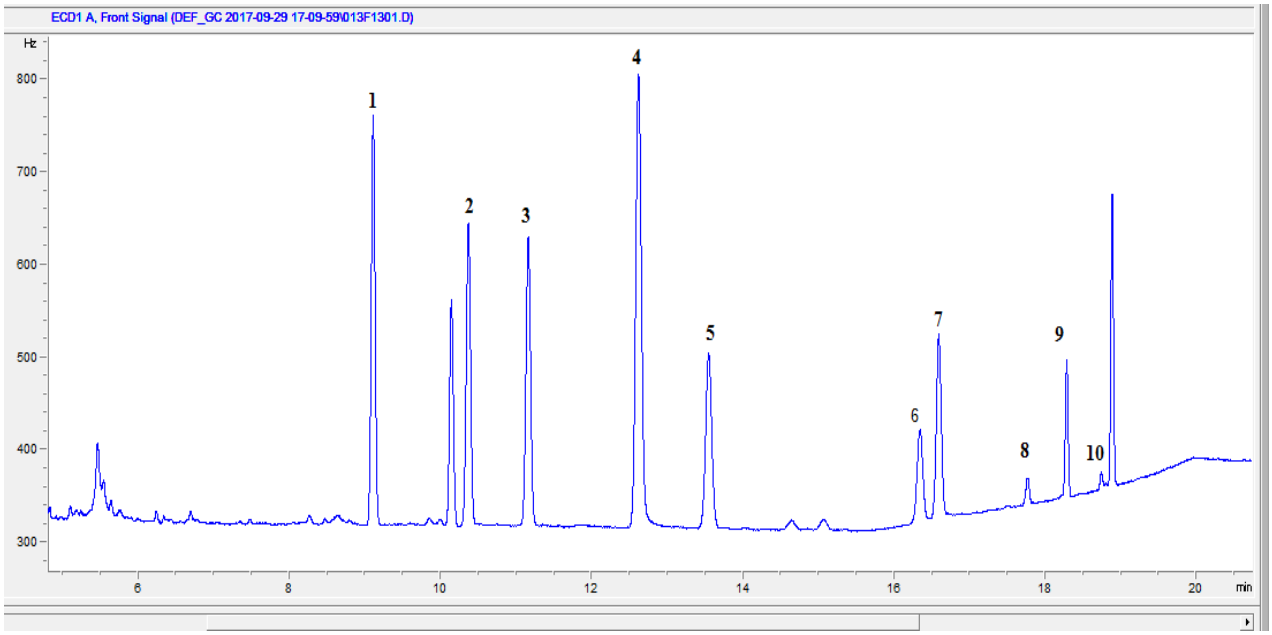

Figure 4. Chromatograms of the target OCPs. Description of anlytes (retention time, t_R, in min): (1) Aldrin (9.157); (2) Dibutyl chlorendate (10.373); (3) 4,4-DDE (12.625); (4) Gamma-chlordane (γ-chlordane) (11.196); (5) Endrin (13.633); (6) Endosulfan sulfate (16.295); (7) Dieldrin (16.634); (8) p,p-DDT (17.701); (9) Methoxychlor (18.305) and (10) Heptachlor epoxide (18.911).

3.5. Sample Collection

The water samples were collected from Gilgle Gibe (I) Hydroelectric Dam on August 28. A purposive (judgmental) sampling method was used to collect the samples. Water samples were collected from five sites using 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 ºC. Before sampling, sampling bottles were flushed three times with the sampled water. Each sample was collected at 10 m distance from the edge of the dam where the four main tributaries, namely, Nada guddaa river (AS_1), Nada xina River (AS_2), Nadi River(AS_3), Gibe River (AS_4) meat the dam. One more sample (AS_5) was collected from the center of the dam. The collected water samples were transported Jimma university chemistry laboratory using ice box and kept below 4 ºC in refrigerator until analysis. Similarly, from the same sampling sites, i.e., meeting sites, Nada guddaa river, Nada xina river, Nadi river, Gibe river and center of the dam sediment samples, A_1, A_2, AB, AD and AE, respectively, were collected in triplicates using stainless steel

spoon at 5 m distance from each other. Triplicate samples collected from the same sites, were thoroughly mixed by dispersing on aluminum foil and then about 1 kg composite sample was taken from each using aluminum foil and transported to laboratory. Then, after air drying in the laboratory, the samples were ground and sieved using a 2 mm mesh pore size sieve, to eliminate pebbles, large particle size materials and debris. Likewise, totally nine fish sample were randomly taken from dam and categorized them in to two were based on their length and weight as smaller (SF) (mean length 11.30 cm and mean weight 246.63 g) and said to be bigger (BF), (if their mean weight 350.80 g and mean length 17.15 cm,) and taken to laboratory by raping in aluminum foil. Then, the samples were slaughtered and the edible parts were separated and air dried for the subsequent procedure.

3.6. DLLME Procedure

For extraction of water samples, the LW-DLLME method reported by Wenting, [20] was used with some modification. Accordingly, 5 mL water sample was taken in to 15 mL centrifuge tube and a mixture of 100 µL of toluene and 600 µL of acetone as extraction solvent and dispersive solvents, respectively, was then rapidly injected using a micro syringe, so cloudy solution was formed. Subsequently, 0.5 g NaCl (i.e., 10%. *m/v*) was added and the mixture was shaken manually until the salt was completely dissolved. Then, the sample solution was vortexes for 30 s to enhance homogeneous distribution of cloudy suspension in the sample solution and thus, to ensure rapid transfer of analyte from aqueous phase to organic phase. Following this stem, the content was centrifuged for 3 min at 4000 rpm to facilitate phase separation. The fine droplet of organic phase pick up by using micro pipette (20-200) from collected organic phase 50µL volume carefully taken and transfer in to insert vial which was housed by 2ml of auto sampler vial after diluting the resulting extract to the total volume of 100 µL by adding 50 µL n-hexane, 1 µL was injected into GC-ECD instrument.

