PESTICIDES IN ENVIRONMENTAL COMPARTMENTS OF AFRAM ARM OF THE VOLTA BASIN IN GHANA

BY

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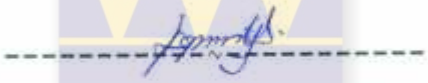
THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF PHD BOTANY DEGREE

2015

DECLARATION

I hereby declare that this Thesis is the result of research work carried out in the Department of Plant and Environmental Biology, University of Ghana, under the supervision of Professor G. K. Ameka and Professor Ebenezer Oduro-Owusu. It has neither in whole nor in part been presented for another degree in this University or elsewhere. All cited works have been fully acknowledged.



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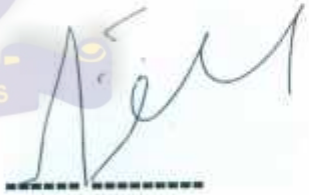

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ACKNOWLEDGEMENTS

The authorship of this work may be credited to me, Samuel S. Koranteng, but I unreservedly acknowledge the special Grace of Jehovah God that enabled its accomplishment.

I wish to express my sincere gratitude to all those who helped to make the completion of this Thesis possible. I am particularly grateful to my supervisors, Professor Gabriel K. Ameka and Professor Ebenezer Oduro-Owusu for their interest, patience, guidance and critical supervision of this research work. Special thanks to Mr. Emmanuel Akorsah, the Driver who took me round in all the field trips. He was more than just a driver; his special knowledge of the study area facilitated selection of appropriate study sites. I do also acknowledge Mr. Emmanuel Klubi of the Department of Marine and Fisheries Science, University of Ghana for assisting me in the field work.

I am under special obligation to express my profound gratitude to the Director and Management of the Ghana Standards Authority for allowing me to use the facilities of the Pesticide Residue Laboratory to carry out all my analyses. I am very grateful to Mr. Paul Osei-Fosu, Head of the Pesticide Residue Laboratory for facilitating access to the use of the laboratory. I am particularly indebted to Mr. Samuel Frimpong, an expert in pesticide residue analyses, who willingly guided and helped in adapting protocols where necessary to suit various aspects of the extraction processes. I cannot forget occasions when we spent entire nights in the laboratory working to get desired results. His assistance is truly invaluable. I also appreciate very much the contribution and friendliness of other staff mainly; Mr. John Danqwah, Ms. Pearl Mensah and Ms. Ernestina Adeennze.

This work benefitted from the Faculty Development Grant of University of Ghana, and it is as such acknowledged.

Sometimes, the challenges were very daunting and prospects of successful completion appeared blurred, however, the moral and prayer supports of Rev. Dr. and Mrs Dzameshie (Department of Linguistics- University of Ghana), Professor Vincent Nartey (Department of Chemistry- University of Ghana), my wife, Mrs Faustine Koranteng, my children: Jessie, Mark-Jonas and Jemimah had been a great pillar of encouragement that urged me on. God bless you all.

Finally, to all who have contributed in one way or the other but have not been mentioned, I say: *Thank you very much.*

ABSTRACT

Inappropriate use of agrochemicals, especially insecticides along the banks of the Afram River in Ghana raises food safety concerns as well as concerns of pollution of the aquatic ecosystem. These environmental compartments along the Afram arm of the Volta Lake are therefore investigated for pesticide residues. Levels of three groups of pesticides (organochlorines, synthetic pyrethroids and organophosphates) were analysed in farmland soils, watermelon, onion and chili pepper as well as some aquatic biota (fish species, and submerged macrophytes), water and surface sediment. In the case of aquatic fauna, pesticide content of three tissues: muscle, gill and liver of *Tilapia zilli*, *Oreochromis niloticus*, *Chrysichthys nigrodigitatus* and *Bagrus bayad* were determined. Two aquatic macrophytes: *Ceratophyllum demersum* and *Nymphaea lotus* were also analysed and bioconcentration factors for quantified pesticides evaluated. Presence of banned pesticides were investigated in the analysed matrices, health risk assessment of pesticide residues in the food commodities for systemic effects conducted, toxicity of sediment investigated and effect of periodic flooding of cultivated lands on pesticide content of water assessed. Approximately 97% of target pesticides analysed were detected and quantified in soil samples of fallow lands; 24% of which exceeded the maximum residue limit (MRL) of the United States Environmental Protection Agency (USEPA). About 88% of total soil samples analysed contained one or more pesticide residues. The Farmland soils temporary left to fallow were therefore found from this study to have high load of residual pesticides. The pesticides that exceeded the USEPA maximum residue limits were *p,p'*-DDT, *p,p'*-DDD, fenvalerate, cypermethrin, permethrin, chlorpyrifos, dimethoate, chlorfenvinphos and methamidophos; and the banned pesticides detected were: aldrin, dieldrin, endrin, gamma-lindane, DDT, heptachlor, parathion and methamidophos. Incidence rate of the pesticides in the food commodities was very high; 100% of target organophosphate and pyrethroids as well as 80% of the organochlorines were quantified in the food commodities (watermelon, onion and pepper samples) analysed. While 38% of the quantified pesticide residues exceeded the European Union (EU) MRL, estimation of health risk associated with pesticide present in the food crops indicate that β-lindane, dieldrin and methamidophos have potential for systemic toxicity in children while heptachlor shows

health risk in both children and adults. Also, comparison of pesticides content of water during flood and recess regimes show significantly high incidence rate and concentration levels of pesticides, as well as high number of pesticides exceeding the WHO MRL during the flood regime. Determination of Afram River bed sediment toxicity identifies γ-lindane, *p,p'*-DDE and dieldrin as contaminants whose concentration levels affect the integrity of the Afram arm of the Volta Lake ecosystem as a whole and pose health risk to benthic organisms in particular. The mean concentration range of pesticides in the four fish species was highest for organochlorines (0.78 µg/kg – 4671 µg/kg), followed by synthetic pyrethroids (1.10 µg/kg – 49.8 µg/kg) and then the organophophates (1.60 – 17.00 µg/kg) while total pesticide load in the fishes follow the order: *C. nigroditatus* > *B. Bayad* > *O. niloticus* > *T. zilli*. Pesticide content was significantly higher ($p \leq 0.05$) in liver than in gill and muscle tissues. The concentration order in the tissues was generally: Liver > Gill > Muscle. Whereas the levels of methoxychlor, aldrin, bifenthrin, permethrin, cyfluthrin, cypermethrin, chlorpyrifos, chlorfenvinphos, ethoprophos and profenofos in the muscle tissues of the fishes exceeded 0.01mg/kg default EU MRL, estimation of health risk indicates that levels of only heptachlor, α-endosulfan and ethoprohos in the fish species present health hazard in children (up to 11 years), while no health risk can directly be imputed or associated with adult consumption of these fishes. While *Ceratophyllum demer*sum and *Nymphaea lotus* have been found to bioconcentrate pesticides, *Ceratophyllum demer*sum in particular was found to fulfill the "very bioaccumulative" criterion since it was able to have bioconcentration factor of approximately 5000 for some pesticides like diazinon and fenitrothion in the aquatic medium. Interaction with the farmers indicates that most of them are well acquainted with the right application of agrochemicals but fail to do so. This attitude has resulted in high pesticide residue levels in various environmental matrices along the Afram River bank of the Volta basin. Most of the farmers, either due to ignorance or deliberate refusal, do not observe the required Pre-harvest interval (PHI) that has the effect of reducing pesticide residues to acceptable levels in food crops before consumption. It is therefore necessary to put appropriate policy and legislative measures in place to ensure compliance to this important requirement that is particularly relevant in improving food security in fruit and vegetable cultivation.

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CHAPTER ONE

INTRODUCTION

1.1 Bacground

The US Environmental Protection Agency defines a pesticide as “any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest” (USEPA, 2006). Pesticides are therefore formulated primarily to control pests and are deliberately introduced into the environment containing the target pests. Due to uncontrolled use of pesticides in some instances, coupled with occasional lack of specificity, the effect of pesticides invariably tend to go beyond their targets, to impacting unintended compartments of the environments with dire consequences on occasions (Carson, 2002). The study of pesticides and their effects has therefore been a thematic research area of much interest.

Historically, pesticides have been in use for about 4500 years (Unsworth, 2010). The first recorded use was of simple sulphur compounds to control insects and mites by the Sumerians (Unsworth, 2010). Since its first use, pesticides of various kinds have been manufactured for various purposes. The first groups of pesticides of major revolutionary importance to be discovered was the synthetic organic pesticides or the organochlorines in the 1940s, such as Parathion, dichlorodiphenyltrichloroethane (DDT), benzenehexachloride (BHC), aldrin, dieldrin and chlordane (History of pesticide, n.d.). This group of pesticides instantly became very popular because of their broad-spectrum activity and also because they were effective yet inexpensive (The History of Pesticides, 2008). The most popular of them, DDT, discovered in 1949 by a Swiss Scientist, Dr. Paul Muller was widely used due to its perceived low mammalian toxicity, to treat insect-borne diseases like malaria, yellow fever and typhus (Delaplane, 2000). From the 1950s through the 60s pesticide use became mostly associated with agricultural activities. There

was over-reliance on the newly found organic pesticides (like DDT, BHC, dieldrin, endrin, chlordane etc) for increase yield of crops (Ganzel, 2007).

Over time, the potential of the organochlorine pesticides for bioaccumulation and long-term toxicity became widely recognized and pest persistence became increasingly evident. Over reliance on the chlorinated hydrocarbons soon led to their indiscriminate use (Carson, 2002) and this resulted in various health and environmental problems. Of particular concern to the international community are the persistent organic pollutants (POPs,) also referred to as the 'dirty dozen' comprising: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, dioxins, hexachlorobenzene, furans, toxaphene and polychlorinated biphenyls (PCBs). They are known to cause an array of adverse effects including diseases and birth defects among humans and animals. The United Nations (UN), through the Stockholm Convention therefore banned the use of the POPs in 2004 (EPA Ghana, 2007). The chlorinated pesticides subsequently fell out of use in preference to the more environmentally friendly ones like orgarnophosphates, carbamates and pyrethroids, which although more acutely toxic, do not persist in the environment.

Discovery of pesticides has brought many benefits to mankind. The most obvious are the economic benefits derived from the protection of commodity yield and quality, and the reduction of other costly inputs such as labour (Thorpe, 1988). Other kinds of benefits include the maintenance of aesthetic quality, the protection of human health from disease-carrying organisms, the suppression of nuisance-causing pests, and the protection of other organisms, including endangered species from pests (Thorpe, 1988).

Despite their many advantages, there are some potential hazards or risks associated with the use of pesticides. Adverse impacts of pesticides use usually stem from a lack of understanding of the

impact of the chemical on the environment and indiscriminate use/overuse of the product. Uncontrolled use can result in the reduction of the population of non-target organisms, including beneficial species. Drift sprays and vapour during application can cause severe damage and residue problems in food resources and present health risks (Pros & cons of pesticide use (n.d.)). Poisoning hazards and other health effects can occur through excessive exposure if safe handling procedures are not followed. There is also the risk of contamination of soil and groundwater as well as development of crop pest populations that are resistant to agrochemical treatment (Pros & cons of pesticide use (n.d.).

1.2 Justification

Ghana is essentially an agricultural country with the sector contributing 31.8%, 29.8%, 25.3%, 22.7% and 21.3% to the nation's Gross Domestic Product (GDP) in 2009, 2010, 2011, 2012 and 2013, respectively (Ghana Statistical Service, 2013). Like all agrarian economies, there is dependence on agro-chemicals for increased yield in every sector of the agricultural enterprise and this has led to an increase in demand for agro-chemicals.

Importation of pesticides into Ghana dates back to the late 1940s when dichlorodiphenyltrichloroethane (DDT), lindane and related pesticides were extensively used for both agricultural and public health purposes (EPA Ghana, 2007). Dichlorodiphenyltrichloroethane was officially used for the control of cocoa capsids as well as malaria and filariasis. However, following the recognition of its adverse effects, the use and importation of DDT, alongside 26 others, was banned in 1985 (EPA Ghana, 2007). As DDT and some organochlorines were phased-out, new and safer alternatives, mainly organophosphates, carbamates and synthetic pyrethroids were introduced. Currently about a total of 380 pesticides

of different categories have been registered or approved for use in Ghana (EPA Ghana, 2013) and accessibility has been facilitated by the rapid and widespread proliferation of agro-chemical retail shops in the country. Dinhan, (2003) reported that about 87% of farmers in Ghana depend on pesticide for increase yield. One particular area that has witnessed intensive use of pesticides is fruit and vegetable farming. This horticultural industry tends to concentrate around water bodies where irrigation occurs all year round. Given the fact that fruits and vegetables are naturally infested by myriads of pests, farmers tend to depend heavily on pesticides for improved yield and aesthetic value of their products for maximum economic benefits. Horna *et al*., (2008) reported that about 90% of vegetable farmers in Ghana apply doses above the recommended rates in single applications. The residue of the pesticides accumulate in the crops, the soil medium, water bodies, sediments and aquatic biota; resulting in the contamination of the food chain and the attendant health hazards.

Presence of pesticides in the following matrices: fish, (Darko, Akoto & Oppong, 2008; Afful, Anim & Serfor-Armah, 2010), water (Ntow, 2001 & 2005), sediment (Ntow, 2001; Darko, Akoto & Oppong, 2008), vegetables (Amoah *et al*., 2006; Ntow, 2008; Darko, 2009; Bempah *et al.*, 2011, 2012) and meat (Darko & Acquaah, 2007), from Ghana have been reported. Evidence of the presence of pesticides in human body is already documented in Ghana (EPA Ghana, 2007; Ntow, 2008; 2001; Osei-Tutu *et al.*, 2011). This occurrence should be of much concern and special attention and concerted effort needs to be directed into investigation of pesticide residue in our environment. There is the need to investigate residues of pesticides in various environmental matrices to provide clues about pathways along which pesticides bioaccumulation occur. Moreover, there is the need to investigate health hazards associated with environmental residue levels. Such data will provide important information needed by the Environmental

Protection Authority (EPA), Food and Drugs Authority (FDA) and Ghana Standards Authority (GSA) for their monitoring and regulatory activities and will as well facilitate assessment of existing policies for the development of alternative pesticide management programmes.

1.3 Problem Statement

According to Gordon and Amatekpor (1999), the Volta River in Ghana was impounded in 1964 following the creation of a dam a year earlier at Akosombo for the main purpose of hydro electricity generation. The impoundment created a lake with a surface area of about 8500 km^2 (3.6% of surface area of Ghana) and a shoreline exceeding 4800 km. It also resulted in reduction in the flow regime and inundation of farm lands and communities in the upper stretches of the Volta River. Since the creation of the Lake some 50 years ago, there have been intensive agricultural activities along its banks that heavily rely on agrochemicals. There is seasonal recession of water from the lake, especially during the drier periods. As water recedes, farmers follow along and cultivate the exposed land, taking full advantage of the residual moisture in the soil hence reducing the burden of irrigation. Communities along the Afram arm of the Volta Lake in particular, apart from fishing, also resort to farming along the immediate banks, taking advantage of the residual moisture in the soil when the lake recedes between the months of November and May (Gordon & Amatekpor, 1999). This type of farming is referred to as 'drawdown farming'. Later, when the moisture of the soil falls below the optimum level, manual or can irrigation is employed. Noticing the economic benefits from 'draw-down' farming, mechanised irrigation equipment has been procured to enable cultivation of large areas upland and further away from the drawdown region. Plates 5a -5c provide some insight into the irrigational facilities that make large scale farming upland of drawdown areas possible. Communities along entire Afram arm of the Volta Lake are now noted for the production of

crops such as tomatoes, okra, pepper, garden eggs, cabbage, lettuce, peas, water melon, sweet potatoes and other short-maturing crops along the banks of the Afram arm of the Volta Lake (Gordon & Amatekpor, 1999).

The most common horticultural crops produced along the bank of the Afram arm of the Volta Lake are the *Allium cepa* L. (red onions), *Capsicum annuum* L. (chilli pepper), *Citrullus vulgaris* Schrad. (Watermelon) and *Abelmoschus esculentus* L. (okra) which have ready market. They are transported to the nearest centres of commercial activities (in this case, Nkawkaw, Kumasi and Accra) for onward retailing to the various parts of the country.

Now, with the introduction of mechanized irrigation, farming goes on all-year round and farmers rely heavily on the use of agrochemicals, including pesticides for increased yield of crops. Reconnaissance visit to the field revealed that the synthetic pyrethroid, organophosphates, fungicides and herbicides are the most commonly used pesticides. Documented evidence of misuse of pesticides and other agrochemicals by the farmers in other vegetable growing areas in Ghana (Ntow, Gijzen, Kelderman & Drechsel, 2006; Ntow et al., 2007; Ntow, 2008; Botwe *et al*., 2011) is enough ground to give cause for similar concerns in the Afram area. The important phenomenon of seasonal flooding of the cultivated areas along the Afram bank increases the probability of the presence of pesticides in water body. Given the dynamics normally involved in pesticide contamination, the farmland soil, the farm produce, the adjoining water body, sediment, and the aquatic flora and fauna, as well as the farmers themselves are invariably exposed to pesticide contamination. Hence these environmental compartments of the Afram arm of the Volta Lake need to be investigated to assess the level of pesticide pollution.

1.4 Objectives of the study

This study seeks to investigate the residual levels of three pesticides groups (organochlorines, synthetic pyrethroids and organophosphates) in farmland soils as well as in fruits and vegetables cultivated along the Afram arm of the Volta Lake. It is anticipated that the Afram arm of the Volta Lake will inevitably receive run-offs from the agricultural fields and hence water, river sediments and some aquatic biota will as well be analysed for pesticide contamination and any possibly associated health hazards.

The specific objectives of this study are to:

- Verify if banned pesticides are still in agricultural use along the bank of the Afram River.
- Investigate pesticide content of fallow farmlands prior to re-cultivation
- Determine pesticide content of Afram water and toxicity in surface sediments
- Ascertain pesticide levels in food crops and fish and the health hazard associated with their consumption.
- Estimate bioconcentration of pesticides by submerged aquatic flora

1.5 Organisation of the Thesis

This study focuses on the analyses of wide range of insecticides from the organochlorine, organophosphate and synthetic pyrethroids groups and their levels in different environmental matrices mainly: soil, water, sediment, fruit and vegetables as well as aquatic biota along the Afram arm of the Volta Lake. The research employed random field sampling. The concentration of pesticides in the samples were evaluated by laboratory measurements using state-of-the-art equipment, mainly gas chromatography equipped with electron capture detector

(ECD) and pulse flame photometric detector (PFPD). Results were analysed and health risk/hazards assessment through dietary exposure determined.

The Thesis starts with an introduction as chapter one and presents background to the entire work. The research outline is presented here and the aim, objectives and strategy defined. Chapter two presents detailed literature review pertaining to the research. Here, current state of work relating to the various aspects of this research, in the country is extensively reviewed. The location and features of the study area are spelt out in Chapter three, and Chapters four to seven present the main body of the research.

In Chapter four, the research presents how soil from agricultural lands on the bank of the Afram River was sampled and analysed for pesticide residues. Chapter five describes the determination of pesticide residues in selected fruit and vegetables from the cultivated fields and the health risk associated with their consumption. Chapter six examines toxicity of surface sediment of the lake and compares pesticide quality of lake water under flood and recess regimes, whilst chapter seven investigates distribution of pesticides in tissues of four selected species of fish in the Afram River. This chapter as well assesses the health risk associated with the consumption of targeted fishery species. Pesticide accumulations in two aquatic plants are also determined in chapter seven and the results used to estimate bioconcentration factors (BCF) for each of the plants. Finally, the Thesis ends with chapter eight presenting the general conclusion, summary and recommendations for future research as well as policy directions for the Ghana Environmental Protection Authority (EPA), Ghana Standards Authority (GSA) and Ghana Food and Drugs Authority (FDA) who are the main stakeholders.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Pesticides

Pesticides of one type or another have been in use for thousands of years. A major motivation for using pesticides is to control diseases. Human deaths due to insect-borne diseases through the ages far exceed those attributable to the effect of warfare (Baird & Cann, 2012). The use of various insecticides has greatly reduced the incidence of diseases transmitted by insects and rodents. Malaria, yellow fever, bubonic plagues and sleeping sickness are but few examples of these diseases. Another principal motivation for insecticide use is to prevent insects from attacking food crops. Even with extensive use of pesticides, about one-third of the world's total crop yield is destroyed by pests during growth, harvesting and storage (Baird & Cann, 2012).

Pesticides as substances that directly kill or control unwanted organisms have been defined by various authours, interest groups and organizations. The United States Environmental Protection Agency (USEPA) defines a pesticide as "*any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest*" (USEPA, 2006). Washington State Department of Agriculture (2009) defines pesticides as: "*Any substance or mixture of substances, including plant regulators, defoliants, desiccants and spray adjuvant, intended to prevent, destroy, control, repel, or mitigate any insect, rodent, snail, slug, fungus, weed, and any other form of plant or animal or virus, except viruses on or in a living person or other animal*".

The European Union prefers a definition for pesticides that covers insecticides, acaricides, herbicides, fungicides, plant growth regulators, rodenticides, biocides and veterinary medicines. It defines pesticides as chemical compounds used to: "*Kill, repel or control pests to protect crops before and after harvest; Influence the life processes of plants; destroy weeds or prevent their*

growth; and Preserve plant products". The Food and Agriculture Organization (FAO, 2002) provides an elaborate and extensive definition of pesticide as: *"any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport"*

The 'US Legal', a legal service industry that offers support and resources such as a legal dictionary, law guides and legal references defines pesticides as: "Any *substance or mixture of substances intended for preventing, destroying, repelling, attracting or mitigating any insects, rodents, nematodes, fungi, weeds or other forms of plant or animal life and/or bacteria and viruses, except bacteria or viruses on or in living man or other animals, which is determined to be a pest". It may also mean any substance or mixture of substances intended for use as a plant regulator, defoliant or desiccant*" (USLegal, 2014). Pests can be insects, mice and other animals, unwanted plants (weeds), fungi, or microorganisms like bacteria and viruses. The U.S. definition of pesticides generally is quite broad and often misunderstood to refer only to insecticides, though the term pesticide also applies to herbicides, fungicides, and various other substances used to control pests as well as any substance or mixture of substances intended for use as a plant

regulator, defoliant, or desiccant (USEPA, 2006). Even though the U.S. definition of pesticides is quite broad, it does have some exclusions such as: drugs used to control diseases of humans or animals; fertilizers, nutrients, and other substances used to promote plant survival and health (are not considered plant growth regulators and thus are not pesticides); biological control agents, except for certain microorganisms, are exempted from regulation by EPA (Biological control agents include beneficial predators such as birds or ladybugs that eat insect pests) and products which contain certain low-risk ingredients, such as garlic and mint oil, have been exempted from the US Federal registration requirements, although State regulatory requirements may still apply (USEPA, 2014). Generally, the USEPA definition has been adopted in Ghana by stakeholder institutions like the EPA, Ghana; Ghana Standard Authority and Ghana Food and Drugs Authority.

Scrutiny of above definitions and others from literature identifies common elements in definitions. According to Young (1987), any accepted definition of pesticides should include key phrases such as "chemical substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest" and "substances intended for use as a plant regulator, defoliant or desiccant". In the light of the above review of pesticides definitions, the one provided by Eldridge, (2008) is quite succinct and aptly summarises the various definitions and therefore adopted for this study. According to him, "*Pesticides are defined as substances or mixtures of substances intended for controlling, preventing, destroying, repelling, or attracting any biological organism deemed to be a pest*".

2.2 Classification of Pesticides

The term *pesticide* encompasses a diverse collection of substances. Appropriate classification of pesticides groups is therefore desirable for easy reference. Different authors adopt different approaches. Irrespective of the approaches however, pesticides are grouped or classified according to the pests they control, their chemical structure, how/when they work, or their mode of action (site of action) (British Colombia Ministry of Agriculture, n.d.).

The most popular way of classifying pesticides is according to the **target pests** they kill; for example, insecticides are pesticides that target insects, herbicides target plants, acaricides target ticks, nematocides target nematodes, etc. Pesticides are also grouped according to their **chemical structure**. Pesticides with similar chemical structures have similar characteristics and usually have a similar mode of action. Most pesticide active ingredients are either inorganic or organic pesticides. Inorganic pesticides do not contain carbon and are usually derived from mineral ores extracted from the earth. Examples of inorganic pesticides include copper sulphate, ferrous sulphater and sulphur. Organic pesticides on the other hand contain carbon in their chemical structure. Most organic compounds are created from various compounds, but a few are extracted from plant material and are called 'botanicals'. The most well known organic group of pesticides include: organochlorines, organophosphates, synthetic pyrethroids and carbamates. (USEPA, 2006)

Pesticides can also be classified **according to how or when they wor**k. On this basis, the following, among others have been identified by the USEPA: Contact pesticides, systemic pesticides, foliar pesticides, selective pesticides, non-selective pesticides, residual and non-residual pesticides.

Contact pesticides generally control a pest as a result of direct contact. Insects are killed when sprayed directly or when they crawl across surfaces treated with a residual contact insecticide. Weed foliage is killed when enough surface area is covered with a contact herbicide. **Systemic pesticides** are pesticides which are absorbed by plants or animals and move to untreated tissues. Systemic or translocated herbicides move within the plant to untreated areas of leaves, stems or roots. They may kill weeds with only partial spray coverage. Systemic insecticides or fungicides move throughout treated plants and kill target pests. **Foliar pesticides** are applied to plant leaves, stems and branches; they may be either a contact pesticide or a systemic pesticide. **Selective pesticides** control only certain pests, while **non-selective pesticides** will control a wide range of pests. **Residual pesticides** do not break down quickly and may control pests for a long time (i.e. several weeks or a year). **Non-residual pesticides** are quickly made inactive after application and do not affect future crops.

Biopesticides are another group of pesticides. They are derived from natural materials such as animals, plants, bacteria, and certain minerals. Biopesticides fall into three major classes: Microbial pesticides, Plant-Incorporated-Protectants (PIPs) and Biochemical pesticides.

Microbial pesticides consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pests. The most widely used microbial pesticides are subspecies and strains of *Bacillus thuringiensis* (Bt). Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some Bt's control moth larvae found on plants, other strains are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular *Bacillus thuringiensis* produces a protein that can bind to a larval gut receptor, thereby

causing the insect larvae to starve (USEPA, 2014). **Plant-Incorporated-Protectants (PIPs)** are pesticidal substances that plants produce from genetic material that has been added to the plant. For example, the gene for the *Bacillus thuringiensis* pesticidal protein can be taken and introduced into the plant's own genetic material. Then the plant, instead of the Bt bacterium, manufactures the substance that destroys the pest. **Biochemical pesticides** are naturally occurring substances that control pests by non-toxic mechanisms. Conventional pesticides, by contrast, are generally synthetic materials that directly kill or inactivate the pest. Biochemical pesticides include substances, such as insect sex pheromones that interfere with mating as well as various scented plant extracts that attract insect pests to traps. Because it is sometimes difficult to determine whether a substance meets the criteria for classification as a biochemical pesticide, USEPA has established a special committee to make such decisions (USEPA, 2014). Pesticides can also be grouped according to **their mode of action** or the way a pesticide destroys or controls the target pest. The pesticide may have a specific site of action, commonly referred to as primary action site. In some instances, one insecticide may affect insects' nerves while another may affect moulting. One herbicide may mimic the plants growth regulators and another may affect the plants ability to convert light into food. One fungicide may affect cell division and another may slow the creation of important compounds in the fungus British Colombia Ministry of Agriculture, n.d.).

2.3 Organochlorine, synthetic pyrethroid and organophosphorus pesticides

The principal pesticides considered in the study area are organochlorines, organophosphates and synthetic pyrethroids **insecticides**. Therefore only these pesticides will be included in the review in this chapter.

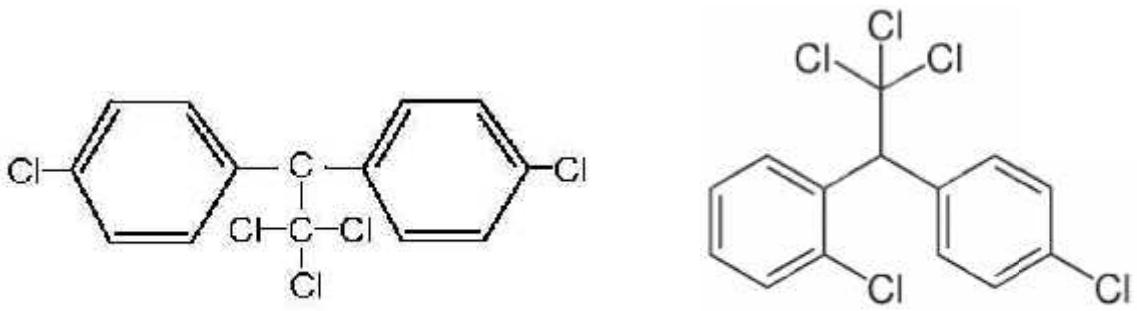
2.3.1 Organochlorine Pesticides (OCPs)

Organochlorine pesticides are insecticides composed primarily of carbon, hydrogen and chlorine. There are four chemically distinct sub-groups of organochlorines – Dichlorodiphenyltrichloroethane (DDT) and its analogues, hexachlorocyclohexanes (HCH), cyclodiene compounds and toxaphene group (Ware, 2000). While each of these sub-groups varies in chemical structure, their properties are mostly typical of all organochlorine pesticides. The organochlorine compounds possess unique physical and chemical properties that influence their persistence, fate, and transport in the environment. Although these properties differ among the organochlorine compounds, they all exhibit an ability to resist degradation, associated with sediments or other solids, and to accumulate in the tissue of invertebrates, fish, and mammals. Their unique properties have contributed to both their efficacy as pesticides and industrial products and their persistence and accumulation in the environment (Organochlorine compounds, n.d). The carbon-chlorine bond of organochlorines is very stable to hydrolysis and the stability increases with greater number of chlorine substitutions. OCPs are typically ring structures with a chain or a branched chain framework. Chlorine attached to an aromatic ring is more stable to hydrolysis than chlorine in aliphatic structures. Due to their high degree of halogenation, they have very low water solubility and high lipid solubility, hence their ability to easily pass through the phospholipid structure of biological membranes and accumulate in fat deposits (Baird & Cann, 2012). Organochlorine pesticides share several notable properties such as: high stability against decomposition or degradation in the environment; very low solubility in water; high lipid solubility; low volatility and relatively high toxicity to insects but low toxicity to humans (Baird & Cann, 1999). Their ability to persist, bioconcentrate, biomagnify and the associated health hazards have made them highly undesirable in the environment.

As insect pests, all organochlorines act by stimulating the nervous system, interrupting the transmission of nerve impulses and signals (Waugh, & Padovan, 2004). In response to their adverse effects seen in wildlife and humans, the production and use of these compounds were banned in industrialised countries during the 1970s or subjected to restrictions in use in many others. However, they continue to be detected in both biological and environmental samples worldwide because of their persistent and bioaccumulative properties (Ntow *et al.*, 2007)

2.3.1.1 Dichlorodiphenyltrichloroethane and its metabolites

Dichlorodiphenyltrichloroethane (DDT) was developed by the German chemist, Othmar Zeidler in 1874, but its insecticidal properties were discovered by the Swiss chemist Paul Hermann Muller in 1939. It was used during the Second World War for control of lice and mosquitoes to combat typhus and malaria, respectively (Snedeker, 2001). The World Health Organization has estimated that malaria reduction programs by the use of DDT, have saved the lives of more than 5 million people (Baird & Cann, 2012). Its insecticidal properties were discovered in 1939 by the Swiss chemist Hermann Muller (Chau & Afghan, 1982) and it was hailed as the "miracle insecticide" (Baskin, 2001) and quickly became one of the most widely used pesticides in the world to control insects on agricultural crops. As a result of its potential for human toxicity and severe ecological effects, DDT was banned in Hungary in 1968, Norway and Sweden in 1970, the United States in 1972 and in the United Kingdom in 1984 (US EPA, 1990). It was subsequently banned for agricultural use worldwide in 2004 under the Stockholm Convention, though it is still being used in some underdeveloped countries for disease vector control.



p,p'-dichlorodiphenyltrichloroethane *o,p'-dichlorodiphenyltrichloroethane*

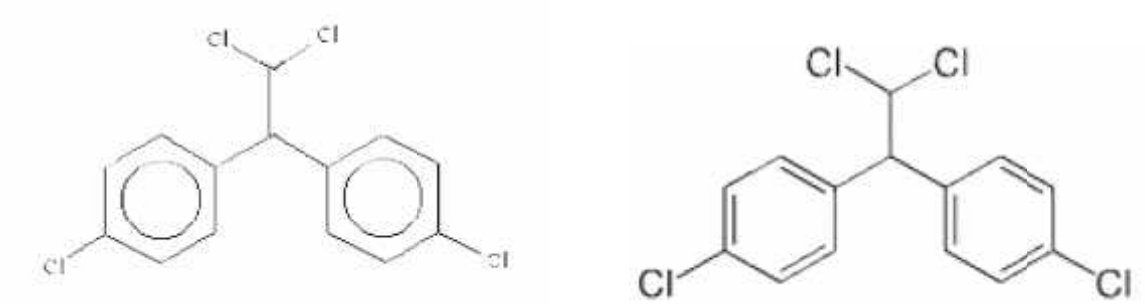
Fig. 2.1: Isomers of dichlorodiphenyltrichloroethane (ATSDR, 2002)

In the environment and in the body, dichlorodiphenyltrichloroethane (DDT) breaks down over time into dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (Figure 2.2). Dichlorodiphenyldichloroethylene and dichlorodiphenyldichloroethane are therefore the major metabolites of DDT. Technical-grade DDT however, is a mixture of some related compounds. The components include the *p,p'*-DDT isomer (85%), *o,p'*-DDT (15%) (Fig. 2.1), and *o,o'*-DDT (trace amounts). Dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) also occur as contaminants (Faroon *et al.*, 2002). The total DDT in a sample refers to the sum of all DDT congeners (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE and *o,p'*-DDD). Dichlorodiphenyldichloroethane was also manufactured and used as an insecticide, but to a much lesser extent than DDT. Dichlorodiphenyldichloroethylene has no commercial use, but is commonly detected along with DDT at concentrations in the environment that often exceed those measured for DDT.

