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PESTICIDE RESIDUE IN STREAMS RECEIVING WET COFFEE PROCESSING EFFLUENT OROMIA NATIONAL REGIONAL STATE, JIMMA ZONE, ETHIOPIA

BY ASHENAFI GEBRE

A THESIS SUBMITTED TO INSTITUTE OF HEALTH SCIENCE, FACULTY OF PUBLIC HEALTH, DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY, JIMMA UNIVERSITY; IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY

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DECLARATION

I declare that the thesis entitled “pesticide residue in streams receiving wet coffee processing effluent in oromia national regional state, at jimma zone, Ethiopia” is part of “Risk Assessment of Coffee Processing Effluent on Human, Animal, and Aquatic Biota in the Receiving Water Bodies: a One Health approach “mega project where the PI is Dr. Argaw Ambelu and submitted to postgraduate studies’ office of Institute of Health Science is original and it has been not be submitted previously in part or fully to any university.

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CERTIFICATE

We certify that Ashenafi Gebre did the thesis entitled “pesticide residue in streams receiving wet coffee processing effluent in oromia national regional state, at jimma zone, Ethiopia ” for the partial fulfillment of Master’s Degree under our supervision.

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Approval sheet of Thesis

As members of the Examining Board of the Final Open Defense, we certify that we have read and evaluated the thesis prepared by Ashenafi Gebre, entitled “pesticide residue in streams receiving wet coffee processing effluent in oromia national regional state, at jimma zone, Ethiopia”, and recommend that it be accepted as fulfilling the thesis requirements for the award of the degree in Master of Science in Environmental Science and Technology.

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Acronyms/Abbreviation

APHA	American public health association
DDT	dichlorodiphenyltrichloroethane
EU	European Union
FAO	Food and Agricultural organization
FEPA	Federal Environmental Protection Agency
g	Gram
GC-ECD	Gas Chromatography with Electron Capture Detector
HCB	hexachlorocychlobenzene
HCH	hexachlorocychlohexane
KAP	Knowledge, Attitude and Practice
LDS-DLLME	Low-density solvent based dispersive liquid- liquid micro extraction
LOD	Limit of Detection
LOQ	Limit of quantification
npic	National pesticide information center
OCPs	organochlorine pesticides
PCB	Polychlorinated Biphenyls
POPs	persistent organic pesticides
rpm	Revolution per minutes
USDHHS	Union States Department of Health and Human Services,
USEPA	United State Environmental Protection Agency
µg /L	Microgram per Liter
µl	Micro liter
µS	Micro Siemens

Abstract

Organochlorine pesticides (OCPs) are synthetic pesticides widely used all over the world. When present in the environment they adversely affect terrestrial and aquatic biodiversity. Thus, there has been a continual demand to monitor the presence of OCPs within the environment. The main objective of this study is to assess pesticide residues in coffee processing effluent receiving stream and biota at Bore, Haro and Agaro, Jimma zone. Farther, 18 each of water, and 12 macro invertebrates samples were collected from the three sites coffee effluent receiving streams. Extraction methods such as Dispersive Liquid-Liquid Micro (DLLME) and Soxhlet Extraction were used for the analysis of pesticides from water and macro invertebrates' sample, respectively. The samples were cleaned up with deactivated silica gel and the final extracts were analyzed using Gas Chromatography with Electron Capture Detector (GC-ECD). The coefficient of determination 9 pesticides showed linear correlation (r^2> 0.99). The performances of the analytical procedures were evaluated using percentage recovery and computed by spiking the blank samples with known concentration of the target analytes. The finding shows, the percent recovery for each analytes were between 81.7% and 109.1% comply with routine analysis detection requirements. Analyzed pesticides such as, Aldrin, g-chlordane, Dibuthylchlor, Endrin, Endosulfan sulfate, Dieldrin, p, p'- DDT, Methoxychlor and chloradate were detected in the water and macro invertebrates (MI) samples with varying concentration 89%, 58% and 37% of g-chlordane, Dibuthylchlor and Aldrin, were detected with the highest mean concentration in MI and water sample at the study area. The lowest detected pesticide was chloradate 0.002% in MI samples. In conclusion, water and aquatic organisms are polluted by organochlorine pesticides and may expose human, aquatic animals or general environment. Therefore, strict regulation is needed in this particular study area as well as in Ethiopia in general.

Keywords: Organochlorines; residues, stream water, Dispersive Liquid-Liquid, Soxhlet, GC-ECD, Jimma Zone.

CHAPTER-ONE

1. INTRODUCTION

1.1 Background

Water supports all forms of life on earth. Fresh water is a vital resource for people all around the world and provides many provisioning (e.g. water for consumptive use), regulatory (e.g., buffering of flood flows), and cultural (e.g., recreation) ecosystem services (De Troyer et al., 2016). . Lack of safe water may affect health, lifestyle, and economic well-being (Sumathi, 2016). The worldwide consumption of pesticides is about two million tons per year: of which 45 % is used by Europe alone, 25 % is consumed in the USA, and 25 % in the rest of the world (De, A., Bose et al., 2014). In most developing countries farmers still use organochlorine pesticides because of their cost-effectiveness and good against controlling insects (Wang et al., 2018). As it is known, pesticides have played a very important role in the development of human agriculture since their invention, and they are still irreplaceable at present. The extreme use of agrochemicals such as, pesticides in farmlands cause health problems of people and cause environmental contamination (Pimentel, 2005; Kafle et al., 2015). Although environmental pollution and degradation is a major global problem, developing countries are at a higher risk for reasons related to lower socio-economic status (Alemayehu et al., 2014).

Use of pesticide especially organochlorine in Ethiopian rift valley region revealed that, farmers had been used DDT as insecticides even though its application for agricultural purpose is banned years ago. In addition, people using this chemical for other applications probably around the home and backyards against ticks, lice, mosquitoes, grain borers. The surveyors also observed that DDT is openly displayed in shops for sale. (Amera and Abate, 2008). However, the agricultural modernizing and the intensive agricultural production system were not beneficial for beekeepers. As the result, the population of honey bee colonies was declining in many parts of the world and even lead to extinction of some of the honeybee species. Organochlorine pesticides such as Endosulfan sulfate, Dieldrin and DDT were detected in honey produced in West Shewa Zone of Oromia Region, According to Mulugeta et al., (2017)

Use in Jimma zone Ethiopia also identified the application of pesticides to improve productivity and protect different food items from various pests before and after harvesting Mekonen et al., (2014). The same study concluded that the application of pesticides particularly persistent organochlorine pesticides contaminate the environment and detected in commonly consumed food items. In addition, indoor residual spraying (IRS) as a major means of malaria vector control to reduce and eliminate malaria transmission including, where indicated, the use of DDT mostly in African country (WHO,2006).

The economy of Jimma Zone and Jimma Town, the former capital of Kaffa Province, largely depends on coffee production. These coffee productions and processing produce large volume of waste which will contaminate different environmental compartments. Water pollution is the gloomy setback for development in coffee producing Countries (Haddis and Devi, 2008). According to Beyene et al. (2011)the untreated effluent direct discharges are of environmental concern due to the depletion in the dissolved oxygen concentration and acidic pH in the water which substantially affect the biotic environment, and can be fatal to fish and other aquatic organisms.

Due to the great demand of coffee, coffee industries are responsible for the generation of large amount of residues, which are toxic and represent serious environmental problems (Solange *et al*., 2011). Nowadays, there is great political and social pressure to reduce the pollution arising from industrial activities. Almost all developed and underdeveloped countries are trying to adapt to this reality by modifying their processes so that their residues can be recycled. Consequently, most major companies no longer consider residues as waste, but as a raw material for other processes (Mussatto *et al*., 2006).

Reduction of the proliferation of pest and increase in food production, has made pesticides application in agriculture inevitable or usual *(Akoto et al. 2013).* Pesticides constitute one of the most hazardous groups of contaminants (*Vega et al. 2005*), posing potential risk to humans and other life forms (*Jeyakumar et al. 2014).* Thus deaths and chronic diseases worldwide are sometimes reported to have resulted from pesticide poisoning *(*Rigotto et al. 2013). There has been a focus on the effect of people who consume large amount of marine food, including products of marine fish (*Bjorn, 2003).* These compounds when discharged into aquatic system play an important role in contaminating such systems. It has been

recognized that the persistent and bioaccumulation tendency of these substances, their metabolites and residues in the environment make them not only remain where they are applied but instead partition between the major environmental compartments in accordance with their physic-chemical properties and may become transported several kilometers from the point of their original release (*Agarwal, 2009*). Such environmental distribution may lead to exposure of living organisms including man that are far removed from intended targets. Many existing studies have provided a strong causal link between agro-chemicals use (particularly fertilizer) and crop yields, new panel data analysis identifies a strong causal relationship between the use of modern agricultural inputs and crop yields and, subsequently, yields and economic growth (McArthur and Mc Cord 2014).

Application of a wide variety of pesticides has been advised to increase the crop productivity in tropical countries where crop loss is severe due to high temperature and humidity, which are conductive to rapid multiplication of pests (Kannan *et al.*, 1993; Lakshmi, 1993). According to a World Health Organization study, 80% of all pesticides are used by developing countries (Veil, 1990). Due to lack of proper legislation, improper market regulations and ignorance shown by people, agricultural workers from developing countries are prone to experience high levels of agricultural chemicals, including pesticides (Smith & Jong, 2001). Among agriculturalists of developing countries, pesticide exposure is the primary occupational hazard (Wasseling *et al.*, 2001; Konradsen *et al.*, 2003; Coronado *et al.*, 2004) which leads to health issues and environmental contamination associated with pesticide use (Mancini, 2005; Remor *et al.*, 2009). Although farmers are considered to be the main risk group, formulators, loaders, mixers, production workers and agricultural farm workers are all extremely susceptible groups. The non-occupational hazards may be due to pollution of the ecosystem or habitat as a whole. An estimate shows that deaths and chronic diseases due to pesticide poisoning amounts to about one million per year worldwide (Environnews, 1999). The overuse or misuse of pesticides is contributing adversely to the environmental health as well as to ecosystem services. Pesticides are reported to affect many aquatic and terrestrial species. Life in aquatic ecosystems such as microorganisms, invertebrates, plants and fish are badly affected by pesticides (Liess *et al.*, 2005; Grande *et al.*, 1994; De Lorenzo *et al.*, 2001; Castillo *et al.*, 2006; Frankart *et al.*, 2003). In the Indian situation, massive use of pesticides

has started since the 1960s when the "Green Revolution" was initiated and maximum agrochemicals were used to achieve high agricultural production.

1.2 Statement of the problem

After harvesting, coffee is processed in dry and wet system. The dry one is processed using solar energy whereas wet processing is held with the help of water to remove the outer red skin and the white fleshy pulp. Wet coffee processing and waste problems are not in compliance with the standards; then contributing to tremendous (great) pollution of the environments (Wintgens, 2009). For instance, the use of agricultural pesticides significantly changes the toxic characteristics of the wastewater (Chanakya and Dealwis, 2004) human health and aquatic life, if discharged directly into the surface waters. People exist in the surrounding area of agro industries utilizing the stream water for domestic purposes suffer from severe health problems (Alemayehu and Rani, 2008).

Sources of pesticide in Ethiopia for malaria control (DDT) absolute pesticide accumulation and its fate and effects. About 75% of the total area of Ethiopia estimated to be malarias and about 65% of the populations of the country are at risk of malarial infection. Ethiopia has implemented indoor residual spraying (IRS) with DDT for malaria control in the past few decades (WHO, 2007). Approximately 400 metric tons of active-ingredient of DDT per year is used for IRS in many Parts of the country including the Rift Valley, a malaria epidemic prone region (Vanden Berg, 2009). In addition, Ethiopia is one of the many African countries burdened with the problem of obsolete pesticides, which have been accumulated since the first imports in the 1960s (Haylamicheal and Dalvie, 2009). These were mostly organochlorine compounds such as chlordane, DDT, Dieldrin that are banned or restricted in most countries. These may increases the chance ecosystems to be exposed by large amounts of pesticides. The use of different types of organochlorine pesticides for different purpose, may lead to stay for decades in different compartments of the environment such as soil, water, sediment, and biota and these results in negative environmental consequences and/or non-target living things including human beings (Spear et al., 2008). According to study conducted by Damalas & Eleftherohorinos, (2011) pesticides residues that enter streams through runoff, leaching and spray drift potential to contaminate surface water and groundwater pose adverse effects on humans, fish, birds, wild animals and plants in the

aquatic habitat. Effects in any non-target species may translate into ecosystem unbalance and food web disruption that ultimately may affect human health and edible species. Organochlorine pesticides are persistence, have lipophilic nature, hydrophobicity, long-range transport, and low biological and chemical degradation rate have led to accumulation in biological tissues and then magnification of concentrations in organisms increase in the food chain. These make high possibility to surface water contamination as it has been documented worldwide and the problem give rise concern at local, regional, national and global scales due to the adverse effects of pesticide on the environment (U.S. Geology Survey, 1999; Huber, 2002).

The study conducted by Mekonen et al., (2014) evaluated the presence of the banned organochlorines pesticides residue (DDT and its metabolites and endosulfan) in the staple food items in the Jimma Zone.