3.7. Extraction of fish and sediment samples

For extraction of OCPs from sediment and fish samples soxhlet extraction procedure which was reported by Farshid [74] was employed. Accordingly, 10 g sediment or fish sample was taken into beaker containing 50 g anhydrous Na_2SO_4 and then, mixed thoroughly. The mixture was then

transferred to an extraction thimble and placed in a Soxhlet extractor. The component was extracted with 150 mL acetone: n-hexane (20:80 v/v) at 50 °C for 4 h. The resulting extract was filtered and then concentrated to 1 mL using a vacuum rotary evaporator. Afterward, concentrated extract was in 5 mL hexane and then the resulting solution was eluted Florisil SPE cartridge for clean-up. The analyte which was trapped in the cartridge was eluted with 0.5 mL hexane (5 times) to quantitatively recover the target analytes. The obtained extract solution was then kept in vial for some time until hexane was evaporated. Finally, dried was re-dissolved in 1 mL hexane, mixed thoroughly with vortex mixer and then placed on auto sampler to inject 1 µL of the prepared extract into gas chromatography. The sample equivalent (mg/g) extract was calculated based on the formula suggested by Schenck and Howard-King [75].

$$Y = a/b \quad x/z$$

where Y is grams of sample equivalent per milliliter of extract, a is the amount of sample analyzed (g), b is the volume of solvent added to extract the sample (mL), x is the amount of the cleaned extract taken after evaporation until dryness (mL), and z is the amount of hexane: acetone (80:20, v/v) added for solvent exchange (mL).

3.8. Method Validation

The efficiencies of the utilized methods were evaluated using recovery studies. Recovery studies were performed by spiking the water, sediment and fishes samples with known concentrations of the standard OCPs. The Spiked and blank samples were extracted in duplicate (experimental replicates) and each extract was then injected in duplicate (instrumental replicates).

3.9 Statistical Analysis

Statistical tests such as student t-test ($p \leq 0.05$) and one-way ANOVA ($p \leq 0.05$) were used to investigate whether concentration of OCPs obtained at different sampling have significant differences in OCPs concentrations or not.

4. RESULTs AND DISCUSION

4.1. Calibration Curves and Analytical Performance Characteristics

For the determination of OCPs in the target samples, five series of standard OCPs solutions were prepared by diluting the intermediate solution containing mixture of all pesticides with hexane. The standard solutions were analyzed with GC-ECD and five points of calibration curves were established by plotting peak areas as a function of concentrations for each pesticide. The obtained calibration curves were exhibited wide linear ranges and excellent coefficient of determination, r^2, and ranging 0.999–1.000. The limits of detection (LOD) and quantification (LOQ) were also determined as the minimum analytes concentrations yielding 3 and10 times the signal-to-noise (S/N) ratio, were ranging from 0.06–0.72 µg/L and 0.30–2.4 µg/L, respectively. The performance characteristics of the utilized method are shown in Table 1.

Table 2. Analytical performance characteristics of the utilized method.

Analyte	LDR	Slop	Intercept	r^2	LOD	LOQ
Aldrin	0.34 – 100	160.6	-29.16	1.0000	0.10	0.34
Dibutyl chlorendate	0.54 – 100	128.9	15.89	1.0000	0.16	0.54
γ-chlordane	0.48 – 100	133.2	-14.17	1.0000	0.14	0.48
DDE	2.40 – 100	265	-110.4	1.0000	0.72	2.40
Endrin	0.50 – 100	104.2	6.633	1.0000	0.15	0.50
Endosulfan sulfate	0.50 – 100	45.37	32.35	0.9997	0.15	0.50
Dieldrin	0.30 – 100	95.26	-29.03	0.9999	0.06	0.30
Methoxychlor	0.50 - 100	35.60	16.26	0.9999	0.15	0.50
Heptachlor epoxide	0.50 - 100	73.22	11.25	1.0000	0.15	0.50

LDR: Linear dynamic range

4.2. The concentration of pesticide in water sample

The concentrations of OCPs in the dam water samples are presented in Table 3.

Table 3. Mean level **(**µg/L ± SD) of OCPs in water samples (n = 4)

OCPS	Sample site					Average	LSD
	AS_1	AS_2	AS_3	AS_4	AS_5		
Aldrin	58.73 ± 1.93	19.29 ± 2.39	14.16 ± 3.64	15.78 ± 1.39	16.93 ± 0.42	24.98 ± 1.95	2.95
Dibutyl chlorendate	146.05 ± 5.66	ND	ND	ND	27.38 ± 2.28	86.69 ± 3.97	
γ-chlordane	178.71 ± 11.83	75.33 ± 4.97	39.14 ± 4.29	40.76 ± 2.91	18.9 ± 2.90	70.57 ± 5.38	8.14
DDE	50.38 ± 3.69	8.46 ± 0.08	2.88 ± 0.05	7.670 ± 1.41	8.64 ± 0.15	15.60 ± 1.08	1.63
Endrin	101.37 ± 5.48	ND	ND	61.74 ± 2.75	ND	81.56 ± 4.12	
Endosulfan sulfate	ND	84.11± 4.58	64.25 ± 9.13	ND	66.89 ± 4.52	53.81 ± 6.08	9.76
Dieldrin	253.36 ± 9.26	26.85 ± 5.67	19.34 ± 2.90	12.95 ± 1.63	ND	78.13 ± 4.86	7.53
Methoxychlor	1161.93 ± 21.93	197.49 ± 3.21	79.66 ± 18.13	80.71 ± 3.62	64.13 ± 3.51	316.78 ± 10.08	15.25
Heptachlor epoxide	1596.96 ± 31.93	1192.8 ± 1.80	388.82 ± 4.32	682.65±7.42	394.51 ± 2.93	851.15 ± 9.68	214.65