2.3.1.2 Environmental fates of DDT, DDE and DDD

Most DDT in the environment is as a result of past use (ATSDR, 2002). It also entered the various compartments of the environment as gas during the production process. DDT still enters

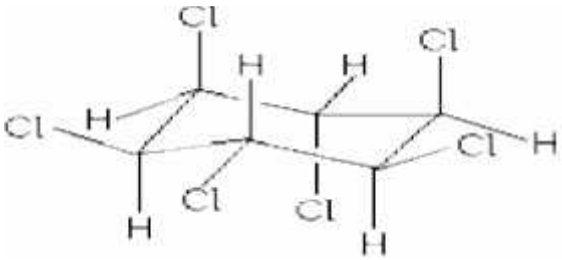
the environment because of its current use in other areas of the world. DDE is only found in the environment as a result of contamination or breakdown of DDT. Dichlorodiphenyldichloroethane was also used as a pesticide to a limited extent in the past. It also enters the environment as a breakdown product of DDT (ATSDR, 2002). Large amounts of DDT were released into the air and on soil or water when it was sprayed on crops and forests to control insects. DDT was also sprayed in the environment to control mosquitoes. DDT, DDE and DDD may also enter the air when they evaporate from contaminated water and soil. DDT, DDE, and DDD in the air will then be deposited on land or surface water. This cycle of evaporation and deposition may be repeated many times. As a result, DDT, DDE, and DDD can be carried long distances in the atmosphere. According to Faroon *et al.*, (2002), these chemicals have been found in regions, far from where they were ever used and some DDT, DDE, and DDD may occur in the atmosphere as a vapor or be attached to solids in air. Vapor phase DDT, DDE, and DDD may break down in the atmosphere due to reactions caused by the sun. The half-life of these chemicals in the atmosphere as vapors has been calculated to be approximately 1.5 – 3 days (ATSDR, 2002). However, in reality, this half-life estimate is too short to account for the ability of DDT, DDE, and DDD to be carried long distances in the atmosphere. DDT, DDE, and DDD remain in the soil for a very long time, potentially for hundreds of years. Their half-life in soil can vary between 2 and 15 years, depending on the soil acidity and temperature (ATSDR, 2002), 350 days in surface waters and 31 years in ground water (Howard, 1991). Most DDT breaks down slowly into DDE and DDD, generally by the action of microorganisms. Figure 2.2 shows the structure of some DDT metabolites.



P,p'-Dichlorodiphenyldichloroethylene (*p,p'*-DDE) *p,p'*-Dichlorodiphenyldichloroethane (*p,p'*-DDD)

Fig. 2.2: Structure of DDT metabolites- *p,p'*-DDE and *p,p'*-DDD (ATSDR, 2002)

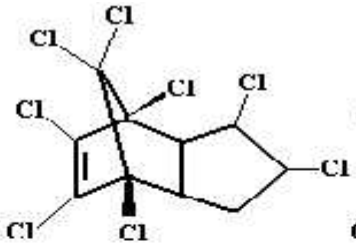
These chemicals may also evaporate into the air and be deposited in other places. They stick strongly to soil, and therefore generally remain in the surface layers of soil. Some soil particles with attached DDT, DDE, or DDD may get into rivers and lakes in runoff. Only a very small amount, if any, will seep into the ground and get into groundwater. The length of time that DDT will last in soil depends on many factors including temperature, type of soil, and whether the soil is wet. DDT lasts for a much shorter time in the tropics where the chemical evaporates faster and where microorganisms degrade it faster. DDT disappears faster when the soil is flooded or wet than when it is dry. Its disappearance is also faster initially when it enters the soil. Later on, evaporation slows down and some DDT moves into spaces in the soil that are so small that microorganisms cannot reach it to break it down efficiently. In tropical areas, total DDT may disappear in much less than a year. In temperate areas, half of the total DDT initially present usually disappears in about 5 years. However, in some cases, half of the total DDT initially present will remain for 20, 30, or more years (ATSDR, 2002). In surface water, DDT will bind to particles in the water, settle, and be deposited in the sediment. DDT is taken up by small organisms and fish in the water. It accumulates to high levels in fish and marine mammals (such as seals and whales), reaching levels many thousands of times higher than in water. In these



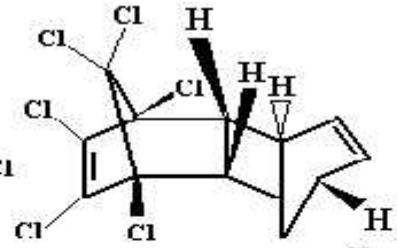
cream, or shampoo for the treatment and/or control of scabies and body lice. In general, HCH isomers and the products formed from them in the body can be temporarily stored in body fat. HCH breaks down in the body to many other substances; these include various chlorophenols, some of which have toxic properties. Among the HCH isomers, β-HCH leaves the body most slowly. α-HCH, δ-HCH, and γ-HCH, and the products formed from them in the body, are more rapidly excreted in the urine; small amounts leave in the feces and expired air (ATSDR, 2005).

According to ATSDR (2005), exposure to Lindane causes the following adverse health effects in humans: neurological effects, liver toxicity, reproductive and developmental effects. Exposure to high doses can cause symptoms such as vomiting, nausea, diarrhea, muscle weakness, seizures, blood disorders and immune deficiencies. Studies have also shown possible associations between lindane exposure in pregnant women and increased risk of spontaneous abortion and premature delivery. Fortunately, ATSDR, (2005) explains that lindane is rapidly broken down and excreted from the body. Long-term exposure to α- HCH, β- HCH, γ- HCH, or technical-grade HCH has been reported to result in liver cancer. It can also result in blood disorders, dizziness, headaches, and possible changes in the levels of sex hormones in the blood.

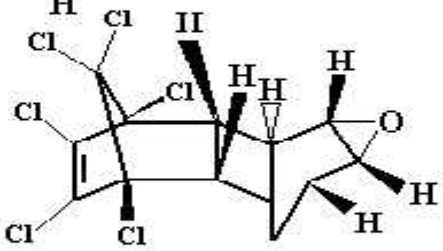
Although technical-grade HCH is no longer used as an insecticide in most developed countries, α- HCH, β- HCH, γ- HCH, and δ- HCH have been found in the soil and surface water at hazardous waste sites because they persist in the environment. In air, the different forms of HCH can be present as a vapor or attached to small particles such as soil and dust; the particles may be removed from the air by rain or degraded by other compounds found in the atmosphere. HCH can remain in the air for long periods and travel great distances depending on the environmental



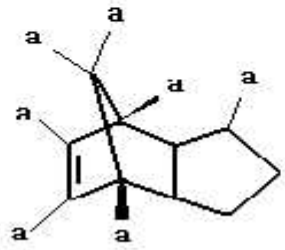
chlordane



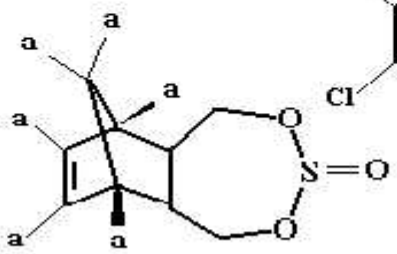
aldrin



dieldrin



heptachlor



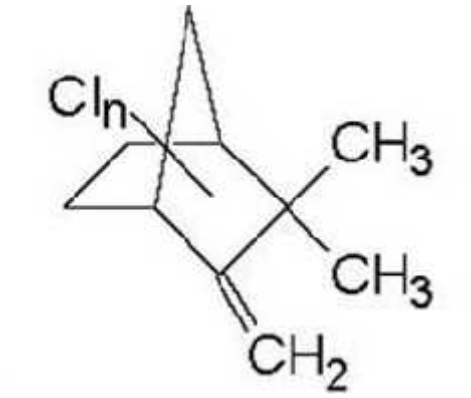
endosulfan

rapidly metabolized by organisms but their metabolites, dieldrin and heptachlor epoxide, respectively are more toxic and persistent than the parent material (Howard, 1991).

The compounds in this group are classified as neurotoxins. In both humans and animals the primary acute toxic effects are on the central nervous system, including hyper excitability and tremors followed by convulsions and possibly death. Acute poisoning from them may be fatal. The compounds also affect the reproductive system, liver and kidney.

2.3.1.5 Toxaphene

Toxaphene (Fig. 2.5) is a mixture of hundreds of similar substances all of which are produced when the naturally occurring hydrocarbon-camphene is partially chlorinated (Baird & Cann, 1999). It was used primarily to control insect pests on cotton and other crops as well as controlling insect pests on livestock and killing unwanted fish in lakes. When toxaphene is released into the environment, it can enter the air (by evaporation), the soil (by sticking to soil particles), and the water (from runoff after rains). It does not dissolve well in water, and hence found mainly in air, soil, or the sediment at the bottom of lakes and streams. Toxaphene is capable of long distances transport by air and has been found in water, soil, sediment, air, and animals in places far from where it has been used. It breaks down very slowly in the environment. It is highly bioaccumulative and even when levels are low or confined to a certain area, it could be high in individual animals. It is also persistent, chronically toxic and harmful to living matter. Toxaphene was therefore banned for all registered uses by 1990 in the US (ATSDR, 2010). Individuals may be exposed to toxaphene through drinking contaminated water, eating fish, shellfish, or wild game from areas contaminated by toxaphene; also through breathing contaminated air or through direct skin contact with contaminated soil. Toxaphene causes damage to the liver and kidney, and is a potential liver carcinogen in mammals. When



reproductive and development effects (Weltman & Norback, 1983) have also been reported as a result of organochlorine contamination.

According to Squipp (n.d.) the carcinogenicity of organochlorine insecticides is not well established, however, some epidemiological studies among agricultural workers suggest an association between organochlorine pesticide use and non-Hodgkin's lymphoma, and long persistent exposure indicates long term damage to the nervous system. (Squibb, n.d.). Exposure to DDT early in life is associated with an increased breast cancer risk later in life (Cohn *et al*., 2007). Many other organochlorine pesticides, such as mirex, chlordane and toxaphene, are known to be carcinogenic as well (Weltman, & Norback, 1983)

2.3.2 Pyrethroids

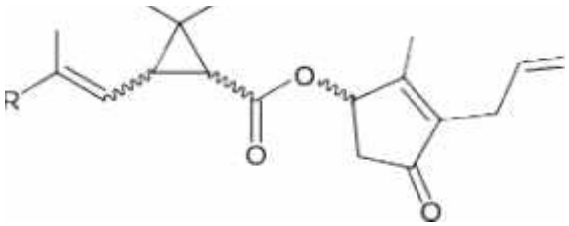
The term *pyrethroid* bears similarity to the name of the natural insecticide mixture known as *pyrethrum* or *pyrethrins (*Pyrethroids and Pyrethrins, 2013). Pyrethrum is one of the oldest known naturally occurring mixtures of chemicals with insecticidal properties; found in two species of asters: *Chrysanthemum cinerariifolium* and *C. coccineum* (ATSDR, 2003). Pyrethrum was first recognized as having insecticidal properties around 1800 in Asia and was used to kill ticks and various insects such as fleas, cockroaches, beetles and mosquitos. Six individual chemicals have active insecticidal properties in the pyrethrum extract, and these compounds are the pyrethrins. The six chemically active pyrethrins are: pyrethrin I and II, Cinerin I and II, and Jasmolin I and II (EXTONET, 1994). Pyrethrins are contact poisons which easily penetrate nerve system of insects and kill them. They however break down quickly in the environment, especially when exposed to natural sunlight.

Pyrethroids are Synthetic chemicals that are very similar in structure to the pyrethrins. While chemically and toxicologically pyrethrins and pyrethroids are similar, the former are extremely sensitive to light, heat and moisture. In direct sunlight, half-life can be measured in hours. The pyrethroids, however, were developed to capture the effective insecticidal activity of their botanical analogues-pyrethrins, with increased stability in light, higher insect toxicity and longer residence time (Gosselin, 1984; Pyrethroids and Pyrethrins 2013). Both pyrethrins and synthetic pyrethroids are sold as commercial pesticides used to control insect pests in agriculture, homes, communities, hospitals and as a topical head lice treatment (Chemicalwatchfactsheet, n.d.).

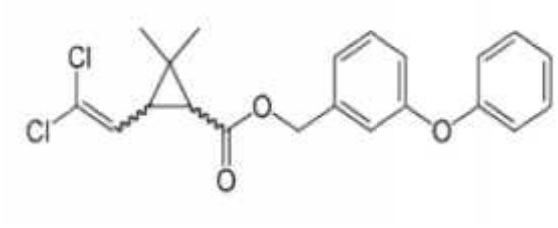
Not all pyrethroids in the same class are equally toxic, however, irrespective of what group they belong, pyrethroid insecticides are observed to be low in toxicity to mammals and birds but high in toxicity to fish; especially if applied directly to water. They are very poorly soluble in water but generally soluble in organic solvents; require very low doses to kill insects (high arthropod toxicity) and are fast-acting. They bind tightly to soil and organic matter and are therefore not effective in penetrating soil to kill pests. Figure 2.6 presents the structure of some pyrethroids.

2.3.2.1 Environmental fate of Synthetic Pyrethroids

Pyrethrins and pyrethroids are primarily released into the air because of their use as insecticides. Pyrethroids are broken down or degraded rapidly by sunlight or other compounds found in the atmosphere. Often, they last only 1 or 2 days before being degraded (ATSDR, 2003). Rain and snow help remove the pyrethroids from air that are not rapidly degraded. Many of these compounds are extremely toxic to fish, and although they are usually not sprayed directly onto water, they can enter lakes, ponds, rivers, and streams from rainfall or runoff from agricultural fields. Pyrethroids bind strongly to dirt and are usually not very mobile in soil, they are not easily taken up by the



Allethrin



Permethrin

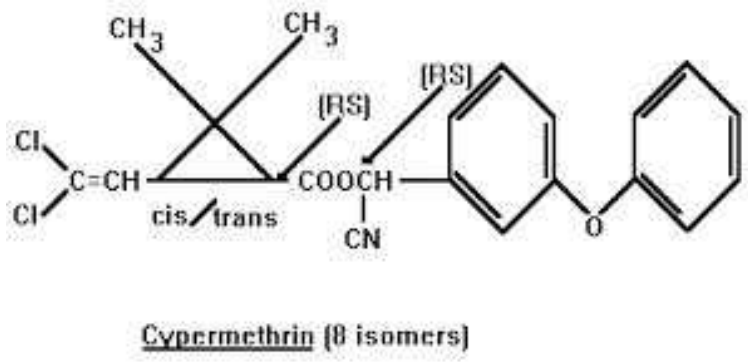


Fig. 2.6: Chemical structures of some pyrethroids. (ATSDR, 2003)

roots of plants and vegetation because they are strongly bound to the soil; however, they are often sprayed directly onto crops and plants so they may be found on leaves, fruits, and vegetables. Because these compounds adsorb so strongly to soil, pyrethroids usually do not leach into groundwater. These compounds are eventually degraded by the microorganisms in soil and water. They can also be degraded by sunlight at the surfaces of water, soil, or plants (ATSDR, 2003). However, some of the more recently developed pyrethroids can persist in the environment for a few months before they are degraded. The extreme toxicity of pyrethroids to aquatic organisms, including fish has been measured to be a lethal concentration (LC_{50}) of 1.0ppb (Chemicalwatchfactsheet, n.d.). These levels are similar for most insects. Because they

are toxic to all insects, both beneficial insects and pests are affected by their application. Pyrethroids are moderately toxic to birds with most LC_{50} values greater than 1000 mg/kg. Birds can however be indirectly affected by pyrethroids because of the threat to their food supply (Mueller-Beischmidt, 1990).

2.3.2.2 Health Effects and Metabolism of Pyrethroids

Pyrethroids are not easily absorbed through the skin, but are absorbed through the gut and pulmonary membranes. They have been proven to be neurotoxic when administered by injection or orally. Reigart and Roberts, (2013) explained that low toxicity can be attributed to limited dermal absorption and inhalation. Although limited absorption may account for the low toxicity of pyrethroids, rapid biodegradation by mammalian liver enzymes is mostly responsible for this phenomenon. They stated further those insects without liver function, exhibit greater susceptibility to the chemical. Signs and symptoms of pyrethroid poisoning, according to Fishel, (2014) may take several forms. Persons, especially children with a history of allergies or asthma are particularly sensitive. Symptoms of acute toxicity due to inhalation include sneezing, nasal stuffiness, headache, nausea, incoordination, tremors, convulsion, numbness, facial flushing, and swelling, burning and itching sensations. The most severe poisonings have been reported in infants who are not able to efficiently break down pyrethroids (Fishel, 2014; Chemicalwatchfactsheet, n.d.). When ingested orally in significant doses, nervous symptoms may occur, which include excitation and convulsion leading to paralysis, accompanied by muscular fibrillation and diarrhea (EXTONET, 1994). Death in these cases is due to respiratory failure. Symptoms of acute exposure may last for two days. Pyrethroids and their metabolites are not known to be stored in the body nor excreted in the milk (Elliot *et al.*, 1972; Hayes, 1982).

They exit the body quickly mainly through urine and faeces. If exposure levels are high or they occur over a long time, pyrethroids can accumulate in the fatty tissue and persist in the body for a little longer. Some type of pyrethroids can also be retained for longer time in the skin and hair (ATSDR, 2003)

2.3.3 Organophosphorus Pesticides (OPs)

Organophosphate compounds were first developed by Schrader shortly before and during the Second World War. They were first used as an agricultural insecticide and later as potential chemical warfare agents (Kazemi *et al*., 2012b). In the late 1990 and early 2000, with the advent of increased awareness of terrorism, the OPs gained prominence as weapons of nerve agents for mass destruction (Kazemi *et al*., 2012a). Organophosphate pesticides (OPs) currently are considered the most widely used in the world (Ross *et al*., 2013) and the most hazardous to vertebrate animals; being responsible for many cases of poisoning worldwide, particularly in the developing countries (WHO, 1990; Soltaninejad & Abdollahi, 2009). Organophosphate pesticides have largely replaced organochlorine insecticides, having the advantage of being more rapidly biodegradable and hence less persistent in various environmental compartments (Soltaninejad & Abdollahi, 2009). Concern about the effects of organophosphates on human health has been growing as they are increasingly used throughout the world for a variety of agricultural, domestic and industrial purposes. Authors such as Karalliedde *et al*., (2001) and Ross *et al*., (2013) stated that organophosphorus pesticides have been used in agriculture and horticulture; in veterinary medicines to prevent ectoparasitic infections of farm animals and domestic pets; some human medicines (e.g. to treat head lice); and in public hygiene products both for use by professional operators and the general public to control insect infestations in public and residential buildings, outside spaces and gardens; and organophosphate compounds

are used in industry as lubricants, plasticizers and flame retardants. Many organophosphates are acutely toxic to those who may come into contact with them. Exposure to these chemicals by inhalation, swallowing or absorption through the skin can lead to health problems (Baird & Cann, 2012). Like the organochlorines, organophosphates concentrate in fatty tissues. The neurotoxic effects due to OPs poisoning are well established and involve inhibition of the enzyme acetylcholinesterase (AChE) causing changes in peripheral, autonomic and central nervous system function (the cholinergic crisis). However, the possibility that long-term low-level exposure to OPs in doses below that causing acute toxicity causes ill health is controversial (Ross *et al*., 2013).

2.3.3.1 Classes of Organophosphates Pesticides

According to Kazemi *et al*., (2012a), organophosphate pesticides are compounds derived from orthophosphoric acid esters in which a central, pentavalent phosphorus atom is doubly bonded to either oxygen (O) or sulphur (S) atoms and singly bonded to two alkyl substitutions(two methoxy ($–OCH_3$) or ethoxy ($–OCH_2CH_3$)) and a third group more labile to hydrolysis (Fig. 2.7). They are put into three main subclasses as follows:

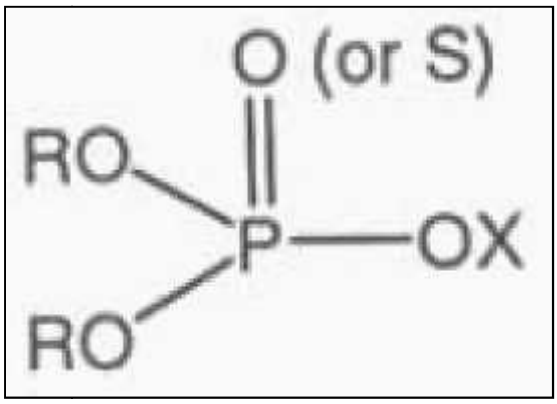
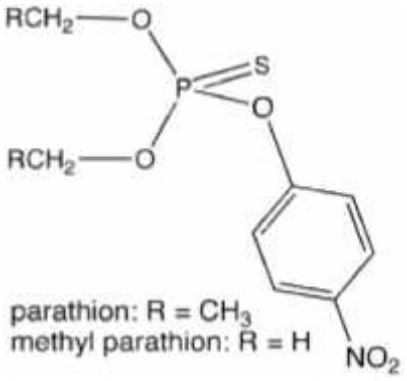


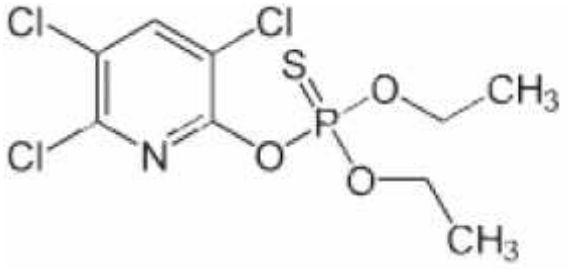
Fig. 2.7: General structure of organophosphates Insecticides (Kazemi *et al*., 2012a)
(R, normally corresponds to methyl or ethyl groups, the oxygen in the OX can be replaced by S)

In **type A** organophosphate pesticides **(Phosphates)**, the phosphorus is doubly bonded to an oxygen atom (P=O). Dichlorvos and Chlorfenvinphos are examples of type A organophosphate pesticides. Dichlorvos in particular is a relatively volatile insecticide that is used as a domestic fumigant to kill flies in the room (Baird & Cann, 1999). **Type B Organophosphates** (Phosphorothioates) contain S atom doubly bonded to P. Examples include Parathion, Chlorpyrifos, Fenitrothion and Diazinon. In type **C Organophosphates** (Phosphorodithioates), there is P-S in addition to P=S bond. Examples of type C organophosphates are Malathion and Dimethoate (Baird & Cann, 1999).

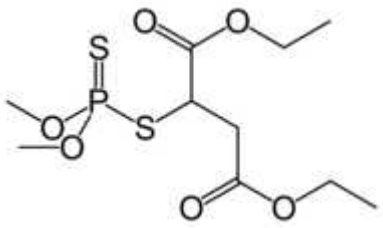
According to Baird & Cann, (1999), organophosphates containing P=S units are converted within insects to the corresponding molecule with P=O unit, which are more toxic but less stable and with lower insect penetration ability. In the presence of water molecules, the P-O bond within the structure splits to yield non-toxic substances such as phosphoric acid and its ions and alcohols (Baird & Cann, 1999). Figure 2.8 presents the chemical structures of some of organophosphorus pesticides mentioned.



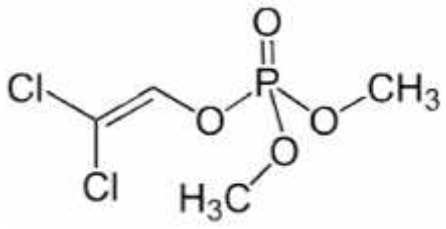
Parathion



Chlorpyrifos



Malathion



Dichlorvos

Fig. 2.8: Chemical structure of some common organophosphate pesticides (inchem.org, 2015)

2.3.3.2 Health Impacts of Organophosphorus Pesticides

The organophosphate compounds are most commonly associated with serious human toxicity. Kazemi *et al*., (2012b) reported that among pesticides, organophosphates are responsible for more than 50% of total poisoning cases whereas Kumar *et al*., (2010) asserted that they account for more than 80% of pesticide-related hospitalizations. In contrast to the past, when chlorinated hydrocarbon compounds such as DDT were commonly used, organophosphate insecticides have become increasingly popular for both agricultural and home use because their unstable chemical structure leads to rapid hydrolysis and little long-term accumulation in the environment (Kumar

et al., (2010). This widespread use, however, has resulted in increased numbers of human poisonings. In the 1970s, the United States Environmental Protection Agency (USEPA) estimated that 3,000 hospitalizations per year were registered for insecticide poisoning in the United States, with a fatality rate of 50% in the paediatric age group and 10% in adults (Kumar *et al*., 2010). In 1983 data from the American Association of Poison Control Centres indicated that the national incidence of insecticide exposures was 77,000, of which 33,000 involved organophosphates (Veltri & Litovitz, 1983).

Concerns have been raised about the increasing levels of cancer incidence and possible links with high levels of pesticide exposure. Kazemi *et al*., (2012a) reported that each year 500 people are recorded as dying from cancer in Golestan province of Iran; 350 from stomach and 150 from throat cancer. In addition, organophosphate compounds have been used as harmful nerve gases and they induce oxidative stress resulting in cell death (necrosis and apoptosis) as well as changes in metabolic and vital functions of the cells that leads to cancers. Many studies reviewed by the Ontario College, Canada show positive associations between solid tumors and pesticide exposure, including kidney cancer and failure (Abdollahi *et al*., 2004; Kazemi *et al*., (2012a). Symptoms of acute organophosphate pesticide poisoning develop during or after exposure, within minutes to hours, depending on the method of application. Exposure due to inhalation results in the fastest toxic symptoms, followed by the gastrointestinal route and finally the dermal route. Some of the most commonly early symptoms of OP poisoning include, headache, nausea, dizziness, hyper secretion (sweating and salivation), muscle twitching, weakness, and tremors, paralysis and starvation (Kazemi *et al.*, 2012a). Some organophosphorus pesticides (OPs) have been found to have endocrine disrupting properties (Kavlock, 2001). Epidemiologic studies have suggested associations between OPs exposure and reproductive disorders

(infertility, birth defects, adverse pregnancy outcomes, perinatal mortality) (Peiris-John & Wickremasinghe, 2008). Organophosphate pesticides are suspected to alter reproductive function by reducing brain acetyl cholinesterase activity and secondarily influencing the gonads.

2.3.3.3 Environmental Fates of Organophosphorus Pesticides

The fate of pesticides once they reach soil or water depends on their chemical and physical characteristics and susceptibility to various transformation and transport processes. The OPs are easily degraded by the hydrolysis of the ester linkage to produce harmless phosphoric acids and alcohols (Baird & Cann, 1999). The OPs may also leach, however, persistence, or longevity, of a pesticide influences the likelihood of leaching (Kazemi *et al*., 2012a). Soil factors that influence leaching include texture and organic matter, in part because of their effect on pesticide adsorption. Soil permeability (how readily water moves through the soil) is also important. A soil that is more permeable is susceptible to leaching of OPs (Abrahams, 2002). Certain physical and chemical properties of the pesticide, such as how quickly it is absorbed by plants or how tightly it is bound to plant tissue or soil, are also important. Typically, heavy rainfalls lead to more leaching. Organophosphate pesticides runoff can cause direct injury to non-target plants. Organophosphate pesticides runoff can also contaminate groundwater and can cause injury to crops, livestock or humans if the contaminated water is used downstream. Environmental conditions, biota, soil and sediment characteristics and water composition also influence fate of OPs residues (Abrahams, 2002). Degradation rates after release to the environment vary extensively between substances, with half life from minutes to many years. Organophosphate pesticides ingested by ruminants by means of residues on forage crops or as dermal or inhalation exposure from spray drifts are exposed to potential metabolic attack by rumen microorganisms.

2.4 Pesticide use in Developing Countries

According to Agrow, (as cited in Williamson *et al*., 2008) pesticides alone account for about US$31 billion in the global market with 2.26 million tons of active ingredients used, as at 2001. Of the world's total production, 25% of pesticides is used in developing countries. Although developing countries use only 25% of the pesticide produced worldwide, 99% of deaths due to pesticides worldwide occur in them (WHO, 2008). Developing countries experience highest death rate due to pesticides because use of pesticides tends to be more intense misused and unregulated, health and education systems are weaker in these countries (WHO, 2008). Recent trend unfortunately indicates that use of pesticides has risen in developing countries and the fastest growing markets are in Africa, Asia, South and Central America and Eastern Mediterranean (Fleischer & Waibel, 2003). It is estimated that average pesticide use per hectare in Africa is low, reported as only 1.23 kg/ha, compared with 7.17 and 3.12 kg for Latin America and Asia (Williamson *et al*., 2008) and tends to be intensively used mostly on cash crops such as cotton, cocoa, oil palm, coffee and vegetables (Matthews, Wiles, & Baleguel, 2003). In the 1980s, Nigeria ranked first among West African countries in terms of quantities of pesticides, importing about 5,346 metric tons of pesticides from the UK. Ghana ranked second with 299.5 metric tons, and Gambia third with 237.6 metric tons. Thus, Nigeria alone accounted for nearly 93% of UK pesticide exports to West African countries (Youm, Gilstrap, & Teetes, 1990).

2.5 Review of Pesticide Use in Ghana

Ghana, just like most developing countries experiences poor and inefficient pesticide practices such as using incorrect/innappropriate dosages, use of unauthorised or banned products, cocktail mixes of products, mixing with bare hands, poorly maintained or leaking application equipment,

splashing pesticides onto crops using brushes or twigs and lack of minimal protective clothing (Ntow *et a.l*, 2006; Fianko *et al*., 2010).

The findings of various researchers all agree that the estimates of the proportion of Ghanaian farmers using chemical pesticides to control insects and diseases on their food and cash crops are above recommended dosages. A 2003 survey by Dinham (2003) estimated 87% of farmers use pesticides whereas a 2008 research by Horna et al, (2008) indicates 86%. Yet still, a recent study by Northern Presbyterian Agricultural Services and Partners (2012) puts the percentage at 90. Inappropriate use has consequences not only for the effectiveness of the intended pest control but also for operator and consumer health, farm livestock, soil organisms, wildlife and vegetation, and contamination of soil, water and air.

2.5.1 Pesticides in Fruits and Vegetables

Fruits and vegetables are known to be excellent sources of dietary fibre, vitamins minerals and other natural substances that help protect the body from chronic diseases (Centers for Disease Control and Prevention, 2012). Health experts therefore recommend daily intake of them to remain healthy. In order to meet market demand, vegetable production in Ghana is on the increase. Production activity can be seen around almost every water body, including utilization of even urban sewage waters in some instances, for irrigation. Since vegetables in particular are naturally infested by myriads of pests, production tends to rely heavily on pesticide input to improve yield and market value. Thus, an attempt to improve health through consumption of fruits and vegetables come with an attendant health hazard of bacterial or/and pesticides contamination. Analysis of pesticide residues in crops in Ghana has been carried out to some extent, with the spectrum stretching across vegetables, fruits, cereals and cash crop like cocoa. Target pesticide in analyses is mainly organochlorines but currently, organophosphorus

pesticides and synthetic pyrethroids, are covered; however, carbamates, fungicides, weedicides and others are beginning to receive some attention. Even though there is enough documented work, review of literature on pesticide residue analyses shows there is no planned systematic temporal and spatial monitoring efforts by the designated authorities like the Ghana Environmental Protection Authority (Ghana EPA) and Ghana Standards Authority (GSA) to investigate levels of pesticide residues in environmental matrices and in food crops in particular on consistent basis. Work in this direction is sporadic and carried out mostly by individual researchers, academic institutions and sponsored cooperate bodies with specific or parochial interest and on ad hoc basis. Pesticide residue levels in fruits and vegetables in particular have been a matter of health and research interest considering their rate of daily consumption.

A recent study by Asiedu (2013) on residue of organochlorine pesticides, organophosphorus pesticides and synthetic pyrethroids in fruits and vegetables from three regions in Ghana recorded the presence of over 30 pesticides with incidence rate between 40-52% of the targeted pesticides. Lindane, chlorpyrifos and cypermethrin were the most frequently encountered. This singular work in three regions of Ghana (Greater Accra, Central and Eastern) reveals how widespread the use of pesticides is in the fruits and vegetable farming industry. Similarly, an earlier work done in 2009 and 2010 by Bempah, Buah-Kwofie and Denutsui (2011), in determining residue level of 7 organochlorine pesticides (OCPs) and 5 synthetic pyrethroids in about 350 locally produced fruits and vegetables in the Kumasi metropolis recorded 19% of the samples registering pesticide residue levels exceeding the European Commission Maximum Residue Limit (EC MRL). The fruits and vegetables investigated included water melon, pineapples, pawpaw, banana, mango, onions, chili pepper, tomato, lettuce, cabbage, cucumber

and okra. Within the same period of 2009 to 2010, Bempah e*t al*., (2012) conducted similar exercise in the Accra metropolitan area and found out that methoxychlor, lindane, endrin and dieldrin recorded residue levels above the EC MRL in crops such as: onions, pineapples, lettuce, cabbage, cucumber, carrot and banana. Chlorpyrifos, was however, the only organphosphate detected to be above the EC MRL; and this was in pineapple. Botwe *et al*., (2011) also recorded OCP levels in the range of 0.1-46.95ug/kg wet weight in tomato, pepper, onions and garden egg from farming communities of Akumadan in the Ashanti Region of Ghana. Even though levels in this case were low, researchers expressed health concerns because of the persistent and bioaccummulative nature of the OCPs. The presence of OCPs in food crops is a matter of much concern since most of the OCPs have been banned from use in Ghana. Their presence is often not only detected with large incidence rate but in many cases, their levels are also above recommended limits. Ntow (2008), Kokroko *et al*., (2012), Owusu-Boateng and Amuzu (2013) and Agyekum *et al*., (2014) all recorded residue levels of OCPs exceeding FAO/WHO and EC MRL guidelines in fruits and vegetables such as mango, cabbage, pineapple and tomato. Locally produced fruit-based soft drinks were also found to contain appreciable levels of OCPs (Bempah *et al*., 2011).