1.3 .Significance of the study

The output of this study will provide data about conventional or traditional wet coffee processing plants effluent, to assessing pesticide residues from wet coffee effluent receiving steam and biota and the results of the present study could be baseline to understand the level of pesticide residues contamination in the study sites, and help to drive management measures.

The analysis of pesticide residues in water and macro invertebrates has proved to be a significant approach in assessing the fate of contaminants in aquatic environments and contamination history of the area (Akan et al., 2014). While the analysis of water samples gives important information on the water quality and the detection of any potential risks, macro invertebrate's analysis enables the detection of pollutants that could not be quantified by the water analysis as explained by Jasem, (2011). These pesticides that are more hydrophobic tend to be detected more frequently in macro invertebrates thus, measuring pesticides in macro invertebrates is important for looking for their fate in the environment and evaluating for potential toxicity for aquatic life. The study may help to characterize the types and content of persistent organic pollutant, i.e. the organochlorine pesticides. In addition, the result obtained by this study may be used as baseline information on the water quality of the streams and establishment of its suitability for aquatic life and human consumption. Finally,

the study will provide update information for policy makers, environmental officers and watershed management experts, helps on designing mitigation measures and strategies to reduce environmental pollution. May also use as an indication for further study in other parts of Ethiopia where intensive wet coffee processing is taken place.

CHAPTER TWO

2. Literature reviews

2.1. General overview of pesticides and its application

Organo-chlorine pesticides (ocps) such as aldrin, endrin, dieldrin, chloridane, heptachlor, DDT, toxaphene, mirex and hexachlorobenzene (HCB)] constitute out of the chemical substances defined under the Stockholm Convention on persistent organic pollutants (pops). These compounds are characterized by high persistent, low polarity, low aqueous solubility and high lipid solubility *(lipophilicity*).They are ecotoxic, non-biodegrable and able to bioaccumulation and biomagnifying in living organisms (*Afful et al., 2010*). The toxicity of ocps has caused them to be banned in developed and many developing nations. Even though, some developing countries still use those (*Adeyemi et al., 2011).* In Nigeria, there have been reports of some levels of pesticide residues in water, sediments and fish (*Ogunfowokan et al., 2012; Idowu et al., 2013)*. The results have continually revealed contamination by OCPs in Nigeria Rivers. Several activities (such as waste from industrial chemical production, pesticides runoff from agricultural areas, sewage and refuse dump) have contributed to the levels of chlorinated hydrocarbon compounds in Nigerian rivers. Because of their influence, efficiency and low cost compared with alternative pesticides, OCPs are still being used by some cocoa farmers (*Idowu et al., 2013).* The use of OCPs such as DDT has been outlawed since 1990 in Nigeria. Meeting the minimum requirements of health standards is generally regarded as one of the elements of sustainable agricultural development. In Nigeria, Federal Environmental Protection Agency (FEPA, (1991) has established criteria, guidelines, specifications and standards for pesticides usage. FEPA standard for maximum allowable limits for water is 0.1 mg/L.

Ethiopia, the second populous nation in Africa with 85% of its population currently estimated to be at 96.6 million individuals living in rural areas, depends on the agricultural sector for necessities and as a source of employment. The agricultural sector currently contributes 47% of the Gross National Product *(Ethiopian economy, 2015).* Though Ethiopia has endorsed or (certified) many proclamations in order to minimize and control occupational and environmental risks in general and pesticides in particular (Pesticide registration and control

proclamation number 674/2010, Labor proclamation number 277/2003 and Environmental pollution control proclamation number 300/2002), previously conducted pesticide-related Knowledge, Attitude and Practice (KAP) studies in Ethiopia have indicated that farm workers had limited knowledge on pesticide hazards, inadequate awareness about safe pesticide management, and poor hygienic and sanitation practices (Mekonnen and Agonafer, 2002; Amera, and Abate, 2008; Karunamoorthi et al., 2011).

2.1.1. Definitions of pesticide

According to the Codex Alimentarius of the Food and Agriculture Organization of United nations (FAO, 2003):- is defined as "any substance intended for preventing, destroying, attracting, repelling or controlling any pest including unwanted species of plants or animals during the production, storage, transport, distribution and processing of food, agricultural commodities or animal feeds or which may be administered to animals for the control of ecto-parasites. The names are derived from the Latin or scientific name for the group. The ending or suffix -*cide* means kill or killer. But not all pesticides end with -*cide*. Examples include: growth regulators, which stimulate or retard the growth of pests; defoliants, which cause plants to drop their leaves; desiccants, which speed the drying of plants for mechanical harvest or cause insects to dry out and die; repellents which repel pests; attractants, which attract pests, usually to a trap; and chemosterilants, which sterilize pests. Pheromones, are *scents* produced by animals to communicate to other members of the same species, they are used as attractants to monitor or trap insects. Finally, the term biocide is often referred to as a pesticide that kills a wide range of organisms and is toxic *to both plants and animal.* A pesticide product is in fact a formulation of an active ingredient (pesticide) in combination with other inert ingredients such as carriers, solvents or propellants *(Helfrich et al., 2009*) Strong reduction of crop losses due to pathogens `(including bacteria and fungi), viruses, animal pests and weeds, mainly through pesticide application. Agricultural pesticide use has increased agricultural production worldwide and thereby contributed to food security (Zhang et al., 2015).

2.2 Classification of Pesticides

Worldwide pesticides are divided into different categories.

2.2.1 Based on target organism or function

Acaricide – controls mites

Algaecide –controls algae

Bactericide- controls plants pathogenic bacteria

Fungicide –controls fungi

Molluscicide- controls slugs or snails

Nematicide- controls nematodes

Virucides –controls viruses

Insecticide –controls insects

Rodenticide – controls rodent

Herbicide –controls weeds and other unwanted plants (Mahmood et al., 2016).

2.2.2 Class according to site interaction with the pest

Contact –go through the pest's integument action within the body

Stomach –enters through the oral way prior to poisoning the pest

Systematic-absorbed and transported to the part of the plant where pests feed (Zacharia, 2011).

2.2.3 Classification of Pesticides Based on Chemical Nature

The classification of pesticides based on the chemical natures are divided into many classes, of which the main are: Organochlorines, Organophosphorous and Carbamates pesticides

2.2.3.1 Organochlorine Pesticides (OCPs)

Organochlorine pesticides are chlorinated hydrocarbons, which are among the oldest and most toxic synthetic pesticides. First introduced in the 1940's, these chemicals were used extensively from the 1940's through the 1960's in agriculture and mosquito control until most of them were banned in the 1970's and 1980's due to their health risks(Solomon, 2016). OCPs show strong hydrophobic nature, which results in their accumulation in fatty tissues, and as consequences it becomes carcinogenic and endocrine disruptor in mammals (Ali et al., 2008).

Organochlorine are pesticides of chlorinated hydrocarbons that generally have long residues action and are very stable chemical compounds, which can resist the action of various environmental factors including the biological system. OC pesticides are characterized by low polarity, low aqueous solubility and high lipid solubility (lipophilicity) and as a result they have a potential for bioaccumulation in the food chain posing a great threat to human health and the environment globally (Stoytcheva, 2011; Kuma ,2013).

2.2.3.2 List of different types of OC pesticides and their uses

The most important pesticides, from the standpoint of environmental and health, are the persistent ones: DDT (and its metabolities DDE and DDD), Aldrin, Dieldrin, Dibutyl Chlorendate, Endosulfan sulfate, gamma-chlordane, Endrin, Methoxychlor, Heptachlor Epoxide.

2.2.3.2.1 Dichlorodiphenyltrichloroethane (DDT)

Dichlorodiphenyltrichloroethane (DDT) is the common name of 1, 1, 1-trichloro-2, 2-di-(4-chlorophenyl) ethane. Technical-grade DDT is a mixture of up to 14 compounds. The active ingredient is p,p'-DDT (65–80%). The other compounds include 15–21% of o,p'-DDT, up to 4% of p,p'-DDD and 1-(p-chlorophenyl)-2,2,2-trichloroethanol, and traces of o,o'-DDT and bis(p-chlorophenyl)sulfone. Othmar Zeidler, an Austrian scientist, first synthesized DDT in 1874. Paul Hermann Müller, a Swiss chemist who received the Nobel Prize in 1948 by the discovery of its insecticidal properties in 1939; it was widely used as an agricultural insecticide after the war. DDT easily degrades into dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), which are more persistent than the parent compound. The half-life of p,p-DDE in humans has been estimated as more than 7 years In the 1970s and 1980s, most countries banned the agricultural use of DDT. It was restricted in the United States in 1972 and finally banned in 1979. Agricultural use continues in some countries, and developing countries use about 4000–5000 tons annually for vector control applications.

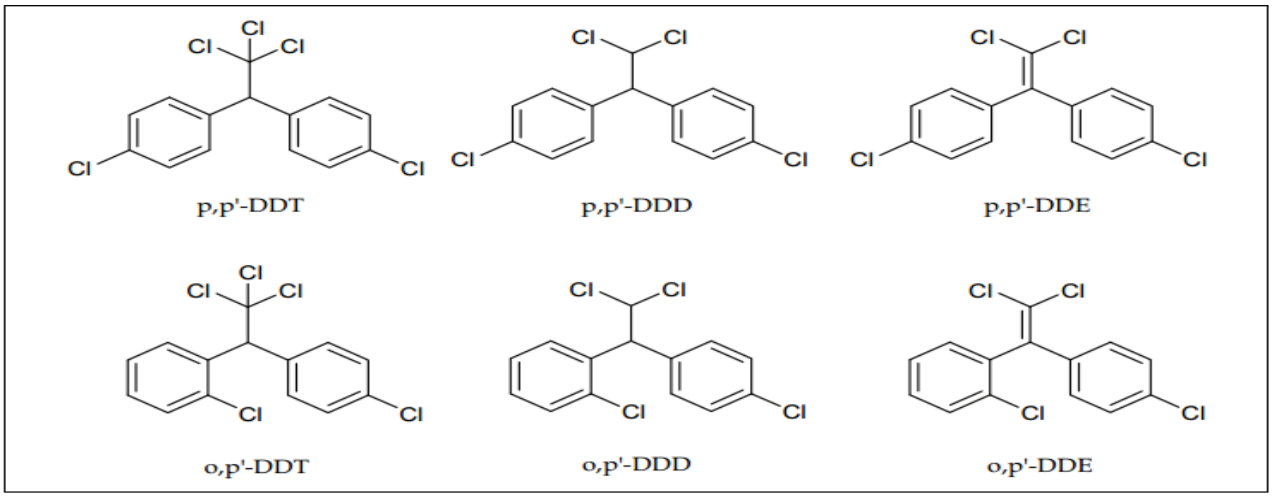

Figure 1: Structures of dichlorodiphenyltrichloroethane analogues

2.2.3.2.2 Gama- chlordane

Chlordane is a broad-spectrum contact insecticide that has been used on agricultural crops including vegetables, small grains, maize, other oilseeds, potatoes, sugarcane, sugar beets, fruits, nuts, cotton and jute. It has also been used extensively in the control of termites. Chlordane is insoluble in water, and is semi-volatile and can be expected to partition into the atmosphere as a result. It binds readily to aquatic sediments and bio-concentrates in the fat of organisms as a result of its high partition coefficient (Ritter et al., 1996). Exposure to high levels of chlordane can harm the human endocrine system, nervous system, digestive system, and liver (Hawai‘i Department of Health, 2011). EPA has also concluded that chlordane is a probable human carcinogen and may cause liver cancer.

2.2.3.2.3 Aldrin Dieldrin and Endrin

Aldrin, dieldrin, and endrin were used as insecticides from the 1950s to the mid-1970s. They have a similar structure (Fig. 3). Aldrin was first synthesized in 1948 and commercially manufactured in 1950. It is the common name of 1,2,3,4,10,10-hexachloro 1,4,4a,5,8,8ahexahydro-exo-1,4-endo-5,8-dimethanonapthalene, and technical-grade aldrin contains 90% of aldrin. Dieldrin, a pesticide product, is the oxygenated metabolite of aldrin. Aldrin easily degrades into dieldrin and is therefore rarely detected in the environment. Endrin was introduced in 1951 and primarily used as a cotton insecticide. It is a stereoisomer of dieldrin and is rapidly metabolized in the environment. Endrin aldehyde and endrin ketone

are its degradation products. In most countries, aldrin, dieldrin, and endrin are banned for agricultural use and severely restricted for nonagricultural applications. Agricultural use of these chemicals was banned in 1970 and all uses were banned in 1987 in the United States.