ND: not detected, SD: standard deviation: LS: least significant difference

As can be seen in the Table 3, the dam water samples contain significant amount OCPs, which far beyond WHO guide line for surface water [47]. In general, of the studied pesticides, relatively the smallest and highest concentrations were observed in all water samples for DDE and Heptachlor epoxide, respectively. Moreover, water samples collected from different sites of the dam also contained different concentrations of OCPs. Specifically, a sample which was collected from, AS1 contains the highest concentrations of the studied pesticides than other sampling sites. This could be attributed to the presence of source of theses OCPs in to the water via water runoff from agricultural activities around the catchment, e.g. farms and domestic gardens.

Aldrin, DDE, γ-chlordane, Methoxychlor and Heptachlor epoxide were detected in all water sample at different concentration but Dibutyl chlorendate and Endrin were not detected in AS_2 and AS_3 , Endosulfan sulfate and Dibutyl chlorendate were not detected in AS_4 , Endrin and dieldrin also were not detected in AS_5 sample .

4.3. The concentration of pesticide in sediment sample

Concentrations of the target OCPs in sediment of the dam are presented in Table 4.

Table 3. Mean level (µg/kg ± SD) of OCPs in sediment samples (n = 4)

Analyte	Sample Sites					Average	LSD
	A_1	A_2	AB	AD	AE		
Aldrin	408 ± 9.90	125 ± 2.12	195 ± 2.12	775 ± 2.69	933.50 ± 6.43	487.30	7.04
Dibutyl chlorendate	103.15 ± 1.50	95 ± 2.12	135 ± 2.12	717.5 ± 1.77	ND	262.66	9.56
γ-chlordane	677 ± 3.39	700 ± 1.41	ND	1460.00 ± 1.56	1679.00 ± 3.21	1129.00	3.70
DDE	770 ± 7.07	500 ± 1.41	700 ± 1.41	885.50 ± 1.62	174.50 ± 2.12	606.00	4.02
Endrin	ND	ND	170 ± 2.83	ND	ND	170.00	
Endosulfan sulfate	5622.5 ± 2.19	250 ± 1.41	185 ± 0.71	855.00 ± 5.23	328.50 ± 14.21	1448.20	7.19
Dieldrin	659.5 ± 1.63	280 ± 2.40	ND	229.50 ± 0.78	142.50 ± 2.19	327.87	2.17
Methoxychlor	2919 ± 3.54	660 ± 5.66	240 ± 15.56	202.50 ± 2.05	661.00 ± 3.25	936.5	9.10
Heptachlor epoxide	7104 ± 9.66	115 ± 4.95	ND	6236.00 ± 0.36	1406.00 ± 4.68	4485	6.08

The result of the analysis of sediment samples has shown the presence of a number of OPCs in most sediment samples, except Endrin. The compound detected were Heptachlor epoxide, Methoxychlor, Dieldrin, Endosulfan sulfate, gamma chlordane, DDE, gamma chlordane, Dibutyl chlorendate and Aldrin.

Out of nine OPCs analyzed, Endrin were not detected in all samples, except in samples taken from AB site. The mean concentration of OPCs determined in sample from AB ranges from 135±2.12 to 700 ± 1.41 µg/ kg. However Dieldrin, gamma chlordane and heptachlor epoxide were not observed in AB with the detection limit of 0.06, 0.14 and 0.15 µg/kg, respectively. These may be attributed to the sampling location were less exposed to movements of these substances. Of all OCPs investigated in sediments, Heptachlor epoxide were the dominant substance determined with the range of ND to 7104 ± 9.66 µg/kg. These may be due to the chemical property which is persistence to degradation in sediments and aquatic environment [76]. The level of Aldrin in the sediment sample taken was in range of 125 ± 2.12 to 933.5 ± 6.43 µg /kg. The concentration of DDE in all sediment samples taken from Gilgle Gibe I dam was more than that of Aldrin, except in AE sample.

The mean level of concentration in sediments of under study ranges from 185 ± 0.71 to 5622.5 ± 2.19 µg / kg. These, high level of 1Endosulfan sulfate in sample A_1 is may be attributed from the adsorption property of the organic pollutants which is dependent on the soil organic matter content [77]. The OCPs level in this study was considerably lower in comparison to another surface water reservoir reported [78]. Chemically aldrin, have a property to rapidly broken to dieldrin [79]. However, in most of samples the concentration of aldrin in the sediment sample is higher than that of dieldrin, which may indicate that the fresh input of aldrin in the study area.

4. 4. The concentration of pesticide in fish sample

Table 5. Mean level (µg/kg -± SD) of OCPs in Fish samples (n = 4)

Analyte	SF	BF	Average
Aldrin	35.50 ± 1.60	500.00 ± 5.60	267.76
Dibutyl chlorendate	7.50 ± 0.20	ND	3.75
γ-chlordane	ND	ND	ND
DDE	35.50 ± 0.70	330.00 ± 1.00	182.75
Endrin	17.00 ± 0.01	95.00 ± 4.95	56.16
Endosulfan sulfate	73.50 ± 2.20	240.00 ± 14.00	156.75
Dieldrin	37.50 ± 3.50	395.00 ± 7.80	216.25
Methoxy chlor	109.50 ± 7.00	205.00 ± 2.10	157.25
Heptachlor epoxide	28.91 ± 0.01	680.00 ± 1.40	354.45

SF: smaller fish; BF: larger fish:

OCPs, including Heptachlor epoxide, Methoxychlor, Dieldrin, Endosulfan sulfate, DDE, Dibutyl chlorendate, Endrin and Aldrin were detected except γ-chlordane, in fish of the present study with varying concentrations, see table 5. Among the OCPs analyzed the level of Methoxy chlor and Heptachlor epoxide were predominant in both small and big fish of under study, with the highest concentrations being 109.5 ±7 and 680 ± 1.4 µg/kg, respectively. Contamination pattern of OCPs in fish was generally in order of Heptachlor epoxide > Aldrin > Dieldrin > DDE > Endosulfan sulfate > Methoxy chlor > Endrin > DDE > Dibutyl chlorendate > gamma chlordane. The higher concentration of these compounds might be due to the higher bio-accumulative properties of these compounds in line with continuous release into aquatic environment, which can be used intensively used in agriculture and for malaria control. The deferent level of these OCPs in fish is because of the variation in age, metabolic capacity and the bio- accumulative in food chain [11]. This fact has been observed from table 4 except dibuthyl chlordane.

This may indicated that most farmers around dam use as agricultural pesticide or possible source poly ethylic rubber and strongly bind in aquatic sediments accumulator rapidly in many species unless it has no natural source in environments [80]. Similarly the concentration of DDE in present study was lower than that was reported earlier in human milks and caw milks of Jimma, Asendabo and Serbo town [81]. These might be due to strong persistence and bio- accumulative character of DDE.

4.5. Bioconcetrations

Bioconcetrations of the detected pesticides were calculated as the ratio of concentration of OCPs in sediment or in fish to the concentration of pesticide in water [76]. Bioconcentrations of the detected pesticides in sediment and fish samples are presented in Table 6

Table 6.Bioconcentration of the detected pesticides

Analyte	C_s/C_w	C_F/C_w
Aldrin	19.15	10.72
Dibuthyl chlorendate	3.03	0.15
Gamma chlordane	15.99	-
4,4,DDE	38.83	11.71
Endrin	2.08	0.69
Endosulfan sulfate	26.91	2.91
Dieldrin	4.19	2.77
Methoxychlor	2.95	0.50
Heptachlor epoxide	5.27	0.42

C_s/C_w: ratio of concentration of the analyte in sediment to water
C_F/C_w: ratio of concentration of the analyte in fish to water

As absorbed from above table .4 in general the bioconcetration of target pesticide increase from water to sediment .the bioconcetration of DDE and endosulphane sulphate were notably high in Gilgle Gibe (I) hydroelectric dam compared to in water and fish.

The order of concentration of pesticide in sediments were DDE > Endosulfan sulfate > Aldrin > gamma chordate > heptachlor epoxide > dieldrin > dibuthyl chlorendate > methoxychlor > endrin. However the bioconcentration of properties of pesticide in fish to water sample was not like as sediment to water. But there was certain indication of bioconcetration characters were clearly seen for DDE, aldrin dieldrin and endosulphane sulphate. In case, some result from above table does not show bioconcetration character this was might be breakdown or transformation of pesticides by microbial agents which normally occurs in water and soil. which means 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 favours microbial activities, such as warm temperature, favorable pH, adequate soil moisture, aeration and high organic matter content and microorganisms participating in biodegradation include fungi, bacteria and other microorganisms that use pesticides as their substrate [83].

4.6. Recovery test

The 100µg/L solution was prepared from intermediate working solution and 100µL was spiked in 10g of fish and sediment sample but 50 µL spiked in 5 mLof water sample . Recovery (%R) study was performed by spiking water, sediment and fish samples to investigate the performance of the utilized methods. Spiked samples were extracted in duplicate (experimental replicates) and each extract was then injected in duplicate (instrumental replicates). The analytes were determined by comparing the peak area of the spiked water, sediment and fish samples with the peak area of the non-spiked water, sediment and fish sample. The obtained results are presented in Table 7

Table 7. Recovery (%R) of the method for the water, sediment and fish sample

Analyte	Water	Sediment	Fish
Aldrin	77	96	105
Dibutyl chlorendate	87	101	92
Gamma chlordane	89	94	83
DDE	114	91	91
Endrin	91	69	118
Endosulfan sulfate	67	78	78
Dieldrin	74	74	97
Methoxychlo**r**	67	86	103
Heptachlor epoxide	73	97	112

As can be seen in the above table 7, recoveries of the target analytes are ranging from 67 – 118 %. With the exception of Endosulfan sulfate and methoxychlor in water sample, recoveries results are in acceptable region for analysis of pesticides residues, indicating the reliability of the obtained results in the study.

4.7 Comparison of the absorbed result with other reported result

The comparisons of obtained result with other reported result were made based on mean concentration in terms of micro gram per kilogram and micro gram per litter in sediment, fish and water sample respectively

Table 8. Comparison mean level (µg/L) of OCPs in water samples

Country	Aldrin	Dibuthylchlorndate	ɣ chlordane	DDE	endrin	Endosulfan sulfate	dieldrin	Methoxychlor	Heptachlor epoxide	Reference
Ethiopia	--	--	--	0.055	--	0.046	--	--	--	Teklit GA et.al 2016
Iran	0.797	--	--	--	--	28.510	1.010	5.444	4.187	Befar. A .et .al 2013
Iran	--	--	--	0.055	--	0.046	--	--	--	Kalfilzadeh.F et.al 2012
Ethiopia	24.980	86.69	70.57	15.60	81.56	53.81	78.13	316.78	851.15	This report

Table 9. Comparison mean level (µg/kg) of OCPs in sediment samples

Country	Aldrin	Dibuthylchlorndate	ɣ chlordane	DDE	endrin	Endosulfan sulfate	dieldrin	Methoxychlor	Heptachlor epoxide	Reference
Ethiopia	--	--	0.074	9.840	--	10.622	--	--	0.081	Teklit GA et.al 2016
Iran	--	--	0.083	8.750	--	12.475	--	--	0.088	Kalfilzadeh.F et. al 2015
Iran	--	--	0.074	9.840	--	10.622	--	--	0.081	Kalfilzadeh.F et. al 2012
Ethiopia	487.30	262.66	1129.00	606.00	170	1448.20	327.87	936.5	4485.00	This report