The presence of organophosphorus and synthetic pyrethroid pesticides has also been detected. Odhiambo, Gbewonyo and Obeng-Ofori (2014) recorded high levels of chlorpyrifos in cabbage from suburbs of Accra and Mampong-Akuapem in the range of 55-124 mg/kg; far above the FAO/WHO MRL; for instance Chlorpyrifos recorded 55-124 fold increases above the recommended MRL of 1mg/kg. Pyrethroid residues from their investigation were also not only above MRL, but 12-18 folds above levels recorded in an earlier study by Ninsin (1997), and 2-15

folds increase above recommended MRL. Gonu *et al*., (2012) investigated pyrethroid residual levels in chili pepper from Northern Region of Ghana. Their report indicated that whereas residual concentration of Fenvalerate equaled the European Union MRL, that of Fenpropathrin was above it. The presence of eight other pyrethroids was registered, though at very low levels. Gbewonyo and Afreh-Nuamah (2008) also detected the presence of cypermethrin, DDT, dimethoate and chlorpyrifos in shallots from the Volta Region of Ghana with residual level of chlorpyrifos in most of the samples above the Codex maximum permissible level. Tordzaglah *et al.*, (2013) investigated specific levels if chlorpyrifos in pineapples from Amasaman District. They analysed the extracts from epicarp and mesocarp and found out that 10% of all samples recorded residue levels above European Commission MRL of 0.05 mg/kg.

Cocoa, a cash crop and a major foreign exchange earner for Ghana has also received a considerable attention in pesticide residue analysis. Boakye (2012) investigated the levels of OCPs, organophosphorus pesticides and synthetic pyrethroids in cocoa beans from two principal producing regions in Ghana; Ashanti and Brong-Ahafo Regions. The research revealed that 45% and 50% of pesticide residue detected in Ashanti and Brong-Ahafo Regions, respectively were above their EU allowable limits, with Chlorpyrifos registering as high as 9.81 mg/kg and 10.55 mg/kg in the former and latter regions, respectively. Endosulfan and delta-lindane recorded the lowest residual concentration of 0.01mg/kg each in both regions. Similar work done by Blankson (2011) in Brong-Ahafo Region alone showed that cocoa beans contained DDT and its isomers as well as Endosulfan, although in lower concentrations. However, organophosphorus pesticides such as ethoprophos, fenitrothion, malathion and profenofos registered residual levels of 0.0573 mg/kg, 0.0537 mg/kg, 0.0581 mg/kg and 0.6368 mg/kg respectively; which were all higher than

the maximum limit set by the EU. Synthetic pyrethroids such as permethrin, cypermethrin and fenvalerate also registered residual concentrations above their EU MRL. Lindane, a pesticide that has been listed among the Prior Informed Consent (PIC) pesticides is heavily dependent upon by cocoa farmers in controlling capsids. Owusu-Ansah *et al.,* (2010) as well as Apau and Dodoo (2010) investigated the levels of lindane in cocoa beans in Ashanti and Central Regions of Ghana respectively. Whereas Owusu-Ansah *et al*., (2010) reported no detectable concentration of Lindane in all samples, Apau and Dodoo (2010) recorded a residue range of 0.055-3.318mg/kg; a concentration range that indicated levels above the EU MRL. Frimpong *et al*., (2012); Frimpong, *et al*, (2012a, 2012b & 2012c); Frimpong *et al*., (2013) and Frimpong, Yeboah, and Fletcher, (2013) monitored over 15 OCPs in cocoa beans ready to be exported from Tema and Takoradi port of Ghana. The OCP residual concentrations in all cases were low, ranging from 0.01-103.0 µg/kg. The residual presence of organophosphorus pesticides such as: dimethoate, pirimophos-methyl, chlorpyrifos and fenitrithion were also low. Methamidophos, profenofos and malathion concentration were however appreciable, being at the threshold of EU MRL. Synthetic pyrethroids were also detected but in traces. Allethrin, fenvalerate and cypermethrin average concentrations bordered on the Japanese MRL threshold.

Work done by researchers in other crops may exist but not easily accessible. The work of Akoto, Andoh, Darko, Eshun, and Osei-Fosu (2013) indicates even cereal crops are not exempted from pesticide contamination. Maize and cowpea from Ejura in the Brong-Ahafo Region were assessed by Akoto et al for the presence of OCPs, organphosphorus pesticides and synthetic pyrethroids. Fifteen OCPs were detected and 5 of them: β-lindane, β-endosulfan, Heptachlor, *p,p'*-DDT and *p,p'*-DDE had levels above their EU MRL. So also levels of synthetic pyrethroids

such as Fenpropathrin and λ-cyholothrin exceeded their respective EU MRL. In maize, with the exception of Chlorpyrifos, levels of organophosphorus pesticides were all within limits.

2.5.2 Pesticides in Fish

According to Akan, *et al.,* (2013a) presence of pesticides in water has the potential to impart objectionable and offensive taste, odours and colours to fish and aquatic plants. Pesticides in water column normally adsorb to sediment particles and the sediment particles in turn adsorb to entities within the aquatic medium. Pesticide-laden sediments are easily adsorbed by fish on the skin and also on the gills; during feeding by ingestion of water. Persistent pesticides like the organochlorines readily accumulate in animal tissues and since they are poorly hydrolysed, they cause serious bioaccumulation and bioconcentration problems in food chains (Nowell *et al.,* 1999). The immediate banks of every fresh water body in Ghana are subjected to human activities of one kind or the other, especially agriculture. Fish constitute a very important protein source in diet all over the globe and it is necessary to have a continuous investigation into levels of pesticides in their tissues, particularly because the water bodies in which they are found invariably receive run-offs from adjacent agricultural fields. The dearth of investigations on pesticide content of fish species in the country has resulted in scanty information. The few researches done in this field have been sporadic over time and results reveal generally low residual levels of pesticides.

Darko, Akoto & Oppong (2008) investigated the levels of OCPs in *Tilapia zilli* from Lake Bosomtwi and registered the presence of endosulfan, aldrin, dieldrin, lindane, *p,p′*-DDE and p,p′-DDT within a concentration range of 0.018 – 5.232 ng/kg. DDE and DDT both recorded highest incidence and concentration. Essuman, Togoh & Chokky (2009) also evaluated the presence of

four OCPs (*o,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT and Propiconazol) and four organophosphorus pesticides (Fenitrothion, Chlorpyrifos, Dichlorvos and Diazinon) in one fish species, *Sarotherodon melanotheron* from Fosu and Etsii lagoons in Central Region of Ghana. Whereas *o,p′*-DDE was not detected in any of the fish sample, the other three OCPs were present, albeit at very low concentration, in a range of 0.0001 – 0.0098 mg/kg. Similarly, only Diazinon and Chlorpyrifos were registered in very low concentration range of 0.0001 – 0.0003 mg/kg. Afful, Anim & Serfor-Armah (2010) investigated pesticide residue levels in six fish species from Densu River with respect to 14 OCPs. The fish species were: *Heterotis niloticus, Channa obscura, Hepsetus odoe, Tilapia zilli, Clarias gariepinus and Chrysichthys nigrodigitatus.* Seven of the targeted OCPs recoded 100% incidence whilst the rest recorded 75%, indicating the ubiquitous nature of these chemicals. Their levels were however low, in a range of 0.3-71.3 ug/kg. Gbeddy *et al*., (2012) in a separate study focused on the presence of 15 OCPs in gills and muscle tissues of only two species of fish; *Tilapia zilli* and *Chrysichthys nigrodigitatus* from the Volta Lake. Thirteen of the targeted OCPs were identified in all the samples. Whereas 100% incidence was recorded for beta-lindane, delta-lindane, *p,p′*-DDD, Heptachlor and Endosulfan sulphate in the muscle; beta-lindane, delta-lindane, *p,p′*-DDD, gama-chlordane, Endosulfan-sulphate and Methoxychlor also registered 100% incidence in the gills. The mean residue concentration ranges in muscle and gill tissues were 0.10-17.35 ng/g and 0.56-37.75 ng/g, respectively. A recent study by Adu-Kumi *et al*., (2010) on edible fish in Lake Volta recorded the presence of DDT compounds, Chlordane compounds, Hexachlorobenzene and Lindane isomers in tilapia and catfish. This research report suggests that the Volta Lake and its environs are possibly experiencing fresh contamination by diverse kinds of organochlorine chemicals; both banned and restricted.

2.5.3 Pesticides in sediments of water bodies in Ghana

Pesticides in aquatic medium can bioconcentrate unto suspended materials and get deposited to the substratum. Sediment serves as a principal reservoir and sink from which pesticides can be released to groundwater, redistributed into the overlaying water column and as well be adsorbed to suspended particulate matter and aquatic flora and fauna (Xue, *et al.*, as cited in Williams, 2013). Investigations carried out on Ghanaian water bodies revealed that sediment contain appreciable levels of pesticides, especially, organochlorine pesticides. Darko et al., (2008) detected lindane, endosulfan, aldrin, *p,p′*-DDE and p,p′-DDT in the sediment of Lake Bosomtwi at concentrations of: 6.755, 9.683, 0.065, 0.072, 8.342 and 4.410 ng/kg respectively; with DDE being widespread, registering occurrence frequency of 98%. Later studies by Afful, Awudza, Osae, and Twumasi, (2013a) on the same water body registered 16 OCPs, and at higher concentration levels; along with 5 polychlorinated biphenyls (PCBs). The concentration range of the 16 detected OCPs were 0.01 – 15.23 ug/kg. Endosulfan sulfate recorded the highest of 15.23ug/kg, followed by methoxychlor and β-endosulfan at 5.95 and 5.60 ug/kg respectively.

Ntow (2005) analysed the presence of six OCPs namely: Lindane, α-endosulfan, β-endosulfan, endosulfan sulphate, *p,p′*-DDT and p,p′-DDE, in the Volta Lake and found out that these chemicals were very widespread, with the least frequency of 86% and individual mean concentration of 2.30, 0.21, 0.17,0.36, 9.00 and 52.3 ug/kg dry weight respectively; indicating the persistence of DDT isomers. Fianko *et al.*, (2011) and Kuranchie-Mensah *et al.*, (2012) investigated the residual levels of 14 OCPs in Densu river sediment and reported high levels. The 14 OCPs included Dieldrin, Endrin, Endrin ketone, Endrin aldehyde, Methoxychlor, Heptachlor, Aldrin, γ-lindane, δ-lindane, DDT, DDE, α-endosulfan and α-endosulfan sulfate. Fianko *et al*, (2011) reported concentration range of 0.10ug/kg – 163.0ug/kg dry weight whilst that for

Kuranchie-Mensah *et al.*, (2012) was 0.03 ug/kg – 10.98 ug/kg dry weight. Alpha-endosulfan and Aldrin registered the highest concentrations by both investigators. The huge difference in concentration range as reported by both researchers could be due to different locations of investigation along the same river. Whereas Fianko *et al.*, targeted the main Densu and its tributaries in the forest zone where industrial crops such as cocoa, coffee, oil-palm and food crops are produced on large scales, Kuranchie-Mensah *et al*, sampled further down at Weija where the Densu River was dammed to form a reservoir for potable water supply. Afful *et al*., (2013) carried the investigation further by assessing levels of synthetic pyrethroids, namely: bifenthrin, fenpropathrin, lambda-cyhalothrin, cyfluthrin, cypermethrin, fenvalerate, deltamethrin, allethrin and permethrin in sediment of Weija Lake. The concentration range was 0.15 – 6.60 ug/kg. Even though the concentration levels were below maximum residue limit set by the EU, the registering of the presence of the wide spectrum of pyrethroids in this investigation indicates increasing diversification in the use of pesticides.

2.5.4 Pesticides in Ghanaian Waters

Researchers have reported presence of pesticides in various water bodies in the country. The Volta Lake, although by far the biggest water body in the country, has not received adequate study into levels of pesticides in its waters. Ntow (2005) monitored the residues of chlorinated pesticides in one hundred and eighty water samples from six locations in the Volta Lake. Out of these, only four organochlorine pesticides were detected, namely: Lindane, α-endosulfan, β-endosulfan and Endosulfan sulphate. About 23% of the samples contained detectable levels of Lindane with a mean concentration value of 0.008µg/L. Alpha-endosulfan was present in the highest concentration, with a mean value of 0.036µg/L, being detected in 16% of samples analysed. B-endosulfan was detected in 18% of the samples while Endosulfan sulphate was

found in 10% of the samples. DDT and DDE were however not detected. Fianko *et al.*, (2011) reported the presence of OCPs in 3.3% of samples of water from River Densu. Concentration of detected OCPs varied between 0.1µg/L and 48.6 µg/L in compounds such as Dieldrin, DDT, Endosulfan sulphate, γ-HCH, Aldrin, γ-chlordane, DDE and Endrin. γ-HCH and γ-chlordane recorded the highest frequency of 4.4% each, and Endosulfan sulphate recorded the highest residual concentration of 48.6µg/L. Afful, Awudza, Osae, and Twumasi, (2013b) detected the presence of seven synthethic pyrethroids in water from Densu around Weija in a low range of 0.1 µg/L – 3.5 µg/L. Cyfluthrin was most ubiquitous, with 100 per cent frequency of occurrence. Agyapong, Lugushie, Fei-Baffoe and Atabila (2013) also recorded the presence of seven OCPs in waters from the River Tano in Brong Ahafo of Ghana. The OCPs were: Lindane (and all its metabolites), Aldrin, Dieldrin, Endrin, Chlordane, Heptachlor and all metabolites of DDT. Their results indicated that the mean concentrations of aldrin, dieldrin, endrin, heptachlor and cis-heptachlor epoxide all exceeded the WHO limits and could pose a threat to human and aquatic life within the watershed.

Darko *et al*., (2008) and Afful *et al*., (2013) studied the water quality of Lake Bosomtwi whereas Darko et al recorded the presence of only 5 OCPs, later investigation of Afful *et al*., (2013) recorded more than 15 organochlorine compounds, including Polychlorinated biphenyls (PCBs). This indicates degeneration of water quality in the lake with time. The reason could only be attributed to the increasing anthropogenic activities around the lake, especially agricultural ones. Botwe, Ntow and Nyarko (2012) investigated 2 streams and 9 ground water sources (wells) in the Ashanti region. The stream water registered the precence of α-endosulfan, β-endosulfan and Endosulfan sulphate at the mean concentrations of 0.027, 0.021 and 0.011 µg/L respectively. None was however detected in ground water.

2.6 Government Policy and Regulation on Pesticides

Ghana is a signatory to a number of international Conventions on chemicals and as such is under obligation to put proper policies and regulations in place for safe importation, management and use of chemicals and pesticides.

2.6.1 Signatory to International Regulations

The main international regulations and legal instruments relating to pesticides use and to which Ghana is a signatory are:

The International Code of Conduct for the Distribution and Use of Pesticides (also known as the FAO Code of Conduct), which is a voluntary international mechanism for countries to regulate the availability, distribution and use of pesticides in their countries. It was revised in 2002. (Northern Presbyterian Agricultural Services and Partners, 2012)

The Rotterdam Convention on Prior Informed Consent is an international treaty designed to facilitate informed decision-making by countries with regard to trade in hazardous chemicals. It establishes a list of covered chemicals and requires parties seeking to export a chemical on that list to first establish that the intended importing country has consented to the import. It also requires that a party seeking to export a chemical that is not listed under the Convention, but is subject to a ban or severe restriction in its own territory, must provide notice to the importing country of the proposed export. The Convention entered into force on February 24, 2004 (UNEP, 2005, 2008). By this provision, exports and imports of banned or severely restricted pesticides is prevented or highly restricted. It also encourages identification of pesticides that cause problems to health or the environment. Ghana signed the Rotterdam Convention in 1998 and ratified it in 2003 (Northern Presbyterian Agricultural Services and Partners, 2012).

The Stockholm Convention on Persistent Organic Pollutants, which aims to protect human health and the environment from persistent organic pollutants (POPs). Parties to the Convention are obliged to develop a National Implementation Plan (NIPs) which identifies the current state of these chemicals and their management and articulates the policy, program, priorities and activities that Parties will take to eliminate the use of these chemicals (FAO, 2006).

The ILO Convention on the Safety of Chemicals at the Workplace

Ghana is a party to this convention. Ghana's response to the needs of this Convention however is a comprehensive piece of legislation on labour. This law is divided into several parts and includes Occupational Health, Safety and Environment. Under this last part employers are enjoined to ensure that their employees work under safe and healthy conditions. Furthermore they are to protect workers from "toxic gases, noxious substances, vapours, dust, fumes and other substances likely to cause risk to safety or health. Failure on the part of the employer to do such or to report any occupational accident is punishable. Labour Inspectors have the right to take or remove for purposes of analysis, samples of materials and hazardous or chemical substances used or handled by workers in the course of their employment (FAO, 2006).

Although Ghana is a signatory to all the aforementioned international regulations, there is little compliance or implementation of them, largely due to insufficient allocation of resources (Northern Presbyterian Agricultural Services and Partners, 2012). For instance the Ghana EPA proposed the creation of a specific Fund for pesticide management to increase resources available for pesticide surveillance and training. This was necessary since the existing Environmental Fund never had any specific allocation for pesticide surveillance. In this direction, industries agreed to contribute 0.1 per cent of the values of their import to the fund, yet

the proposal was rejected by the Ministry of Finance (Northern Presbyterian Agricultural Services and Partners, 2012). The government of Ghana, as a matter of fact, is aware of the dangers of pesticides and has taken a number of measures to ensure their safer use. One important step is the establishment of the legislative framework to manage and control the use of all manner of chemicals.

2.6.2 National Legislative Framework for Pesticides Management

Even though there are various laws on chemical management in existence, there is no comprehensive piece of legislation on chemicals management in Ghana (EPA Ghana, 1997, 2007). Laws on Chemicals Management are scattered in various pieces of Legislation. Together, these laws provide a framework for the management of all chemicals and pesticides in Ghana. They include Principal Acts, Subsidiary legislation, guidelines, codes of conduct and practice (FAO, 2006).

2.6.2.1 The most important Act is the **Environmental Protection Act of 1994 (Act 490**). This Act forbids any pesticide imports except where they have been registered, requires the Ghana EPA to keep a list of registered pesticides and requires all importers and sellers to keep a record of the pesticides imported or sold. The Act also allows for EPA inspections to take place on grounds of reasonable suspicion that illegal pesticides are being stored and for pesticides and equipment to be seized. The EPA is the key Agency responsible for the management of all Chemicals in Ghana. The Act establishes a Committee of the Environmental Protection Agency Board known as the Hazardous Chemicals Committee. The functions of the Hazardous Chemicals Committee are to monitor the use of hazardous chemicals by collecting information on the importation, exportation, manufacture, distribution, sale, use and disposal of such

chemicals; advise the Board and the Executive Director on the regulation and management of hazardous chemicals; and perform such other functions relating to such chemicals as the Board or the Executive Director may determine (EPA Ghana, 1999). To be able to perform its duties under the Act, the EPA has as one its Divisions, the Chemicals Control and Management Centre (CCMC) which plays a vital role in the management of Chemicals in Ghana. The CCMC has as its primary objective, to protect human health and the environment from the possible effects of chemicals The CCMC issues Chemical Clearance Permits to importers of Industrial Chemicals. One permit is given per each import. The CCMC also supervises the disposal of obsolete chemicals.

2.6.2.2 The Pesticides Control and Management Act, 1996 (Act 528)

Act 528 is the only piece of legislation that addresses the manufacture, classification, labeling, importation, exportation, and use of pesticides in Ghana. This Law has very broad application and affects government, companies, manufacturers, users, vendors, importers, exporters, advertisers and formulators. The law focuses on the registration of pesticides, the restriction and suspension of the use of pesticides, the licensing of pesticide dealers and the penalties for non-compliance. Act 528 stipulates that no person shall import, export, manufacture, distribute, advertise, sell or use any pesticide in Ghana unless the pesticide has been registered by the EPA. The Agency will only register a pesticide if it is satisfied that the pesticide is safe to use under Ghana's local conditions and effective for the use for which it is intended (FAO, 2006).

2.6.2.3 The Food and Drugs Law, 1992 (Act 305B)

This Act aims at regulating the manufacture, preparation, sale or supply, export or import and use of food, drugs, cosmetics and chemical substances so as to protect the health of consumers.

The food and Drug Law Act established a body known as the Food and Drugs Board (FDB), which has the responsibility of performing the administrative functions under the Law. Though this law does not make specific reference to banned or severely hazardous chemicals, the word "chemical substance" is defined under the Law as "any substance or mixture of substances prepared, sold or represented for use as a germicide, an antiseptic, a disinfectant, a pesticide, an insecticide, a rodenticide, vermicide, or detergent" (FAO, 2006). The Law prohibits a person from manufacturing, preparing, selling or supplying, exporting or importing into Ghana any chemical substance unless it has been registered with the FDB. There seems to be an overlap in the regulatory functions of FDB and EPA. In practice, however, the FDB stays clear of the registration and regulation of pesticides use. Despite this fact, there is still the need to rectify this obvious overlap so as to ensure that the functions of each body are clarified. With regard to enforcement, it is an offence for any person to sell any chemical substance (that has in or on it any substance) which when used according to the directives on the label accompanying such pesticide may cause injury to the health of the user. The Act empowers authourised officers of the FDB at any hour reasonable for the proper performance of their duty to open and examine any receptacle or package, which he believes, contains any pesticide. Such officers also have the power of seizure of such products. Furthermore, it an offence for any person to use or dispose of any chemical substance (pesticide) in a manner likely to cause contamination of food or water for human consumption or in a manner likely to be injurious or dangerous to the health of any person. It is an offence under this law for anyone to label, package, sell or advertise any chemical substance in a manner that is false, misleading or deceptive as regards its character, constitution, value, potency, quality, composition, merits or safety. The Law prescribes the penalty for offences committed as either the payment of a fine or a term of imprisonment or both.

2.6.3 Control of Imports and Exports

2.6.3.1 Export and Import Act, 1995 (Act 503)

This Act seeks to regulate the efficient import and export of all goods, including chemicals, into and from Ghana. The Customs, Excise and Preventive Service (CEPS) play an important role in the enforcement of this Law. The Law stipulates that where an importer or exporter is required under any other enactment to obtain a permit or license or certificate, in addition to other certification for any category of goods, certified copies of same shall be given to the Commissioner of CEPS (FAO, 2006).

2.6.3.2 The Customs, Excise and Preventive Service (Management) Law of 1993

The Customs, Excise and Preventive Service (Management) Law of 1993 regulates all imports into and exports from Ghana, including chemicals. CEPS currently performs certain duties on behalf of EPA. It examines documents and as well as certificates and permits granted by the EPA to ensure that they cover the particular importation or exportation that the bearer of permit claims. Records of chemical import returns are submitted by CEPS to the EPA on a quarterly basis. The law gives CEPS officers the power to search persons, premises and baggage and seize prohibited goods, including chemicals, and they are required to prevent the illegal import into Ghana of any pesticide. In order to implement this law, CEPS officers are given the same powers as the police. They are also required to keep a list of all pesticides imported into the country for onward submission to the EPA. The Plant Protection and Regulatory Services Directorate of the MOFA, (PPRSD) also has staff – quarantine staff – at border posts looking for illegal imports of pesticides, among other things (FAO, 2006).

CHAPTER THREE

STUDY AREA

3.1 Background and Reconnaissance survey of the study area

The River Afram is a major tributary of the Volta Lake in Ghana. Gordon and Amatekpor (1999) noted that after the creation of the Volta Lake following its damming in 1964, there was flooding of large area that affected about 80,000 people. The villages and farmlands of these people were inundated by water and the Ghana government had to put these people in new resettlement townships further upland beyond areas demarcated for the newly created lake. The Afram tributary was one of the major outlets of the then River Volta and as such experienced serious rise in water levels, leading to flooding of its banks and consequent relocation of communities. It is worthy of note that during the creation of the Volta Lake, potential for irrigation farming on large tracts of lands in the Afram plains in particular was recognized. As such, experimental irrigation farming of vegetables was set up in three communities, namely Ampem, Dedeso and Amartey (Gordon and Amatekpor, 1999).

The study area occupies part of the Eastern Region that lies along the lower reaches of the Afram tributary of the Volta Lake (Fig. 3.1) and for the purpose of this research, it will be referred to as the Afram arm of the Volta Lake (AVL). This area extends across three administrative districts (Fanteakwa, Kwahu South and Kwahu East) of the region. In the quest to select the appropriate sites for the study, a stretch of land area and communities bordering on the lower reaches of Afram arm of the Volta Lake (AVL) was surveyed from lower part of Fanteakwa District, through Kwahu South to the northern part of Kwahu East districts, lying within the coordinates: N 06^0 34′ 124″ - 06^0 43′ 696″ and W 000^0 14′ 089″ - 000^0 37′ 810″ (Fig. 3.1). Many of the

communities within this area are involved in food crop production along the banks of the AVL, but of particular interest is the predominance of irrigation farming of fruits and vegetable crops; both on small holder and large commercial basis. The farm size of the small-holder farmers was generally less than one acre while others are much bigger. Sampling sites near six communities on the lower banks of the AVL were chosen for the study. These are: Odortom, Dedeso, Kwahu Amanfrom, New Jerusalem, Adawso and Kotoso. Access roads to the communities are very deplorable and barely motorable. The communities were therefore chosen considering relative ease of accessibility to them; and also because farming along the banks here is done on large scale compared to other bank. The location of these sampling sites along the AVL enables the study to span the entire or the greater length of the Afram tributary (Fig. 3.1) and hence can be considered to be fairly representative of the entire region along both banks of the Afram tributary that engage in fruits and vegetable farming.

3.2 Ethnicity and livelihood of people in the study area

Eastern Region has a heterogeneous population made up mostly of the Akans and the Ga-Adangbes. Other major ethnic groups are Ewes, Guans and ethnic mix from the Northern Region of Ghana. Ewes, and to a lesser extent, the Ga-Adangbes are however the predominant inhabitants along the AVL as fisher folks. Dependence on fishing solely is no longer sustainable hence the practice of irrigation farming which over the years has turned out to be more economically profitable.

Available records indicate that as at 2013, an estimated 5,753 and 4,700 farmers were actively engaged in market-oriented farming along the Afram arm in Kwahu East and Fanteakwa Districts respectively; and these figures constitute about 72% and 75% of the farming population

of the respective Districts (Fanteakwa and Kwahu East District Profiles). Cultivated food crops along the banks include sweet potatoes, maize, cassava, cowpea and fruits and vegetables. Due to the short maturing period of fruits and vegetables, they are cultivated all-year round with reliance on water from the Afram tributary for irrigation. The main fruits and vegetables cultivated are pepper, onions, okra, tomato, watermelon and egg plants. As at 2013, the estimated annual production in metric tonnes of pepper, egg plant, onions water melon and cowpea respectively were: 25,292.95, 12,673.50, 1,077.79, 10,425.24 and 787.15 in the Fanteakwa District alone. These productions came from farmlands of average area of about 0.4 hactare per holder (Fanteakwa District Assembly, 2014). Similarly, the average crop production per holder in the Kwahu East District with about 0.75 hactares for pepper and water melon; and 0.25 ha for okra and tomato were 456, 480, 90 and 273.60 metric tones respectively (Kwahu East District Assembly, 2014).

3.3. Geology, soil, topography and drainage, of the study area

The topography of the study area is generally hilly and rugged with undulating landforms. The average height of the land is about 8762 feet above sea level. Underlying these land masses are several rocks or parent rocks from which several different soils have developed. These parent rocks include the Birimian foundation and Voltaian Metamorphosed sediments, with their associated rocks such as phyllites, schist and granites. Most of the hills are capped with iron pans, bauxite and kaolin. Gold and bauxite are also embedded within these rocks. The area is well drained with several rivers portraying the trellised drainage pattern system. Some of the important rivers are the Afram, Pra, Akrum, Osubin, Amanfuesua and Dede rivers. Most of these rivers are seasonal with most of them overflowing their banks during the rainy season and drying up during the dry season (Fanteakwa District Assembly, 2014). The river Afram is a major tributary of the Volta and flows through the northern border of the Kwahu South District within the Afram Plains while the Pra River take its source from Kwahu Twenedruase and flows through Akwasiho and Kwahu Praso where it leaves the district.

The Volta Lake is an important resource which provides employment to many fishermen and fish mongers who have settled along the lake (Kwahu South District Assembly, 2014). The underlying Birimian formation in the Kwahu South District is an important geological formation since it contains most of the valuable mineral exported from the country for foreign exchange such as gold, bauxite, diamonds etc. Gold is believed to be in the district but currently remains unexploited. Soils developed over these rocks in the district are the Atewa-Anum simple formation (association) Nzima-Bekwai/Oda compound association, Atewiredu-Katie Simple Association, Bediesa-Yaya/Asuansi-Atewa complex association, Nankesi-Akrosi/Nta Offin compound association (Fanteakwa District Assembly, 2014). Soils belong to the forest ochrosols and consist of fine sand

loams, congregational loams, non-gravel sandy clay loams and iron pan soils. These soils possess good chemical properties of clay and appreciable amount of humus, making them generally fertile for the production of both cash and food crops such as cocoa, coffee, plantain, cassava and yams.

3.4 Climate and Vegetation

Information from the three district assembly offices (Kwahu East District Assembly, 2014; Kwahu South District Assembly, 2014 and Fanteakwa District Assembly, 2014) within the study area presents the same account of climate and vegetation. The study area lies within the wet-semi equatorial region with mean annual rainfall between 150.0mm to about 2000mm. The area experiences all year round sunshine with an average temperature of 24° C. During the rainy season there are brief interruptions of the sun by thick cloud cover. There is a double maxima rainfall during the period of June and October in each year with just slight deviations. Rainfall intensity however, decreases towards the Voltaian basin. Mean monthly temperature ranges from high of 30^{o}C in the dry season to about 26^{o}C in the wet season. It is worthy of note that the relatively higher altitude has moderating influence on the local temperature. Relative humidity ranges between 75% and 80% (Kwahu South District Assembly, 2014). The study area lies within the Semi-Deciduous forest zone. The vegetation is dense in terms of tree coverage with most trees shedding off their leaves in the dry season. Trees of economic value like Odum, Wawa and Sepele are found in the forest. The forest is made of three layers namely the upper, middle and lower layers. A greater part of the natural vegetation has been altered due to anthropogenic activities. The forests however, remains in their natural state in the five (5) reserve areas namely the Southern Scarp Forest, Oworobong South, Abisu, Northern Scarp West, Oworobong South, Northern scarp West and Oworobong North Forest reserves. Together, the reserves cover a total of 37, 070 hectares of land.

CHAPTER FOUR

PESTICIDE CONTENT OF FALLOW LANDS

4.1 Background

The banks of the Afram arm of the Volta Lake are intensively cultivated for all kinds of crops, owing to availability of water for irrigation. In particular, fruit and vegetable cultivation which is much dependent on water availability is carried out all-year round. In order to increase yield and maximize economic gains, there is over-reliance on agro-chemicals and in particular, pesticides. Due to the persistence, bioaccumulation and health effects of the organochlorine pesticides, their use has been discouraged in favour of the less persistent new generation pesticides like the organophosphates and the synthetic pyrethroids. Wide range of pesticides belonging to these groups is indiscriminately used to control insect pests. Although it has generally been assumed that this new generation pesticides rapidly degrade in the environment, they are known to be more toxic and reports indicate that a good number of them are sufficiently persistent to contaminate the environment (Schimmel *et al.*, 1983; Kjolholt, 1985; Barcello *et al.*, 1990; Readman *et al.*, 1992; Ragnarsdottir, 2000). For instance Readman *et al.*, (1992) found chlorpyrifos and parathion to be stable and sufficiently persistent and hence contributed to the contamination of tropical marine sediments. Bifenthrin has been established to have a half live of up to 8 months in soils (Extension Toxicology Network, 1995); cypermethrin, up to 8 weeks (Extension Toxicology Network, 1996a) and permethrin up to 43 days (Extension Toxicology Network, 1996b).

With the introduction of mechanized irrigation, farming goes on all-year round along the Afram banks on crop and land rotation bases. Lands on the immediate banks of the river are keenly competed for since irrigation efforts on such lands are reduced. Here, crop rotation is practiced mostly as a way of maximizing the use of a piece of land, rather than cultural means of managing

pests. Nevertheless the practice of land rotation also occurs. In the land rotation system however, previously cultivated lands do not have enough fallow period. Typically, they are left uncultivated temporarily for a maximum period of one vegetable growing season, i.e. 3 months. The lands are therefore under pressure of cultivation all-year round. Since cultivation is heavily dependent on agro-chemicals (Gordon and Amatekpor, 1999), it is pertinent to know the pesticide content; both the organochlorines and the new generation pesticides, of the abandoned farmlands at the time of their recultivation. The objective of this chapter therefore is to assess pesticide content of fallowed fields prior to recultivation, as well as to determine if the presence of banned organochlorine pesticides was due to current or historical inputs.