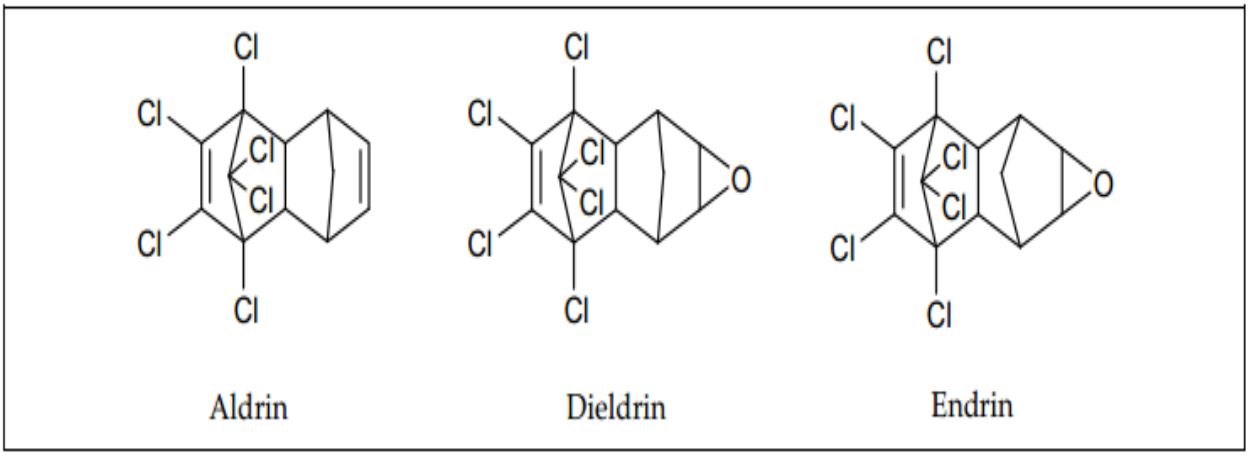

Figure 2: Structures of Aldrin, Dieldrin, and Endrin

(EPA, 2003)

2.2.3.2.4 Endosulfan Sulfate

Endosulfan sulfate is the metabolite of Endosulfan. It used in controls a wide range of sucking and chewing insects' pest like termites, mites, tsetse fly, beetles and aphids. Used in non-food crops such as coffee plantation, corns, vegetables, cotton, tobacco.

2.2.3.2.5 Methoxychlor

Methoxychlor registered for use on agricultural crops, in forestry, eradicating mosquito, on ornamentals. Methoxychl or an insecticide generally used for direct application to animals, humans, cockroaches and human clothing (Steffens et al., 2007).

2.2.3.2.6 Heptachlor epoxide

Heptachlor epoxide is the primary degradation product of heptachlor. Bacteria and animals break down heptachlor to form heptachlor epoxide; therefore, heptachlor epoxide is more to be expected to be found in the environment over time than is heptachlor. Heptachlor used in the past for killing insects in homes, in buildings, and on food crops.

2.2.3.2.7Chlordane

Chlordane was used as a contact insecticide for agricultural crops and lawns, and for termite control in buildings. It has been commercially produced since 1947. Technical-grade chlordane is a mixture of at least 23 compounds and typically consists of 15% cis-chlordane, 15% trans-chlordane, 9.7% trans-nonachlor, 3.9% heptachlor, 3.8% cis-nonachlor, other chlorinated hydrocarbons, and by-products (Fig. 3). Nonachlor is an impurityies of technical chlordane. Oxychlordane is an oxidized form of chlordane. In the United States, the use of chlordane on food crops was ceased in 1978 and all uses were banned after 1988.

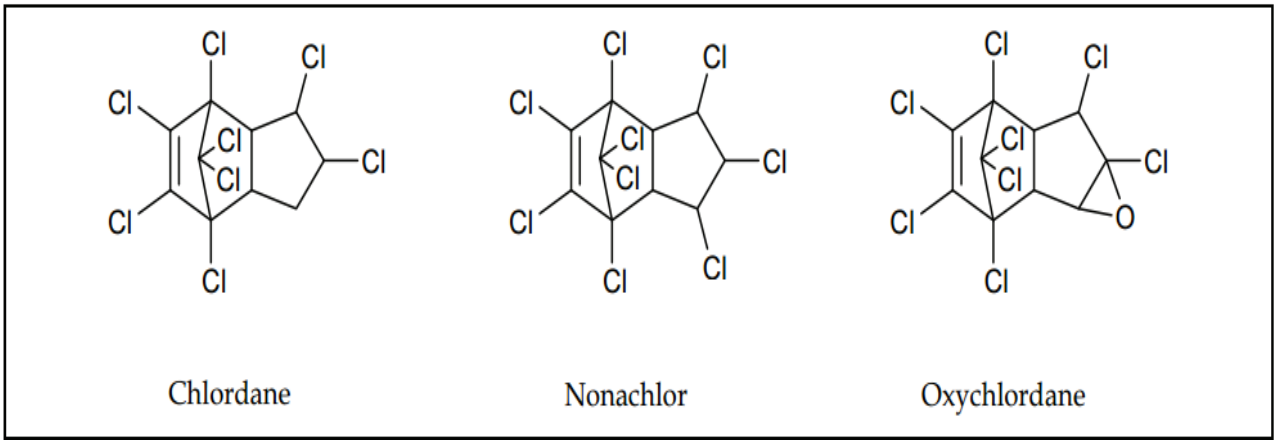

Figure 3: Structures of chlordane, nonachlor, and oxychlordane

2.3 Organochlorine Pesticides Residue in Water

Environmental contamination of natural water by pesticide residue is at present of great concern and because of the wide spread use, the residue were detected in both surface and ground water (Hamilton et al., 2003).

2.4 Organochlorine Pesticides Residue in Biota

Aquatic biota also important in the food web of terrestrial organisms with some aquatic biota, such as fish, being consumed by people and wildlife, the pesticides eroded from applied field deposited on the riverbanks or inside the water body. Leena et al., (2012) reported that the degradation of DDT in soil and sediment is 75-100% in 4-30 years. When bed sediments of fresh water bodies contaminated causes effect on aquatic life (Mobeen et al., 2012).

The sediment stands for the habitat of the benthic fauna, a source and mechanism for removal of some specific contaminants from and to the aquatic ecosystem and transporters of

contaminants in the ecosystems (Elizabeta et al., 2011). Those pesticides that are more hydrophobic tend to be detected more frequently in sediment; thus, measuring pesticides in sediment is important for tracking their fate in the environment and evaluating for potential toxicity (Wayne, 2012).

2.5 Ecological effects of organochlorine pesticides

Organochlorines pesticides are chemically stable lipophilic molecules which stay for a long time in the environmental compartments and are known as ecotoxic molecules (Coat et al., 2006). Runoff due to rainfall is one of the major sources of non-point pesticide contamination of surface water. Among many chemicals that enter to water body through non-point source, insecticides considered as pollutant for aquatic life due their relative toxicity. Insecticides play an important role in aquatic ecosystems as documented by the accumulated data on their detrimental effects to Community structure, reproduction, and developmental processes among several taxa including macro invertebrates, amphibians, birds, fish and other wildlife (Schulz, 2004). The principal pathway that causes ecological impacts is that of water contaminated by pesticide runoff. All organochlorines are relatively insoluble, persist in soils and aquatic sediments, can bio-concentrate in the tissues of invertebrates and vertebrates from their food, move up trophic chains, and affect top predators. The fact that a compound bio-accumulate at all is an indication that it resist biodegradation and is biologically stable and persistent (Castillo et al, 1997)

2.6 Organochlorine pesticides Effects on the Terrestrial Environment

Pesticides have had some of their most striking effects on birds, particularly those in the higher tropic levels of food chains, such as bald eagles, hawks, and owls. These birds are often rare, endangered, and susceptible to pesticide residues such as those occurring from the bio concentration of organochlorine insecticides through terrestrial food chains. Populations of insect eating birds such as partridges, grouse, and pheasants have decreased due to the loss of their insect food in agricultural fields with insecticides. Bees killed by pesticides, resulting in the considerably reduced yield of crops dependent on bee pollination. Populations of beneficial insects such as bees and beetles can significantly decline by the use of broad-spectrum insecticides such as carbamates, organophosphates and pyrethroids. Insect population has also been found to be greater on organic farms compared to non-organic ones.

Synergistic effects of pyrethroids and triazole or imidazole fungicides are harmful to honey bees (Mahmood et al, 2016*).*

2.7 Organochlorine pesticide effects on humans

Consumption of biota from contaminated aquatic body is considered an important route of exposure to persistent organochlorine pesticides. The most important aspect of pesticides is how they affect humans. There is increasing anxiety about the importance of small residues of pesticides, considered as disrupting endocrine activities or carcinogen, in drinking water and food. In spite of severe regulations by national and international regulatory agencies, reports of pesticide residues in human foods, from homemade and imported pesticides.

Exposure to organochlorine pesticides adversely affect male and female reproductive abnormalities, immune inhibition, disruption of hormone, cancer, damage of the liver behavioral/developmental outcomes are some human health effects have been reported by ATSDR, (2007) ; HEER, (2011) ; Rahbar et al., (2016) ; Jokha et al., (2014).

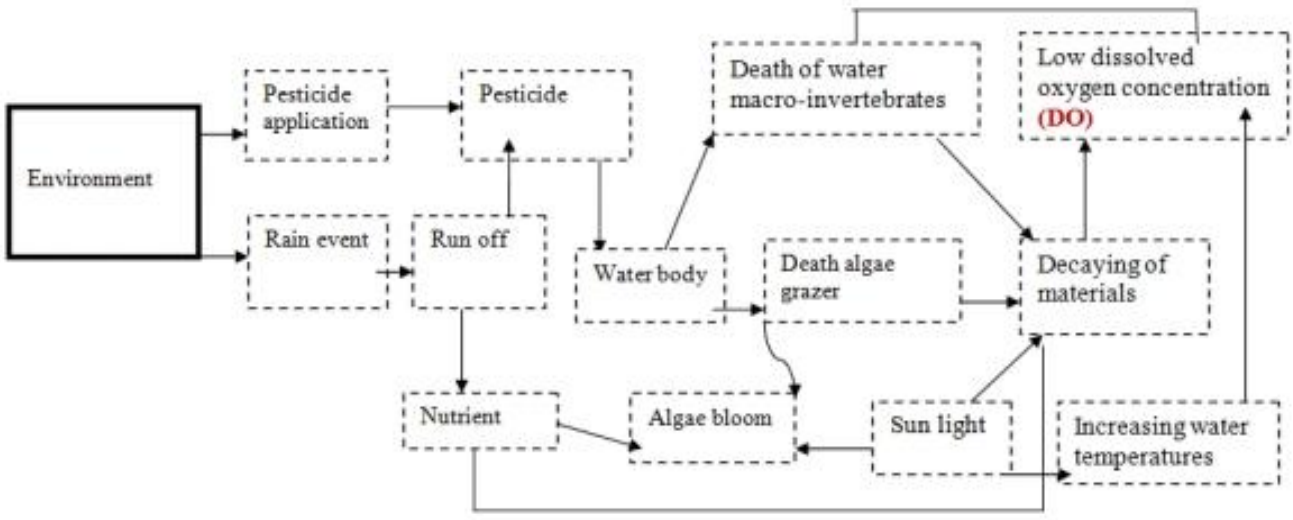

Figure 4: conceptual models of some environmental processes of pesticide application and its impact

CHAPTER THREE

3. Objectives

3.1 General objective

The main objective of the study is to assess pesticide residues in coffee processing effluent receiving stream and biota at Bore, Haro and Agaro (Masifine site) oromiya region, jimma zone.

3.2. Specific objective

- To identify pesticide residue in water receiving wet coffee processing effluent
- To determine physicochemical parameters of coffee processing effluent receiving stream water
- To identify and quantify the pesticides residue in macro invertebrates found in streams
- To compare concentration of pesticides that detected from water and macro invertebrates samples.

3.3. Hypothesis

In view of achieving the stated objectives, the study focused on the following research questions:

1 .What type of pesticides or agro-chemicals has been used at the study area?

2. What physicochemical properties does the effluent have?

CHAPTER FOUR

4. Materials and Methods

4.1. Study Area

The study was conducted in Haro, Gembe and Agaro districts and near to Jimma Zone.). Its Altitude was 1672m, 1558m and 1672m above sea level was situated 245680E and 863290N (Haro), 242178E and 867481N (Gembe) and 231841E and 867897N (Agaro) respectively. Distance of the study area from Addis Ababa around 397 kilo meters southwest of in Oromia National Regional State. As might be expected, water quality in Haro, Gembe and Agaro district rivers ranges from absolutely spotless to dangerously poor because at the area number of traditional wet coffee processing Plant. Water samples were taken from the rivers that receive wastewater from coffee processing at upstream and downstream of the discharge points (minimum of 100 m above and below the coffee processing plant) (Dejen .Y et al. 2015).