Table 10. Comparison mean level (µg/kg) of OCPs in fish samples

Country	Aldrin	Dibuthylchlorndate	ɣ chlordane	DDE	Endrin	Endosulfan sulfate	dieldrin	Methoxychlor	Heptachlor epoxide	Reference
Ethiopia	--	---	0.024	4.864	--	0.816	--	--	0.041	Teklit GA et.al 2016
Ghana	8.63	---	13.39	11.19	3.37	--	2.23	--	13.56	Mensah.H.K et.al 2011
Iran	--	--	0.024	4.864	--	0.816	--	--	0.041	Kalfilzadeh.F et.al 2012
Ethiopia	267.76	7.50	ND	182.75	56.16	156.75	216.25	157.25	354.45	This report

5. Conclusion and Recommendations

5.1. Conclusion

In this study, the analysis of persistent OCPs in water, sediment and fish sample were conducted by using efficient method GC-ECD with DLLME and soxhlet extraction .The results were indicated that the presence of OCPs with different concentration in Gilgle Gibe I hydroelectric dam. This shown us there is contamination of the environment by these pesticides from recent of historical use. The concentration levels of most residues in sediment were higher than those found from fish and water. In all sample maximum concentration was achieved by heptachlor epoxide in water and sediment of sample area one .the concentration of pesticide in sediment to water ratio higher than fish to water ratio this may be used as an indication of the sediment is the most important sinker for pesticide.

5.2. Recommendations

The following recommendations are made as a result of the outcome of this study

- In view of this study further investigation were needed for persistent organochlorine pesticides to elucidate future pollution trends and to assess especially human in particular to children health risk at study area.
- It is better to develop appropriate management, monitoring and control strategies on pesticide user by authorized body
- Monitoring of OCPS at different environmental sample should be done at regular interval to determine the extent of the release of this compound to environment.