4.2 Materials and Method

4.2.1 Collection of Soil Samples

Cultivated lands that were temporarily left uncultivated for a maximum of three months were the target for sampling (Plate 1). Sixty-six composite soil samples were taken from farmlands on the banks of the Afram arm of the Volta Lake (four sampling sites: Odortorm, Kwahu Amanfrom, Adawso and Kotoso – Fig. 3.1) between March to October 2012. The number of farmlands sampled in each of these sites depended upon the willingness of the farmers to permit sampling on their lands. At Odortom site, 14 samples were obtained; 17 from Kwahu Amanfrom; 19 from Adawso and 16 from Kotoso. One composite sample consisting of nine sub-samples was collected from each farm by random sampling within a grid according to British Crop Protection Council, (2003). Three transects were established in each farm, with the middle transect approximately equidistant from the other two. A margin of 1 to 2 metres was demarcated from the edges of each

farm and this margin was not included in the sampled area. This was done to ensure all samples were taken within the main farm area. With the help of hand trowel, samples were taken at three point along each transect at a depth of 0 – 20 cm. The subsamples were placed in a thoroughly cleaned plastic bucket, well mixed with the hand trowel and sieved through a 2 mm brass soil sieve to separate liter, roots large stones and other large exogenous objects. Approximately 1 kg of the sieved soil was then placed in a zip-locked plastic bag and labeled and tightly enclosed in another polythene bag. One extra soil sample was collected in each community from a seemingly pristine and uncultivated field and used as matrix blank sample. All samples were stored on ice transported within 24 hours to the Ghana Standards Authority laboratory where they were subsequently stored in a freezer until analysis. Enclosure of samples in the second plastic bags was a precautionary measure to prevent samples from being soaked by melting ice blocks during transportation.

4.2.2 Chemicals and Reagents

All chemicals and reagents used in this study were of purest grade and are listed in Appendix I

4.2.3 Preparation of soil samples for pesticide extraction

Wet soils were used for extraction. This approach is more convenient and desirable because it minimizes potential contamination and possible volatilization loses due to drying (Jayaraman, 2001; Muir & Sverko, 2006).

Soil samples from storage freezers were remove and allowed to thaw. The percentage of water in each sample was determined by accurately weighing 10 g, drying at 105^{o}C and reweighing to a constant dry weight. A wet weight equivalent to 10 g dry sample was calculated for each sample

after determining the percentage of water. Therefore, wet soils equivalent to 10 g of dry soil were extracted per sample.

4.2.4 Extraction of pesticides from soil samples

Comminuted homogenous wet weight soil samples equivalent to 10 g dry samples were weighed into 100 mL separating flasks and 10 mL acetonitrile added to each. The flasks were stoppered and sonicated for 5 minutes in an ultrasonic bath, Decon FS400B (Plate 10a). A further 10 mL acetonitrile was added to each flask, placed on a horizontal mechanical shaker and set to shake continuously for 30 minutes at 300 motions per minute. The mixture in each flask was allowed to stand for 10 minutes to separate layers, as demonstrated by plates 7a and 7b. The organic phase (top layer) was decanted and dried over 5 g anhydrous magnesium sulphate through No. 1 Whatman filter paper. Ten mL aliquots of the filtrate were introduced into 50 mL round-bottomed flasks and evaporated to 1 mL concentrate using Buchi rotary vacuum evaporator (Plate 11).

4.2.4.1 Clean-up of extracted samples

Two grammes of silica gel that has previously been activated at 130^0C for 2 hours was carefully packed into 10 mL polypropylene cartridge columns and capped with a 1 g anhydrous sodium sulfate. This was conditioned with 10 mL acetonitrile. The 1 mL extract concentrates were loaded onto cartridges by washing with 5 mL acetonitrile and the flow rate adjusted to 3 mL per minute, with the help of visiprep vacuum manifolds. The eluates were collected into 50 mL pear-shaped flasks. The cartridges were then further eluted with another portion of 5 mL acetonitrile. The combined eluates were then evaporated just to dryness at 39^oC and 77 mbarr pressure, using vacuum evaporator (Buchi R.210). The concentrate was re-dissolved in 1 mL ethyl acetate and

transferred into a 2 mL standard opening vial prior to analysis by gas chromatography equipped with electron capture detector (GC-ECD) for quntitation of organochlorine and synthetic pyrethroid pesticides. And also chromatography equipped with pulse-flame photometric detector (GC-PFPD) for organophosphorus pesticides quantitation. Until the GC determinations, the extracts were stored frozen. On occasions when a final 1mL extract appeared a bit turbid, it was introduced into a 15 mL centrifuge tube, frozen for one hour and centrifuged for 5 minutes at 3000 rpm (revolutions per minute) (Plate 10b). The top clear liquid is then taken into a 2 mL standard vial, ready for gas chromatographic quantitation.

4.2.5 Preparation of stock pesticide mix standards solution

Pesticides stock solutions (1000 µg/mL) of individual standards were prepared by dissolving 25 mg corrected by purity of the individual pesticide in 25 mL of ethyl acetate. 0.25 mL aliquot of each prepared pesticide stock standard solutions was accurately pipetted into 25 mL volumetric flask and the volume made up to 25 mL mark with ethyl acetate. This gives a concentration of 10 µg/mL for each pesticide in the mixture. An aliquot of 2.5 mL of the prepared mixed standard solution was pipetted into another 25 mL flask and again the volume made up to the 25 mL mark with ethyl acetate to give a solution with a resultant concentration of 1 µg/mL. The mixed pesticides standards solution for organochlorines contained 15 targeted organochlorine pesticides; that for organophosphates contained 13 pesticides and that for synthetic pyrethroids contained 9 pesticides. The list of pesticides is presented in Appendix I.

4.2.6 Calibration of Instrument (Gas chromatograph)

From the stock standard mix solution, calibration standards of 0.005 µg/mL, 0.01 µg/mL, 0.02 µg/mL, 0.05 µg/mL, 0.1 µg/mL and 0.5 µg/mL were prepared to check the linearity of the determination. 1.0 µL of each of the standard mix organochlorine pesticides (OCPs) and synthetic pyrethroids pesticides (SPs) was injected into the injection port of the GC-ECD and the responses recorded. (For OPs, 2.0 µL of each concentration was injected into the GC-PFPD injection port). Calibration linear curves were constructed by employing Star Workstation software in-built to plot the concentrations against their respective peak areas. Callibration curves had R2 = 0.995 – 0.998. Recalibration curves were run with esch batch of 30 samples to ensure that correlation coefficient was kept above 0.99.

4.2.7 Recovery test of extraction method

Recovery studies were undertaken to evaluate the efficiency of the extraction procedure used. Ten grammes of soil matrix blank samples taken from the field were extracted as previously described to determine the background concentration levels of any residual pesticide. An aliquot of 0.5 mL of the standard stock solution (1µg/mL) was added to fresh 10 g of the soil matrix blank (This is equivalent to a fortification or spiking level of 0.05 mg/kg) and taken through the entire extraction process. These were then analysed by gas chromatograh to compare detected levels with the initial concentration injected into samples prior to extraction. The recovery was done in duplicates, at another fortification level of 0.01 mg/kg. The values, expressed in percentages were calculated from chromatograms as follows:

$$\% \text{ Recovery} = \frac{C2 - C1}{C} \text{ X } 100$$

Where: C_1 = Concentration (mg/kg) of pesticide residue in the matrix blank

C_2 = Concentration (mg/kg) of pesticide residue in the spiked matrix blank

C = Concentration (mg/kg) of pesticide added

The recoveries of the pesticides were within acceptable range of 70% - 120% for all of the pesticides analysed.

4.2.8 Identification and Quantification of pesticide residues

Pesticide residues were identified using reference standards and relative retention time techniques. Residue levels were determined by the external standard method by comparing the peak heights of samples with the corresponding peak heights of the reference standards of known concentrations. Measurements were carried out within the linear range of the detectors. Peak areas whose retention times coincided with that of the standards were extrapolated on their corresponding calibration curves to obtain concentrations.

4.2.9 Limit of detection (LOD) and Limit of quantitation (LOQ)

There are several conceptual approaches to the subject of LOD, each providing somewhat different definition, and consequently the methodology used to calculate the LOD differ. LOD defined by Chang (2011) as “the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value and LOQ as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOD for pesticides reported in this determination was based on the lowest analyte concentration that could consistently and reliably yield 70% or more recovery from fortified samples (Scholtz & Flory, 1999). Standard pesticide mix solutions were serially diluted and lowest concentration whose recovery from fortified samples was greater than 70% and also gave a signal to noise ratio of 3 was run 10 times and the standard deviation of the signals calculated. The standard deviation

(SD) was multiplied by 3 to get the LOD (i.e. SD x 3 = LOD). The standard deviation for the LOD determination was multiplied by 10 to get the LOQ (i.e SD x 10 = LOQ), following Scholtz and Flory (1999). The LOD and LOQ for the determined pesticides in soil were set as 0.15µg/kg and 0.5µg/kg respectively. The standard pesticide mix solutions for LOD and LOQ determination contained all target pesticides however, LOD and LOQ were determined for only certain individual pesticides (markers) that represent the various pesticide groups. The markers for the various pesticide groups are as follows: Organochlorine pesticides (γ-chlordane, *p,p'*-DDE and endosulfan sulphate); Organophosphorus pesticides (diazinon, fenitrothion and parathion-ethyl); Synthetic pyrethroids (bifenthrin, lambda-cyhalothrin and permethrin).

4.2.10 Gas Chromatographic quantification of extracted pesticides

The final extracts were analyzed for organochlorine and synthetic pyrethroid pesticides by Gas Chromatograph- Varian CP-3800 (Varian Association Inc. USA) equipped with combiPal autosampler and ^{63}Ni electron capture detector (ECD) (Plate 9 on page 94) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/kg range) from the matrix to which other detectors do not respond. The GC conditions used for the analysis were capillary column coated with VF-5 (30 m + 10 m guard column x 0.25 mm i.d, 0.25 µm film thickness). The injector and detector temperature were set at 270 ^{o}C and 300 ^{o}C respectively. The oven temperature was programmed as follows: 70^{O}C held for 2 min, ramp at 25 ^{o}C/min to 180 ^{o}C, held for 1 min, and finally ramp at 5 ^{o}C/min to 300 ^{o}C. Nitrogen was used as carrier gas at a flow rate of 1.0 mL/min and detector make-up gas of 29 mL/min. The injection volume of the GC was 1.0 µL. The total run time for a sample was 31.4 min.

Organophosphorus pesticides on the other hand were analyzed by Gas Chromatograph- Varian

CP-3800 (Varian Association Inc. USA) also equipped with combiPal autosampler and pulse flame photometric detector (PFPD) that allowed the detection of contaminants even at trace level concentrations (in the lower µ g/g range). The GC conditions used for the analysis were capillary column coated with VF-1701 (30 m x 0.25 mm i.d, 0.25 µ m film thickness). The injector and detector temperature were set at 270 ^{o}C and 280 ^{o}C respectively. The oven temperature was programmed as follows: 70 ^{o}C held for 2 min, ramp at 25 ^{o}C/min to 200 ^{o}C, held for 1 min, and finally ramp at 20 ^{o}Cmin^{-1} to 250 ^{o}C maintained for 3.3 min. Nitrogen was used as carrier gas at a flow rate of 2.0 mL/min and detector make-up gases (17.0, 14.0 and 10.0 mL/min) for hydrogen, air-1 and air-2, respectively. The injection volume of the GC organophosphorus pesticide determination was 2.0 µL. The total run time for a sample was 14 min. Each sample underwent duplicate analyses.

4.2.11 Quality Assurance and Control of Method

In Order to minimize potential contamination from laboratory air, as well as loses due to volatilization, drying in the laboratory and freeze-drying were avoided. Further quality was assured through the analysis of solvent and matrix blanks. For each batch of 20 samples analysed, a solvent and matrix blanks were prepared. The reagents (ethyl acetate and acetonitrile) were of high quality and were exposed to the same extraction procedures and subsequently run to check that no interfering substances were present. No analytes were detected in the blanks. Recalibration curves were run with each sample batch to ensure that correlation coefficient was kept above 0.99. Strict cleaning procedures were adhered to viz: all glassware were washed with hot water and detergents and copiously rinsed with distilled water. After drying, the glass wares were further rinsed with acetone. Recoveries of spiked standards were all within acceptable range (70% -

120%) for all pesticides.

4.2.12 Data Analysis

Detected pesticides were put into their pesticide groups and the mean concentration of each pesticide was calculated based only on the number of samples in which quantifiable detects were made. Incidence rate (IR) however was calculated as percentage frequency of each pesticide based on the total number of samples. Standard deviations were computed to show the dispersion or distribution of the data points around the mean.

$$IR = \frac{Fi \times 100}{Ts}$$

Where

IR = the incidence rate or percentage frequency of occurrence of an individual pesticide

Fi = frequency of detection of an individual pesticide **i** in all the soil samples and

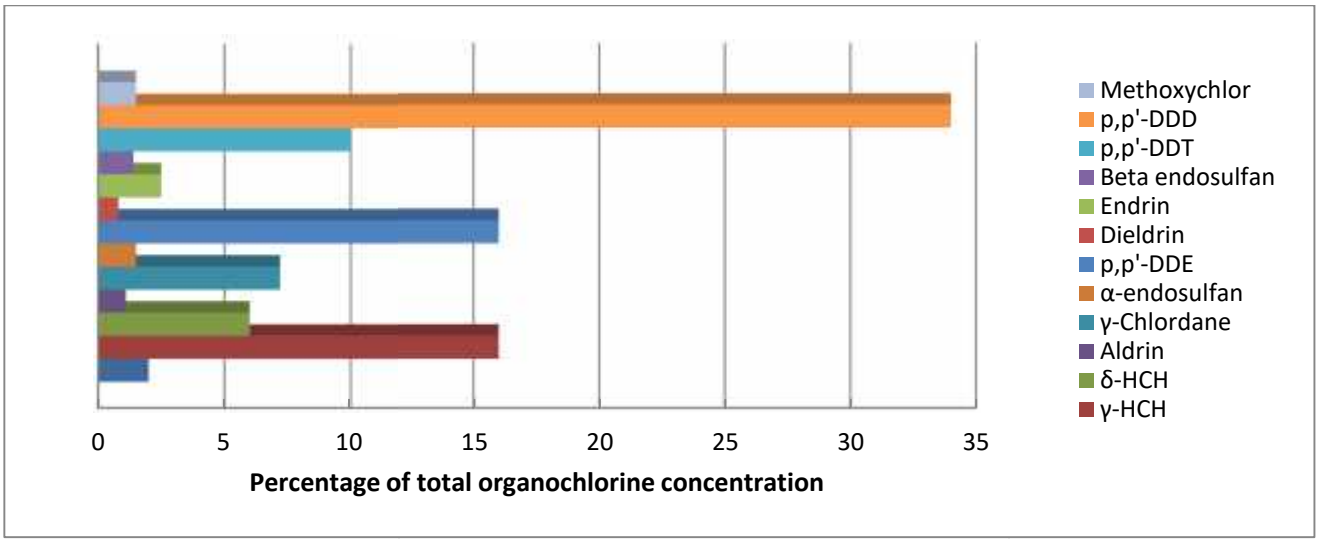
Ts = total number of soil samples taken.

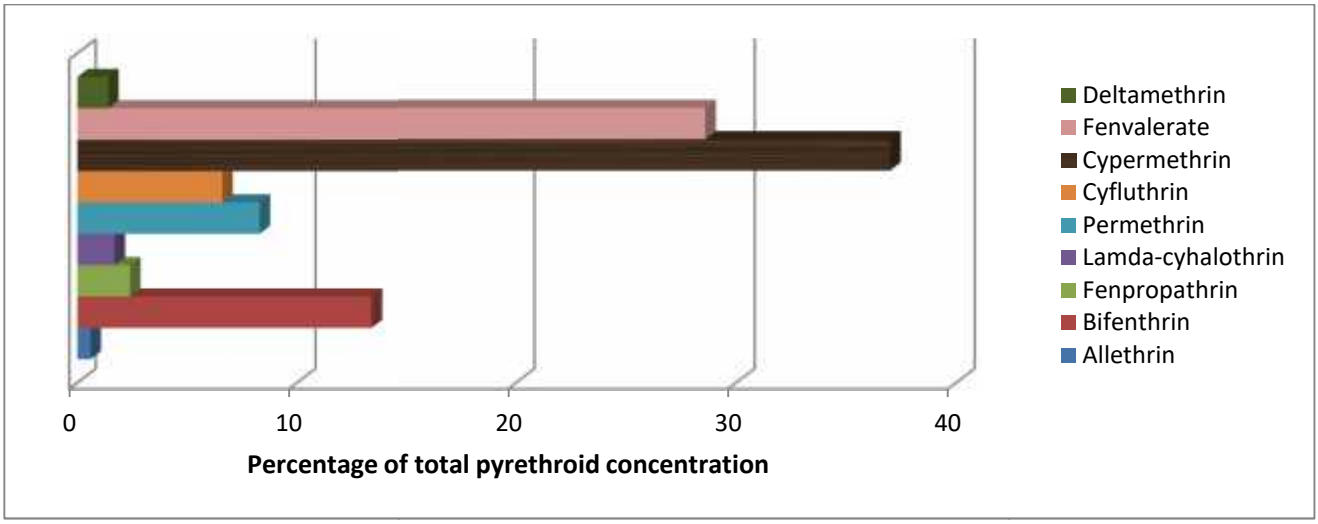
Percentage concentration of each pesticide in a pesticide group was also calculated and illustrated as figures in the form of column and bar charts.

4.3 Results

4.3.1 Overview of the results

Figure 4.1 illustrates the percentage composition of each detected pesticide in their various pesticide groups. It was observed that some single pesticides contributed more than a third of the entire concentration of their respective chemical/pesticide groups. For instance chlorpyrifos alone accounted for 51% of the total organophosphorus concentration in the soil samples while cypermethrin and *p,p'*-DDD contributed 37% and 34%, respectively to pyrethroid and organochlorine pesticide residue concentrations. The ten chemicals with the highest residues are presented in Figure 4.2. Tables 4.1, 4.3 and 4.4 summarise the concentrations and frequencies of organochlorine, synthetic pyrethroid and organophosphorus pesticides respectively in farmland soils of the study area. On the average, the OCPs had the highest incident rate or frequency of occurrence of 34.4% followed by the synthetic pyrethroids with 30% and the organophosphates with 13%. The mean concentrations of residues in the pesticide groups however follow the order: Synthetic pyrethroids > organophosphates > organochlorines. Table 4.5 enlists most of the insecticides employed by the farmers in the study area to combat insect pests in fruits and vegetable cultivation.





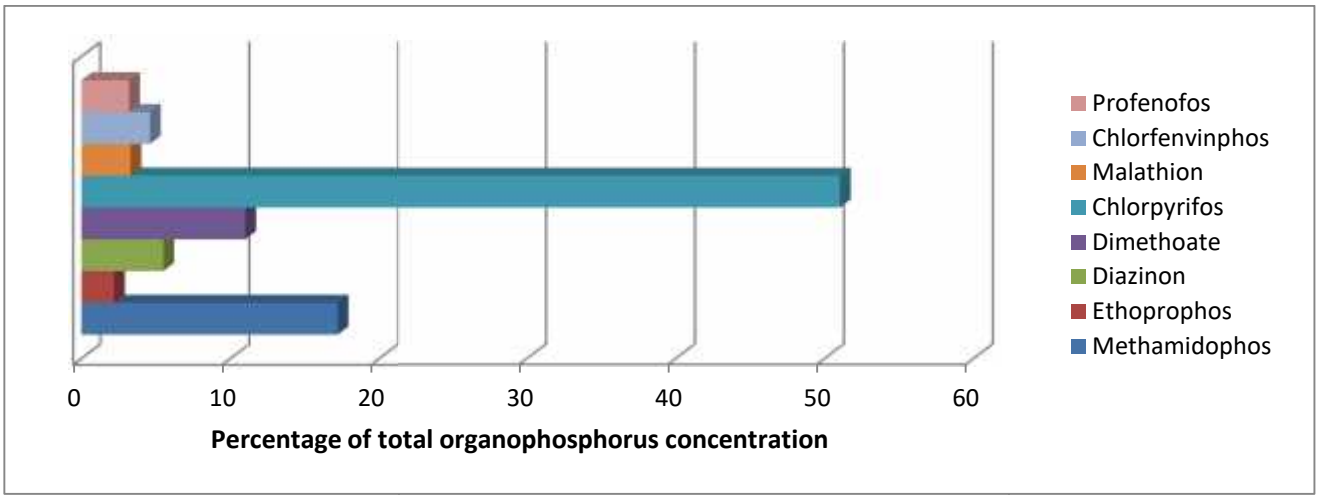


Fig. 4.1: Percentage total concentration of individual chemicals in each group of pesticide

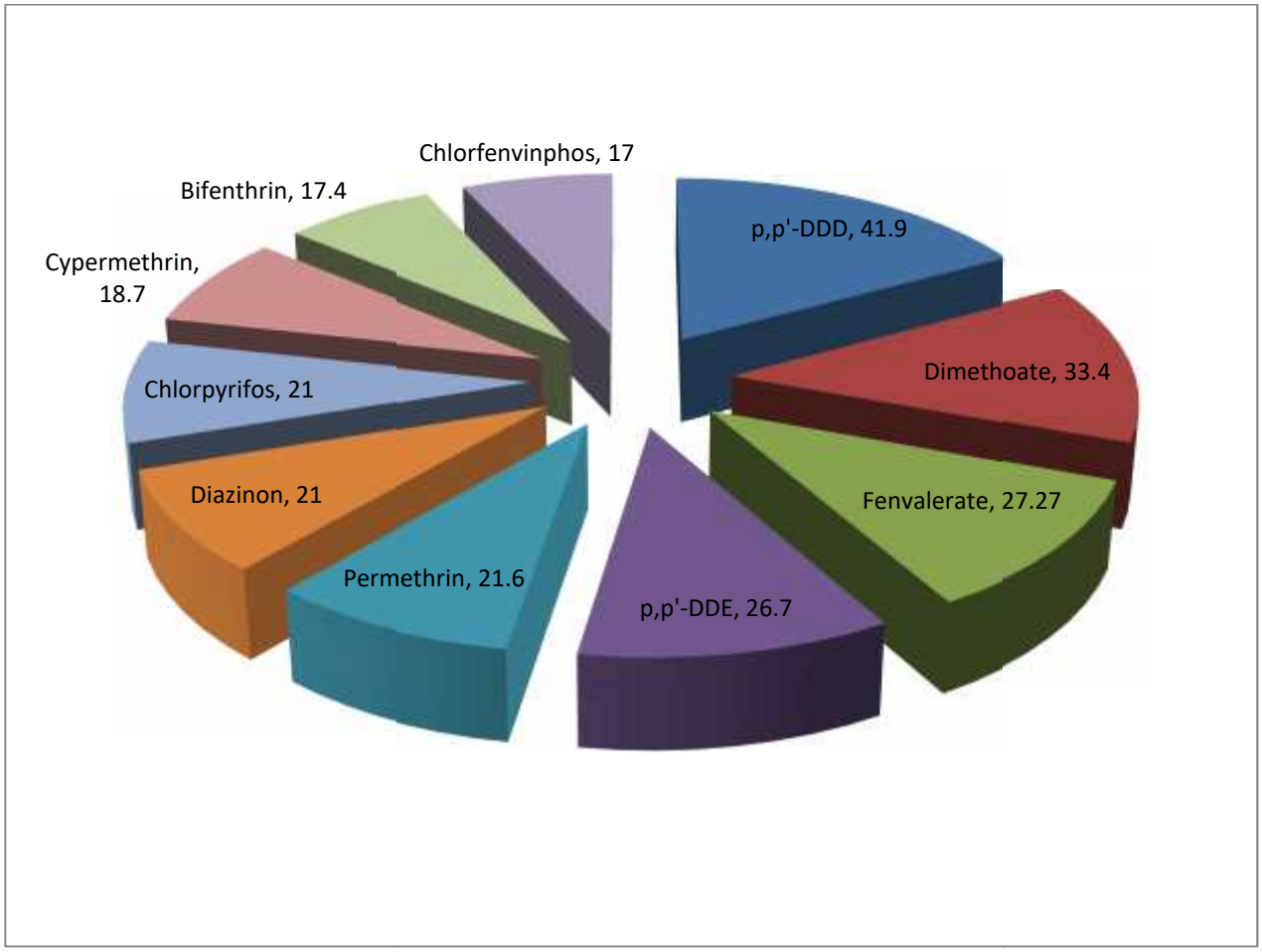


Fig. 4.2: Top ten chemicals with highest mean concentration (µg/kg) in soils from the study area

4.3.2 Organochlorine pesticide residues in soils

The targeted OCPs, with the exception of heptachlor were encountered and the range of concentration of detected pesticides was 0.47 – 91.0 µg/kg. Averagely, the pesticides had frequency of occurrence of 33%. The most frequently encountered was p,p'-DDT, with incidence rate of 88.3%. The least encountered was endosulfan sulphate, with frequency of occurrence of 1.7%. Para, para-DDD (*p,p'*-DDD) registered the highest average concentration of 41.9 µg/kg, followed by *p,p'*-DDE, γ-lindane, γ-chlordane and β-endosulfan with respective concentrations of 26.77, 14.85, 11.93 and 7.86 µg/kg. Endosulfan sulphate recorded both the lowest concentration (0.47 µg/kg) and incidence (1.7%). It is also noted that of the quantified chemicals, *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD recorded levels above the USEPA maximum residual levels (Table 4.1). As much as 38% of total samples analysed recorded *p,p'*-DDD residue levels above the USEPA maximum residual levels while 10% and 3.8% were registered for *p,p'*-DDE and *p,p'*-DDT respectively. The ratios of DDD/DDT and DDE/DDT were 6.7 and 4.2 respectively. Concentrations of parent compounds and their metabolites are captured as ratios in Table 4.2. for evaluation of degradation pathways and new inputs of pesticides.

Table 4.1: Mean concentration of organochlorine pesticides in soils samples from farmlands

Pesticide	Mean ±SD (µg/kg)	Range (µg/kg)	IR (%)	% of samples exceeding USEPA MRL	USEPA MRL (µg /kg)
β-HCH	3.15 ±1.5	2.1-9.5	33.3	0	30.0
γ-HCH	14.85 ±5.3	0.5-28.5	60.0	0	40.0
δ-HCH	6.51 ± 1.2	3.7-8.2	33.3	0	30.0
Heptachlor	nd	-			30.0
Aldrin	1.72 ± 0.3	1.3-2.5	33.3	0	40.0
Dieldrin	1.39 ± 0.2	1.1-1.6	33.3	0	20.0
Endrin	4.68 ± 0.2	1.2-5.2	30.0	0	40.0
γ-Chlordane	11.93 ± 9.5	4.6-39.1	33.3	0	250.0
α-endosulfan	2.48 ±0.4	1.8-3.4	33.3	0	50.0
Beta endosulfan	7.86 ± 2.2	4.4-10.4	10.0	0	40.0
Endosulfan sulfate	0.47	0.47	1.7	0	40.0
p,p'-DDE	26.77 ±9.4	12.7-47.1	33.3	10	40.0
p,p'-DDT	6.3 ±14.6	0.75-91.0	88.3	3.8	50.0
p,p'-DDD	41.89 ± 29.1	1.6-67.0	45.0	38	40.0
Methoxychlor	6.38 ±1.2	5.4-9.0	13.3	0	50.0
Grand mean	**9.74**				
Load	**136.36**				

N = 66 nd = not detected

IR = Incidence rate

Grand mean = Mean of the concentrations of all organochlorine pesticides quantified in the analysis

Load = Total of the means of all OCP residues in soil samples

IR = Percentage frequency of occurrence of an individual pesticide in total sample

Table 4.2: Ratios of metabolites to parent compounds in soils from farmlands

Pesticides	Ratio value
p,p'-DDE/*p,p'*-DDT	4.24
p,p'-DDD/*p,p'*-DDT	6.65
p,p'-DDD/*p,p'*-DDE	1.57
Endrin/Dieldrin	3.37
Endrin/Aldrin	2.72
Dieldrin/Aldrin	0.81
β-HCH/ γ-HCH	0.21

4.3.3 Synthetic pyrethroid pesticide residues in farmland soils

Nine synthetic pyrethroid pesticide residues were targeted in this study and all of them were detected and quantified in the analysed soil samples. The incidence rate ranged from 10% for allethrin to 78.3% for cypermethrin (Table 4.3). The general concentration ranged from 0.50 µg/kg for permethrin to 139.30 µg/kg for cypermethrin. The mean concentration range however was 1.36 µg/kg – 27.27 µg/kg. Fenvalerate recorded the highest mean concentration of 27.27 µg/kg followed by permethrin (21.55 µg/kg) and then cypermethrin (18.70 µg/kg). Allethrin mean detect was the least; 2.20 µg/kg. Residues of three pesticides recorded levels higher than the USEPA MRL and these are fenvalerate, cypermethrin and permethrin, with respective percentages of 10, 10 and 3.3 of total samples analysed exceeding the maximum residue limit (Table 4.3).

Table 4.3: Mean concentration of synthetic pyrethroid pesticides in soils sample from farmlands

Pesticide	**Mean ±SD** (µg/kg)	**Range** (µg/kg)	**IR (%)**	**% of samples Exceeding USEPA MRL**	**USEPA MRL** (µg /kg)
Allethrin	2.20 ± 1.5	0.78-3.30	10.0	0	10.00
Bifenthrin	17.40 ±2.6	12.00-21.10	30.0	0	40.00
Fenpropathrin	8.93 ±1.1	1.0-50.60	28.3	0	20.00
Lamda-cyhalothrin	3.67 ±0.4	3.20-4.00	18.3	0	20.00
Permethrin	21.55 ±32.6	0.5-95.00	15.0	3.3	10.00
Cyfluthrin	9.11 ±5.1	0.50-18.00	28.3	0	150.00
Cypermethrin	18.70 ±30.2	0.50-139.30	78.3	10.0	50.00
Fenvalerate	27.27 ±27.4	1.95-76.00	28.3	10.0	30.00
Deltamethrin	1.36 ±1.4	0.51-6.00	38.3	0	50.00
Grand mean	**12.24**				
Load	**110.16**				

N = 66; Grand mean = Mean of the concentrations of all pyrethroid pesticides quantified
Load = Total of the means of all OCP residues in soil samples

4.3.4 Organophosphorus pesticide residues in farmland soils

The presence of all the target organophosphorus pesticides was registered except pirimiphos-methy (Table 4.4). The concentration range of the detected residues was 1.0 – 58.5 µg/kg. The least concentration of 1.0 µg/kg was recorded for ethoprophos, fonofos, malathion, fenitrothion and profenofos, while the highest concentration of 58.5 µg/kg was registered for chlorfenvinphos. The frequencies of occurrence here was very low, with a mean of 13% and a range of 3.0 - 63.3%. Chlorpyrifos had the highest rate of occurrence while parathion had the lowest. Here too, the levels of four pesticides, namely chlorpyrifos, dimethoate, chlorfenvinphos and methamidophos exceeded the USEPA MRL in 16.7%, 5.0%, 1.6% and 1.6% of total samples analysed.

Table 4.4: average concentration of organophosphorus pesticides in surface soils samples from farmlands

Pesticide	Mean ±SD (µg/kg)	Range (µg/kg)	IR (%)	% of samples Exceeding USEPA MRL	USEPA MRL (mg/kg)
Methamidophos	11.75 ±6.8	4.00-28.65	33.0	1.6	0.03
Ethoprophos	5.40 ±2.3	1.00-7.48	9.1	0	0.05
Phorate	1.71 ±0.4	1.28-2.00	4.5	0	0.05
Diazinon	20.50± 8.9	7.12-25.00	6.1	0	0.03
Fonofos	1.33 ±0.5	1.00-2.00	4.5	0	0.05
Dimethoate	33.42 ±24.0	3.10-57.00	7.6	5	0.03
Pirimiphos-methyl	Nd	-	-		0.03
Chlorpyrifos	20.70 ±13.0	3.12-46.00	63.3	16.7	0.03
Malathion	10.0 ±12.9	1.00-31.21	7.6	0	0.05
Fenitrothion	2.66 ±1.6	1.00-4.50	9.1	0	0.05
Parathion	2.50 ±0.7	2.00-3.00	3.0	0	0.04
Chlorfenvinphos	17.24 ±2.2	1.10-58.50	6.1	1.6	0.05
Profenofos	8.00 ±6.3	1.0-15.12	9.1	0	0.05
Grand mean	**11.02**				
Load	**132.24**				

N = 66 nd = not detected

Grand mean = Mean of the concentrations of all OPs quantified in the analysis

Load = Total of the means of all OCP residues in soil sample

Table 4.5: Inventory of some insecticides used in the study area

Trade Name	Active ingredient(s)	Function
Cydim EC	Dimethoate Cypermethrin	Systemic and contact insecticide for vegetables, Soybeans, pineapple and ornamental plants
Cydim Super EC	Cypermethrin Dimethoate	Insecticide for the control of aphids, caterpillars, whitefly, grasshoppers, bollworms in vegetables and cotton
Plan DEC 25	Deltamethrin	Contact insecticide for the general treatment of of insects of various categories
Agricombi	Fenitrothion (30%) Fenvalerate (10%)	Broad-spectrum insecticide for treating caterpillars, mites and aphids
Akate	Bifenthrin	Broad-spectrum insecticides used for all crops
Decis EC 12.5	Deltamethrin	Contact and ingestion pesticide use to treat vegetables against caterpillars, thrips, cutworms, leafworms and bollworms.
Thiodan	Endosulfan	For the control of bollworms
Akape (Anty Ataa)	Imidacloprid	Broad-spectrum systemic insecticide used on vegetables, fruits cereals and ornamentals
Acetar star EC	Bifenthrin Acetamiprid	Contact and stomach insecticide for the contrl of whiteflies, aphids, bollworms on vegetables & fruits.
Attack	Emmectin benzoate	Non-systemic insecticide that controls cabbage moths, aphids, whitefly, caterpillars and leaf miners.
Sumitex 40EC	Dimethoate	Insecticide for the control of mealybugs, mites, thrips, and larvae borers in vegetables and pineapples
Sumitox	Fenvalerate	Broad spectrum insecticide with contact and stomach action against sucking and boring pests
Actellic 50 EC	Pirimiphos-methyl	For the control of aphids
Confidor SL 200	Imidacloprid	For the control of aphids, thrips, whitefly and termites
Ceres (cerox)	Dimethoate	Broad spectrum insecticide
Alphacp 10 SC	Alpha-cypermethrin	Insecticide for the control of insect pests in vegetables and fruits.
Conquer super 2.5EC	Lambda-cyhalothrin	Insecticide for the control of insect pest in vegetables and pulses
Conpyrifos 48EC	Chlorpyrifos-ethyl	Insecticide for the control of borers in vegetables, cereals and for public health purposes
Contihalothrin 2.5EC	Lambda-cyhalothrin	Insecticide for the control of insect pest in vegetables and pulses
Fastrack 10SC	Alpha-cypermethrin	Insecticide for the control of insect pests in vegetables and fruits crops
Perfekthion	Dimethoate	Insecticide for the control of mealybugs, mites, thrips, and larvae borers in vegetables and pineapples
Vector 30%WP	Beta-cyfluthrin and Imidacloprid	Insecticide for the control of insect pests in vegetables
Sumicombi	-Fenitrothion -Fenvalerate	Insecticide for the control of pests on vegetables and for public health

4.4 Discussions

4.4.1 Organochlorine pesticide Residues in soil samples

The single highest Organochlorine pesticide (OCP) concentrations measured in the samples were recorded for *p,p'*-DDT (91.0 µg/kg), followed by p,p'-DDD (67.0 µg/kg) and *p,p'*-DDE (47.1 µg/kg) as can be observed from their respective concentration ranges in Table 4.1. Except for the DDTs (*p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD), the level of other OCPs were low and below the USEPA maximum residue levels. Apart from γ-HCH whose frequency of occurrence was 60%, the DDTs were the most frequently encountered with *p,p'*-DDT detected and quantified in 88.3% of the samples. Wang *et al.*, (2006) recorded 8.55 µg/kg, 22.78 µg/kg and 11.50 µg/kg for *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD respectively in a paddy agricultural region along Taihu Lake in China while Manz *et al.*, (2001) also recorded up to 90 µg/kg, 100 µg/kg and 20 µg/kg for the respective compounds in agricultural fields in Germany. Values from this study are higher than what Wang *et al* reported but far lower than recorded figures by Manz *et al.*, (2001). Whereas the order of decreasing magnitude of the residue concentration in this study was *p,p'*-DDD > *p,p'*-DDE > *p,p'*-DDT, that in Wang *et al.*, (2006) and Manz *et al.*, (2001) were *p,p'*-DDE > *p,p'*-DDD > *p,p'*-DDT and *p,p'*- DDE > *p,p'*-DDT > *p,p'*-DDD respectively.