Table 1: Description of the Sampling Sites of study area

S/N	Sampling site Name	GPS data			Site characteristics
		Altitude	Easting	Northing	
1	Haro up 01	1627	245277	862722	Farming activities, animal drinking ,washing clothes and bathing
2	Haro up 02	1674	245438	863154	Farming activities (coffees and livestock) and domestic activities ,washing clothes and bathing
3	Haro Ef 03	1672	245680	863290	Wet coffee processing effluent discharged
4	Haro Ef 04	1663	245681	863312	

5	Haro D 05	1651	245530	863476	Farming activities (coffee plantation , sugar cane, banana and vegetables such as cabbage, tomatoes, green vegetables)
6	Haro D 06	1639	245545	863610	
7	Bore UP 07	1561	242332	866964	Human settlements, farming activities (maize, banana, and livestock), children swimming, bathing and washing clothe.
8	Bore Up 08	1560	242270	867182	
9	Bore Ef 09	1558	242178	867481	Coffee processing effluent ,Human settlements and farming activities
10	Bore Ef 10	1550	242212	867501	
11	Bore D 11	1549	242222	867753	Stone creation and road building
12	BoreD 12	1555	242208	867845	Grazing activates
13	Masifine MUp 13	1710	231761	867862	Human settlements, farming activities (maize, banana, livestock), children swimming, bathing
14	Masifine MUp 14	1717	231717	867690	Cattle grazing , farming activities and vegetation coverage
15	Masifine MEf 15	1840	231841	867897	Coffee husk deposition and discharged of coffee processing waste water
16	Masifine MEf 16	1672	231847	867922	
17	Masifine MD 17	1670	231779	867957	Coffee husk deposition
18	Masifine MD18	1658	231975	868001	Agricultures and water abstracting for agricultures or irrigation

4.2 Chemical and Reagents

All chemicals and reagents used were analytical grade and solvents were HPLC grade. The organic solvents; acetone, n-hexane and methanol were obtained from BDH Chemicals Ltd (Poole, England). Sodium chloride (NaCl), anhydrous sodium sulfate (Na2SO4) was BDH Chemicals Ltd. 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 (Maid stone, England) and was used for filtration of the water, and macro invertebrates samples.

Analytical standards of Aldrin, Dibutylchlor, Chlordane, γ-chlordane, Endrin, Endosulfan sulfate, Dieldrin, p, p'-DDT, Methoxychlor, and Sigma Aldrich (St. Louis, MO, USA) products were used).

4.3 Equipment and Apparatus

Agilent Gas Chromatography equipped with an electro capture detector (GC-ECD) auto sampler (model ,of 7980A Vacuum rotary evaporator, sieve 250μm, Orbital shaker(type sm30A), beakers, a multi-probe meter (HQ30d Single-Input Multi-Parameter Digital Meter, Hach) , ice box, kick net, aluminum foils, GPS, Centrifuge (model PLC-02), Oven, Furnace (Nabertherm GmbH, model L15/11/P320), Analytical Balance, crucibles auto sampler vials, conical and round bottom flasks, micropipette, syringes, desiccators, measuring cylinders and surgical gloves, were used.

4.4 Sampling

Sample collection and Extraction were performed using standard methods described by (Robinson and Mansingh, 1999; APHA, 1999; EPA, 2007; Jasem 2011). The sample identification code, date, stream name, and sampling location, were labeled properly and placed into the sample containers.

4.4.1 Water sample collection

Composite water samples were collected in one-liters sampling polyethylene bottles by using grab sampling technique. Before sampling, the sampling bottles were washed with detergents, rinsed with tap water and distilled water, and finally with acetone followed by dried in oven.

Samples were then transported in a cooler box with ice to the laboratory where they were stored in a cold room at 4°C until they were analyzed. A total of 18 composite water samples were collected from the three sites of the streams (Haro, Bore and Agera).

4.4.2 Macro invertebrates sample collection

Macro invertebrate sampling were done by active kick-net sampling, according to the standardized procedure described in Gabriels et al. (2010). The hand net have a mesh size of 500µm, a 0.15 m by 0.15 m metal frame and a long handle of 1.9 m. Approximately 10-20 m distance was sampling for 5 minutes, covering the different macro-invertebrate habitats were represented at 12 sampling site. The sediment and vegetation were kicked by walking backwards upstream the river holding the hand net in front of the disturbing zone. Macro invertebrates were sorted onsite, preserved in a vials containing 70 % ethanol for further identification process (Stark et al., 2001; Mereta et al., 2012; Ambelu et al., 2013).

Then all water and MI samples were transported immediately to the laboratory of the department of Environmental Health Science and Technology, Jimma University and stored in deep refrigerator until preparation for the further experiments (Jokha et al., 2014).

4.4.3 Preparation of standard solution of pesticides

The stock solution of 1000ppm of the individual pesticide standards were prepared by accurately weighing 10 mg of each analyte in volumetric flasks and dissolving in 10 mL methanol. These were stored in a refrigerator at $4^{o}_{C.}$ Then an intermediate and working standard solution were prepared by using dilution formula. An intermediate standard solution of 100ppm was prepared by mixing the appropriate quantities of the individual stock solutions followed by requisite volume makeup. A working standard solution of required concentration was prepared by diluting the intermediate standard solution, and finally 0.05, 0.1, 0.5, 2.5,10, 50 and 100 ppb standards solutions were prepared for calibration curve.

The following equation was used to prepare diluted standard solutions.

C 1 V 1 = C 2 V 2 ---------- eqa-1

Where; C1 = concentration of analyte in the stock or intermediate standard solution to be diluted

V1 = volume of stock or intermediate standard to be used

C2 = final concentration of the standard solution to be prepared

V2 = total volume of the standard solution to be prepared (Chem. soft, 2011)

4.5 Extraction

4.5.1 Extraction from water samples using Dispersive Liquid Liquid Micro Extraction (DLLME)

First, the water samples were filtered with Whitman filter paper in order to remove particulate matter from the samples. After homogenization and filtration of water samples, the DLLME extraction procedure was used. DLLME is one of the best sample preparation method because of it provides considerable advantages including easy operation, easy handling, cost-effectiveness, rapidity, limited consumption of organic solvents, and high enrichment capabilities (Zacharis et al., 2010).

A 5 mL of the water sample was placed in a 15 mL screw-cap glass test tube with conic bottom. A mixture of 100 μL of n-hexane (extraction solvent) and 500 μL acetone (dispersive solvent) was injected rapidly into the sample solution through a 5 mL syringe and a cloudy solution (water, disperser solvent, and extraction solvent) was formed in the test tube. The mixture was let stand for 2 min to stable cloudy state, and then centrifuged for 4 min at 4 000 rpm, causing the dispersed droplets of the extraction phase to settle to the top of the conical test tube. An emulsion formed in vial was centrifuged at 4000 rpm for 4 min to separate the mixture into two phases. Then, the upper layer containing the organic extract (∼50 μL) was removed using syringe and micropipette and transferred in to insert vial which was housed by 2mL of auto sampler as indicated by Guo and Lee, (2011) and Xu et al., (2017). Finally, 1.0 μL was injected into GC ECD system.

4.5.2 Extraction and clean up from macro invertebrates samples

In the laboratory the stored macro invertebrates were gently washed with distilled water, to remove ethanol traces, before being identified (Bizzotto et al., 2009). MI were identified to family level using a stereomicroscope (10× magnification) and the identification key (Gerber and Gabriel, 2002 and Bouchard, 2004). Again, after identification the samples were washed with distilled water followed by acetone. Macro invertebrates sample were dried at room

temperature for 3 days. The samples were ground with a mortar and pestle, mixing with anhydrous sodium sulfate to a free flowing powder. 1 g sample and 5 g anhydrous sodium sulfate were placed into a beaker, mixed thoroughly. Then, sample mixture were transferred to an extraction cellulose thimble and placed in a Soxhlet 40 extractor.

The mixture was extracted with 30ml of acetone: n-hexane (20:80 v/v) at 50°C for 4 h. The extracts were filtered, concentrated to 1 ml using vacuum rotary evaporator.

After extraction, samples were subjected to column chromatography using pre packed silica gel topped with anhydrous sodium sulfate, and extracts were eluted with 2 x 10 mL of n-hexane. After purification, all samples were further concentrated to dryness using a vacuum rotary evaporator at 45 ºC and reconstituted with n-hexane and stored in GC vials at 4 ºC waiting GC analysis (Bizzotto et al., 2009). Then 1.0 µL was injected into the GC.

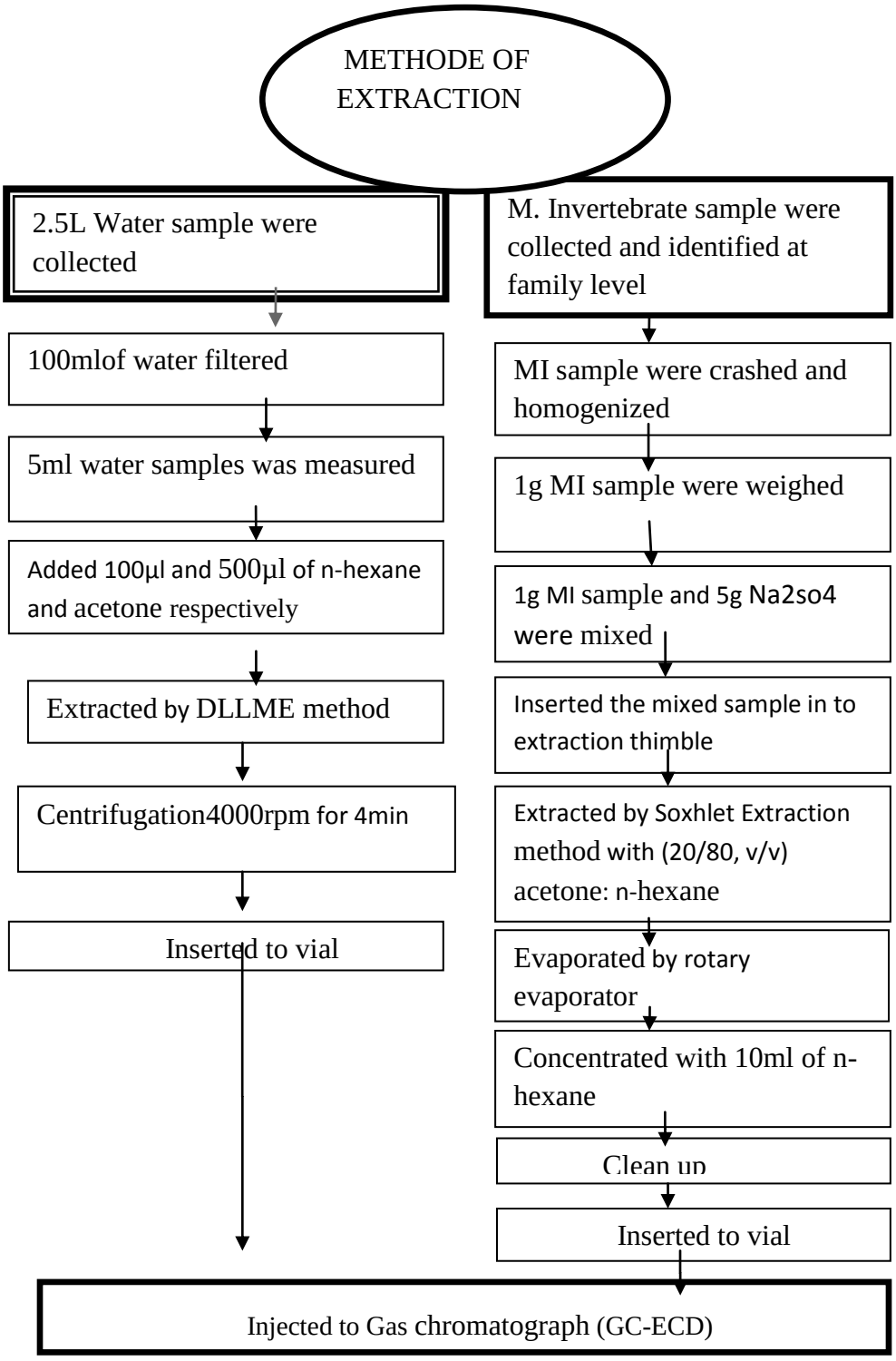

Figure 5: Flow chart for determination of pesticides from water and macro-invertebrate sample

4.6 Detection and quantification of the pesticides

4.6.1 Gas Chromatography Electro Capture Condition

Determinations of organochlorine pesticides from final extracts were carried out by Gas Chromatography with Electron Capture Detector (GC-EC, Agilent Technologies 7890A) equipped with auto sampler. The GC conditions used for the analysis were HP-5 capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness) coated with 5% phenyl methyl siloxane (model 19091J-433; Agilent) was used. The oven temperature was programmed: initial temperature 80 °C, ramped at 30°C to 180°C held for 2 min, ramp at 30°C/min to 205°C, held for 4 min, ramped at 20°C /min to 290°C ,held for 8 min, ramped at rate of 50°C /min and finally to 325°C. The carrier gas was Nitrogen (99.999%) purity at rate of 20 ml/min and detector make up gas at flow rate of 60 ml/min with ECD operated at a temperature of 300°C. An aliquot of 1 μL were injected under in split mode at split ratio of 10:1 and injection temperature of 280°C. For the detection of pesticides each sample was injected in triplicate in the GC and the concentration of each sample was calculated from the average of the three analyses (Fosu-Mensah et al., 2016).