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Appendix

Appendix .A

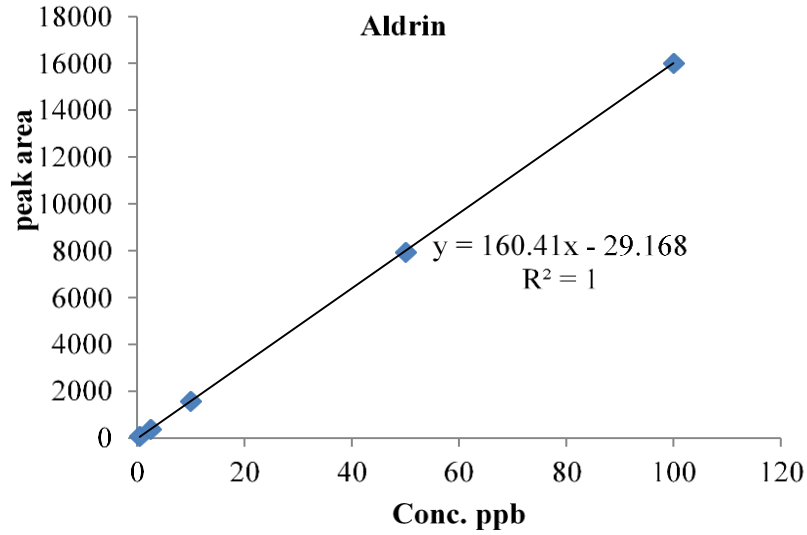
Sample collection time

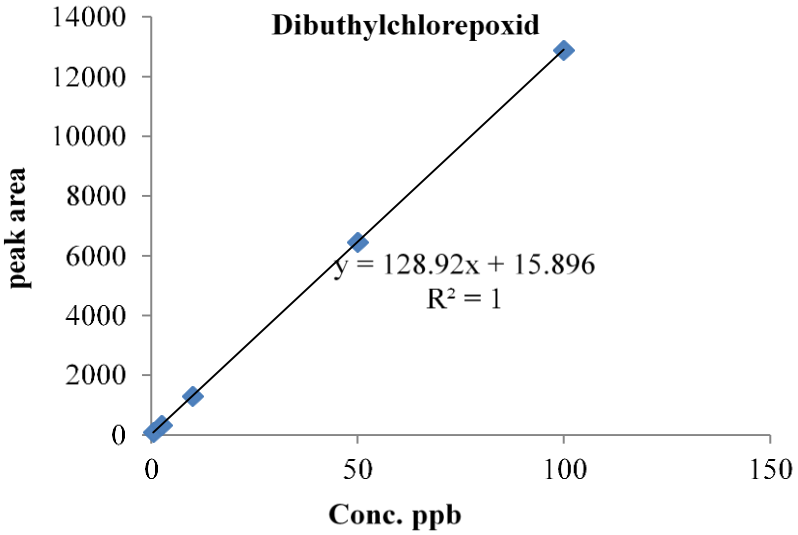
Water sample

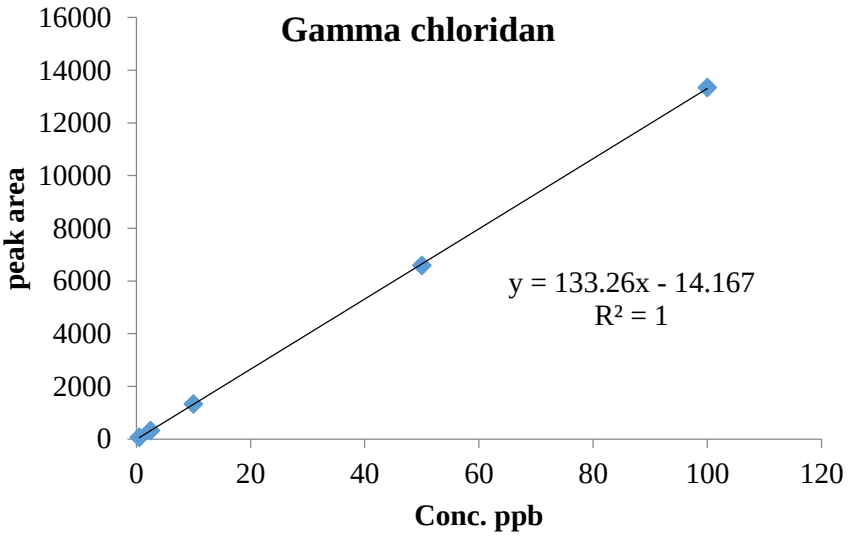
Sediment sample fish sample

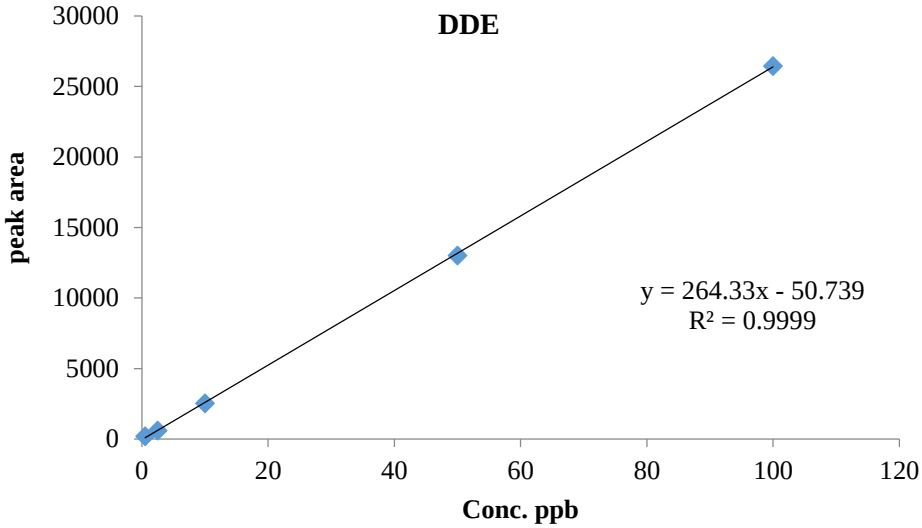
Appendix .B

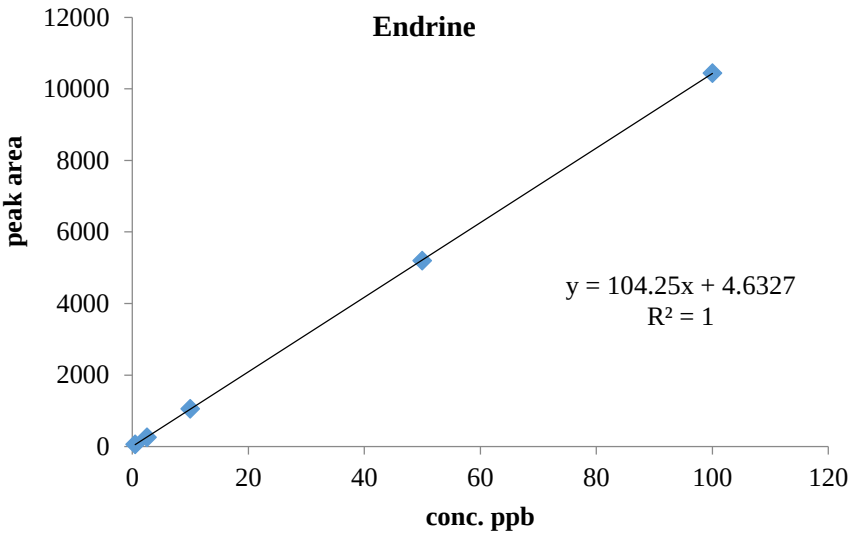
Calibration graph each target analyte

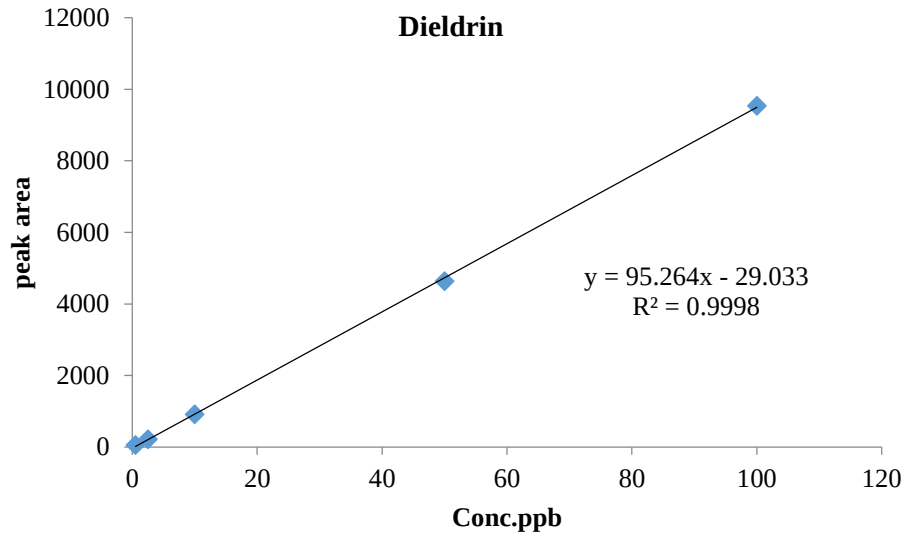


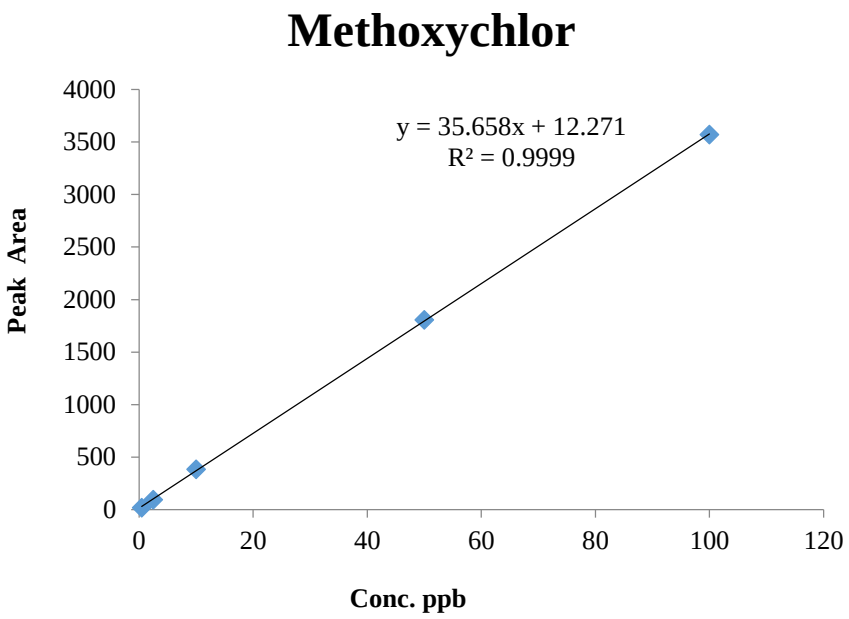


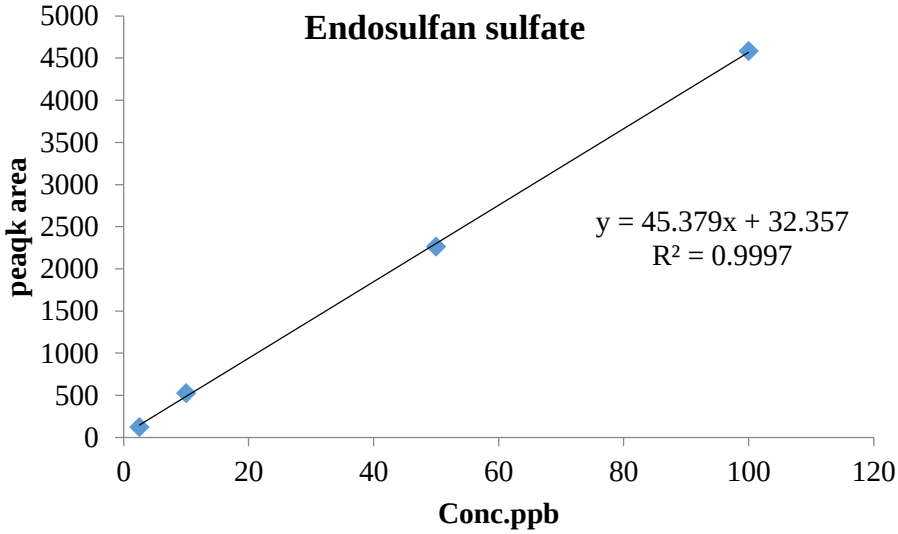