For the lindanes, β, γ and δ isomers were quantified. Alpha-HCH was not recorded in any sample. Gamma-HCH was the most frequently encountered (60% incidence rate) and also had the highest mean concentration (14.85 µg/kg), followed by δ-HCH (6.51 µg/kg) and then β-HCH (3.15 µg/kg). Relative to the DDTs, the lindanes and the drins (aldrin, dieldrin and endrin) recorded lower residue levels and incidence rates. The mean concentration of aldrin (1.72 µg/kg), the parent compound, was higher than that of dieldrin (1.39 µg/kg) but lower than the endrin concentration (4.68 µg/kg).

Of the endosulfan group, the parent, β-endosulfan had the highest mean concentration (7.86 µg/kg) but α-endosulfan was more frequently encountered. The metabolite, endosulfan sulfate was virtually absent, being recorded in only one sample. On the whole, the concentrations of OCPs in the soils were in the following order: DDTs > HCHs > endosulfans > drins.

4.4.2 Degradation pathways and evaluation of input of new organochlorine pesticides

The predominance of the DDTs, both in concentration and incidence rate on the surface suggests possible fresh introduction of technical DDT. According to Manz *et al*., (2001), studying the isomeric ratio and metabolite/parent compound ratios enables past and present pollutant emission sources to be distinguished. If there was no other pollution source, the metabolite/parent compound ratio generally increases with time. Thus the ratio of metabolites to parent compounds could be used as a rough estimate of the period of their application as well as to infer pollution sources (Karina *et al*., 2003; Gao *et al*., 2005).

P,p'-DDT is easily metabolized into the more stable and persistent isomers *p,p'*-DDE and *p,p'*-DDD in the environment (Bossi *et al.,* 1992). In this study, both metabolites were present in appreciable quantities, though *p,p'*-DDD recorded a higher concentration. The degradation of DDT in the environment could be explained as a result of anaerobic process of reductive dechlorination to DDD and dehydrochlorination to DDE respectively (Quensen *et al*.,1998; Yao *et al*., 2006). Generally, a value of metabolite**:**parent compound greater than 1.0 indicates aged or historical use of pesticide while a value less than 1.0 indicates new pesticide application (Karina *et al*., 2003; Zhang *et al.,* 2006). The ratios of *p,p'*-DDE**:** *p,p'*-DDT, *p,p'*-DDD**:** *p,p'*-DDT and endrin**:**dieldrin as well as endrin**:**aldrin were all greater than 1.0 (Table 4.2), indicating no new inputs of these

pesticides (DDT, aldrin and endrin) in the field. However, the ratio of dieldrin**:**aldrin was less than 1.0 (0.8), suggesting there might be a current input of aldrin pesticide, to a lesser extent. The degradation pathway of DDT to DDD could be direct and indirect. The indirect path was from DDT to DDE, then further to DDD (Wenzel *et al*., 2002). The ratios of DDD**:**DDE, DDD**:**DDT and DDE**:**DDT were employed to deduce the possible dechlorination pathway (Wenzel *et al*., 2002) in the farmland soils along the Afram bank in the current study. It was possible that DDT to DDD was the main degradation route and as a result, the ratio sequence was DDD:DDT > DDE:DDT > DDD: DDE (Table 4.2).

Technical Lindane contains 60%-70% α-HCH, 5%-12% β-HCH, 10%-12% γ-HCH, 6%-10% δ-HCH and 3%-4% ε-HCH. If technical lindane is the pollution source, the ratio of α-HCH to γ-HCH should be 5.3-6.3 (Fu *et al*., 2001). However, if lindane (γ-HCH) is the pollution source, the ratio of β-HCH/ γ-HCH increases in the soil during the degradation process (Wang *et al*., 2006) and a value of 1.0 or greater will indicate aged or historical use of lindane (pure γ-HCH) while a value less than 1.0 will imply current use. From Table 4.2, β-HCH/ γ-HCH value is less than 1 (0.21), implying the source of lindane in the farmland soils might be due to current input of Lindane (γ-HCH) other than technical lindane. This view is reinforced by the fact that there was no detection of α-HCH; and γ-HCH concentration was far higher than the other isomers.

Technical endosulfan is a mixture of two isomers: α-endosulfan (64-67%) and β-endosulfan (29-32%) (British Crop Protection Council, 2003). Their degradation product is endosulfan sulfate, the presence of which indicates the residue might have originated from historical use of endosulfan (Dem *et al*., 2007). The virtual absence of endosulfan sulfate in the soil samples from this study therefore suggests the detected residues came from recent applications. It is interesting to note that

the, DDTs, aldrin, dieldrin and endrins whose usage has been banned in the country have also been found, (per their metabolite-parent compound ratios in this study) not to be of current use. It should also be noted that even though the presence of the DDTs in the fallow lands does not suggest current inputs, their concentrations levels are all above the MRLs of the USEPA. They therefore still pose as sources of contaminants to crops that will subsequently be cultivated on those lands (even without further application of pesticides) and health hazard in food chains. Lindane and endosulfan are the only OCPs whose presence in the soil suggests that they are still being used. Whereas the source of endosulfan may come from the use of **thiodan and thionex** to control bollworms in fruits**,** vegetables and cereal crops**,** the source of Lindane cannot be easily traced since it has not been listed in the inventory of pesticides used in the study area (Table 4.5); neither was any pesticide with Lindane as an active ingredient chanced upon by personal observation. Lindane being a popular and effective broad-spectrum insecticide was probably one of those illegal pesticides that were covertly peddled from house to house to farmers, knowing very well that their use has been banned. Heptachlor, a pesticide whose use has not only been banned, but listed by the EPA as completely not in use in the country has also not been detected in this study.

4.4.3 Residues of Pyrethroid pesticides

All the target pyrethroid pesticides (SPs) were found in soil samples. Cypermethrin was found in 78.3% of the samples, deltamethrin in 38.3%, befenthrin in 30.0%, fenvalerate, cyfluthrin and fenpropathrin in 28.3%. Permethrin was measured in 18.3% and allethrin in only 10% of the total of 66 soil samples. Generally, pyrethroids are known to be the least persistent among the pesticides. The detection and quantification of all the target pesticides in samples therefore underscore the high dependence and suggests indiscriminate use of this group of pesticides in

agricultural activities in the study area. As indicated in Table 4.3, pyrethroids are mostly broad spectrum insecticides effective against a wide range of insect pests of the orders *Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera and Thysanoptera* (Thatheyus & Selvan, 2013). Their low mammalian toxicity but high arthropod toxicity makes them very effective and therefore popular and desirable in the treatment of all kinds of insect pests. As can be inferred from Table 4.3, they constitute the main insecticides found from the study. Indeed information from farmers in the study area indicate that although they are aware of the requisite pre harvest interval (PHI) (a period prior to harvesting, during which there should be no pesticide application to food crops), yet most of them still spray their crops very close to harvest time in order to increase their aesthetic and economic values.

The general concentration range of detected pyrethroids in the soil samples was 0.25 µg/kg – 139.3µg/kg. Even though the mean of each of the three highest pesticides measured follow the order fenvalerate > permethrin > cypermethrin, the highest concentrations measured in single samples follow the order cypermethrin > permethrin > fenvalerate (Table 4.3). The percentage contribution of each pesticide to the total/cummulative pyrethroid concentration in the area however is of the order cypermethrin > fenvalerate > bifenthrin (Figure 4.1). It is also worthy of note that the levels of some of the pesticides, mainly cypermethrin, permethrin and fenvalerate in some of the samples exceeded the maximum residue levels (MRL) set by USEPA. Of the three pesticide groups under study, pyrethroids registered the highest total average concentration of 12.24 µg/kg, followed by the organophosphates (11.02 µg/kg), and then the organochlorine pesticides (9.74 µg/kg). This is interesting since OCPs are known to be most persistent, followed by the OPs. This information is indicative of the fact that there is more reliance on SPs for pest

control relative to the other two and this is corroborated by facts gathered through interviews and observations from the field (Table 4.5).

Pyrethroids are largely insecticides and are normally sprayed on crops, rather than on soils, to control pests. Considering this fact, and also that they are supposed to be readily degradable, within few days to weeks of their application, there is cause for concern noting that they still have some appreciable levels in soils of farmlands that have been left temporarily uncultivated for at least three months. This could be due to abuse of the chemicals, mostly over dosage, as discovered by Dinham *et al.*, (2003) with the resultant high residual concentration irrespective of their short half lives. This finding may underscore some level of environmental persistence of this group of pesticides that may have been underestimated and must be re-evaluated on the basis of the individual chemicals. Indeed, as pointed by some authors (Lee *et al.*, 2003; ATSDR, 2003; Thatheyus & Selvam, 2013), pyrethroids can persist in soils for few hours to some few years, depending on a number of factors such as the type of pyrethroids in question, soil type, climate, species of microbes present in the soil and their population size. Whereas allethrin and resmethrin are known to be among the fastest degrading pyrethroids; taking few minutes to hours after application to degrade, others like permethrin, cyfluthrin, bifenthrin, fenvalerate and cypermethrin have given indication- though uncertain- of their persistence in soils up to 5 years, 9 months, 8 months, 3 months and 2 months respectively (Pyrethroids and pyrethrins, 2013).

4.4.4 Residues of Organophosphorus pesticides

Chlorpyrifos and methamidophos were the most frequently detected with incidence rates of 63.3% and 33.0% respectively. The rest had frequency rates below 10%. Even though Pirimiphos-methyl

was listed among pesticides found in use in the study area (Table 4.5), it was not detected in any of the 66 samples analysed. The presence of dimethoate, fenitrothion and chlorpyrifos in soil samples agree with organophosphorus pesticides listed as being in use during reconnaissance survey. The levels of three of them, dimethoate, chlorpyrifos and chlorfenvinphos exceeded the MRLs while the level of methamidophos in some samples (38.65 µg/kg) almost equaled MRL of the USEPA. The concentrations recorded in this study were generally higher than those reported by Kumari *et al*., (2008) and Bhupander *et al*., (2011) from northern India and Delhi Region in India respectively. Reported mean concentrations for parathion by Velasco *et al.*, (2014), from agricultural soils in Rio Verde Region of Mexico were however much higher (25.2 µg/kg - 47.48 µg/kg) than what is found in this study. Unlike pyrethroids, OPs are very toxic to humans as well as other organisms and their presence in the environment has been associated with many health consequences (Ragnarsdottir, 2000). Even though they are also known to be less persistent in the environment, their persistence is higher relative to the SPs. Species like chlorpyrifos and parathion, and indeed many more OPs, are known to persist in the environment for up to more than 1 year (Ragnarsdottir, 2000; Kazemi *et al*., 2012b).

The findings from this study established the facts that 37 out of 38 (97.4%) targeted pesticides for investigation in soils of temporally abandoned (fallowed) farmlands were detected and quantified. Ninety-four percent of the targeted OCPs were detected in 88.3% of the samples; 100% of targeted synthetic pyrethroids were detected in 78.3% of the samples while 69% of the organophosphates were detected in 63.3% of all the samples. Of all the detected pesticides, 9 (*p,p'*-DDT, *p,p'*-DDE**,** *p,p'*-DDD, permethrin, cypermethrin, fenvalerate, dimethoate, chlorpyrifos and chlorfenvinphos), representing 24% exceeded the USEPA maximum residue limit. The presence of 8 banned

pesticides, namely: aldrin, dieldrin, endrin, γ-HCH, *p,p'*-DDT, heptachlor, parathion and methamidophos was also identified and among them, the presence of only γ-HCH have been assessed to be due to current input. It can, therefore, be concluded that the residual load of pesticides on fallowed cultivated lands along the banks of the Afram arm of the Volta Lake are heavy enough to potentially contaminate new crops that will be cultivated on them, even without further application of insecticides.

1a

1b

4a

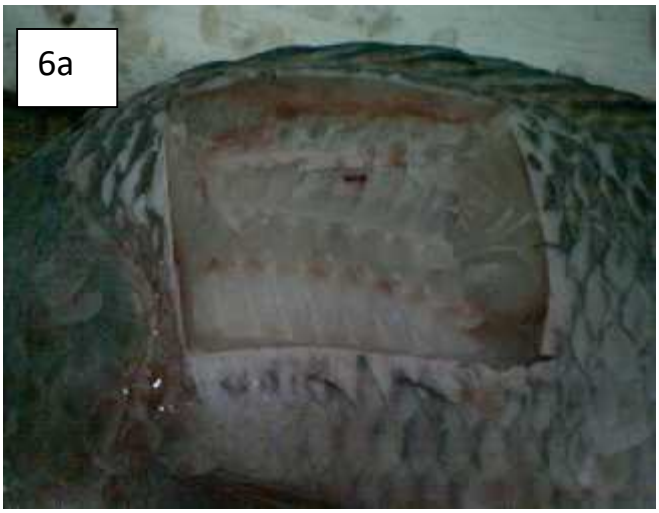
4b

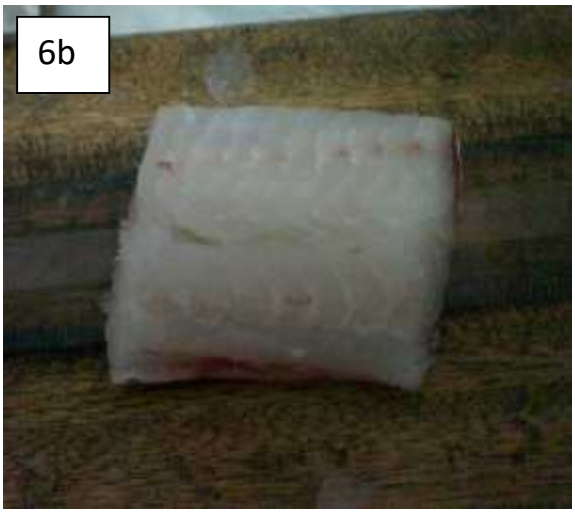
4c

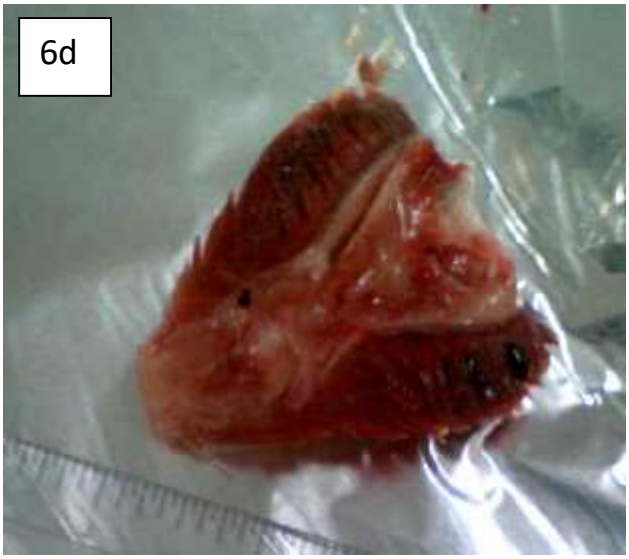
5a

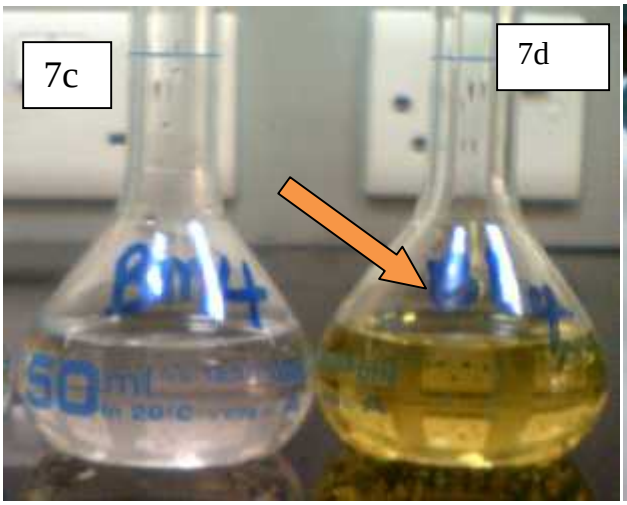
5b

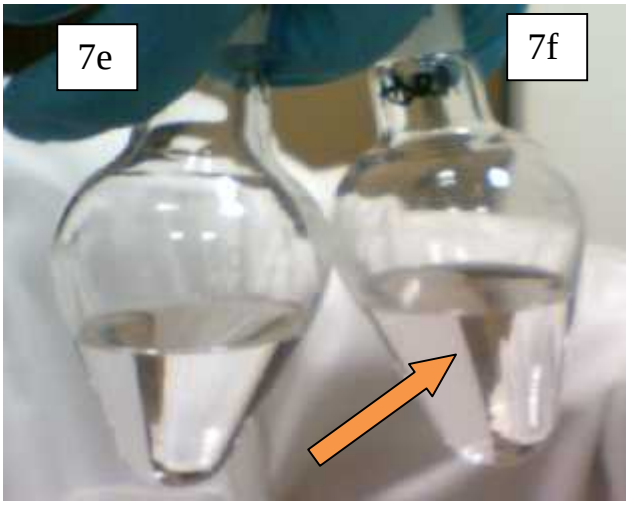
5c

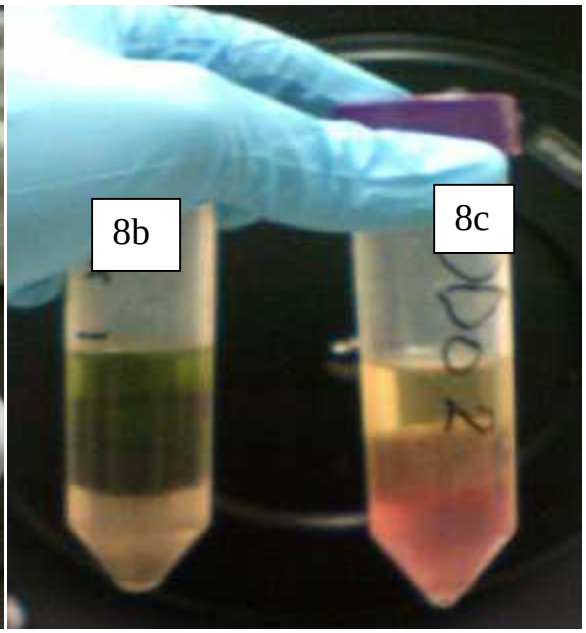


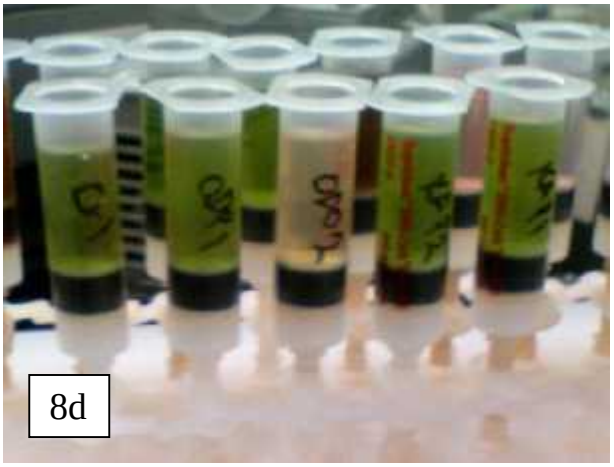


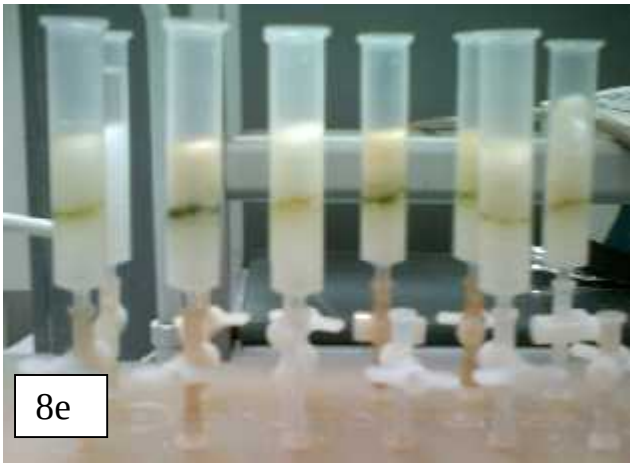


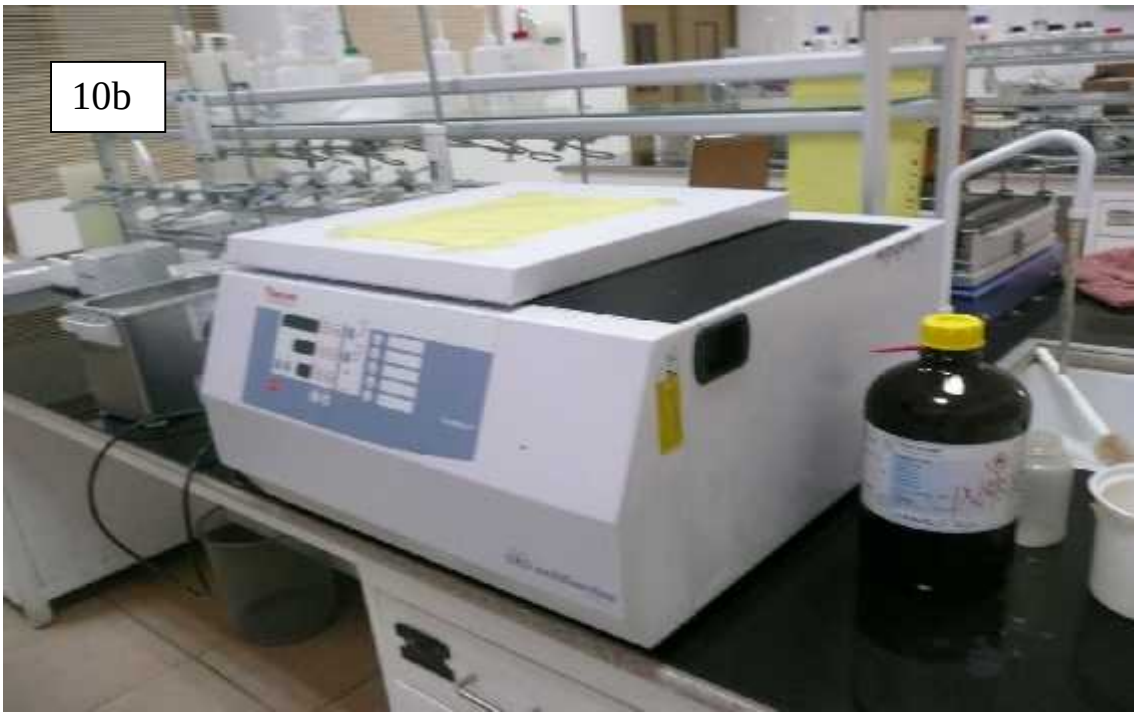












CHAPTER FIVE

PESTICIDE CONTENT AND HEALTH RISK ASSESSMENT OF WATERMELON, PEPPER AND ONION FROM THE CULTIVATED BANKS OF AFRAM ARM OF THE VOLTA LAKE

5.1 Background

The varieties of crops cultivated along the Afram arm of the Volta Lake may, out of convenience, be put into two categories, based on their demand for agro-chemical input for increased yield. Crops like cassava, maize, sweet potato and peanuts are mainly for subsistence and do not require much input of agro-chemicals, especially insecticides. Their cultivation virtually does not require irrigation and application of insecticides. Others like onions, water melon, pepper and okra are intensively cultivated for cash and highly dependent on regular irrigation and insecticide application for good yield. It is the latter group of crops that are the target for investigation for multiresidue analysis of pesticides. This group of crops is short-maturing, taking on the average, three months to mature. Thus, it is possible to have at least three cultivation seasons in a year. In order to make maximum economic gain, most farmers continuously cultivate fruits and vegetables throughout the year.

The intensity of farming has implications for pest persistence and hence the need to intensify insecticide application, sometimes including cocktail applications and experimentation with unfamiliar or inappropriate pesticides. Continuous application of insecticides may not only tend to produce resistant species of insect pests but may also contaminate crops by their residual concentrations. In order to produce crops of aesthetic quality (that is so much desired by consumers), pesticide application sometimes goes on through to the harvest time, sometimes ignoring the required pre-harvest interval (period prior to harvesting when crops are mandatorily

not required to be treated with pesticides to prevent contamination). The aim of this chapter is therefore to assess the presence and residual level of a spectrum of insecticides in water melon, onions and chili pepper cultivated along the banks of the Afram arm of the Volta Lake and their associated health hazard.

5.2 Materials and method

5.2.1 Sampling of fruit and vegetable samples

Large quantities of water melon fruits, onions and fresh chili peppers were bought from the sampling sites in the field (Fig. 3.1) from farmers at the time when farmers were harvesting for sale and crops were ready for immediate consumption. Plate 2 shows some of the samples. The sampling was done at different times between January – November, 2013 to ensure diversity and consistency with Codex sampling method (FAO/WHO, 2000). The onion and pepper samples after purchase were kept on ice. All samples were transported to the pesticide laboratory at the Ghana Standards Authority in Accra and were immediately processed and stored in deep freezers until extraction is done within 24 hours.

5.2.2 Chemicals and Reagents

All the chemicals and reagents used are listed in Appendix I

5.2.3 Processing of samples for pesticide extraction

Each of the onion and pepper samples from all the sampling sites were put together and thoroughly mixed in the laboratory. Fifty gramme (50 g) composite portions were separately weighed, thoroughly washed in two changes of distilled water and homogenized in a warring blender food

processor. Water melons samples weighed between 3 kg to 7 kg per fruit. Each fruit was thoroughly washed with distilled water and sliced into six cones. The edible part of a number of cones weighing at least 1kg was homogenized. The homogenized samples were then put into plastic bags and kept in deep freezer until extraction was done within a maximum of 24 hours.

5.2.4 Extraction and clean-up of pesticides from samples

Annastassiade *et al.,* (2003) developed a novel method for extraction of pesticide multiresidues in food commodities and described it as Quick, Easy, Cheap, Effective Rugged and Safe (QuEChERS). It is an effective extraction and cleanup approach for the analysis of diverse analyte residues in food matrices that requires minimum input of chemicals and reagents (cost), time and expensive equipment to accomplish. So far, it has achieved excellent results and its application has been extended to analyses in other fields including pharmaceuticals and veterinary (Wilkowska and Biziuk, 2010). Over the years, the original method has been modified for improved efficiency and also adapted to achieve specific results. Takatori *et al*., (2009) described rapid and easy multiresidue methods for the determination of pesticide residues in various foods matrices. In this study, their method (Takatori *et al*., 2009) was modified for extraction and clean-up of pesticides in the fruit and vegetable samples.

Frozen homogenized samples were allowed to thaw. Ten gramme portions of samples were put into 50 mL polypropylene tubes and 10 mL acetonitrile added. The content of the tube was homogenized by vortex mixer (Thermolyne-Maxi Mix-Plus) (Plate 8a) at high speed for 1 minute. Four grammes of anhydrous magnesium sulphate and 1g of sodium chloride were then added and vigorously shaken for another minute. The mixture was then centrifuged for 5 minutes and 4 mL supernatant organic layer taken. Supelclean Envi-Carb/LC-NH_2 SPE cartridge (500mg/500mg, 6ml

size) was conditioned with 10 mL acetonitrile and allowed to run down but the cartridge was not allowed to dry. The 4 mL supernatant from the centrifuge process was then loaded onto the conditioned solid phase extraction cartridge. This was then eluted with two portions of 5 mL acetonitrile into a pear-shaped 50mL flask, using a 12 port visiprep vacuum manifold. The elution was done at the rate of 3 drops per second. Plate 8 illustrates some stages of the extraction process. The eluate was evaporated to dryness using Buchi rotary vacuum evaporator (Buchi Rotovapor R.210) at 40^{o}C and 77 mbar with recirculation chiller, (Buchi, B-740). The concentrate was re-dissolved in 2 mL ethyl acetate and transferred into a 2mL standard opening vial for Gas chromatogram equipped with electron capture detector (GC-ECD) and pulse flame photometric detector (GC-PFPD) analyses.

5.2.5 Recovery test of extraction method

Duplicate of samples that have already been analysed were spiked with 0.05 mg/kg of standard pesticide mixtures and subjected to the same extraction process in order to determine recovery efficiency of the extraction method. The recovery was done in duplicates, at another fortification level of 0.01mg/kg. The values, expressed in percentages were calculated from chromatograms as follows:

$$\% \text{ Recovery} = \frac{C2 - C1}{C} \text{ X } 100$$

Where: C_1 = Concentration (mg/kg) of pesticide residue in the matrix blank

C_2 = Concentration (mg/kg) of pesticide residue in the spiked matrix

C = Concentration (mg/kg) of pesticide added

The recoveries of the pesticides were within acceptable range of 70% - 120% for the pesticides analysed. Reagent or procedural blanks were also extracted similarly as the test samples and found to be devoid of any interfering agents. Calibration of gas chromatogram, quantification of pesticide residues and limits of detection and quantification as well as calibration standards were all set as described in chapter four. The limit of detection (LOD) and limit of quantification (LOQ) of the GC for all the pesticides in fruits and vegetables were 0.15 µg/kg and 0.5 µg/kg respectively,

5.2.6 Gas Chromatographic quantification of extracted pesticides

The final extracts were analyzed for organochlorine and synthetic pyrethroid pesticides by Gas Chromatograph- Varian CP-3800 (Varian Association Inc. USA) equipped with combiPal autosampler and ^{63}Ni electron capture detector (ECD). The GC conditions used for the analysis were capillary column coated with VF-5ms (30 m + 10 m guard column x 0.25 mm i.d, 0.25 µm film thickness). The injector and detector temperature were set at 270°C and 300°C respectively. The oven temperature was programmed as follows: 70°C held for 2 min, ramp at 25°C /min to 180°C, held for 1 min, and finally ramp at 5 °C/min to 300°C. Nitrogen was used as carrier gas at a flow rate of 1.0 mL/min and detector make-up gas of 29 mL/min. The injection volume of the GC was 1.0 µL. The total run time for a sample was 31.4 min.

Organophosphorus pesticides on the other hand were analyzed by Gas Chromatograph- Varian CP-3800 (Varian Association Inc. USA) also equipped with combiPal autosampler and pulse flame photometric detector (PFPD) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/g range) from the matrix to which other detectors do not respond. The GC conditions used for the analysis were capillary column coated with VF-1701 (30 m x 0.25 mm i.d, 0.25 µm film thickness). The injector and detector temperature

were set at 270 ^{o}C and 280 ^{o}C respectively. The oven temperature was programmed as follows: 70 ^{o}C held for 2 min, ramp at 25^{o}Cmin^{-1} to 200 ^{o}C, held for 1 min, and finally ramp at 20 ^{o}C /min to 250 ^{o}C maintained for 3.3 min. Nitrogen was used as carrier gas at a flow rate of 2.0 mL/min and detector make-up gases (17.0, 14.0 and 10.0 mL/min) for hydrogen, air-1 and air-2, respectively. The injection volume of the GC organophosphorus pesticide determination was 2.0 µ L. The total run time for a sample was 14 min. Each sample underwent duplicate analyses.