4.6.2 Identification and Quantification of Pesticide Residues

Identification and quantification of pesticides were undertaken based on retention time and peak area of organochlorine pesticides, respectively. The pesticides residue level were determined by comparing and extrapolating on their corresponding calibration curves of the peak area of the analyte of unknown concentration with those of reference (external) standards of known concentrations run at the same analytical conditions with samples (Ali et al., 2008)

$$CAN = \frac{P_{AS} \times C_{Std}}{P_{Astd} \times M_{V}} \quad \text{----------Eqa 2}$$

Where;

Can = concentration of the analyte

Pas = peak of the sample

PAstd = peak of the standard

Cstd= concentration of standard

Mv= mass volume ratio

= mass of sample extracted/final volume of extract

4.7 Quality Assurance and Analytical Performance

4.7.1 Calibration curve

For calibrating individual OC pesticides, multipoint external standard calibration for single compound curves were produced by analyzing the 0.05-100 ppb calibration standard solutions (Mary and Duane, 2004)

A calibration for each organochlorine pesticide were drawn and used in quantification of the pesticides and data processing was done using MS Excel. As shown below figure 4,5 and Appendix.1 for the rest calibration curves for the standard organochlorine pesticides were obtained by plotting the GC-ECD peak areas, as instrumental response as a function of the analytes concentrations shows best line fit of coefficient of determination (r^2) of 0.9999 to 1 were achieved.

4.7.2 Recovery

To evaluate the performance of the analytical procedures and the accuracy, recover (% recovery), studies were undertaken for each pesticide as stated by Mekonen et al., (2015).

Recovery studies were determined by spiking the previously analyzed samples with the pesticides standard (Williams, 2013). For water samples, 0.5 ppb of mixed standard solution was spiked in deionised water of blank matrices then, spiked samples were then analysed using the same analytical procedures as field sample. A mixed standard solution with concentration of 50 ppb was added in macro-invertebrates samples respectively to inquire the spike recovery percent of the nine organochlorine pesticides. The test results showed that spike recovery amount for each analytes are between 81.7% and 109.1% and fulfill with routine analysis detection requirements.

$$\text{Recovery}(\%) = \frac{CS_2 - CS_1}{CS} \times 100$$ ----------Eqa- 3

Where, CS_1 = concentration of pesticide residues in the sample,

CS_2 = concentration of pesticide residues in the spiked sample,

CS = concentration of added pesticide standard.

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

LOD: the lowest concentration of analyte in a sample that can be detected but not necessarily Quantified as an exact value.

LOQ: minimum concentration of the analyte in a sample that can be quantified with acceptable accuracy and precision.

LOD and LOQ were determined as the lowest concentrations yielding a signal-to-noise (S/N) ratio of 3 and 10, respectively.

LOD = 3 S/N

LOQ = 10 S/N

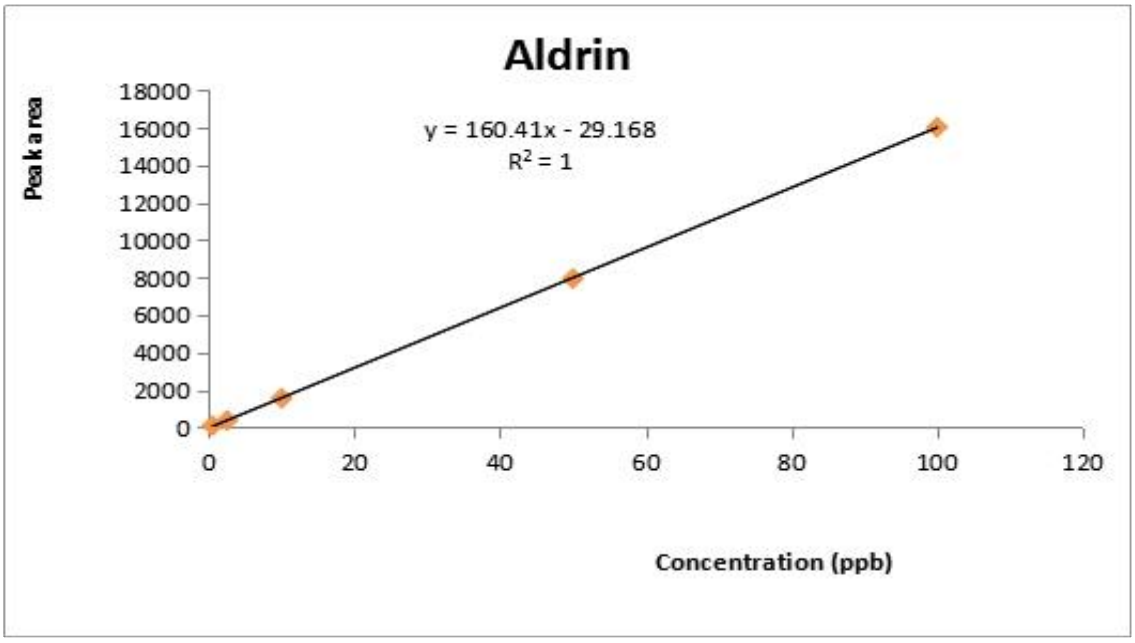

Figure 6: Calibration curve of Aldrin

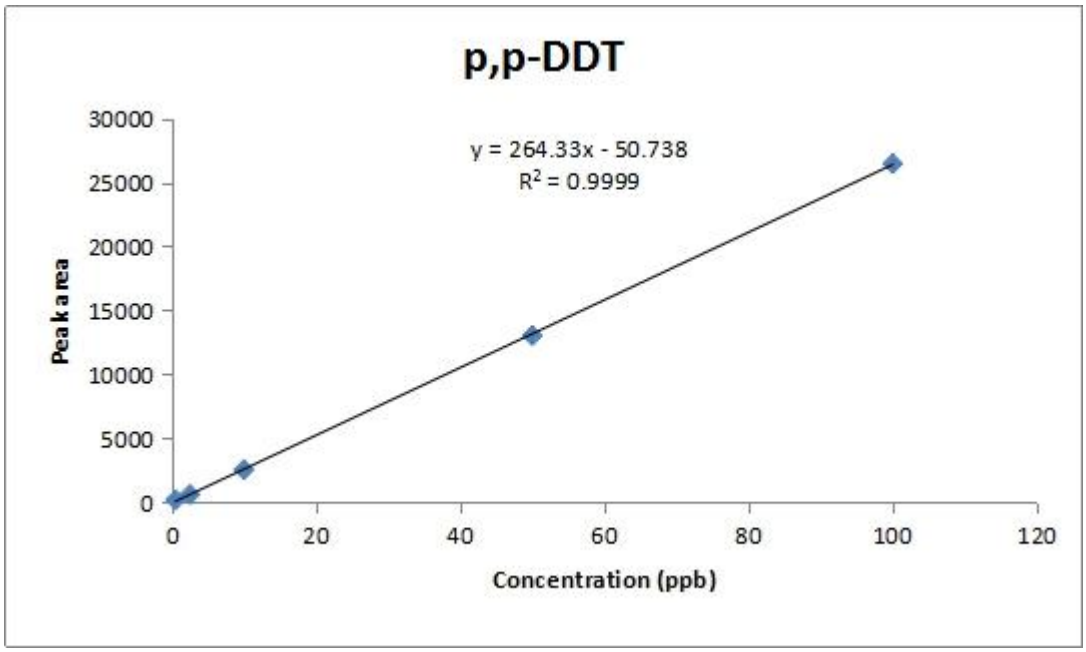

Figure 7: Calibration curve of p,p-DDT

Table 1: Shows the data of calibration curve of the standards

List of analyte	RT	Equitation	Correlation Coefficient (r^2)
Aldrin	9.157	Y=160.4x-29.16	1
Dibutylchlor	10.363	Y=128.9x+15.89	1
Epoxide			
γ-chloridane	11.196	Y=133.2x-14.17	1
P'P-,DDT	12.662	Y=264.3x-50.73	0.999
Endrin	13.633	Y=104.2x+4.633	1
Endosulphane	16.395	Y=45.37x+32.35	0.999
Dieldrin	16.634	Y=95.26x-29.03	0.999
Methoxychlor	18.305	Y=35.60x+16.26	0.999
Chloridate	18.911	Y=4.672x+10.50	0.999

4.8 Determination of Physico-chemical parameters of water samples

Acidity and alkalinity were determined by titration whereas total suspended solid (TSS) and total dissolved solid (TDS) determined by gravimetric methods. Electro conductivity, pH, water temperature and dissolved oxygen (DO) were determined in situ immediately after collecting the water sample. A cleaned plastic bucket was filled with water samples. The tip of the probe was first washed with distilled water and rinsed with the water sample before being dipped into it and readings taken directly from the meter. The electrodes were rinsed with distilled water, wiped dry with clean tissue paper next with water samples after each sample measurement. After the stabilization of the signal, readings were recorded. The test was duplicated for each sample and the mean were taken. Their detail procedures and calculation formulae attached in Appendix.5.

4.9 Statistical data analysis

Descriptive Statistical analysis such as frequency, Mean, percentage and standard deviation were used to analyse by using Microsoft Excel 2007 software and Statistical Package for Social Sciences (SPSS) software version 20.

CHAPTER FIVE

5 .Results and discussion

5.1 Physico-chemical parameters of a water samples

5.1.2 DO, EC, PH and water temperatures

Water temperature plays significant role in influencing the quality and ecology of streams and rivers. Physico-chemical characteristics of water and the metabolic activities of organisms depend on water temperature (Sirajudeen et al., 2013). The degradation of pesticides residue is dependent of temperature, the molecules in the solution have more energy causing them to move and react faster when the temperature increases. This causes hydrolysis reactions to occur at fast rate (Clark D. Jinde, 1994). At the study area of haro, bore and masifine site of downstream and at discharged point respectively with 19-27.8^{o}c, 21.4-25.7 ^{o}c and 18-20.7 ^{o}c and the mean value was respectively 22.8^{o}c, 23.5^{o}c, and 19.1^{o}c.

The amount of dissolved oxygen at Haro, Bore and Masifine wet coffee processing site 4.3 to 7.73 (6.3) mg/L, 0.3 to 5.3(2.31) mg/L and 0.9 to 7.3 (3.9) respectively. The average recorded value of dissolved oxygen was 6.3±2.31 mg/L. This result does not fit requirement for surface water quality standards for the protection of the aquatic ecosystem, which is recommended by the basic environmental water quality standards guidelines > 6 mg/L for a small river in Flanders (De Troyer et al., 2016). The depletion of dissolved oxygen resulted because of breaking down of organic matter by microorganisms in the water that brought by surface runoff can adversely affect aquatic life. DO below 5.0 mg/L adversely affect aquatic life (Gebreyohannes et al., 2015).

The high conductivity results in increase of concentration of salts, organic and inorganic materials. The permissible limit for electrical conductivity (EC) is 300 µS cm-1 EC of the collected samples from Haro, Bore and Masifine site ranged 286 to 74.6, 111 to 2110 and 71 to 924 µS cm-1 and the Mean values of each site 142.15, 846.67 and 232.9 µS cm-1 respectively. This showed that the EC values of water samples at Bore site were above permissible limits and the potable water was not safe in terms of EC.

Hydrogen ion concentration (pH) indicates acidic and basic status of water. It is very important variables in water quality assessment and chemical and biological process may

affect due to pH. The amount of dissolved CO2 which form carbonic acid water determines the pH. Organic acids from decaying vegetation can lower the pH of water (Yosef and Alemayehu., 2016). The range of 6.5 to 8.5 recommended by WHO, However pH at the study site of haro, bore and masifine wet coffee processing site were 6.53 to 7.41(7.1), 5.12 to 7.13(6.23) and 5.2 to 7.23(6.62) mg/l respectively minimum, maximum and mean value.

5.1.3 Alkalinity, TSS, TDS

The presence of bicarbonate, carbonate and hydroxide in the water indicates that alkalinity. The acceptable limit of alkalinity is 200 mg/L and in the absence of alternate water source, alkalinity up to 600 mg/L is acceptable for drinking. Alkalinity in Haro, Bore and Masifine site of study area ranged 26 to 93.3mg/L, 33.3 to 113.3mg/L and 26.7 to 166.7mg/L and also the mean value of the site 46.67, 68.85 and 70.0 respectively. It was below the acceptable limit. Total dissolved solids of water sample the minimum, maximum and mean values of TDS at Haro, Bore and Masifine wet coffee processing sites were 3-16 (7.1), 1.4-168.8 (37.9) and 6-44.6 (20.5) mg/l respectively and also TSS values at Haro, Bore and Masifine wet coffee processing site were 0.06-0.9 (0.26), 0.04-1(0.42) and 0.004-0.74(0.3) mg/l and mean value 0.26, 0.42 and 0.3 respectively.