Gamma chloridan
peak area
Conc. ppb
y = 133.26x - 14.167
R² = 1
16000
14000
12000
10000
8000
6000
4000
2000
0
0
20
40
60
80
100
120

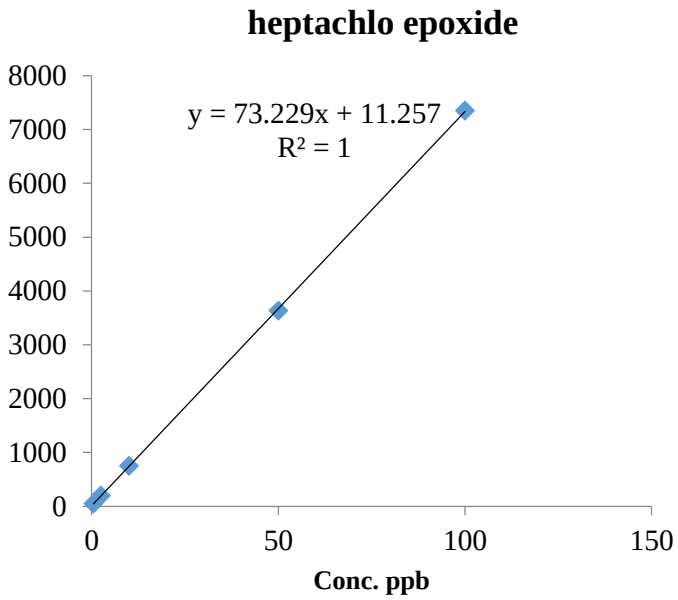

DDE
peak area
Conc. ppb
y = 264.33x - 50.739
R² = 0.9999
30000
25000
20000
15000
10000
5000
0
0
20
40
60
80
100
120


Endrine
peak area
conc. ppb
y = 104.25x + 4.6327
R² = 1
12000
10000
8000
6000
4000
2000
0
0
20
40
60
80
100
120


Dieldrin
peak area
Conc.ppb
y = 95.264x - 29.033
R² = 0.9998
12000
10000
8000
6000
4000
2000
0
0
20
40
60
80
100
120