5.2.7 Quality assurance and control of method

For each batch of 20 samples, a procedural blank, a spiked blank and a pair of matrix spiked sample/duplicate were processed. Extraction was done in duplicate for each sample. The spiked samples contained all the 15 OCPs, 13 OPs and 9 SPs target analytes. All reagents used during the analysis were of high quality (Appendix I) and were exposed to the same extraction procedures and subsequently run to check that no interfering substances were present. No analytes were detected in the blanks. Recalibration curves were run with each sample batch to ensure that correlation coefficient was kept above 0.99. Strict cleaning procedures were adhered to viz: all glassware were washed with hot water and detergents and copiously rinsed with distilled water. After drying, the glass wares were further rinsed with acetone.

5.2.8. Determination of per capita consumption in the study area.

The per capita fruit and vegetable consumption in Ghana, as stated by WHO (2005) are 0.0644 kg/person/day and 0.137 kg/person/day respectively. It is assumed that the per capita consumption of vegetables in the cultivated area will be higher (following observations by Ntow, 2008) than that at the national level and so also will be the associated health risk. Specific per capita consumptions of onions, watermelons and pepper in the study area were therefore estimated. In the estimation, face-to-face interaction was held with one hundred and fifty healthy adults from four farming communities along the Afram arm of the Volta Lake (Dedeso, Kwahu Amanfrom, Adawso and Kotoso). The information mainly elicited was daily frequency and quantity of consumption of the three food items over 12 months. For each food item, individuals indicated the quantity and frequency of consumption within a day. In determining the quantity, they were presented with samples of the food items and asked to indicate how much of it is consumed at an instance. The quantity indicated was immediately weighed on a portable scale and recorded. Among the 150 people interviewed, 55% (82) were female and 45% (68) were male. By age categorization, 60% (90) were adults (grown-ups of 12 years and above) while 40% (60) were children between the ages of 6-11 years. There was a gender-specific difference in the rate of consumption of onion in particular; women consumed more (180 g/day) than men (166 g/day). There was however no gender difference in consumption of watermelon and pepper. Similarly, there was no age-specific difference in the consumption of the food items. In most cases, the amounts of pepper and onion consumed by children were determined by the adult since they normally prepare the meals for consumption.

5.2.9 Data Analysis

5.2.9.1 Dietary exposure and Health Risk Assessment

The dietary exposure to pesticides is calculated in order to assess the chronic (long-term) consumer health risk. The Codex Alimentarius Commission Procedural Manual (Codex Alimentarius Commission, 2006) defines exposure assessment as "the qualitative and/or quantitative evaluation of the likely intake of biological, chemical, and physical agents via food as well as exposures from other sources if relevant". For each type of exposure, the estimated lifetime exposure dose (mg/kg/day) is obtained by multiplying the residual pesticide concentration (mg/kg) in the food of interest by the daily food consumption rate (per capita consumption) (kg/day), and dividing the product by the body weight (kg). The per capita consumption of fruits and vegetables in Ghana, according to a WHO-sponsored survey by Ruel *et al*., (2005) are 23.5 kg/person/year and 50.1 kg/person/year respectively. The general equation for dietary exposure is expressed as follows:

$$\text{Dietary exposure (mg/kg/day)} = \frac{\text{Food chemical concentration (mg/kg)} \times \text{Daily Food consumption (kg/day)}}{\text{Body weight (kg)}}$$

Hazard index (HI) is used as a measure of the risk associated with the exposure. In HI assessment, the estimated dietary exposure is compared with the toxicological reference value, Acceptable Daily Intake (ADI) or reference dose (RfD). The acceptable daily intake (ADI) is a level of intake of a chemical that can be ingested daily over an entire lifetime without any appreciable risk to health (WHO, 2001).

$$\text{HI} = \frac{\text{Exposure dose}}{\text{ADI}}$$

Two hazard Indices were calculated; one based on national per capita consumption and the other based on the estimated per capita consumption in the study area. Hazard indices were also determined for two categories of people: children between the ages of <1-11 years and grown-ups (12 years and above). In the health risk estimation, this study adopts the U.S. Environmental Protection (1989,1996) assumption that: the hypothetical body weights of 0 – 1year children is 10 kg; >1 – 11 years old children is 30kg and 70-kg for adults.

5.2.9.2 Treatment of Non-Detect samples

To account for residue levels in commodities that were below limit of method detection, three assumptions were considered. The first assumption that content of pesticides not detected have been assumed to be 0 (zero). The limitation of this model is that the calculated results might be underestimated, since non-detected residues are ignored. The second assumption is that actual concentrations of Non-detect (ND) samples were equal to limit of detection. This model tends to overestimate concentrations if there were no contamination of the sampled vegetables. The third assumption that if a pesticide has not been detected at all in a commodity, then non-detect samples of that commodity are assumed to be zero. If however, pesticide has been detected in some samples of the commodity, then the non-detect samples are assumed to be 50% of the limit of detection (Petersen *et al.,* 2013). A correction factor is sometimes applied to the third assumption since it is assumed that a small margin of overestimation can still occur because the summation of very small frequencies of detection that may sometimes occur may still be high enough to be significant. The correction factor is normally applied when the food commodity of interest undergoes processing or part of it (for instance the outer part) will have to be rid off before consumption. In this study concentration means, rather than summation will be used and means computed based on the second

or third models/approaches will underestimate detected levels hence the first assumption adopted. Furthermore, there is no need for correction factor since pepper and onion are consumed whole and only the edible part of melon is extracted.

5.3 Results

5.3.1 Overview

The identities of target chemicals of the three groups of pesticides (organochlorines – OCs, organophosphates – OPs and Synthetic pyrethroids – SPs) detected in water melon, fresh chili pepper and onion are presented in Tables 5.1 – 5.3. Means of residue are those of detect-samples only while incidence rates are based on total number of samples. The pesticide concentrations are determined on wet weight basis and compared with the maximum residue levels (MRL) allowed by European Union (EU) countries in the food commodities.

5.3.2 Pesticide residues in watermelon samples

Out of the 16 organochlorine pesticides analysed, 5 of them; β-HCH, γ-HCH, heptachlor, γ-chlordane and p,p'-DDT were detected and quantified in water melon samples in respective mean concentrations of 0.081 mg/kg, 0.061 mg/kg, 0.0101 mg/kg, 0.0124 mg/kg and 0.001 mg/kg. Their concentration ranges are also listed in Table 5.1. With the exception of *p,p'*-DDT, the mean concentrations of all the other registered organochlorines exceeded the maximum residue limit (MRL) set by the European Union (EU). Even though the incidence rate of β-HCH, γ-HCH were low (27% and 20% respectively), they recorded residue levels above that recommended by the EU in all samples in which they were quantified.

Out of the 9 targeted synthetic pyrethroid pesticides, 4; allethrin, fenpropathrin, cypermethrin and fenvalerate had measureable quantities in any of the water melon samples and at very low frequency of occurrence; below 20% in all of them. Their respective mean concentrations were 0.06 mg/kg, 0.004 mg/kg, 0.003 mg/kg and 0.003 mg/kg. Of the synthetic pyrethroids, only allethrin had residue level (0.06 mg/kg) exceeding the EU limit of 0.01mg/kg.

Table 5.1: Pesticide concentrations in Watermelon (n=30)

Pesticide	Mean ± SD (mg/kg)	Range	Incidence Rate (%)	% of detect samples above EU MRL	EU MRL(mg/kg)
Organochlorines					
β-HCH	0.0810 **±0.031**	0.0337-0.1096	26.7	100	0.01
γ-HCH	0.0610 **±0.003**	0.0572-0.0637	20.0	100	0.01
Heptachlor	0.0101 **±0.007**	0.0006-0.0276	50.0	40	0.01
γ-Chlordane	0.0124 **±0.005**	0.0029-0.0189	30.0	67	0.01
p,p'-DDT	0.0010 **±0.001**	0.0007-0.0014	6.7	0	0.05
	Load=0.1655				
	G-mean =0.0331				
Pyrethroids					
Allethrin	0.0604 **±0.023**	0.0402-0.0805	13.3	100	0.01
Fenpropathrin	0.0041 **±0.000**	0.0041	6.7	0	-
Cypermethrin	0.0033 **±0.000**	0.0033	6.7	0	0.20
Fenvalerate	0.0030 **±0.000**	0.003	6.7	0	0.02
	Load=0.0708				
	G-mean =0.0177				
Organophosphates					
Methamidophos	0.0041 **±0.001**	0.0022-0.0078	70.0	0	0.01
Ethoprophos	0.0012 **±0.0**	0.0006-0.0016	13.3	0	0.02
Phorate	0.0018 **±0.002**	0.0004-0.0078	26.7	0	0.05
Diazinon	0.0005*	0.0005	3.3	0	0.01
Dimethoate	0.0072 **±0.001**	0.0039-0.0095	83.3	0	0.02
Chlorpyrifos	0.0018 **±0.001**	0.0005-0.0037	20.0	0	0.05
Malathion	0.0015 **±0.001**	0.0005-0.0033	36.7	0	0.02
Fenitrothion	0.0031 **±0.001**	0.0005-0.004	16.7	0	0.01
Chlorfenvinphos	0.0005*	0.0005	3.3	0	0.02
Profenofos	0.0110 **±0.007**	0.0026-0.0267	66.7	0	0.05
	Load=0.0327				
	G- mean.=0.003				

Load : Total of the means of all residues in a pesticide group

G-mean : Grand mean; i.e. mean of the concentrations of all pesticides of a pesticide group

*** :** Detects in single samples

Incidence Rate: Percentage frequency of occurrence of an individual pesticide in total sample

Of the three groups of pesticides, the oraganophosphorus were the most encountered in the water melon samples analysed. Ten out of the targeted 13 OPs were encountered. Pirimiphos-methyl, fonofos and parathion were not quantified (below limit of detection) in any of the samples. Even though most encountered, the OPs had very low residue levels, with the mean concentration range of 0.0005–0.011 mg/kg. None exceeded their corresponding EU stipulated residue limit. Individual pesticides like methamidophos, dimethoate and profenofos recorded high incidence rates of 70%, 83% and 67% respectively; while others like diazinon, fonofos and chloefenviphos were encountered in single samples only.

5.3.3 Pesticide residues in chili pepper samples

Table 5.2 presents residual concentrations of organochlorine (OC), synthetic pyrethroid (SP) and organophosphorus (OP) pesticides in chili pepper. The results show that 10 out of the targeted 15 OCs were encountered in the fresh samples of chili pepper analysed. Methoxychlor recorded the highest mean concentration of 0.053 mg/kg, followed by γ-HCH (0.025 mg/kg), δ-HCH (0.022 mg/kg), p,p'-DDD (0.016 mg/kg), with p,p'-DDE and p,p'-DDT recording the lowest of 0.0014 mg/kg among the OCs. Delta HCH and p,p'DDT recorded incidence rate of 100%, followed by γ-HCH (90%) and p,p'DDE (72%). γ-Chlordane recoded the lowest frequency of 15%. Methoxychlor, though with a lower incidence rate (45%), had all its samples, as well as the mean concentration (0.053 mg/kg) above the EU permissible limit of 0.01 mg/kg. Delta HCH also has its mean (0.022 mg/kg), as well as 50% of its samples recording residue levels above EU limit (0.02 mg/kg). So also γ-HCH had 11% of its sample recording residue concentrations above the EU limit, though the average concentration of 0.025 mg/kg is below the EU limit of 0.5 mg/kg.

Table 5.2: Pesticide concentrations in Chili pepper (n = 20)

Pesticide	Mean ± SD (mg/kg)	Range	Incidence Rate (%)	% of detect samples above EU MRL	EU MRL (mg/kg)
Organochlorines					
β-HCH	0.0017 ±0.0004	0.0011-0.0023	45	0	0.5
γ-HCH	0.0248 ±0.017	0.0080-0.0539	90	11	0.5
δ-HCH	0.0221 ±0.02	0.0009-0.0531	100	50	0.02
γ-Chlordane	0.0032 ±0.002	0.0011-0.0058	15	0	0.02
p,p'-DDE	0.0014 ±0.001	0.0005-0.00396	80	0	1.0
Endrin	0.0049 ±0.004	0.0008-0.0123	70	0	0.1
Beta endosulfan	0.0072 ± 0.003	0.0023-0.0115	60	0	-
p,p'-DDT	0.0014 ±0.001	0.00037-0.003	100	0	1.0
p,p'-DDD	0.0163 ±0.011	0.0019-0.0274	65	0	-
Methoxychlor	0.0534 ±0.002	0.0512-0.0575	45	100	0.01
	Load=0.1364				
	G- mean =0.0136				
Pyrethroids					
Allethrin	0.0066 ±0.002	0.0044 - 0.0104	40	12.5	0.01
Bifenthrin	0.0031 ±0.002	0.0007- 0.0086	45	0	0.10
Fenpropathrin	0.0044 ±0.001	0.0040 - 0.0051	30	0	-
Lamda-cyhalothrin	0.0086 ±0.003	0.0020 - 0.0137	85	0	0.05
Permethrin	0.0156 ±0.012	0.0027 - 0.0330	70	0	0.10
Cyfluthrin	0.0071 ±0.002	0.0032- 0.0118	100	0	0.10
Cypermethrin	0.0031 ± 0.002	0.0005 - 0.0052	65	0	0.10
Fenvalerate	0.0012 ±0.001	0.00047-0.0020	65	0	0.05
Deltamethrin	0.00055 ±0.0	0.0005 - 0.0006	10	0	0.05
	Load=0.0501				
	G-mean =0.0056				
Organophosphates					
Methamidophos	0.0014 ± 0.001	0.0005-0.0004	25	0	0.10
Ethoprophos	0.0058 *	0.0058	5	0	0.02
Phorate	0.0017 *	0.00171	5	0	0.10
Diazinon	0.0015 *	0.00152	5	0	0.10
Fonofos	0.0022 ±0.002	0.0005-0.0063	35	0	0.01
Dimethoate	0.0056 ±0.003	0.0015-0.0114	45	0	0.50
Pirimiphos-methyl	0.0040 ±0.004	0.0007-0.0147	35	0	0.10
Chlorpyrifos	0.0087 ±0.003	0.0044-0.0150	50	0	1.00
Malathion	0.01 ±0.001	0.0090-0.0110	15	0	0.02
Parathion	0.004 ±0.0	0.0037-0.0042	15	0	0.10
Profenofos	0.0033 ±0.001	0.0024-0.0046	30	0	0.10
	Load=0.0482				
	G-mean =0.0044				

Load = Total of the means of all residues in a pesticide group

G-mean = Grand mean; i.e. mean of the concentrations of all pesticides of a pesticide group

***** = Detects in single samples

All 9 targeted SPs were encountered in the analysis of the chili pepper samples. Permethrin recorded the highest residue concentration of 0.0156 mg/kg, followed by lamda-cyhalothrin (0.008 mg/kg), whilst deltamethrin recorded the lowest of 0.00055 mg/kg. The most encountered of the SPs were cyfluthrin (100%), lamda-cyhalothrin (85%) and permethrin, (70%). Of all the SPs encountered in chili pepper, only allethrin had 12.5% of total samples recording concentrations above the EU permissible limit of 0.01 mg/kg.

With the exception of fenithrothion and chlorfenviphos, all the targeted OPs were encountered in the chili pepper. However, the frequency of occurrence of the individual pesticides was very low. For instance, chlorpyrifos with the highest incidence had a rate of only 50%, followed by dimethoate (45%). Ethoprophos, phorate and Dimethoate all registered as low as 5% occurrence rate. None of the concentration means as well as individual sample residue concentration also registered values that equals or exceeds the EU limit. Malathion that was encountered in only three samples, with incidence rate of only 15% recorded the highest mean concentration of 0.01 mg/kg, followed by chlorpyrifos (50%).

5.3.4 Pesticide residues in onion samples

Table 5.3 presents concentrations of OCPs, SPs and OPs in onion samples. Among the OCs, β-HCH recorded the highest level of 0.084 mg/kg, followed by γ-HCH (0.056 mg/kg) and Beta endosulfan (0.051 mg/kg). The most widely encountered OCPs in onion samples were γ-HCH, γ-Chlordane, Endrin, β-HCH and Beta endosulfan with occurrence rate of 80% - 100%. As many as 8 organochlorine pesticides: β-HCH, γ-HCH, δ-HCH, γ-chlordane, p,p'-DDE, dieldrin, endrin and methoxychlor had concentration in samples above their respective EU permissible limits; while 4

of these β-HCH, γ-HCH, endrin and methoxychlor had mean concentrations also above the permissible limits. All the samples had β-HCH levels above maximum limit of 0.01 mg/kg.

The range of mean concentration of SPs in onion samples was 0.0014 mg/kg – 0.0512 mg/kg. Allethrin and deltamethrin recorded highest levels of 0.0512 and 0.02 mg/kg respectively. Only allethrin registered mean concentration above the EU permissible limit and frequency of occurrence was highest in fenpropathrin, lambda-cyahalothrin and deltamethrin; all recording 75% incidence.

Organophosphorus pesticide occurrence was highest in onion samples, both in concentration and incidence. Four OPs; methamidophos, ethoprophos, diazinon and dimethoate all recorded incident rate of 100%. The OPs concentration range was 0.071 mg/kg – 0.0036 mg/kg, with dimethoate at the apex and malathion at the rear of concentration range.

In summary, concentration and incidence of pesticides was highest in onion samples, followed by pepper and water melon. The number of samples that had pesticide concentrations exceeding their corresponding EU limits follows the order: onion > water melon > pepper. In this study, OCPs registered the highest mean concentration in each of the three food items analysed; being

Table 5.3: Pesticides concentrations in Onions (n = 20)

Pesticide	Mean ± SD (mg/kg)	Range	Incidence rate (%)	% of detect samples above EU MRL	EU MRL (mg/kg)
Organochlorines					
β-HCH	0.0842 ±0.02	0.0432-0.3150	80	100	**0.01**
γ-HCH	0.0561 ±0.027	0.0015-0.1017	100	90	**0.01**
δ-HCH	0.0069 ±0.005	0.0005-0.0182	40	12.5	**0.01**
γ-Chlordane	0.0091 ± 0.004	0.0050-0.0191	100	25	**0.01**
p,p'-DDE	0.0163 ±0.031	0.0005-0.0855	35	14	**0.05**
Dieldrin	0.0091 ±0.002	0.0076-0.0109	20	50	**0.01**
Endrin	0.0125 ±0.009	0.0040-0.0566	85	23	**0.01**
Beta endosulfan	0.0513 ±0.031	0.005-0.11526	80	0	**0.1**
p,p'-DDT	0.0216 ±0.017	0.0021-0.0456	45	0	**0.05**
Methoxychlor	0.0209 ±0.018	0.0005-0.0624	60	53	**0.01**
	Load=0.288				
	G-mean =0.0288				
Pyrethroids					
Allethrin	0.0512 ±0.005	0.0470-0.0600	25	100	**0.01**
Bifenthrin	0.0018 ±0.0	0.0014-0.0023	55	0	**0.05**
Fenpropathrin	0.0179 ±0.007	0.0099-0.0315	75	0	-
Lamda-cyhalothrin	0.0132 ±0.013	0.0015-0.0400	75	0	**0.2**
Permethrin	0.0088 ± 0.006	0.0020-0.0181	45	0	**0.05**
Cyfluthrin	0.0015 ± 0.0	0.0009-0.0019	40	0	**0.02**
Cypermethrin	0.0016 ± 0.001	0.0006-0.0027	35	0	**0.1**
Fenvalerate	0.0014 ± 0.001	0.0005-0.0029	15	0	**0.02**
Deltamethrin	0.0200 ±0.019	0.0014-0.0554	75	0	**0.1**
	Load=0.1174				
	G-mean =0.013				
Organophosphate					
Methamidophos	0.0550 ±0.034	0.0145-0.1087	100	100	**0.01**
Ethoprophos	0.0138 ±0.008	0.0029-0.0246	100	40	**0.02**
Diazinon	0.0123	0.0008-0.0205	100	0	**0.05**
Fonofos	0.0080 ±0.005	0.0056-0.0099	15	0	**0.01**
Dimethoate	0.0711 ±0.002	0.0037-0.1323	100	75	**0.02**
Pirimiphos-methyl	0.0067 ±0.046	0.0008-0.0165	80	0	**0.05**
Chlorpyrifos	0.0410 ±0.004	0.0089-0.0600	75	0	**0.2**
Malathion	0.0036 ±0.019	0.0005-0.0066	25	0	**0.02**
Fenitrothion	0.0300±0.012	0.0021-0.0445	40	87.5	**0.01**
Parathion	0.0096 ±0.0	0.0094-0.0100	20	0	**0.05**
Chlorfenvinphos	0.0178 ±0.004	0.0126-0.0270	45	0	**0.05**
Profenofos	0.0046 ±0.0	0.0040	10	0	**0.05**
	Load=0.2735				
	G-mean =0.023				

Load = Total of the means of all residues in a pesticide group

G-mean = Grand mean; i.e. mean of the concentrations of all pesticides of a pesticide group

highest in water melon (0.033 mg/kg), followed by onion (0.0288 mg/kg) and pepper (0.0136 mg/kg). Total residue concentrations (Load) in each of the pesticide groups in the three food items are as follows: OCs > SPs > OPs (for watermelon and fresh chili pepper) and OCs > OPs > SPs (for onions) (Tables 5.1 - 5.3).

5.3.5 Health risk analysis

The daily consumption of onion and watermelon was found to be higher in the study area, compared to the national per capita consumption. Per capita consumption of pepper in the study area was however lower than at the national level (Table 5.4).

Table 5.4: Daily consumption of food items in the study area compared to national per capita consumption for Ghana

Food item	Daily consumption in study area (g/person/day)	National per capita consumption (g/person/day)
Onion	173 ± 35	137.3
Pepper	14 ± 5	137.3
Watermelon	900 ± 150	64.4

Analysis of health risk associated with consumption of each of the food commodities was carried out and the results presented in Tables 5.5 – 5.7. Residues were selected based on those concentrations exceeding the EU MRL, reference dose values and relative high concentration levels. Smaller reference dose values have serious/greater impact on the calculated hazard index and hence residues with very small reference values are of important concern, even though their residual concentrations in the food crops may be small. Health risk was calculated for the year 1-11

and adult categories (12 years and above). Children belonging to the 0-1year group were not considered for the risk determination since their consumption of these food items is insignificant.

Watermelon registered residues associated with health risk (Table 5.5), and these are heptachlor and β-HCH. So also dieldrin level in onion is associated with some level of health risk. No risk was associated with residues in pepper samples since all the Hazard Indexes were less than unity (Tables 5.6 and 5.7). It is also observed that health hazard was mainly associated with dietary exposure among both adults and children in the study area only.

Table 5.5: Health risk estimates for systemic effects associated with pesticide residues in Watermelon

Pesticide	ADI mg/kg/day	Exposure dose- mg/kg/day (based on Per capita consumption in study area)	Exposure dose- mg/kg/day (base on national Per capita consumption)	HI_1	HI_2
β-HCH	0.005	1.04×10^{-3} - Adults	7.5×10^{-5} - Adults	0.21	0.015
		2.43×10^{-3} – Children	1.7×10^{-4} – Children	0.50*	0.03
γ-HCH	0.005	7.8×10^{-4} – Adults	5.6×10^{-5} – Adults	0.16	0.011
		1.8×10^{-3} – Children	1.3×10^{-4} – Children	0.37	0.03
Heptachlor	0.0001	1.3×10^{-4} – Adults	9.3×10^{-6} – Adults	1.30*	0.09
		3.0×10^{-4} – Children	2.2×10^{-5} – Children	3.03*	0.22
γ-Chlordane	0.001	1.6×10^{-4} – Adults	1.14×10^{-5} – Adults	0.16	0.01
		3.7×10^{-4} – Children	2.7×10^{-5} – Children	0.37	0.03
Allethrin	0.008	7.8×10^{-4} – Adults	5.5×10^{-5} – Adults	0.10	0.007
		1.8×10^{-3} – Children	1.3×10^{-4} – Children	0.23	0.02
Fenpropathrin	0.030	5.3×10^{-5} – Adults	3.8×10^{-6} – Adults	0.002	0.0001
		1.2×10^{-4} – Children	8.8×10^{-6} – Children	0.004	0.0003
Dimethoate	0.001	9.3×10^{-5} – Adults	6.6×10^{-6} – Adults	0.09	0.01
		2.2×10^{-4} – Children	1.5×10^{-5} – Children	0.22	0.02
Profenofos	0.030	1.4×10^{-4} – Adults	1.0×10^{-5} – Adults	0.005	0.0003
		3.3×10^{-4} – Children	2.4×10^{-5} – Children	0.01	0.001
Methamidophos	0.001	5.3×10^{-5} – Adults	1.0×10^{-5} – Adults	0.05	0.01
		1.2×10^{-4} – Children	8.8×10^{-6} – Children	0.12	0.01

HI_1: Hazard Index based on per capita consumption in the study area.

HI_2: Hazard Index based on national per capita consumption

*** :** Hazard index significant enough to indicate possible health implication

Table 5.6: Health risk estimates for systemic effects associated with pesticide residues in Chili pepper

Pesticide	ADI mg/kg/day	Exposure dose- mg/kg/day (based on Per capita consumption in study area)	Exposure dose- mg/kg/day (base on national Per capita consumption)	HI_1	HI_2
γ-HCH	0.005	5.0×10^{-6} – Adults	4.9×10^{-5} – Adults	0.001	0.01
		1.2×10^{-5} – Children	1.1×10^{-4} – Children	0.002	0.02
δ-HCH	0.005	4.4×10^{-6} – Adults	4.3×10^{-5} – Adults	0.001	0.01
		1.0×10^{-5} – Children	1.0×10^{-4} – Children	0.002	0.02
Methoxychlor	0.005	1.0×10^{-5} – Adults	1.0×10^{-4} – Adults	0.002	0.02
		2.5×10^{-5} – Children	2.5×10^{-4} – Children	0.005	0.05
Permethrin	0.05	3.1×10^{-6} – Adults	3.0×10^{-5} – Adults	0.00006	0.0006
		7.3×10^{-6} – Children	7.3×10^{-5} – Children	0.0001	0.001
λ-cyahalothrin	0.005	1.7×10^{-6} – Adults	1.7×10^{-5} – Adults	0.0003	0.003
		4.0×10^{-6} – Children	4.0×10^{-5} – Children	0.001	0.01
Allethrin	0.008	1.3×10^{-6} – Adults	1.3×10^{-6} – Adults	0.0001	0.002
		3.1×10^{-6} – Children	3.1×10^{-5} – Children	0.0004	0.004
Malathion	0.030	2.0×10^{-6} – Adults	1.9×10^{-5} – Adults	0.00006	0.0006
		4.7×10^{-6} – Children	4.7×10^{-5} – Children	0.0002	0.002
Chlorpyrifos	0.010	1.7×10^{-6} – Adults	1.7×10^{-5} – Adults	0.0002	0.002
		4.1×10^{-6} – Children	4.1×10^{-5} – Children	0.0004	0.004
Dimethoate	0.001	1.1×10^{-6} – Adults	1.0×10^{-5} – Adults	0.001	0.01
		2.6×10^{-6} – Children	2.6×10^{-5} – Children	0.003	0.03
Ethoprophos	0.0001	1.1×10^{-6} – Adults	1.0×10^{-5} – Adults	0.01	0.11
		2.7×10^{-6} – Children	2.7×10^{-5} – Children	0.03	0.30

HI_1: Hazard Index based on per capita consumption in the study area.

HI_2: Hazard Index based on national per capita consumption

Table 5.7: Health risk estimates for systemic effects associated with pesticide residues in Onions

Pesticide	ADI mg/kg/day	Exposure dose- mg/kg/day (based on Per capita consumption in study area)	Exposure dose- mg/kg/day (base on national Per capita consumption)	HI_1	HI_2
β-HCH	0.005	2.1×10^{-4} – Adults	1.6×10^{-4} – Adults	0.04	0.03
		4.8×10^{-4} – Children	3.9×10^{-4} – Children	0.10	0.08
γ-HCH	0.005	1.4×10^{-4} – Adults	1.1×10^{-4} – Adults	0.03	0.02
		3.2×10^{-4} – Children	2.6×10^{-4} – Children	0.07	0.05
δ-HCH	0.005	1.7×10^{-5} – Adults	1.4×10^{-5} – Adults	0.003	0.003
		4.0×10^{-5} – Children	3.0×10^{-5} – Children	0.008	0.006
Dieldrin	0.0001	2.3×10^{-5} – Adults	1.8×10^{-5} – Adults	0.23	0.18
		5.3×10^{-4} – Children	4.2×10^{-5} – Children	5.30*	0.42
Endrin	0.0002	3.1×10^{-5} – Adults	2.5×10^{-5} – Adults	0.15	0.12
		7.2×10^{-5} – Children	5.7×10^{-5} – Children	0.36	0.29
β-HCH	0.005	2.1×10^{-4} – Adults	1.6×10^{-4} – Adults	0.04	0.03
		4.8×10^{-4} – Children	3.9×10^{-4} – Children	0.10	0.08
γ-chlordane	0.001	2.3×10^{-5} – Adults	1.8×10^{-5} – Adults	0.02	0.02
		5.3×10^{-5} – Children	4.2×10^{-4} – Children	0.05	0.04
Methoxychlor	0.005	5.1×10^{-5} – Adults	4.1×10^{-5} – Adults	0.01	0.008
		1.2×10^{-4} – Children	1.0×10^{-4} – Children	0.02	0.02
Allethrin	0.008	1.3×10^{-4} – Adults	1.0×10^{-4} – Adults	0.02	0.01
		3.0×10^{-4} – Children	2.3×10^{-4} – Children	0.04	0.03
Methamidophos	0.001	1.4×10^{-4} – Adults	1.0×10^{-4} – Adults	0.14	0.11
		3.2×10^{-3} – Children	2.5×10^{-4} – Children	3.20*	0.25
Dimethoate	0.001	1.8×10^{-4} – Adults	1.4×10^{-4} – Adults	0.18	0.14
		4.1×10^{-4} – Children	3.3×10^{-4} – Children	0.41	0.3
Fenitrothion	0.005	7.4×10^{-5} – Adults	5.9×10^{-5} – Adults	0.01	0.01
		1.7×10^{-4} – Children	1.4×10^{-4} – Children	0.04	0.03
Chlorfenviphos	0.0005	4.4×10^{-5} – Adults	3.5×10^{-5} – Adults	0.09	0.07
		1.0×10^{-4} – Children	8.2×10^{-5} – Children	0.21	0.16

HI_1: Hazard Index based on per capita consumption in the study area.

HI_2: Hazard Index based on national per capita consumption

*** :** Hazard index significant enough to indicate possible health implication

5.4 Discussions

5.4.1 Organochlorine pesticide residues in fruit and vegetables

Twelve organochlorine pesticide residues (OCPs), namely: β-HCH, γ-HCH, δ-HCH, *p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, dieldrin, endrin, heptachlor, γ-chlordane, β-endosulfan and methoxychlor, representing 80% were detected in the fruit and vegetables (watermelon, pepper and onion) samples analysed. The range of mean concentration was 0.001 – 0.084 mg/kg. The highest concentration was measured for β-HCH in onion (0.084 mg/kg) while the lowest (0.001 mg/kg) was recorded for p,p'-DDT in watermelon (Tables 5.1 – 5.3). Fifty percent of the watermelon samples registered the presence of one or more OCP residues while residues were measured in 100% samples of both pepper and onion. The limit of detection of OCPs was 0.15µg/kg (0.00015 mg/kg). The total of the mean of all OCP residues (load) was highest in onion (0.288 mg/kg), followed by watermelon (0.166 mg/kg) and then pepper (0.136 mg/kg). The incidence rates of the OCPs were lower in watermelon but higher and similar in pepper and onion (Tables 5.1 – 5.3). In all, nine pesticides (: β-HCH, γ-HCH, δ-HCH, *p,p'*-DDE dieldrin, endrin, heptachlor, γ-chlordane, and methoxychlor), representing 24.3% exceeded the European union maximum residue level (EU MRL). Eight of them (21.6%) exceeded in onion (Table 5.3), four (10.8%) in watermelon (Table 5.1) and 3 (8%) in pepper (Table 5.2). Twenty one and a third percent (21.3%) of watermelon samples contained organochlorine pesticide residues whose concentrations were above the EU MRL; 35% of pepper samples contained pesticides above MRL while in onion it was 29.4% of samples. Meanwhile, 100% of the samples registered the presence of one or more OCP pesticides.

Botwe *et al.*, (2011) measured concentrations of DDT, lindanes and methoxychlor in pepper and onion samples from Ashanti region in a range of 0.01 – 7.43 µg/kg and 0.02 - 46.95 µg/kg respectively. These figures are lower than what is reported in this study for the same vegetables. In

the same way, reported concentrations for vegetables by Owago *et al*., (2009) for β-HCH, γ-HCH, δ-HCH, DDE, DDD and DDT for vegetables in Deyang and Yantin areas in China were comparably lower than findings in this study. However, residue concentrations of 0.041 mg/kg, 0.023 mg/kg and 0.035 mg/kg measured by Bempah *et al*. (2012) respectively for methoxychlor, *p,p'*-DDE and *p,p'*-DDT in onion samples from the Greater Accra Region of Ghana were observed to be higher than corresponding concentrations from the current study. Bempah *et al*., (2012) also recorded higher levels of DDT (0.008 mg/kg) in watermelon samples but lower lindane (0.004 mg/kg), methoxychlor dieldrin and endrin levels. Whereas their (Bempah *et al*., 2012) investigation of pesticide levels in fruits and vegetables from markets in Greater Accra recorded 4 OCPs (methoxychlor, lindane, dieldrin, and endrin) exceeding the European Commission (EC) maximum residue limits, their earlier similar investigation (Bempah *et al*., 2011) in Kumasi metropolis reported 5 (γ-lindane, methoxychlor, dieldrin endrin and *p,p'*-DDE) exceeding MRL.