Table 2: A recovery of pesticides in water and macro invertebrate's samples, LOD, and LOQ in ppb

		Recovery %			
Analytes	RT	Water	Macro Invertebrates	LOQ	LOD
Aldrin	9.157	99.4	81.7	0.10	0.34
Dibutylchlor Epoxide	10.363	108.24	80.6	0.16	0.54
γ-chloridane	11.196	109.1	86.3	0.14	0.48
P,p,DDT	12.662	99.3	82.5	1.3	4.3
Endrin	13.633	105.03	96.4	0.15	0.50
Endosulphane	16.395	82.6	104.9	0.15	0.50
Dieldrin	16.634	97.8	96.1	0.06	0.30
Methoxychlor	18.305	84.1	103.8	0.15	0.50
Chloridate	18.911	84.3	89.7	0.15	0.50

5. 2 Concentration of organochlorine pesticides residue in water samples

Nine different types of pesticide residues were detected in the water samples with varying concentrations from the 18sampling sites of Bore, Haro and Masifine site streams

The results of organochlorine pesticides in the steam water samples collected from Bore, Haro and Masifine wet coffee processing area are reported below in (**Table 3**). Almost all pesticides analyzed, Aldrin, gama-chlordane, dibutylchlor, Dieldrin, p, p'- DDT, Methoxychlor chloradane and endosulfan sulfate except Endrin were detected in the water samples. concentration of pesticides in water has slightly higher than macroinvertebrates sample recorded in this study may due to the effluent receiving streams has slow velocity and stagnant nature of water in some site of the study area .

Aldrin were detected with mean concentration of 0.13±0.21mg/l and ranged from 0.01 to 0.68mg/l, were detected **(Table 3)**. The mean level of concentration of Dibutylchlor were

ranged from <LOD to 7.01mg/l (mean 0.15mg/l), it was detected in this study was above the level of concentration reported by Abdullah et al., (2015) and Abdulrazaq et al.,(2017) which were ranged from <LOD to 0.018 µg/L (mean 0.011 µg/L) and <LOD to 0.201 µg/L respectively.

The concentrations level of Dieldrin in water samples were detected in ranged <lLOD to 1.56mg/l, with a mean value concentration of 0.15±0.01mg/L which is above the WHO guideline value of for drinking water. The existence of Dieldrin may imply a possible degradation of Aldrin to Dieldrin in the environment from pervious application in agricultural activities and in home to control termites.

The mean concentrations level of p, p'- DDT were observed in water samples with minimum of <LOD and a maximum of 0.33mg/l which is higher than the concentration ranged from 0.190 to 4.714 µg/L in the study conducted by Azab et al., (2013).The above concentrations of the p, p'- DDT was above the acceptable limits in water 2 µg/L as per WHO guidelines but this study results were listed in mg/l >µg/L. The level of Methoxychlor was detected with the range <LOD to 0.27mg/l and mean value was 0.1mg/l. This values were higher than the level concentration investigated by Imo et al., (2007) and Abdulrazaq et al., (2017) were ranged from <LOD to 0.056 and <LOD to 0.252 µg/L respectively. However, the detected values of Methoxychlor were below the WHO guideline value of 20µg/L for drinking water.

Chlordane concentration ranged from <LOD to 0.42 and the mean concentration of 0.15mg/L were detected at coffee effluent receiving stream. The lowest concentration of Chlordane were detected in the study conducted by Abdulrazaq et al., (2017) that was ranged from <LOD to 0.086 µg/L when compared to this study. The value is above the WHO minimum residual limit (MRL) of 0.03µg/L for drinking water. The highest occurrences from the nine organochlorine pesticides across the 18 sites were observed for γ-chloridane and Dibutylchlor epoxide has the highest mean concentration levels of 0.58 and 0.17mg/L. The detection of organochlorine pesticides residue in water samples indicates the recent use both indoor and outdoor activities in pest control in the study area.

Table 3: The minimum, maximum, mean and standard deviation of every physico-chemical parameters of water samples (n=18) and WHO the standard guidelines for water quality

Parameters	Unit	HARO				BORE				MASIFINE			
		Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
Temp	O_c	19	27.8	22.8	3.82	21.4	25.7	23.5	1.87	18	20.7	19	0.95
PH	-	6.53	7.41	7.1	0.49	5.12	7.13	6.23	0.85	5.2	7.23	6.63	0.74
EC	µs/cm	74.6	286	142.1	102	111	2110	846	939	71	924	232	339
DO	mg/L	4.3	7.43	6.3	1.6	0.3	5.3	2.31	2.17	0.9	7.3	3.9	2.64
TSS	mg/L	0.06	0.96	0.26	0.35	0.04	1	0.42	0.38	0.004	0.74	0.3	0.26
TDS	mg/L	3	16	7.1	4.83	1.4	168.8	37.9	65	6	44.6	20.5	14.23
Alkalinity	mg/L	26.7	93.3	46.67	29.2	33.3	113.3	68.85	25.9	26.7	166.7	70	53.95
Acidity	mg/L	26.7	40	32.23	6.54	6.67	40	24.44	14.4	26.7	80	40.12	20.22
Depth	M	0.02	0.35	0.22	0.13	0.15	0.4	0.28	0.1	0.1	0.35	0.22	0.1
Width	M	0.2	3	1.19	1.02	0.5	1.5	1.08	0.38	0.35	2	1.25	0.72
Velocity	m/s	0.15	0.45	0.31	0.12	0.2	0.45	0.31	0.09	0.1	0.56	0.32	0.17

Table 4: Types and mean levels of organochlorine pesticide residues detected (mg/L± SD) in Water Samples (N=18)

Site Name	Aldrin	Dibutylchlor Epoxide	Γ-Chloradane	DDT	Endrin	Endosulphane	Dieldrin	Methoxychlor	Chlordane
HU01	0.03	0.04	<LOD	0.04	<LOD	N	0.04	<LOD	0.06
HU02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.15
HE 03	0.59	7.01	0.70	0.07	<LOD	<LOD	1.56	0.10	<LOD
HE04	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HD05	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.02	0.01	<LOD
HD06	<LOD	0.23	0.25	0.02	<LOD	0.14	<LOD	<LOD	0.27
BE07	0.21	0.19	0.14	0.15	<LOD	0.07	0.15	<LOD	0.05
BU08	0.07	0.08	0.04	0.04	<LOD	0.35	0.30	<LOD	<LOD
BE09	0.68	0.65	0.72	0.06	<LOD	0.10	<LOD	0.11	<LOD
BU10	0.04	0.06	0.04	0.02	<LOD	0.14	0.13	0.06	0.01
BD11	0.39	0.36	0.41	0.33	<LOD	<LOD	0.06	<LOD	<LOD
BD12	0.30	0.45	0.44	0.33	<LOD	<LOD	0.04	<LOD	<LOD
ME13	0.04	0.06	0.03	0.02	<LOD	0.11	0.09	<LOD	<LOD
ME14	0.05	0.07	0.05	0.02	<LOD	0.20	0.05	0.12	0.42
MU15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.06	<LOD
MU16	<LOD	0.12	<LOD	0.08	<LOD	<LOD	0.07	0.11	0.16
MD17	0.03	0.02	0.03	0.02	<LOD	0.01	<LOD	0.27	<LOD
MD18	0.01	0.01	0.02	0.01	<LOD	0.05	0.01	0.03	0.11
Mean	***0.13***	***0.58***	***0.17***	***0.07***	***0.00***	***0.11***	***0.15***	***0.10***	***0.15***
STD	***0.21***	***1.72***	***0.25***	***0.10***	***0.00***	***0.10***	***0.37***	***0.07***	***0.13***

B= Bore site, E= effluent site, H= Haro site, U =up site, M =Masifine site, D =down site
<LOD = Limit of Detection

Table 5: Comparison of levels of organochlorine pesticides in water with guideline value of World Health Organization (WHO) and Australia (mg/L)

Analyte	This study (mg/L)	Ethiopia (mg/L)	WHO (mg/L)	Australia (mg/L)
Aldrin	0.13	0.03	0.03	0.01
G-chlordane	0.17	n.a	0.02	0.01
Dibutylchlor Epoxide	0.58	n.a	2	0.06
Endrin	n.a	n.a	n.a	n.a
Dieldrin	0.15	0.03	0.03	0.01
P,p-DDT	0.07	2	2	0.06
Methoxyclor	0.1	20	20	0.2
Chloradane	0.15	0.03	0.03	0.05
Endosuphane	0.11	n.a	n.a	n.a

n.a = Not available

Source: (Kuranchie-mensah et al., 2012; ESA, 2013

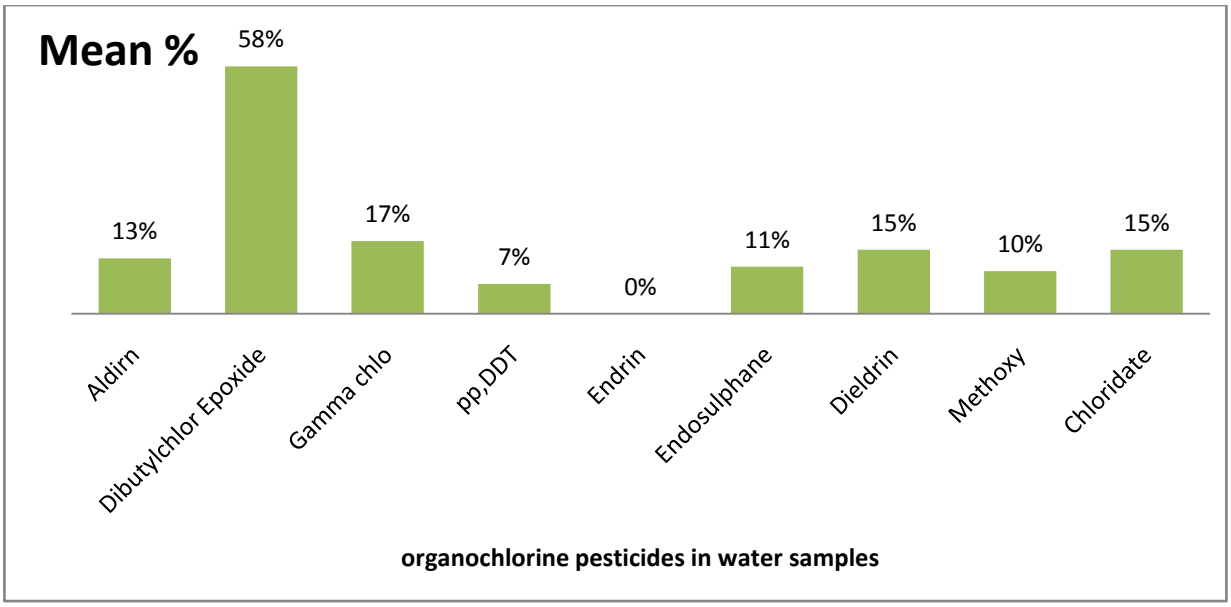

Figure 8: Frequency of detection for each analyte in water sample

The above graph (figures .8), showed that the mean concentration level of pesticides at each site (Haro,Bore And Agaro (Masifine Site) comparing the results from graph Dibutylchlor Epoxide, g-chlordane, Dieldrin and chloridate have 58%,17% ,15% and 15% respectively.

P, P-DDT, Methoxychlor, Endosulphane, Aldrin were 7%, 10%, 11% and 15% respectively. The result indirect those in the study area were highly detected all type of pesticides comparing with Ethiopia, WHO and Australia guideline value except Endrin. Therefore, in the study area concentration of pesticides in water sample ranged <LOD to7.01mg/l and mean value 0.58 ±0.07mg/l Methoxychlor and Methoxychlor were detected.

5.3 Organochlorine pesticide residues in macro invertebrates samples

As shown in (Table-6) below from 9 organochlorine pesticides residue were detected in macro invertebrates samples that was collected from 12 sampling sites by varying concentration. The concentration of Aldrin was ranged from 0.01 to 0.61mg/L with mean concentration of 0.37±0.29mg/L or 37%. The detection of γ-chlordanewas ranged from minimum <LOD and maximum 1.0 mg/g with mean concentration of 89.1%. The concentration of Endrin was <LOD to 0.58mg/L and mean percentage value 0.34mg/L or 34% was detected. This value less when compared to other concentration of organochlorine pesticides which was detected in macro invertebrates of this study may be due to less lipophilic natures. Dibutylchlor Epoxide and p, p'-DDT detected in lowest frequency with 5% and 29% respectively, followed by Endosulfan sulfate detected with low frequency of 0.94%. Aldrin and g-chordate were detected with the highest frequency of 37% to 89.1% and Dieldrin was distributed equally at all sites 37%. Methoxychlor and chloridate were lower level of concentration but from all above pesticide, chloridate was nearly zero at all site of the study area in macro invertebrate samples.

The persistence, hydrophobic and lipophilic natures provides the necessary conditions for organochlorine pesticides to bioaccumulation and biomagnifications in all living organisms. These chemicals have high tendency toward lipid, and they will concentrate in tissues with high lipid content.