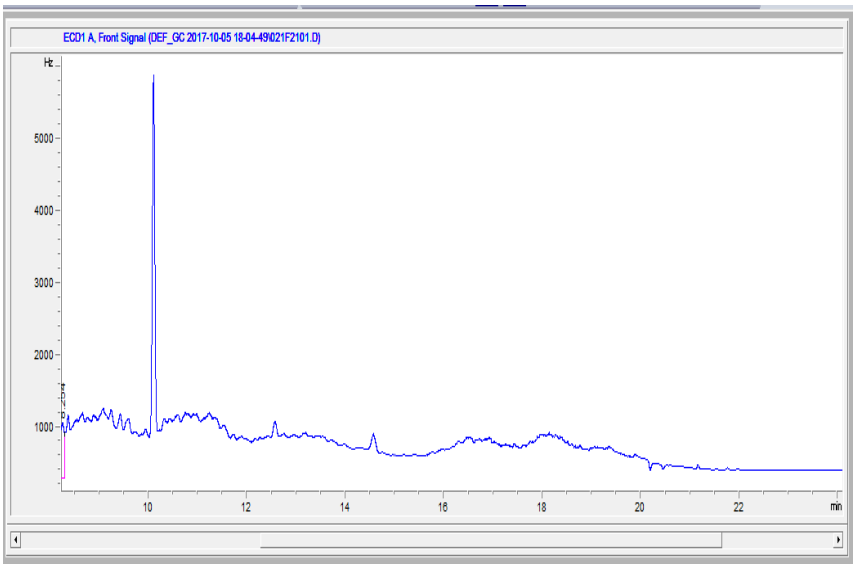

Methoxychlor
y = 35.658x + 12.271
R² = 0.9999
Peak Area
Conc. ppb
0
500
1000
1500
2000
2500
3000
3500
4000
0
20
40
60
80
100
120


Endosulfan sulfate
y = 45.379x + 32.357
R² = 0.9997
peaqk area
Conc.ppb
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
0
20
40
60
80
100
120

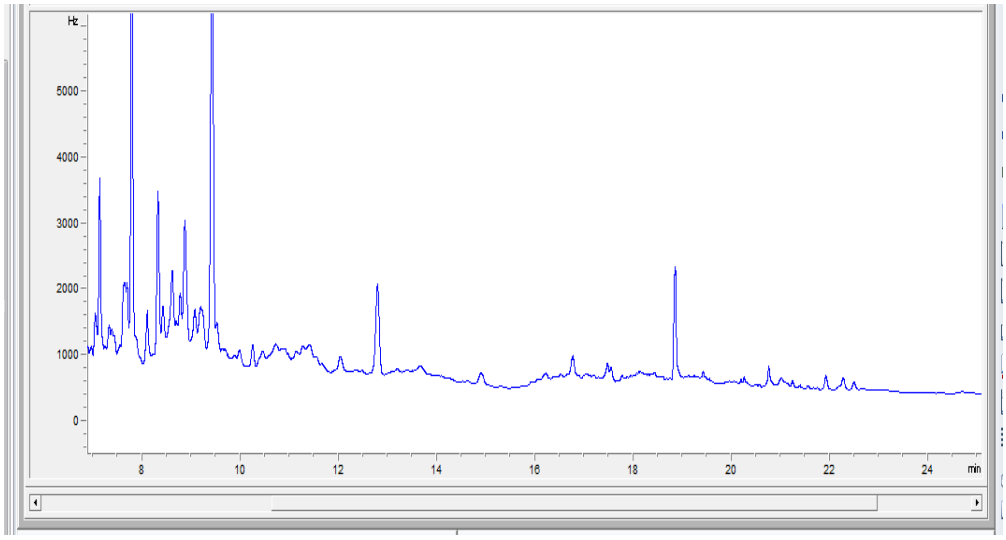

heptachlo epoxide
y = 73.229x + 11.257
R² = 1
8000
7000
6000
5000
4000
3000
2000
1000
0
0
50
100
150
Conc. ppb

Appendix C

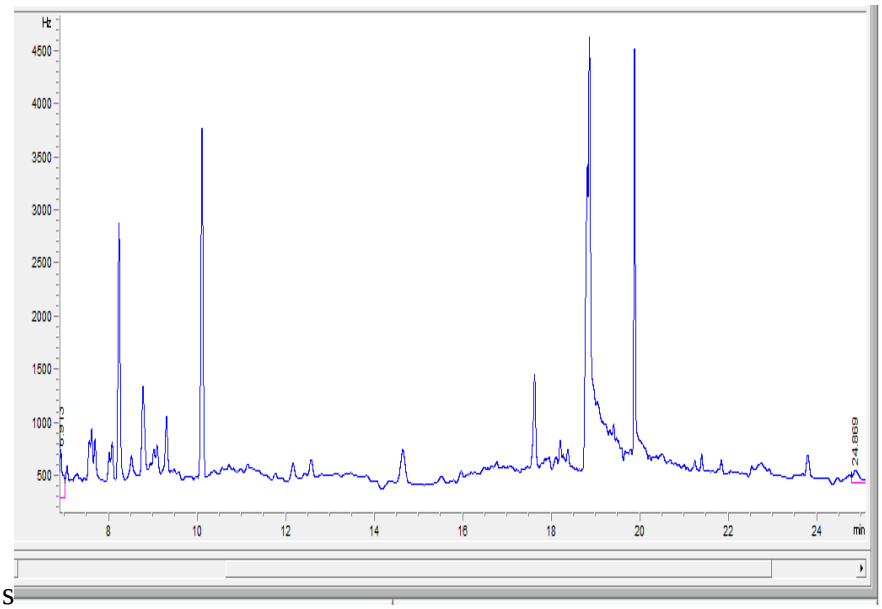
Chromatograms’



Chromatogram of sediment sample



chromatogram of fish sample



s

chromatograme of water sample