Asiedu (2013) who also investigated pesticide residues in fruits and vegetables in three regions of Ghana (Eastern, Central and Greater Accra) recorded far higher mean concentration range of OCPs (0.01 – 1.27 mg/kg), as compared to 0.001 – 0.084 mg/kg in the present study. He also listed 7 OCPs (β-HCH, γ-HCH, heptachlor, aldrin, dieldrin, endrin and p,p'-DDE) (as against 9 in the present study) as exceeding the MRL. Comparison of the present results with that of the other local studies quoted above reveal that β-HCH, γ-HCH, heptachlor, dieldrin, endrin, *p,p'*-DDE and methoxychlor always seem to exceed MRL in fruits and vegetables. Meanwhile, all of them are listed among pesticides that have been banned from agricultural use in this country. Although their levels may not necessarily be due to current inputs, there is still therefore the need for stakeholder agencies like Ghana EPA and Standard Authority to redouble their efforts in monitoring. Whereas the consistent similar concentration levels of β-HCH and γ-HCH fruit and vegetables analysed in

this study could imply both new input and biotransformation of γ-HCH to β-HCH, *p,p'*-DDE is known to be a degradation product of DDT that is energetically more stable and therefore persistent in matrices of many commodities Manz *et al*., (2001). Endrin is also a conversion product of both aldrin and dieldrin. From the current study, endrin dieldrin and aldrin were not detected in watermelon while only endrin was recorded in chilli pepper. In onion however, both endrin and dieldrin were recorded with endrin: dieldrin ratio (1.4) being greater than unity, implying source of the drins to be from old inputs. Prevalence of methoxychlor on the other hand is difficult to account for, particularly because field survey does not indicate its use. Bempah and Donkor (2010), however, suggested that methoxychlor is technically a component of DDT during its formulation and therefore as historically used DDT is being degraded, level of methoxychlor should be expected to rise.

5.4.2 Synthetic pyrethroid residues in fruit and vegetables

Synthetic pyrethroids (SPs) were detected in watermelon, chili pepper and onion samples and in a mean concentration range of 0.0005 mg/kg – 0.0604 mg/kg. The highest was measured for allethrin in watermelon, while the lowest was recorded for deltamethrin in pepper. Residues were measured in 13.3% of watermelon samples, 75% in onion and 100% in pepper. Forty-four percent of the SPs were measured in watermelon and 100% of them measured in both onion and pepper samples. The limit of detection and quantitation were 0.00015 mg/kg (0.15 µg/kg) and 0.0005 mg/kg (0.5. µg/kg), respectively. Pyrethroid load was highest for onion (0.117 mg/kg), followed by watermelon (0.0708 mg/kg) and then pepper (0.0501 mg/kg). Only allethrin exceeded the MRL in all the three food crops analysed. Whereas all samples contained residues, only 14.3% of them recorded pyrethroid pesticides with levels above MRL.

Bempah *et al*., (2011) analysed pyrethroid levels in fruits and vegetables in Kumasi, Ashanti Region of Ghana. They quantified levels of only permethrin, cyfluthrin and deltamethrin in watermelon. These pyrethroids were, however, below limit of detection in watermelon samples from this study. They also quantified levels of permethrin, cyfluthrin, fenvalerate and deltamethrin in pepper and onion in a range of 0.009 mg/kg – 0.10 mg/kg which was higher, compared to 0.0005 mg/kg – 0.02 mg/kg for the same pesticides in this study. In another study, the same nine SPs under investigation in this study were investigated by Gonu *et al*., (2012) in pepper samples from an irrigation field in the Northern Region. They established a concentration range of 0.0023 mg/kg – 0.1428mg/kg. Their range was higher than the 0.0005 mg/kg – 0.0156 mg/kg recorded for pepper in the current study. Whereas allethrin and deltamethrin recorded the lowest and the highest concentrations respectively in their study, deltamethrin and permethrin recorded the highest and the lowest concentrations, respectively in this study.

Comparison with works from other regions of the world reveals that findings in this study are in good agreement with that of Zawiya *et al*., (2007) in Malaysia, and Masud and Hassan (1992) in Pakistan. As noted by Bempah *et al*., (2011), the increasing presence of pyrethroid insecticide residues in food crops is an indication of a shift from the use of organochlorine pesticides to the easily degradable groups of pesticides. If the pyrethroids are non-persistent yet they are found to have high detection rate in food crops, then the misuse and abuse of them are likely and this should be cause for serious concern and therefore more investigation.

5.4.3 Organophosphorus pesticide residues in fruit and vegetables

All the thirteen organophosphorus pesticides (OPs) under study, i.e. 100% of the OPs were measured in the sample. Ten, eleven and twelve OPs were measured in watermelon, pepper and onion samples, respectively. These values represent 77%, 84% and 92.3% of OPs respectively detected in the food crops (Tables 5.1-5.3). The mean concentration range recorded for the OPs in the analysed fruit and vegetables was 0.0005 – 0.0711 mg/kg. Dimethoate recorded the highest value in onion while the least was recorded by chlofenvinphos in watermelon. Onion recorded the highest OP load of 0.2735 mg/kg; followed by pepper (0.048 mg/kg) and then watermelon (0.0327 mg/kg). Whereas none of the watermelon and pepper samples recorded OP level above MRL, onion recorded levels in four OPs (methamidophos, ethoprophos, dimethoate and fenitrothion) exceeding MRL (Table 5.3). Methamidophos for instance, apart from having 100% incidence rate, also exceeded MRL in 100% of the samples.

The level of OPs in food crops in this study agrees with local findings by Bempah *et al*., (2012) and Botwe *et al*., (2011) as well as studies outside Ghana by Sanghi and Tewari (2001) and Paeveen, Khuhro and Rafiq, (2004). Dimethoate levels in pepper and onion were however higher than those reported by Botwe *et al*., (2011). The fact that every single sample contained one or more pesticide residue indicates most farmers did not follow good agricultural practices of observing a pre-harvest interval (PHI); a minimum period of time allowed between the last application of pesticide on food crops and final harvesting. The PHI is specific for each pesticide and is necessary to allow enough time for pesticides to degrade to an acceptable level (Baig *et al.*, 2009).

5.4.4 Health Risk Estimate

Health risk estimates for systemic effects associated with pesticide residues in watermelon, pepper and onion are presented in Tables 5.5 -5.7. The tables comprise acceptable daily intakes (ADIs), computed average daily intakes based on national per capita consumption, estimated per capita consumption for the study area and corresponding hazard indices for children (1-11 years) and grown-ups (12 years and above). The hazard indices showed that heptachlor, β-HCH, dieldrin and methamidophos showed health risk association. Examination of the results in Tables 5.5-5.7 shows that only watermelon and onion contain pesticides with hazard indices indicating health risk association. Heptachlor and β-HCH have significant hazard indices in watermelon while dieldrin and methamidophos had significant hazard indices in onion. The indices of heptachlor indicate potential for systemic toxicity in both adults and children.

It should be noted that apart from heptachlor indicating toxicity in both adults and children, the other pesticides with significant hazard indices (β-HCH, dieldrin and methamidophos) point to potential toxicity in children only, who constitute the most vulnerable subgroup of the population. It is also worthy of note that the hazard indices indicating potential health risk are all based on per capita consumption estimates for the study area. This trend was also observed by Ntow (2008) in vegetable producing community of Akumadan in the Ashanti Region of Ghana. Ntow explained that the trend is not surprising since the consumption of food crops is normally higher in the producing communities. In children, hazard indices computed for β-HCH, dieldrin and methamidophos (based on field per capita consumption) were less than unity (0.50, 0.53 and 0.32 respectively) (Tables 5.5 and 5.7). They are however significant and associated with health risk since their respective estimated doses (0.0044, 0.0053 and 0.0032 mg/kg/day) are either higher or close to their corresponding ADIs (Tables 5.5 and 5.7).

Analysis of 37 pesticide residues of watermelon, chili pepper and onion from cultivated fields along the banks of the Afram arm of the Volta Lake established the facts that 92% of the pesticides were recorded in 92.9% of the total samples. Hundred percent of target organophosphorus and pyrethroid and 80% of organochlorine pesticides were quantified in the samples. The ranges of mean concentrations of the three groups of pesticides, OCPs, SPs and OPs were 0.001 – 0.084 mg/kg, 0.0005 – 0.0604 mg/kg and 0.0005 – 0.0711 mg/kg respectively. The pesticide residual load in the three food commodities follows the order: onion > watermelon > pepper. Levels of 37.8% of the pesticides (β-lindane, γ-lindane, δ-lindane, γ-chlordane, dieldrin, endrin, p,p'-DDE, methoxychlor, alletrin, methamidophos, ethoprophos, diamethoate and fenvalerate) exceeded the EU MRL in one or more of the food crops, and as many as 10 detected pesticides are among those that have been banned from use by the EPA, Ghana. These are: β-lindane, γ-lindane, δ-lindane, γ-chlordane, dieldrin, endrin, p,p'-DDT, methamidophos, heptachlor and parathion. Health risk analysis indicates that heptachlor, β-lindane, dieldrin and methamidophos may be of public concern since their concentration levels exceeded the reference doses in watermelon and onion samples analyzed indicating a great potential for systemic toxicity to consumers in Ghana.

It must be noted that fruits and vegetables are now important commodities for Ghana's non-traditional export market. Standards and quality control measures demanded by European Union (EU) countries on these commodities are very high and exportation from Ghana has of recent been particularly problematic since pesticide quality standard of most of the commodities, especially pineapple has not been met. When this happens, the country loses foreign exchange earnings, farmers who made huge investment into the production fail to meet production cost, labourers on plantation farms are either laid off or unpaid and the entire economy is affected in one way or the other. Above all, the risk of systemic toxicity to local consumers become real concern since

chronic health effects as a result of long-term dietary pesticide exposures become difficult to deal with.

CHAPTER SIX

PESTICIDE CONTENT OF WATER AND SEDIMENT TOXICITY OF THE AFRAM ARM OF THE VOLTA LAKE

6.1 Background

Globally, there is widespread chemical pollution of fresh water bodies. This is of much concern since fresh water bodies tend to be main sources of potable water for many communities. A major source of chemicals like pesticides in water bodies is from run-offs from agricultural fields. The persistence and mobility of pesticides in soils, coupled with their ability to disperse in water greatly contribute to their potential to contaminate water (Nowell *et al.,* 1999). Pesticides may be in the dissolved phase or associated with soil particles when they enter surface water column (*Nowell et al.,* 1999). The particle-associated pesticides may subsequently be deposited to the bed sediment and remain in this domain for a very long time. Sediments therefore represent a principal long-term environmental sink of pesticides from which food chains, overlying water column as well as ground water could be contaminated (Nowell *et al.,* 1999; Xue *et al.,* 2006). Sediments are important in the functioning of aquatic ecosystems because they serve as reservoirs of contaminants for bioaccumulation and trophic transfer (Burton, 2002). For this reason, once chemical contamination level reaches a point at which it causes adverse effect to biota, sediment is considered polluted and hence merits remediation intervention. To determine whether sediment is contaminated or not, its quality is first determined and compared with internationally established sediment quality criteria or guidelines. Decisions are then made whether it presents risk or not. According to Fluck *et al.,* (2010), sediment quality criteria for instance can be depended on to classify sediment samples with regard to their toxic potential, identify problematic contaminants, put priorities on areas based on the frequency and degree of which values are exceeded. Risk

assessment of sediments is a relatively new and rapidly developing field that has been embraced by international research outfits worldwide (Burton, 2002).

The major inland water bodies in Ghana tend to be epicenters of numerous anthropogenic activities, particularly, agriculture. Existing literature search reveals all or most the water bodies are associated with some levels of pesticide residues. The Volta Lake in particular has experienced a sizeable increase in farming, industrial and cultural activities along its entire course. According to Ntow, (2005), the short distances between agricultural fields and waterways increase the probability of agrochemicals reaching the waterways via agricultural run-offs. The water level in the Volta Lake experiences occasional rise and fall. Currently, due to climate variability, the recession and flooding times are highly unpredictable. Nevertheless, there is still occasional flooding of the banks when there is sufficient recharge from the upper courses of the Volta Lake. When the level of water in the lake recedes, there is intense farming along the immediate banks to take advantage of the residual moisture in soil to grow short maturing crops such as vegetables and fruits. When water level rises again, the previously farmed lands, or lands still under cultivation are covered with water. It is pertinent to know the implication of occasional flooding of farmlands on pesticide water quality of the Afram waters, particularly since the river is the only source of potable water for the majority of the riparian communities.

In keeping with the worldwide interest in quantifying and controlling pollution of surface waters (Brown *et al.,* 2002), this chapter assesses pesticide content of surface sediment of Afram arm of the Volta Lake and evaluates its toxicity by using established sediment quality criteria (with particular respect to organochlorine pesticides). In addition, quality of water from the Afram arm of the Volta Lake during the recession period, and the period of water rise when cultivated lands are flooded is compared.

6.2 Materials and Methods

6.2.1 Sampling of water and sediment

Water samples were collected in December 2011 and July 2012. The December samples were taken at a time when water level had risen and flooded the banks while the July samples were taken when it was observed that water level had receded. A total of eighty water samples were collected from four sites (Odortom, Kwahu Amanfrom, Adawso and Kotoso sites – Fig 3.1) along the Afram arm of the Volta Lake. Water samples were taken approximately 0.5 m below the surface at midstream positions to approximate the mean concentrations of water from the river. The samples were taken with a 3 L Goflon water sampler into 1 L amber glass bottles that were previously washed with detergent, rinsed with distilled water, dried and finally rinsed with acetone. Prior to sample collection, each bottle was rinsed with sample water three times. Twenty samples per site were collected.

Sediment samples were taken from sites of water collection points after water samples had been taken. Grab samples of fine textured lake sediment were collected from the bottom at the mid-stream of the river. As much as possible, sediment samples were taken at mid-stream points where the Afram River was not very broad; otherwise, samples were generally taken at an approximate distance of 50 metres from the recession bank mark. Composite samples were pooled from three subsamples and properly homogenized. The composite samples were wrapped in aluminium foils and placed in zip-locked plastic bags. Twelve composite samples per site were taken; thus a total of forty-eight sediment samples were taken. Water and sediment samples were separately stored on ice at approximately 4°C during transport until they were brought to the Pesticide Laboratory at the Ghana Standard Authority where sediment samples were stored in a deep freezer at -4°C and water samples in refrigerator at 4°C pending analyses.

6.2.2 Chemicals and Reagents

Full list of Chemicals and Reagents needed for the study is presented in Appendix I

6.2.3 Extraction of samples

6.2.3.1 Extraction of pesticides from water samples

Water samples were removed from fridge and allowed to equilibrate with the ambient temperature. The water samples were extracted according to Mathur *et al.,* (2003) and as done routinely in the Ghana Standards Authority pesticides Laboratory. Water samples were well shaken and filtered through whatman no.1 filter paper. After filtration, 1 litre water sample was taken in a 2 litre capacity separatory funnel and 30 mL of saturated sodium chloride solution was added. The water sample was partitioned with 100 mL of methylene chloride (thrice) by shaking the separatory funnel vigorously for 2-3 minutes and releasing the pressure intermittently. The layers were allowed to stand for 10 minutes to separate. The three extracts of methylene chloride layers were combined and passed through anhydrous sodium sulphate and concentrated to about 2 mL using rotary vacuum evaporator.

6.2.3.2 Extraction of pesticides from sediment samples

Sediment was wet extracted and followed the method of Muir & Sverko, (2006). Sediment samples were removed from storage freezers and allowed to thaw. The percentage of water in each sample was determined by accurately weighing 10 g, drying at 105°C and reweighing to a constant dry weight. A wet weight equivalent to 10 g dry sample was calculated for each sample after determining the percentage of water. Equivalent of 10 g dry sediment, samples were therefore extracted per sample. Comminuted homogenous wet weight sediment samples equivalent to 10 g dry samples were weighed into 100 mL separating flasks and 10ml acetonitrile added to each. The

flasks were stoppered and sonicated for 5 minutes in a Decon sonicator model FS 400B. A further 10 mL acetonitrile was added to each flask, placed on a horizontal mechanical shaker and set to shake continuously for 30 minutes at 300 motions per minute. The mixture in each flask was allowed to stand for 10 minutes to separate layers. The organic phase (top layer) was decanted and dried over 5 g anhydrous magnesium sulphate through No. 1 Whatman filter paper. Ten mililitre aliquots of the filtrate were introduced into 50 ml round-bottomed flasks and evaporated to 1 mL concentrate using Buchi rotary vacuum evaporator. Duplicate of previously extracted sediment samples were spiked with standard pesticide mix solutions and taken through the entire extraction process in order to determine recovery efficiency.

6.2.4 Clean-up of pesticide extracts from water and sediment samples

Clean-up of the extracted samples was done by column chromatography. Ten mililitres polypropylene cartridge was packed with activated silica gel 2 g (heated for 2h at 130°C) packed between two layers of sodium sulphate (1 g each) and the column was conditioned with 10 mL of methylene chloride and not allowed to dry. The 2 mL concentrate from the water extraction was then loaded onto the conditioned cartridges. The flask was rinsed with 10 mL methylene chloride and used to elute the column into a pear-shaped flask. The elution was repeated with another 10 mL portion of methylene chloride. The combined eluate was collected and evaporated to dryness using rotary vacuum evaporator. A final sample of 2 mL was prepared in ethyl acetate (HPLC grade) and analyzed by Gas Chromatograph. Cleaning of sediment extracts was done exactly in the same manner except that acetonitrile, instead of methylene chloride was used for the elution.

6.2.5 Recovery test of extraction efficiency

Recovery tests for water analysis was carried out prior to analysis by fortifying de-ionized water samples with 5 ml of 0.05 µg/L standard mixture solutions of organochlorines, organophosphorus and synthetic pyrethroid pesticides to determine the efficiency of the analytical techniques. Similarly, 5 ml of 0.05 µg/L standard mix pesticide solution was spiked into previously extracted sediment samples. These fortified samples were then extracted in manners similar as their corresponding matrix samples.

$$\% \, Recovery = \frac{C2 - C1}{C} \, X \, 100$$

Where: C_1 = Concentration (mg/kg) of pesticide residue in the matrix blank

C_2 = Concentration (mg/kg) of pesticide residue in the spiked matrix

C = Concentration (mg/kg) of pesticide added

Recovery for OCs and SPs was in the range of 75-95 % and 70-80 % for OPs.

Reagent or procedural blanks were also extracted in the same manner and found to be devoid of any interfering agents. Calibration of instruments, quantification of pesticide residues and limits of detection and quantification as well as calibration standards were all set as described in chapter 4. The limit of detection (LOD) and limit of quantitation (LOQ) for the determined pesticides in water were 0.003 µg/L and 0.01 µg/L respectively; and 0.15 µg/kg and 0.5 µg/kg respectively in sediment.

6.2.6 Quality assurance and control

For each batch of 20 samples, a procedural blank, a spiked blank and a pair of matrix spiked sample/duplicate were processed. The spiked samples contained all the 15 OCPs, 13 OPs and 9 SPs target analytes. All reagents used during the analysis were of high quality and were exposed to the same extraction procedures and subsequently run to check that no interfering substances were present. No analytes were detected in the blanks. Recalibration curves were run with each sample batch to ensure that correlation coefficient was kept above 0.99. Strict cleaning procedures were adhered to viz: all glassware were washed with hot water and detergents and copiously rinsed with distilled water. After drying, the glass wares were further rinsed with acetone.

6.2.7 Gas Chromatographic quantification of extracted pesticides

The final pesticide extracts were analyzed for organochlorine and synthetic pyrethroid pesticides by Gas Chromatograph- Varian CP-3800 (Varian Association Inc. USA) equipped with combiPal autosampler and ^{63}Ni electron capture detector (ECD) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/kg range) from the matrix to which other detectors do not respond. The procedure follows the Ghana Standard Authority pesticide laboratory routine process of conditioning the Gas Chromatography for pesticide quantification. The GC conditions used for the analysis were capillary column coated with VF-5 (30 m + 10 m guard column x 0.25 mm i.d, 0.25 µ m film thickness). The injector and detector temperatures were set at 270°C and 300°C respectively. The oven temperature was programmed as follows: 70°C held for 2 min, ramp at 25°C/min to 180°C, held for 1 min, and finally ramp at 5°C/min to 300°C. Nitrogen was used as carrier gas at a flow rate of 1.0

$mLmin^{-1}$ and detector make-up gas of 29 mL/min. The injection volume of the GC was 1.0 µ L. The total run time for a sample was 31.4 min.

Organophosphorus pesticides on the other hand were analyzed by Gas Chromatograph- Varian CP-3800 (Varian Association Inc. USA) also equipped with combiPal autosampler and pulse flame photometric detector (PFPD) that allowed the detection of contaminants even at trace level concentrations (in the lower µ g/g range) from the matrix to which other detectors do not respond. The GC conditions used for the analysis were capillary column coated with VF-1701 (30 m x 0.25 mm i.d, 0.25 µ m film thickness). The injector and detector temperature were set at 270°C and 280°C respectively. The oven temperature was programmed as follows: 70°C held for 2 min, ramp at 25 °C/min to 200°C, held for 1 min, and finally ramp at 20 °C/min to 250°C maintained for 3.3 min. Nitrogen was used as carrier gas at a flow rate of 2.0 $mLmin^{-1}$ and detector make-up gases (17.0, 14.0 and 10.0 mL/min) for hydrogen, air-1 and air-2, respectively. The injection volume of the GC organophosphorus pesticide determination was 2.0 µ L. The total run time for a sample was 14 min. Each sample underwent duplicate analyses.

6.2.8 Data Analysis

6.2.8.1 Water data analysis

Simple arithmetic means, range and frequencies were employed in analyzing the lake water data. Means of concentrations, concentration ranges and frequency of occurrence of various pesticides were compared under flood and recession states. Number of pesticides whose residues concentrations exceeded the WHO maximum residue limits under flooding and recession situations were also compared.

6.2.8.2 Determination of sediment toxicity

In determining toxicity, sediment quality criteria, which provide scientific benchmarks, or reference points, for evaluating the potential for observing adverse biological effects in aquatic systems (Fluck *et al.,* 2010) is employed in this study. The approach of Fluck *et al.*, (2010), is adopted. Quotient of pesticide between its concentration in sediment and its quality criteria is calculated as follows:

$$RQsi = MECi \,/\, SQCi \qquad (6.1)$$

RQs*i* = Risk quotient for pesticide *i* in sediment
MEC*i* = measured environmental concentration (in total sediment) for pesticide *i*
SQC*i* = sediment quality criteria for pesticide *i*

Calculation of mean quotient for more than one contaminant, according to Fluck et al, (2010) is also considered as follows:

$$RQm = \Sigma RQsi \,/\, n \qquad (6.2)$$

RQm = mean risk quotient in sediment
ΣRQs*i* = sum of RQs*i*
n = number of calculated quotients

Decision rule

- If RQm or RQs*i* < 0.1, the risk is said to be negligible
- If 0.1 < RQm or RQs*i* < 0.5, the risk is said to be low, but the non-hazard of sediments has to be checked.
- If RQm or RQs*i* > 0.5, the risk is non-negligible and there is the need for a detailed diagnosis.

Approaches to sediment quality assessment generally employ two concentration threshold values; a lower limit of sediment quality value below which effects rarely occur and an upper value, above which harmful effects on sediment-dwelling organisms are likely to occur (Burton, 2002). According to Fung *et al.*, (2005), values of the two thresholds can be used to calculate risk quotients under the best-case (RQb) and the worst-case (RQw) scenarios as follows:

$$RQb = \frac{\text{Lowest measured concentration of a chemical in sediment}}{\text{Upper limit (threshold) of sediment quality value } (SQV_{UL})} \quad (6.3)$$

$$RQw = \frac{\text{Highest measured concentration of a chemical in sediment}}{\text{Lower limit (threshold) of sediment quality value } (SQV_{LL})} \quad (6.4)$$

Various institutions and nations have developed guidelines by setting their own threshold values. A combination of two different guidelines, as a matter of propriety and legitimacy, is often used to assess potential risks. Ghana has no established/interim national or institutional guidelines; therefore the guidelines employed here are the protocols of Canadian Council of Ministers of the Environment (1999) for the derivation of Canadian Sediment Quality Guidelines - CSQG (at Threshold effect level [TEL]) and Probable effect level [PEL]); and the United States National Oceanic and Atmospheric Administration – NOAA [at the Effects range-low (ERL) and Effects range-median (ERM) levels, (Burton, 2002)]. TEL and ERL represent the lower threshold values while PEL and ERM represent upper limits in their respective guidelines.

The determination of RQb and RQw present a simple way of characterizing the risk associated with a pesticide in the sediment. In principles, if the RQb and RQw calculated values are greater than 1, then the pesticides in question could pose risk and therefore require remediation attention. Conversely if the value of RQb and RQw are less than 1, the chemical is probably of little concern and thus could be accorded a lower management or remediation priority attention. In situations

where both risk quotients do not present the same interpretations, (e.g. RQb < 1 and RQw >1) then a more refined risk assessment must be undertaken to ascertain the risk due to specific chemicals (Fung *et al*, 2005). The toxicity assessment in this study is limited to only the organochlorine pesticides since quality criteria are not yet developed for the other pesticide groups. Even then, not all OCPs have established guidelines.

6.3 Results

6.3.1 Pesticide content of lake water

There were differences in the number of pesticides recorded under flood and recession conditions. For instance 95% of target pesticides were detected and quantified under flood condition while 70% were detected under recession situation (Appendices II and III). The reduction occurred much more in organochlorine pesticides (OCPs) and synthetic pyrethroids (SPs), while the Organophosphorus pesticides (OPs) detects remained approximately the same.

Frequencies of occurrence of quatified pesticides were very high under the flood condition, especially among the OCPs and the SPs, almost all of which registered 75% and above (Appendices II and III). Curiously however, the OP incidence during the recession was higher than during the flood condition.

It is also noted that the concentration levels during the flood state were far higher, with as many as 12 pesticides residues exceeding the maximum level prescribed by the WHO/GSA in samples, as against only 5 during the recession. Table 6.1 presents a vivid detail of specific and general differences in quality of water between the two regimes. Total of mean concentrations, frequency of occurrence and number of pesticides exceeding WHO recommended limits for the pesticide groups under study were all higher during the flood regime. Fourteen OCPs, 9 SPs and 12 OPs were quatified during the flood, as against 10, 4 and 11 respectively, during the recession. It is however observed that the recess regime recorded higher frequency of occurrence of OPs. Some of the organophosphates like dimethoate, malathion, chlorfenvinphos and profenofos also recorded higher residue levels during the recession (Appendices I and II). However, total OP load under flood regime (0.91µg/L), just like those of OCPs and SPs, was still higher, compared to that under the recession regime (0.38 µg/L).

Table 6.1: Comparison of pesticide water quality during occasional flooding of farmlands and period of recession

Pesticide group	Flood				Recession			
	MC_T (µg/L)	Range (µg/L)	Average Frequency (%)	MRLe	MC_T (µg/L)	Range (µg/L)	Average Frequency (%)	MRLe
OCPs	15.220	0.01-16.79	89.6	6	0.497	0.01-0.269	17.5	2
SPs	1.470	0.01-2.135	85	6	0.127	0.01-0.37	23.8	1
OPs	0.910	0.01-1.12	20	0	0.382	0.01-0.158	31	2
Total	17.60	0.01-16.79	NA	12	1.01	0.01-0.370	NA	5

MC_T = Total of mean concentrations of all residues in a pesticide group

MRL_e = Number of residues Exceeding WHO MRL

Flood = Period during which water level in the Afram Lake rises and covers part of the bank that is cultivated for crop production.

Recess = Period during which water in the Afram Lake assumes normal level following flood regime, exposing land with high moisture content suitable for cultivation of short-maturing crops.

NA = Not applicable

6.3.2 Pesticide content and toxicity of sediment

Concentration of pesticide residues in sediment are presented in Table 6.2. The OCPs recorded the highest average concentration, with the average of the concentration of all OCP pesticides as 11.26 µg/kg. Followed by the organophosphates (7.85 µg/kg) and pyrethroids (5.84 µg/kg). Dimethoate, though less frequently encountered recorded the highest concentration of 58.9 µg/kg. Gamma chlordane recorded the next highest concentration of 42.69 µg/kg, followed by p,p-DDD, p,p-DDE, γ-lindane, and δ-lindane with respective concentrations of 35.76 µg/kg, 31.00 µg/kg, 14.67 µg/kg and 9.39 µg/kg. Organophosphorus pesticide residues, with the exception of dimethoate registered only trace concentrations. They were however more frequently encountered than the other group of pesticides. Concentration ranges of OCPs, SPs and OPs are 1.55-42.69 µg/kg, 1.96-13.18 µg/kg and 1.41-58.90 µg/kg respectively. The top ten most frequently encountered pesticides were γ-lindane, chlorpyrifos,profenofos, cyfluthrin, chlorfenviphos, fenitrothion, ethoprophos, p,p-DDD, fenvalerate and parathion, with incidence rate ranging from 71% - 97%.

The calculated risk quotients of some OCPs are presented in Table 6.3 The risk quotient under best-case scenario (RQb) estimates of p,p-DDD, γ-chlordane, dieldrin, and endrin were all below 1 based on both CSQG and NOAA. Gamma-lindane was the only chemical whose risk quotient under best case scenario was more than 1 based on both CSQG and NOAA. It is also noted that, with the exception of dieldrin, the risk quotients of all the pesticides in question, under worst case scenario were greater than 1 based on both CSQG and NOAA. The risk quotients of *P,P'*-DDD, *p,p*-DDE, γ-chlordane, and γ-lindane under the worst case scenario had very high values (greater than 10) based on both CSQG and NOAA.

Table 6.2: Concentration of pesticide residues in sediment

Pesticides	Mean ± SD (µg/kg)	Range (µg/kg)	Frequency (%)
Organochlorines			
β-HCH	2.16 ± 0.9	0.50-3.50	53.0
γ-HCH	14.67 ±8.4	1.52-38.95	97.0
δ-HCH	9.39 ±12.9	0.50-55.00	47.0
Heptachlor	4.10	4.10	3.0
Aldrin	2.88 ±4.6	1.40-17.60	37.5
γ-Chlordane	42.69 ± 27.3	1.60-95.00	47.0
α-endosulfan	2.18 ±0.5	1.70-3.50	37.5
p,p'-DDE	31.00 ±9.7	14.80-49.80	37.5
Dieldrin	1.55 ±0.4	1.20-2.50	37.5
Endrin	4.55 ±0.1	4.40-4.80	25.0
Beta endosulfan	5.20	4.00-6.40	6.0
p,p'-DDT	4.60 ±2.0	0.50-8.80	37.5
p,p'-DDD	35.76 ±29.5	3.69-91.55	72.0
Endosulfan sulfate	2.12 ±0.7	1.00-3.30	50.0
Methoxychlor	6.10 ±1.8	5.00-9.60	19.0
Pyrethroids			
Allethrin	6.35 ±3.5	0.77-9.30	15.6
Bifenthrin	13.18 ±1.0	12.00-15.00	37.5
Fenpropathrin	3.43 ±0.8	2.48-5.00	44.0
Lamda-cyhalothrin	4.35	4.40	6.0
Permethrin	7.67 ±3.6	1.71-11.70	15.6
Cyfluthrin	6.15 ±5.1	0.50-16.20	78.0
Cypermethrin	5.41 ±3.2	1.00-12.60	67.0
Fenvalerate	4.08 ±2.5	0.50-8.20	72.0
Deltamethrin	1.96 ±1.8	0.50-8.10	44.0
Organophosphate			
Methamidophos	5.35 ±3.1	0.98-9.00	13.5
Ethoprophos	2.26 ±1.0	1.17-5.30	73.1
Phorate	2.57 ±1.9	0.52-8.80	71.0
Diazinon	1.43 ± 1.1	0.50-5.00	69.2
Fonofos	1.41 ± 1.7	0.50-7.50	61.5
Dimethoate	58.90 ± 3.5	57.00-63.00	5.8
Pirimiphos-methyl	1.48 ±0.9	0.50-3.58	57.7
Chlorpyrifos	9.50 ±14.8	0.51-69.00	94.0
Malathion	1.73 ±1.1	0.50-3.00	32.7
Fenitrothion	3.00 ±1.5	0.85-6.60	77.0
Parathion	3.40 ±2.5	0.52-8.42	71.0
Chlorfenvinphos	6.00 ±8.4	0.56-50.00	77.0
Profenofos	5.00 ±3.9	0.51-17.45	80.8

Table 6.3: Estimated risk quotients of organochlorine pesticides concentrations in sediments (OCPs) based on Canadian Sediment Quality Guideline (CSQG) and National Oceanic and Atmospheric Administration NOAA) guidelines.

Pesticide	OCPc	NOAA (µg/kg)		CSQG (µg/kg)		1>RQb>1	0.1<RQw<1	1<RQw<10	RQw>10
		ERL	ERM	TEL	PEL				
p,p-DDD	3.69-91.55	2.0	20	3.54	8.51	○●			○●
p,p-DDE	14.8-48.8	2.0	27	1.42	6.8	● ○			○●
p,p-DDT	0.50-8.80	1.0	7	-	-	●		●	
γ-chlordane	1.60-95.0	0.5	6	4.5	8.9	○●			○●
Dieldrin	1.20-2.50	0.02	8	2.85	6.67	○●	○		●
Endrin	4.40-4.80	0.02	8	2.67	62.4	○●		○	●
γ-Lindane	1.52-49.0	0.32	1	0.94	1.38	○●			○●

Risk quotients were calculated based on two sediment quality criteria (○ = CSQG; ● = NOAA)

6.4. Discussion

6.4.1 Variation of pesticide content of Lake Water under flood and recession regimes

Details of pesticide content of lake water from the Afram arm of the Volta Lake (AVL) during periods when lake flooded agricultural fields and when it receded to its normal level in the lake are presented in Appendices I and II. Comparison of pesticide water quality during both regimes is summarized in Table 6.1. Hundred per cent of water samples taken under both flood and recess regimes contained more than one pesticide residue. All pesticides under investigation were detected in water samples (except alpha-lindane). Thus 37 different pesticide residues were quantified in the water samples and this underscores the application of a wide range of pesticides in the agricultural fields along the banks of AVL.