Table 6 : Types and levels of organochlorine pesticide residues detected (mg/L) in macro invertebrates samples (N=12)

Site name	Aldirn	Dibutylchlor	Gchloridane	pp,DDT	Endrin	Endosulphane	Dieldrin	Methoxy	Chloridate
BD01	0.61	<LOD	1	0.0078	<LOD	<LOD	0.37	<LOD	<LOD
BD02	0.01	0.009	1	0.0056	0.011	<LOD	0.37	0.027	0.0003
BU03	0.61	0.0028	1	0.0051	<LOD	<LOD	0.37	0.0048	<LOD
BU04	0.61	<LOD	1	0.011	<LOD	<LOD	0.37	0.103	<LOD
HD05	0.011	0.011	<LOD	0.013	0.013	<LOD	0.37	0.0026	0.00017
HD06	0.023	0.016	<LOD	0.011	<LOD	<LOD	0.37	<LOD	<LOD
HU07	0.15	0.305	<LOD	0.58	<LOD	<LOD	0.37	<LOD	<LOD
HU08	0.61	0.01	<LOD	0.58	0.013	<LOD	0.37	0.0022	<LOD
MD09	0.61	<LOD	0.126	0.58	0.58	<LOD	0.37	<LOD	0
MU10	0.029	0.024	1	0.58	0.58	0.009	0.37	<LOD	0.0008
MU11	0.61	<LOD	1	0.58	0.58	<LOD	0.37	<LOD	0
MD12	0.61	0.00	1	0.58	0.58	<LOD	0.37	<LOD	0
MEAN	0.37	0.05	0.89	0.29	0.34	0.01	0.37	0.03	0.0002
STD	0.29	0.10	0.31	0.30	0.30	0	0.00	0.04	0.00

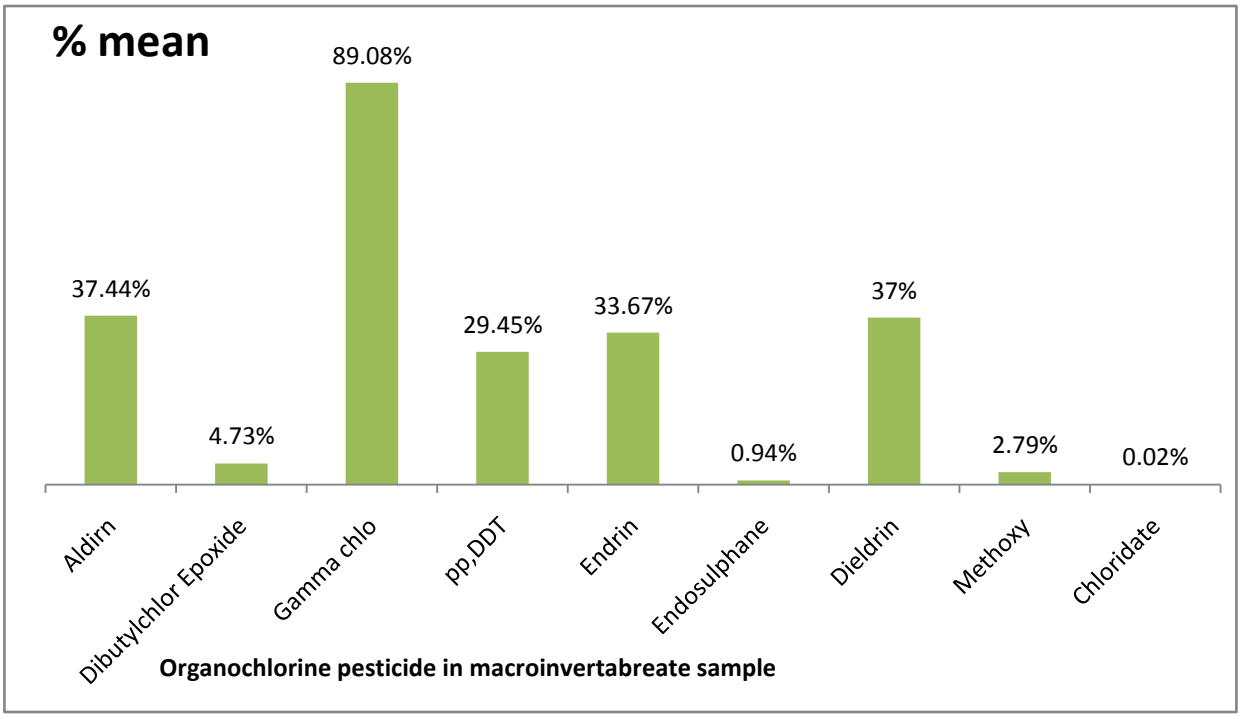

Figure 9: Frequency of detection for each analyte in macro invertebrate's sample

5. 4 Correlations of levels of pesticides in water and Macro invertebrates

To examine the association, the direction and strength of the Physico-chemical properties and pesticide residues concentrations measured in the samples, Pearson's product moment correlation coefficient used.

Pearson's correlation of Organochlorine pesticide residues with physicochemical parameters was done using Pearson correlation from SPSS tool. Pearson's correlation co-efficient (r) is a measure of linear dependence between two variables X and Y giving a value of +1, 0and -1. Where1 indicates a strong positive correlation (association), 0 it means that there is no linear association or any correlation and -1 is strong negative correlation (association). Positive value indicates that the changes are in the same direction while negative values indicate inverse variation relationships. Correlation coefficients above 0.5 are considered strong whereas below 0.5 are considered weak. The significant of correlations were indicated by the P value. Correlations are significant if $p<0.05$ and are not significant if $p>0.05$(John Wiley & Sons, 2003). There were moderate positive correlations (Pearson correlation coefficient

significant at P ≤ 0.01) a correlation pH with (p, p'- DDT). Similarly, a strong positive correlation also existed between electro conductivity and Endrin at ($p<0.05$). A high positive ($p < 0.01$) correlation also existed between total TSS, TDS and EC are strongly correlated with most organochlorine pesticide. The organic pollutants like p, p'- DDT, Aldrin and others are non degradable natures and the strong positive correlations verify that TSS and total suspended solids of water could have enhanced the adsorption of these pesticide compounds. (John Wiley & Sons, 2003)

Table 7: Mean concentration OCP in water and Macro invertebrates (n=18)

Analytes	Water (mg/L)			Macro invertebrates(mg/L)		
	Range	Mean	SD	Range	Mean	SD
Aldrin	0.01-0.68	0.13	0.21	0.01-0.61	0.37	0.30
g-chlordane	<LOD- 0.72	0.17	0.25	<LOD- 1	0.89	0.31
Dibutylchlor-Epoxide	<LOD -7.01	0.58	1.72	<LOD- 0.305	0.05	0.0
Endrin	<LOD	0	0	<LOD -0.58	0.34	0.3
Endosulfan	<LOD -0.35	0.1	0.1	<LOD -0.01	0.01	0
Dieldrin	<LOD -1.56	0.15	0.37	<LOD -0.37	0.37	0
pp-DDT	<LOD -0.33	0.07	0.1	0.01-0.58	0.29	0.3
Methoxychlor	<LOD -0.27	0.1	0.07	<LOD -0.103	0.03	0.04
Chloradate	<LOD -0.42	0.15	0.13	<LOD -0.0008	0.0002	0

<LOD = Limit of detection

The mean concentrations of organochlorine pesticide residues in water and macro invertebrates' samples are represented as shown above in **(Table 7).** The mean concentrations of organochlorine pesticides residue detected are higher in water than macro invertebrates.

The concentration of organochlorine pesticide residues ranged <LOD to 7.01mg/L in water sample and <LOD to 0.61mg/L in macro invertebrate samples Dibutylchlor Epoxide and Aldrin respectively. The mean concentration level 0.58mg/L Dibutylchlor Epoxide in water and 0.89mg/L g-chlordane in macro invertebrates were highly detected comparing with others compounds .Endrin were not detected at all in the study area in water sample but minimum amount of p,p,DDT were detected in water .In macro invertebrates g-chloradate and Dieldrin were highly detected comparing with other 0.89mg/L and 0.37mg/L respectively and Endosulphane was not detected at all in macro invertebrates.

CHAPTER SIX

6 .Conclusions and Recommendation

6.1 Conclusions

6.1.2 Pesticide residue in stream water and macro invertebrates and physico-chemical parameters of water were determined.

The results of this study showed that water sources from the study area were contaminated with different types of organochlorine pesticides at various concentrations. Nine organochlorine pesticides residues consisting of Aldrin, g-chlordane, Dibutylchlor Epoxide Endrin, Endosulfan sulfate, Dieldrin, p, p'-DDT, Methoxychlor and Chlordane was detected different concentrations in water and macro invertebrates samples. The detection of organochlorine pesticides residue in the water and macro invertebrate's samples may be due to application of pesticides in different types of activities, misuse of persistent organic pollutant by farmers and outdated pesticides in the area. In addition, it indicates that some rural agricultural farms especially coffee and khat lands located in the study area are applied substantial dosage on the crop subsequently initiate the movement of pesticides into the streams. The other reason may be Ethiopia is one of the many African countries loaded by the problem of obsolete pesticide stocks. These introduced into the environment section through the atmospheric transport of volatilized pesticides, direct spillage, leaching, erosion and run-off from application field and surrounding areas for the period of and after pesticide applications.

The study highlights all most all of the organochlorine pesticides residues detected in water were more concentrated in coffee effluent waste receiving site of the stream and down site of the stream. The findings from the study site compared with WHO and Australia maximum residue limit (MRLs) were above the water quality guideline for drinking water. Therefore the results suggests that concentrations of pesticides in streams from which samples were collected for this study pose health problems to a society, animals and aquatic life who utilize water and fish from these sources.

Similarly, in the biota (macro invertebrates) the persistence, hydrophobic and lipophilic natures provide the necessary conditions for organochlorine pesticides to bioaccumulation. These chemicals have high resemblance toward lipid, and they will concentrate in tissues with high lipid content instead of in an aqueous environment that why significant amount was detected.

Even though the use of pesticides has supported to increase agricultural yield and generated broad health benefits through the management of pests and disease, there are serious risks to human health and environmental sustainability.

6.2 Recommendations

- A significant level of organochlorine pesticides residue detected in water and macro invertebrates, the stakeholders need to confirm and implement regulations on the use of banned pesticides as these pesticides were found to be used in the study area.
- Additional work for evaluating the temporal variation of organochlorine pesticide residues in water and biota from the study should be needed because of the residue of pesticides was detected in substantial amounts during the study period.
- Advise growers to use biodegradable pesticides and encouraging to use integrated pest management to control pest that are considered as environmental friendly activities
- Buffering riparian land by planting grass and policies to control clearing forests are some of good land management approaches, which can strength the catchments area of river and that of the streams in order to reduce the nutrient and pesticides loading to the water bodies.
- Macro invertebrates a suitable indicator for biological monitoring of pollution in streams and rivers contamination with organochlorine pesticides and other nutrients, so continuous monitoring measures need to be put into place for sustainably environmental protection and human health.

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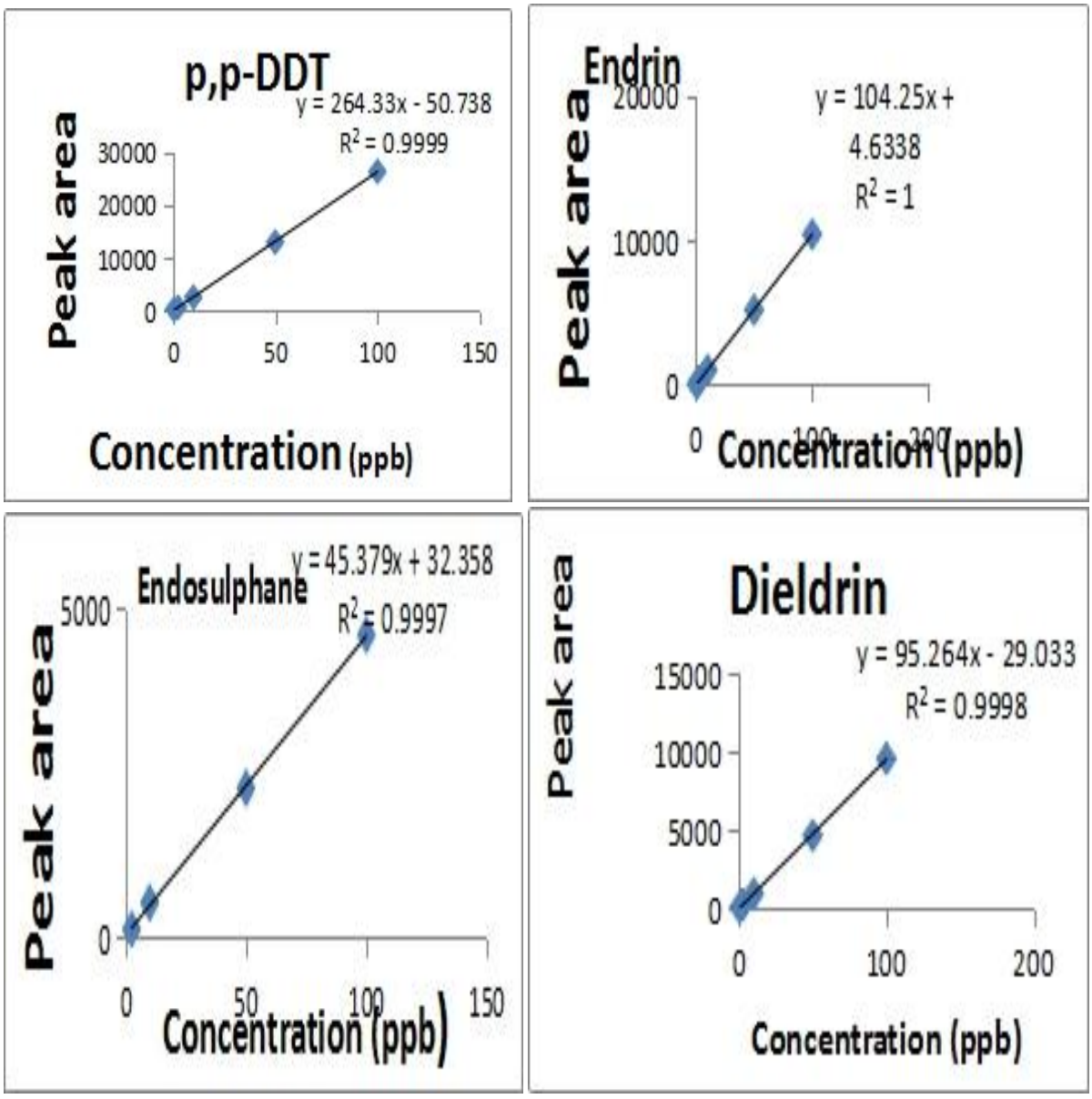
Appendixes

Appendix 1: Calibration curve of Standard



Appendix 2: Definition of terms

Analyte: a pesticide or its metabolite in which concentration to be determined.

Matrix blank: Material (a sample, or a portion or extract of a sample) known not to contain detectable Levels of the analyte(s) sought.

Calibration: determination of the relationship between the observed signal (response produced by the detection system) from the target analyte in the sample extract and known quantities of the analyte prepared as standard solutions.

Calibration standard: a solution of a standard prepared from working standards and used for calibration of the determination system. .

Residue: the pesticides that remains in the environment after an application

Spike/spiking: addition of analyte for the purposes of recovery determination

Standard: general term, this may refer to a "pure" standard, stock standard, working standard, or calibration standard.

Stock standard: the most concentrated solution (or solid dilution, etc.) of the "pure" standard or internal standard, from which aliquots are used to prepare working standards or calibration standards

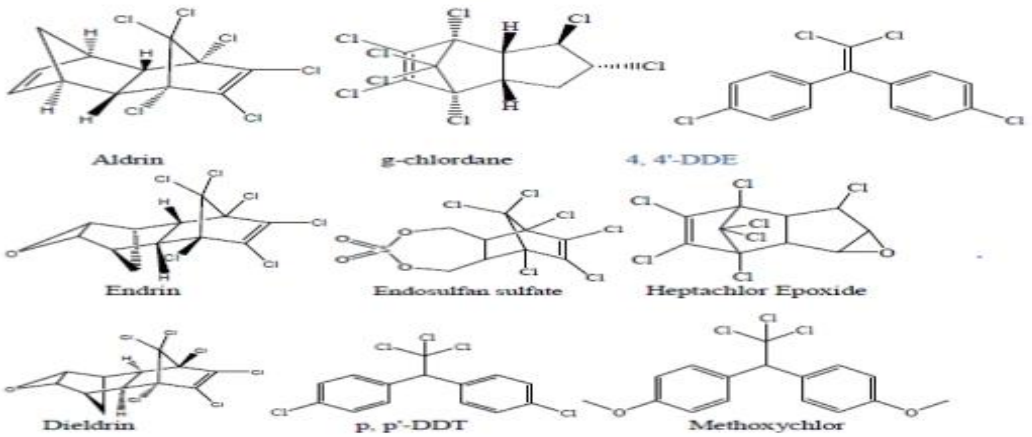
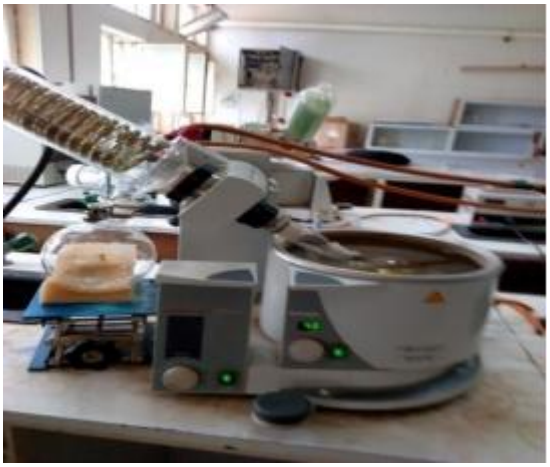


Figure1: The chemical structures and common names of the organochlorine pesticides

Appendix- 3 Pesticide extraction and clean up procedures

Soxhlet extraction clean up with column chromatographs

Chemicals/Reagents



Rotary evaporator

Figure 4: pesticide extraction and clean up procedures in lab

Appendix 4: Chromatograms of the target OCPs

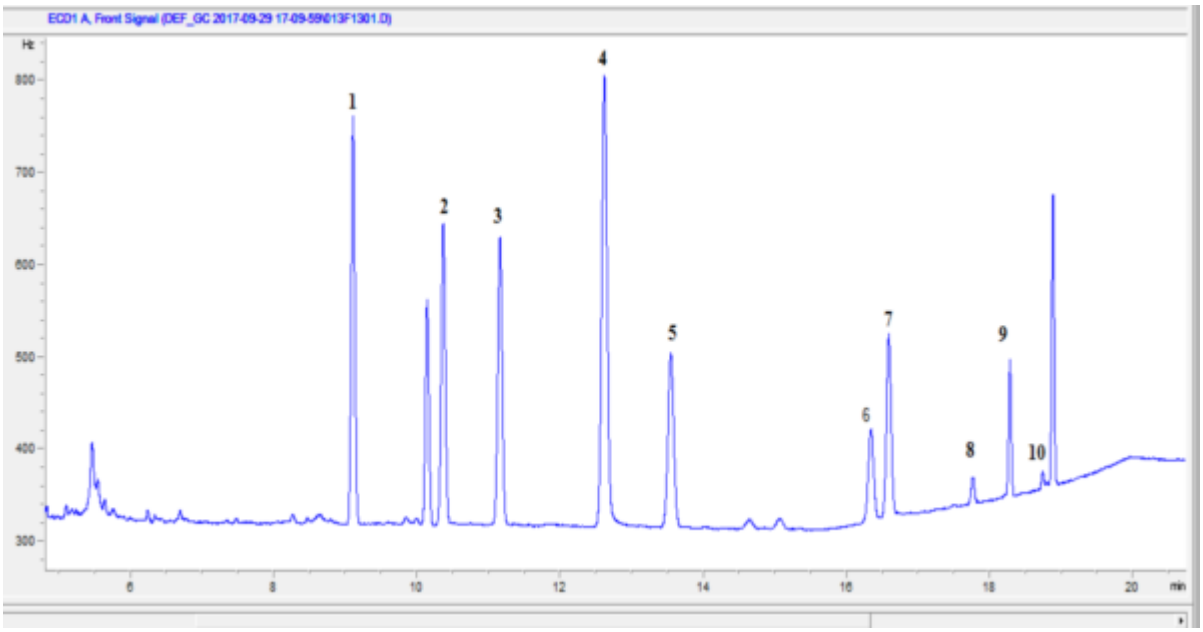


Figure 11: Sample chromatograms of the target OCPs

Appendix-5 Procedure for determination of TDS, TSS, Alkalinity

Determination of Total Dissolved Solids

Determined by filtrating the water sample then evaporation and finally drying the water sample

Total Dissolved Total (mg/l) = $\frac{(A-B)*1000}{\text{ml of sample}}$ Equation-------1

Where

Total Suspended Solids (TSS)

For total suspended solid (TSS), 100 ml of the water sample of each was filtered through a pre weighed filtered paper. The filtered papers were dried at 103 – 105°C in oven and TSS was determined by the following formula.

A well-mixed water sample was filtered through a pre weighed what man filter, 70 mm id under vacuum and filtration apparatus. The residues retained on the filter paper were dried to

constant weight at 105 0C. The weight difference was used for calculating the TSS in the water samples.

$$\text{Total suspended solids mg/l} = \frac{(A-B))*1000}{\text{ml of sample}} \qquad \text{Equation-------1}$$

Determination of Alkalinity of water samples (titrimetric method)

To 50 mL of each of the water samples, 3 drops of phenolphthalein indicator was added. The sample was titrated with 0.02N H_2SO_4 to pH 8.3 and phenolphthalein alkalinity was estimated (phenolphthalein indicator was changed color from pink to colorless at pH 8.3). Finally, the phenolphthalein alkalinity of water was calculated as follows, 50 ml of water samples was transferred to an Erlenmeyer flask. Two drops of phenolphthalein indicator solution were added and mixed. Since the sample was, remain the same or there was no color change, 2 drops of methyl red indicator solution were added to the same sample and titrated sulfuric acid until the color changes from greenish blue to light pink, then the amount of sulfuric acid consumed were recorded.

$$\text{Alkalinity (mg/l)} = \frac{V*N*50}{\text{ml of sample}} \text{x}1000$$

Where V= volume of H_2SO_4 in mL; N= Normality of standard acid

Determination procedure of Acidity of water

A 50 ml of water samples were transferred to an Erlenmeyer flask. 0.15m1(3 drops) Phenolphthalein indicator solution were added and titrated against a white background with standard 0.02 N sodium hydroxide to the appearance of the faint pink color characteristic of pH = 8.3. Then, the amount of NaOH consumed during titration was recorded.

$$\text{Acidity (mg/l)} = \frac{V*N*50}{\text{ml of sample}} \text{x}1000$$

Where V= volume of NaOH in mL; N= Normality of standard acid (Reda, 2016).

Appendix .6 Table of the value of physico-chemical parameters recorded in three study area at 18 sampling sites.

Bore district Phsico-chemical parameters of water

Sample site	Depth M	Width M	Velocity m/s	T°c	EC(µS/cm)	PH	DO mg/l	TSS mg/l	TDS mg/l	Alkalinity mg/l	Acidity/ mg/l
BE1	0.15	1	0.2	25.7	2000	5.12	0.3	1	2.4	60	6.67
BE2	0.2	0.5	0.25	25.8	2110	5.19	10.4	0.7	1.4	113.3	6.67
BU1	0.4	1	0.35	21.4	111	7.13	5.3	0.2	7.2	66.6	33.3
BU2	0.35	1.5	0.3	22.3	296	6.62	4.82	0.5	17.6	33.3	26.7
BD1	0.35	1	0.45	22.3	283	6.7	0.81	0.08	168.8	73.3	33.3
BD2	0.25	1.5	0.3	23.4	280	6.59	1.22	0.04	30	66.6	40
Mean	**0.28**	**1.08**	**0.31**	**23.48**	**846.67**	**6.23**	**3.81**	**0.42**	**37.90**	**68.85**	**24.44**
Sd	**0.10**	**0.38**	**0.09**	**1.87**	**939.09**	**0.85**	**3.87**	**0.38**	**65.03**	**25.88**	**14.39**

Haro District Phsico-chemical parameters of water

Sample site	Depth m	Width m	velocity m/s	Toc	EC (µs/cm)	PH	DO mg/l	TSS mg/l	TDS mg/l	Alkalinity mg/l	Acidity mg/l
HE1	0.1	0.22	0.2	27	262	6.51	4.3	0.08	3	73.3	33.3
HE2	0.02	0.2	0.15	27.8	286	6.53	4.24	0.96	3.4	93.33	26.7
HU1	0.25	1.3	0.45	19.1	75.6	7.1	7.73	0.12	16	26.7	40
HU2	0.3	1.1	0.4	19	74.6	7.74	7.63	0.14	4.8	33.3	26.7
HD1	0.3	1.3	0.35	21.4	77.3	7.41	6.82	0.06	8.4	26.7	26.7
HD2	0.35	3	0.3	22.5	77.4	7.31	7	0.22	7	26.7	40
Mean	**0.22**	**1.19**	**0.31**	**22.80**	**142.15**	**7.10**	**6.29**	**0.26**	**7.10**	**46.67**	**32.23**
SD	**0.13**	**1.02**	**0.12**	**3.82**	**102.42**	**0.49**	**1.60**	**0.35**	**4.83**	**29.19**	**6.54**

Agaro District (Masifine site) Phsico-chemical parameters of water

Sample site	Depth m	Width m	Velocity m/s	T_{oc}	EC (µS/cm)	PH	DO mg/l	TSS mg/l	TDS mg/l	Alkalinity mg/l	Acidity mg/l
ME1	0.15	0.35	0.1	19.6	924	5.19	0.9	0.36	44.6	166.67	80
ME2	0.1	0.46	0.4	18.4	150.4	6.92	2.42	0.24	6	100	26.7
MU1	0.3	1.2	0.56	19	74.5	7.23	7.28	0.22	28.6	33.3	40.7
MU2	0.15	2	0.4	19	71	7.07	7.02	0.74	11	26.7	33.3
MD1	0.25	2	0.2	20.7	89	6.55	2.53	0.004	20.4	46.67	33.3
MD2	0.35	1.5	0.25	18	88.3	6.73	3.05	0.1	12.4	46.67	26.7
MEAN	**0.22**	**1.25**	**0.32**	**19.12**	**232.87**	**6.62**	**3.87**	**0.28**	**20.50**	**70.00**	**40.12**
SD	**0.1**	**0.72**	**0.17**	**0.95**	**339.81**	**0.74**	**2.64**	**0.26**	**14.2**	**53.95**	**20.22**