Occasional flooding of the agricultural fields by the lake water is perceived to have implication on the occurrence and level of concentration of pesticide residues of water hence the decision to assess pesticide content of water under both regimes. From the results in Appendices I and II, 35 different pesticides were quantified during flood regime, as compared to 25 under recess regime. An average frequency of occurrence of any pesticide during flood regime was 65% compared to 24.1% during recess (Table 6.1). The range of concentration and pesticide load (total of mean of concentrations of all pesticides) under the two regimes were 0.01 µg/L – 16.79 µg/L and 17.60 µg/L respectively for flood period; and 0.01 µg/L – 0.37 µg/L and 1.01 µg/L respectively for the recess (Table 6.1). Whereas as many as 12 pesticide residues in water samples exceeded the WHO MRL during the flood, only 5 exceeded during the recess. Higher frequency of occurrence and concentration of pesticide residues implies associated higher health risk with water consumption during the flood regime. Unfortunately this is the only source of potable water for most of the communities along

the AVL. Even the communities in which there is provision of boreholes, individuals still prefer water from the lake since majority of them complain about high salt content of borehole water.

Recession of water in the lake was previously predictable hence farmers could plan when to cultivate the immediate banks of the lake ('drawdown' areas) and harvest in time before water levels rose. Within the past decade however, farmers have confirmed that periods of rise and fall in the Afram arm of the Volta Lake is no longer predictable; obviously due to climate variability. Farming in the 'drawdown' areas has therefore become a risky commercial and subsistence venture since farms could be flooded before maturity of food crops. Plates 4a - 4c present scenarios of events in typical drawdown areas. In such situations, agrochemicals on farmlands dissolve in the water causing their levels to be elevated. This primarily explains high incidence and concentration levels of even non-persistent second generation pesticides like pyrethroids and organophosphates. For instance, methamidophos recorded 0.75 µg/L; cypermethrin, 0.637 µg/L; fenpropathrin, 0.341 µg/L; deltamethrin, 0.234 µg/L; permethrin, 0.066 µg/L and allethrin 0.051 µg/L during flooding, all exceeding their WHO maximum residue limits (MRL). The most ubiquitous of the pesticides under flood regime were allethrin, bifenthrin, permethrin, deltamethrin, β-HCH, γ-HCH, δ-HCH, aldrin endrin *p,p'*-DDD, endosulfan sulfate and methoxychlor, all of which registered 100% incidence rate. Dieldrin and endrin, degradation compounds of aldrin had higher concentrations than their parent compound, aldrin; suggesting the presence of aldrin was due to old inputs and confirming the ban on use of this pesticide in Ghana. However, the general high concentrations of both parent and metabolites seem to indicate some level of current inputs that needs to be further investigated.

The concentration of some pesticides (chlorfenvinphos, dimethoate and cypermethrin), even during the recess regime exceeded MRLs and gives cause for concern since water from the lake is the only

reliable source for all purposes, including drinking. Water from the Afram arm as well as water from the upper reaches of the Volta Lake all flow down into the Kpong water head where water is treated and distributed for domestic consumption in the greater part of Greater Accra Region. Therefore, quality of water from the Kpong water works, by implication is a function, to some extent, of the quality of water that it receives and is treated. It is therefore necessary that the Volta Lake as a whole is protected from potential pollution and contamination that can result from anthropogenic activities taking place along its course. The results from this study under the recess regime agree with earlier investigation into OCP levels in other parts of the Volta Lake by Ntow (2005). So also are the results in agreement with other local works done by Darko *et al*., (2008) on Lake Bosomtwi, Kuranchie-Mensah *et al*., (2012) and Fianko *et al*., (2011) on Densu River. However, results under flood regimes are several folds higher than their reported values. Pesticide pollutions of fresh water bodies in Ghana are primarily due to agricultural activities in their vicinity as reported by various researchers including Darko *et al*., (2008); Adu-Kumi *et al*., (2010); Botwe, Ntow and Nyarko (2012); Fianko *et al*., (2012) and Afful *et al*., (2013). Pollution of the water bodies, according to Ntow (2005), is facilitated by the short distances between agricultural fields and waterways that increase the probability of agro-chemicals reaching the water bodies via run-offs.

6.4.2 Level of organochlorines in sediment

Fifteen organochlorine pesticide residues were detected in sediment samples from AVL (Table 6.2). The general concentration range was 0.5 µg/kg - 95.0 µg/kg, while the mean concentration range was 1.55 µg/kg – 42.69 µg/kg. The highest concentration recorded in a single sample as well as for the mean was for Gamma-chlordane. The most frequently encountered OCPs were γ-HCH (97%), p,p'-DDD (72%), β-HCH (53%) and endosulfan sulfate (50%). Frequencies of occurrence of the rest were below 50%. While the mean concentration range in the current study lie between that quoted by Fianko *et.al.*, (2011) for 14 OCPs in sediment from River Densu (0.10 µg/kg - 163.00 µg/kg) and that of Kuranchie-Mensah *et al*., (2012) for Lake Bosomtwi (0.03 µg/kg - 10.98 µg/kg, it was below what was reported by Fung *et al*., (2005) for Pearl River Delta in China (1.4 µg/kg - 600 µg/kg).

6.4.2.1 DDT and its metabolites (*p,p'*-DDE and *p,p'*-DDD) in Lake bed sediment

The mean and range of concentrations of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD as shown in Table 6.2 are 4.60 µg/kg, 0.50-8.80 µg/kg; 31.00 µg/kg, 14.80-49.80 µg/kg and 35.76 µg/kg, 3.69-91.55 µg/kg respectively. The result is an indication of the rate of degradation of DDT in typical tropical water with hot climatic conditions (Jiries, *et al*., 2006). The current trend of higher *p,p'*-DDE and *p,p'*-DDD relative to *p,p'*-DDT was also observed by Ntow (2005) in sediment from the Volta Lake and Darko *et al.*, (2008) in sediment from Lake Bosomtwi, as well as by Pazou *et al*., (2014) from sediment of Nokoue and Cotonou Lagoons in Benin. Whereas the concentration levels recorded in this study were lower than those reported by Ntow (2005), they were higher than those reported by Darko *et al.*, (2008). The low DDT:DDE and DDT:DDD ratios of 0.15 and 0.13, respectively is an indication that current exposure levels primarily come from previous

contamination and environmental persistence rather than from recent applications. DDT in the environment gradually breaks down into DDD and DDE which are more stable. As a result, when the input of DDT into the environment ceases, the levels of its metabolites; DDE and DDD will be higher than that of the parent DDT (Bossi, 1992) as it is in the present study. The higher concentration of *p,p'*-DDD, relative to *p,p'*-DDE in sediments from the Afram arm of the Volta Lake indicates the degradation pathway is from DDT to DDD by reductive dechlorination (Quensen *et al*.,1998; Yao *et al*., 2006).

Due to its environmental persistence and health hazards, DDT and formulations containing its metabolites have been banned from agricultural use under the Stockholm convention (FAO, 2006) to which Ghana is a signatory. The present investigation to a large extent, confirms the restricted use of DDT for agricultural purposes in Ghana. There are however challenges with enforcement of the ban in the country due to lack of adequate logistics for training and proper monitoring programme (EPA Ghana, 1999). There is therefore evidence of current use of DDT in isolated cases in the country. For instance, Fianko *et al*., (2011) reported high DDT concentrations in both water and sediment from parts of the Densu River basin. Williams, (2013) also recorded extremely high concentrations of DDT in sediment of Tarkwa bay lagoon in Lagos, Nigeria, indicating the challenge effective banning of the use uf DDT goes beyond the boundaries of Ghana.

6.4.2.2 Endosulfan and its metabolite endosulfan sulfate in Lake Sediment

Endosulfan was present in the Afram arm of the Volta Lake sediment mainly as α-endosulfan. Though its mean concentration was lower (2.18 µg/kg), compared to the β-isomer (5.20 µg/kg), it was frequently encountered (37.5% incidence rate) more than the β-isomer (6%). The metabolite endosulfan sulfate had a mean concentration of 2.12 µg/kg and its incidence rate of 50% was

higher than that of the parent compounds. The result indicates that historically used endosulfan pesticides are being converted to the main metabolite endosulfan sulfate (Dem *et al*., 2007) meanwhile, there are still sporadic new inputs of endosulfan; mainly α-endosulfan. The range of concentration of endosulfan sulfate in the sediment of AVL (1.00 – 3.30 µg/kg) is compared to that of Lake Victoria (0.82 µg/kg – 6.62 µg/kg,) as reported by Wasswa *et al*., (2011) and was lower. The mean concentration of each of the compound was however observed to be 10 to 30 folds higher than what was reported by Ntow (2005) in sediments from some other parts of the Volta Lake. Current higher values compared to what was reported by Ntow (2005) signifies intensification of agricultural activities along the banks of the Volta Lake in general, and AVL in particular; as well as overreliance and inappropriate use of agro-chemicals for maximum output. This view was confirmed by the farmers who explained that their primary occupation has shifted from fishing to fruits and vegetable farming since no amount of fishing efforts produces corresponding yield. Cultivation of fruits and vegetable is possible all-year round and yields better economic benefits. Fishing has therefore become a supplementary livelihood. Results in this study however agree with recorded values from Densu River sediment by Kuranchie-Mensah *et al*., (2012).

6.4.2.3 Concentration of lindanes and the drins (aldrin, dieldrin, endrin) in sediment

The mean concentrations of γ-HCH, δ-HCH and β-HCH were 14.67 µg/kg, 9.39 µg/kg and 2.16 µg/kg respectively. This trend of concentration follows that recorded in July, 2002 by Fernandez-Bringas *et al.*, (2008) in Mexico (γ-HCH-0.43 ng/g; δ-HCH-0.37 ng/g and β-HCH- 0.16 ng/g) and locally by Kuranchie-Mensah *et al*., (2012) at Weija (γ-HCH -0.555 µg/kg and δ-HCH – 0.140 µg/kg) and Nsawam (γ-HCH -0.608 µg/kg and δ-HCH – 0.095 µg/kg). The range of concentration of lindanes in the sediment of AVL appears high (Table 6.2) when compared with work done by

Doon *et al.*, (2002) in Taiwan (0.57 µg/kg – 14.1 µg/kg) and Sun *et al.*, (2010) in Huaihe River, China (1.95 µg/kg – 11.05 µg/kg).

The use of lindanes in general is banned in Ghana due their environmental resistance and health implications. Gamma HCH in particular was marketed in Ghana as Gammalin 20 until 2007 when its use was discontinued (Kuranchie-Mensah *et al*., 2012). Awumbila and Bokuma, (1994) reported it was the most extensively used pesticide on farms and for animal husbandry in Ghana. The high concentration of γ-HCH therefore continues to persist in the environment. In the case of the AVL, the absence of α-HCH, coupled with the high γ-HCH**:** β-HCH (6.8) suggests that either pure lindane (γ-HCH), rather than technical lindane is still in use or the degradation of Lindane to the more stable β-HCH in the sediment is very slow.

The ratios of endrin:aldrin (1.6) and endrin:dieldrin (2.9) suggest the presence of these pesticides in the sediment was due to old inputs. The use of the drins, just like the lindanes and the DDTs have been banned. It is therefore not surprising to encounter degradation products of aldrin (dieldrin and endrin) in significant concentrations in the environment. The low dieldrin:aldrin ratio (0.5) however indicates the possibility of fresh aldrin usage. Aldrin itself strongly adsorbs to sediment particles for a long time (Nollet, 2000). This probably also accounts for its high concentration in the sediment.

6.4.3 Concentrations of organophosphorus pesticides and synthetic pyrethroids in sediment

The concentration levels of organophosphorus pesticide and synthetic pyrethroid residues detected in the sediment of the AVL are listed in Table 6.2. All 9 target pyrethroids (SPs) and 13 organophosphorus pesticides (OPs) were detected. The general concentration range for the OPs was 0.50 µg/kg – 63.00 µg/kg while the mean concentration range was 1.41 µg/kg – 58.00 µg/kg.

Whereas the highest concentration measured in the samples (63.00 µg/kg) was for chlorpyrifos, the highest mean concentration (58.00 µg/kg) was recorded for dimethoate. Comparing their incidence rates, chlorpyrifos had 94% as against only 5.8% for dimethoate. For the pyrethroids, the general concentration range was 0.50 µg/kg – 16.2 µg/kg while the mean concentration range was 1.96 µg/kg – 13.18 µg/kg. The highest SP level measured in the sediment (16.20 µg/kg) was recorded for cyfluthrin whilst the highest mean concentration (13.18 µg/kg) was recorded for bifenthrin. Cyfluthrin was the most frequently encountered, with incidence rate of 78%, followed by fenvalerate (72%). The mean concentration range of 1.41 µg/kg – 58.00 µg/kg recorded for OPs in Afram sediment in this study was higher than that reported by Afful *et al.,* (2013) in sediments of Weija Lake in Ghana (0.15 µg/kg – 6.6 µg/kg). In their case, Afful *et al*., (2013) measured the highest mean concentration for permethrin while bifenthrin recorded the highest in this study. In Ghana, SPs and OPs concentrations are rarely studied in water and sediments matrices as they are in cash crops like cocoa where their levels have been studied by many researchers including Blankson,(2011), Boakye, (2012), Frimpong *et al.*, (2012a, and 2012b), and Frimpong *et al.*, (2013). With the exception of the pyrethroid levels recorded by Frimpong *et al.,* (2012), all reported concentration levels for SPs and OPs in the cocoa beans were either in the same range or higher than the levels reported for sediment in this study. Even though the OPs and SPs have higher mammalian toxicities compared to the OCPs, these second generation pesticides (OPs and SPs) have become the recommended and preferred agro-pesticides because of their efficacy, short persistence and ease of degradability in the environment. For instance, only a dose of 10 -40 g of active pyrethroid ingredient is needed to be efficacious on a land area of one hactare (Afful *et al*., 2013). The presence of a wide spectrum of pyrethroids organophosphorus pesticide residues in

sediments from AVL in this study underscores increasing diversification in the use of these pesticides.

6.4.4 Lake Sediment risk and toxicity assessment

A summary of the result of an assessment of potential environmental risk associated with organochlorine pesticide residues measured in sediment of AVL in this study is presented in Table 6.3. The sediment quality values used in the calculation of risk quotients were the Canadian Sediment Quality Guidelines (CSQG) and the United States National Oceanic and Atmospheric Administration guidelines (NOAA) (Burton, 2002). Only contaminants for which lake sediment quality values have been established were assessed.

Scrutiny of the results in Table 6.3 reveals that pesticides with clearly defined risk status are *p,p'*-DDE, γ-HCH and dieldrin. For γ-HCH, risk quotients based on both CSQG and NOAA are greater than one (1) under best and worst case scenarios (RQb and RQw respectively). For *p,p'*-DDE, the risk quotient (RQ) is greater than one under best and worst case scenarios based on CSQG only. Concentrations of these contaminants can therefore be said to reach toxicity levels whereby they can impact on health of sediment and benthic organisms in particular, and affect the integrity of the AVL ecosystem as a whole. These pesticides in the sediment may therefore require priority attention for some control measures. Similar studies carried out by Fung *et al*., (2005) on sediment of Pearl River in China listed DDT (and its metabolites), dieldrin and endrin as serious contaminants with RQs under best case scenarios greater than 10. Nemr *et al.*, (2012) also listed DDT, chlordane and endrin with RQs above 10 in sediment from Egyptian Mediterranean coast.

In the case of dieldrin, the RQ based on CSQG is less than unity under both best and worst case scenarios. Hence this pesticide is of little concern and therefore needs no priority remediation attention. The risk quotient under worst case scenario (RQw) for *p,p'*-DDT, *p,p'*-DDD and endrin were all above 10 (with the exception of *p,p'*-DDT whose RQw is less than 10 but greater than 1) indicating these pesticides may also be of concern to the integrity of the Afram arm of the Volta Lake ecosystem. It is observed, however, that the risk quotients of these pesticides were all less than one under best case scenarios. Hence the actual risks posed by these contaminants (*p,p'*-DDT, *p,p'*-DDD and endrin) need further investigation.

CHAPTER SEVEN

PESTICIDE DISTRIBUTION IN TISSUES OF FISH SPECIES, HEALTH RISK ANALYSIS AND BIOCONCENTRATION OF PESTICIDES IN AQUATIC FLORA

7.1 Backgound

A high proportion of pesticides developed for agricultural and other uses have been recognized to be highly toxic to fish and other aquatic species (Bhalchandra *et al*, 2001; Visvanathan *et al.*, 2009; Muthukumaravel *et al*., 2013). Although the aqueous concentrations may not be directly toxic, these chemicals, especially the organochlorine pesticides (OCPs) may bioaccumulate and biomagnify in aquatic organisms to present health concerns. Generally, fish have the ability to bioconcentrate pesticides from aquatic medium and is therefore an important bioindicator of level of pesticide pollution in aquatic media. Pollution of fish also contaminates the food (Zhou *et al.*, 2007). Since fish is an important source of protein globally, its health is also of global concern. Consumption of contaminated fish is reported to be one important pathway of human exposure, particularly to the OCPs (Muralidharan *et al*., 2008). Due to its availability and affordability, fish constitutes an important source of animal protein in Ghana as well. Literature, however, indicates fish in Ghana are not always free from appreciable levels of pesticides (Adu-Kumi *et al*., 2010). Many studies on exposure of pesticides to humans suggest health implications. For instance health effects associated with OCPs include reproductive failures, birth defects, endocrine disruption, immune system dysfunctions and carcinogenicity (Bouman, Coetzee & Schutte, 1990; Olea *et al.*, 1999; Ayedemi *et al.*, 2008). Pyrethroids, among their numerous health effects, are known to be both neuropoisons and endocrine disruptors. They have been linked to disruption of the endocrine system that adversely affect reproduction and sexual development, interference with immune

system and increased chances of breast cancer (Chemicalwatchfactsheet, n.d). Epidemiological studies on organophosphorus pesticides (OPs) have suggested associations between OPs exposure and reproductive disorders like infertility, birth defects, adverse pregnancy outcomes and perinatal mortality (Peirris-John & Wickremasinghe, 2008). Organophosphorus pesticides, as a matter of fact are known to be highly toxic and hence human exposure to them is undesirable. They are mutagenic, teratogenic and a large number of modern-day diseases of nervous and immune system of mammals, like mad cow disease, Gulf war syndrome, Parkinson's disease and multiple sclerosis can be linked to pesticides of this group (Ragnarsdottir, 2000).

Environmental concentrations of pesticides other than OCPs have rarely been studied in Ghana. Most studies only analyse OCPs distribution in fish muscle. However, distribution of pesticides in tissues such as liver, gills and skin; which could provide more clues about the pathways along which pesticide bioaccumulation occurs, and reflect environmental conditions has not received adequate attention. This chapter therefore examines the distribution of OCPs, OPs and SPs residues in tissues of four fish species from the Afram arm of the Volta Lake as a reflection of the state of pesticide pollution of the river. Also, the potential health risk posed to humans upon consumption of these fish is evaluated. Furthermore, pesticide content of two most common submerged/partially submerged aquatic weeds *Ceratophyllum demersum* and *Nymphaea lotus* are determined. According to Lass, (2015) and FAO (2015) these two aquatic plants are excellent sources of plant food for fish. Toowomba, (2000) reported that *Ceratophyllum* sp. was second among aquatic macrophytes consumed by fishes in Polish inland waters and most abundant in the gut of fishes caught in Sri Lanka. In view of this, it is important to assess pesticide content of these plants and determine their bioconcentration factors. Bioconcentration factor (BCF) represents the ratio of pesticide concentration in an aquatic plant relative to that in the aqueous medium and gives an

indication of the ability of an aquatic plant to concentrate chemicals into its system from the surrounding water.

7.2 Materials and Methods

7.2.1 Collection of fish and aquatic plant samples

Four species of fish: *Tilapia zilli, Oreochromis niloticus, Bagrus bayad and Chrysichthys nigrodigitatus* were freshly bought from fishermen upon their immediate return from fishing expedition on the Afram arm of the Volta Lake (Plates 3a – 3d). These species were selected based on their abundance and availability all-year round. Moreso, they are a delicacy and have high consumption rate among Ghanaians. The purchases were done at four different locations along the Afram arm of the Volta Lake (Dedeso, New Jerusalem, Adawso and Kotoso) on 7th and 8th of January, 2014. The fish samples were wrapped in pre-cleaned aluminium foil, packaged into zip-locked plastic bags, stored on ice and transported to the Department of Marine and Fisheries Sciences of the University of Ghana where they were identified by fish taxonomic experts. Samples were subsequently washed thoroughly with distilled water and promptly taken through pre-extraction preparation.

Ceratophyllum demersum and *Nymphaea lotus* (Plates 3e and 3f respectively) were encountered at only one location. Whereas *Ceratophyllum demersum* is completely submerged *Nymphaea lotus*, is a floating leaved plant. For *Nymphaea lotus*, only submerged parts of were harvested. This was done to ensure that source of pesticide to these aquatic plants was wholly from the aquatic medium. The two aquatic plants were identified in the Ghana Herbarium, Department of Botany of University of Ghana. Thereafter, they were thoroughly washed in three changes of distilled water,

put in zip-locked plastic bags and stored in a deep freezer at the laboratory of Ghana Standards Authority.

7.2.2 Chemicals reagents

All chemicals and reagents used in this study were of purest grade and are listed in appendix I

7.2.3 Preparation of fish tissue samples for pesticide extraction

The fish sample preparation was done at the Department of Marine and Fisheries Sciences. All fish samples were weighed on a weighing balance, and their total length (snout to end of tail) taken with a metre rule. The standard lengths (snout to fork of tail) of fish samples with forked tails (*Bagrus bayad* and *Chrysichthys nigrodigitatus)* were also measured. Scales of *Tilapia zilli,* and *Oreochromis niloticus* were removed while the skin of *Bagrus bayad* and *Chrysichthys nigrodigitatus* were taken off. Three body tissues: muscle, gills and liver were taken from individual fishes of each species. Using dissecting kits, each fish was dissected with a scalpel and the liver carefully removed and weighed. The operculum was then cut off with a pair of scissors and the gill subsequently removed and weighed. Finally, a sizeable filleted muscle of each fish was taken by dissecting between dorsal and ventral portions of the fish and minced using a warring blender. Plates 6a -6d show some of the the fish tissue sample. The minced muscle tissue was then weighed. By this means, a total of one hundred and twenty tissue samples were obtained from selected forty whole fish samples comprising: 10 each of *Chrysichthys nigrodigitatus, Bagrus bayad, Tilapia zilli,* and *Oreochromis niloticus.* All prepared samples were wrapped in pre-cleaned aluminium foils, labeled and stored in a deep freezer at -20^0C at the Ghana Standard Authority laboratory.

7.2.4 Extraction and clean-up of extracted Samples

7.2.4.1 Extraction and clean-up of pesticides from fish samples

A modified method of Sun *et al*., (2005) for the extraction of fish tissue samples was used. Ten grammes wet weight of a fish tissue sample was weighed into a nalgelene jar and 40 mL of acetonitrile added (In the case of liver, when a tissue sample was not up to 10 g, 4 mL of solvent for each gramme was used for extraction). The mixture was marcerated for 2 minutes using IKA ultra Turrax homogenizer and then centrifuged at 3000 revolutions per minute (rpm) (Hermle Z 300, jouan CR3i multifunction) for 3 minutes. The supernatant was filtered through 2 g of anhydrous magnesium sulphate and acetonitrile was added to make up to 50 mL in a round bottomed flask. This was then condensed in a rotary evaporator to about 1 mL. Silica SPE cartridge, (1000mg/6 mL) capped with 2g anhydrous magnesium sulphate was conditioned with 10 mL acetonitrile and the cartridge not allowed to dry. Two portions of 10 mL acetonitrile were then used to wash the concentrated extract onto the SPE cartridge mounted on visiprep vacuum manifold and the eluate collected at a flow rate of 3 drops per second into a 50 mL round bottomed flask. Differences in colour of liver extract (in flask 7d) and the eluate (in flask 7f) of plate 7 emphasises the effectiveness of solid phase extraction (SPE) cleaning process. The eluate was concentrated just to dryness by rotary film evaporator (Buchi Rotovapor R.210) at a pressure of 77 mbar and at a temperature of 44^0C. The concentrate was re-dissolved in 1 mL ethyl acetate, frozen for 1 hour and centrifuged at 3000 rpm for 5 minutes in order to settle out any fat/oil left in the sample. The supernatant was then taken into a 1 mL standard opening vial for quantitation by GC-ECD and GC-PFPD.

7.2.4.2 Extraction and clean-up of pesticides from aquatic plants

The aquatic weeds were removed from freezer and allowed to thaw. Five hundred gramme sample sizes each of *Ceratophyllum demersum* and *Nymphaea lotus* were separately weighed and homogenized in a conventional food processor. Ten grammes sub-samples were then taken for extraction of pesticides.

The method by Takatori *et al*., (2009) was adapted for extraction and clean-up of pesticides. Ten grammes sub-samples of homogenized aquatic weeds were put into a 50 mL polypropylene tubes and 10 mL acetonitrile added. The content is homogenized by vortex at high speed for 1 minute. Four grammes of anhydrous magnesium sulphate and 1 g of sodium chloride were then added and vigorously shaken for another minute. The mixture was then centrifuged for 5 minutes and 4 mL supernatant organic layer taken. Supelclean Envi-Carb/LC-NH_2 SPE cartridge (500mg/500mg, 6 mL size) was conditioned with 10 mL acetonitrile and allowed to run down but the cartridge was not allowed to dry. The 4 mL supernatant from the centrifuge process was then loaded onto the conditioned solid phase extraction cartridge. This was then eluted with two portions of 5 mL acetonitrile into a pear-shaped 50 mL flask, using a 12 port visiprep vacuum manifold. The elution was done at the rate of 3 drops per second. The eluate was evaporated to dryness using Buchi rotary vacuum evaporator at 40^{o}C and 77 mbar. The concentrate was re-dissolved in 2 mL ethyl acetate and transferred into a 2 mL standard opening vial prior to GC-ECD and GC-PFPD analyses.

7.2.5 Recovery test of the extraction method

A duplicate of samples that have already been analysed were spikes with 0.05 mg/kg of standard pesticide mixtures and subjected to the same extraction process in order to determine recovery. The

recovery was done in replicates, at another fortification level of 0.01 mg/kg. The values, expressed in percentages were calculated from chromatograms as follows:

$$\% \text{ Recovery} = \frac{\text{Pesticide (mg/kg) recovered from fortified sample}}{\text{Amount of pesticide (mg/kg) added}} \times 100$$

The recoveries of the pesticides in fish matrices were within the range of 75% - 110% for most of the pesticides analysed whilst that for the aquatic weeds was 70% - 105%. Reagent or procedural blanks were also extracted same and found to be devoid of any interfering agents. Calibration of instruments, quantification of pesticide residues and limits of detection and quantification as well as calibration standards were all set as described in chapter 4. The limit of detection (LOD) and limit of quantification (LOQ) of the GC for the pesticides in fish and aquatic plants were 0.15 µg/kg and 0.5 µg/kg.

7.2.6 Quantification of extracted pesticides by Gas Chromatograph

The final extracts were analyzed for organochlorine and synthetic pyrethroid pesticides by Gas Chromatograph- Varian CP-3800 (Varian Association Inc. USA) equipped with combiPal autosampler and ^{63}Ni electron capture detector (ECD) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/kg range) from the matrix to which other detectors do not respond. The GC conditions and the detector response were adjusted so as to match the relative retention times and response as spelt out by Japanese analytical methods for agricultural chemicals. The GC conditions used for the analysis were capillary column coated with VF-5 (30 m + 10 m guard column x 0.25 mm i.d, 0.25 µm film thickness). The injector and detector temperature were set at 270°C and 300°C, respectively.

The oven temperature was programmed as follows: 70°C held for 2 min, ramp at 25°C min^{-1} to 180°C, held for 1 min, and finally ramp at 5°C min^{-1} to 300°C. Nitrogen was used as carrier gas at a flow rate of 1.0 $mLmin^{-1}$ and detector make-up gas of 29 $mLmin^{-1}$. The injection volume of the GC was 1.0 µL. The total run time for a sample was 31.4 min.

Organophosphorus pesticides on the other hand were analyzed by Gas Chromatograph- Varian CP-3800 (Varian Association Inc. USA) also equipped with combiPal autosampler and pulse flame photometric detector (PFPD) that allowed the detection of contaminants even at trace level concentrations (in the lower µg/g range) from the matrix to which other detectors do not respond. The GC conditions used for the analysis were capillary column coated with VF-1701 (30 m x 0.25 mm i.d, 0.25 µm film thickness). The injector and detector temperature were set at 270°C and 280°C respectively. The oven temperature was programmed as follows: 70°C held for 2 min, ramp at 25°C min^{-1} to 200°C, held for 1 min, and finally ramp at 20°C min^{-1} to 250°C maintained for 3.3 min. Nitrogen was used as carrier gas at a flow rate of 2.0 $mLmin^{-1}$ and detector make-up gases (17.0, 14.0 and 10.0 $mLmin^{-1}$) for hydrogen, air-1 and air-2, respectively. The injection volume of the GC organophosphorus pesticide determination was 2.0 µL. The total run time for a sample was 14 min. Each sample underwent duplicate analyses.

7.2.7 Quality assurance of method

For each batch of 20 samples, a procedural blank, a spiked blank and a pair of matrix spiked sample/duplicate were processed. The spiked samples contained all the 15 OCPs, 13 OPs and 9 SPs target analytes. All reagents used during the analysis were of high quality and were exposed to the

same extraction procedures and subsequently run to check that no interfering substances were present. No analytes were detected in the blanks. Recalibration curves were run with each sample batch to ensure that correlation coefficient was kept above 0.99. Strict cleaning procedures were adhered to viz: all glassware were washed with hot water and detergents and copiously rinsed with distilled water. After drying, the glass wares were further rinsed with acetone.

7.2.8 Data Analysis

7.2.8.1 Health Risk Estimation

Health risk assessment was done as described in chapter 5. The estimated lifetime exposure dose (mg/kg/day) was obtained by multiplying the residual pesticide concentration (mg/L or mg/kg) in the food of interest by the daily food consumption rate (L/day or kg/day) in a country, and dividing the product by the body weight (kg). The lifetime exposure dose is then divided by the Acceptable Daily Intake (ADI) to obtain a health index (HI) that is a parameter in judging health risk associated with consumption of food with certain level of pesticide. The daily food consumption rate of a food item is equivalent to the per capita consumption of that food item. The per capita consumption of fish in Ghana, as reported by the Ministry of Food and Agriculture, MoFA (2011) is 30.2 kg/head/year which is equivalent to 0.082 kg/person/day.

$$\textit{Dietary exposure (mg/kg/day)} = \frac{\textit{(Food chemical concentration (mg/kg)} \times \textit{Food consumption (kg/day))}}{\text{Body weight (kg)}}$$

and

$$HI = \frac{Exposure\ dose}{ADI}$$

The hazard indices for children and adults were estimated separately using the hypothetical body weight of 10 kg for children (0 - 1 yr), 30 kg for children (1 - 11 yrs) and 70 kg for adults. Hazard

index was calculated for the 0-1 year group since a survey conducted during this study showed that their fish consumption was almost the same as that of the adults.

Bioconcentration Factors (BCF) of aquatic plants were determined as ratios of pesticide concentrations in the respective tissues and the surrounding aqueous medium, ie:

$$BCF = \frac{Concentration\ of\ pesticide\ in\ tissue}{Concentration\ of\ pesticide\ in\ water}$$

The concentration of pesticides in aqueous medium used above refers to the site at which the aquatic plants were harvested.

7.2.8.2 Analysis of residues in fish tissues

Residues that were detected in all tissues in each fish species were identified. One-way analysis of variance was carried out to assess whether pesticide residues varied significantly between tissue samples. Possibilities less than 0.05 ($p < 0.05$) were considered statistically significant. Where there were significant differences Tukey's HSD (honestly significant diference) test was used for mean separation.

Calculation of mean: Only detect samples were used in mean calculations. Incidence rate however was based on total number of samples, as previously described on page 69 of chapter Four.

7.3 Results

7.3.1 General trend of pesticide levels in fish tissues

Table 7.1 presents general trend of pesticides concentrations and distribution in various organs of the four fish species analysed. For each fish species, average of the mean concentration of all residues is highest in the liver followed by gill (except in *Bagrus bayad* where) and then the muscle. Mean concentrations (load) of pesticide residues in tissues across all fish species also follow the order: Liver > Gill > Muscle (Table 7.1). It is also noted that the total pesticide body load in the fish species is highest in *Chrysichthys nigrodigitatus* (536.59 µg/kg), followed by *Bagrus bayad* (241.66 µg/kg), *Oreochromis niloticus* (85.86 µg/kg) and *Tilapia zilli* (69.06 µg/kg). *Bagrus bayad* registered the highest mean residue concentrationof all pesticides in the flesh (20.10 µg/kg) whereas C. *nigrodigitatus* had the highest liver residue of 508.77 µg/kg. The residue concentrations in the gills across the species however did not differ very much (Table 7.1). Total number of detected pesticides in tissues of each fish species follows the order: Liver > Gill > Muscle as illustrated by Fig. 7.1

Table 7.1: Comparison of mean concentration (µg/kg) of total detected pesticides in tissues of four fish species

Fish species	**Total pesticide concentration in Tissues**			**Total Body Load**
	Muscle	Gill	Liver	
Tilapia zilli	7.42	10.03	44.07	61.52
Oreochromis niloticus	9.90	16.71	61.25	85.86
Chrysichthys nigrodigitatus	11.71	16.11	508.77	536.86
Bagrus bayad	20.10	15.80	203.33	241.66
Pesticide load in tissues	**49.13**	**58.64**	**817.42**	

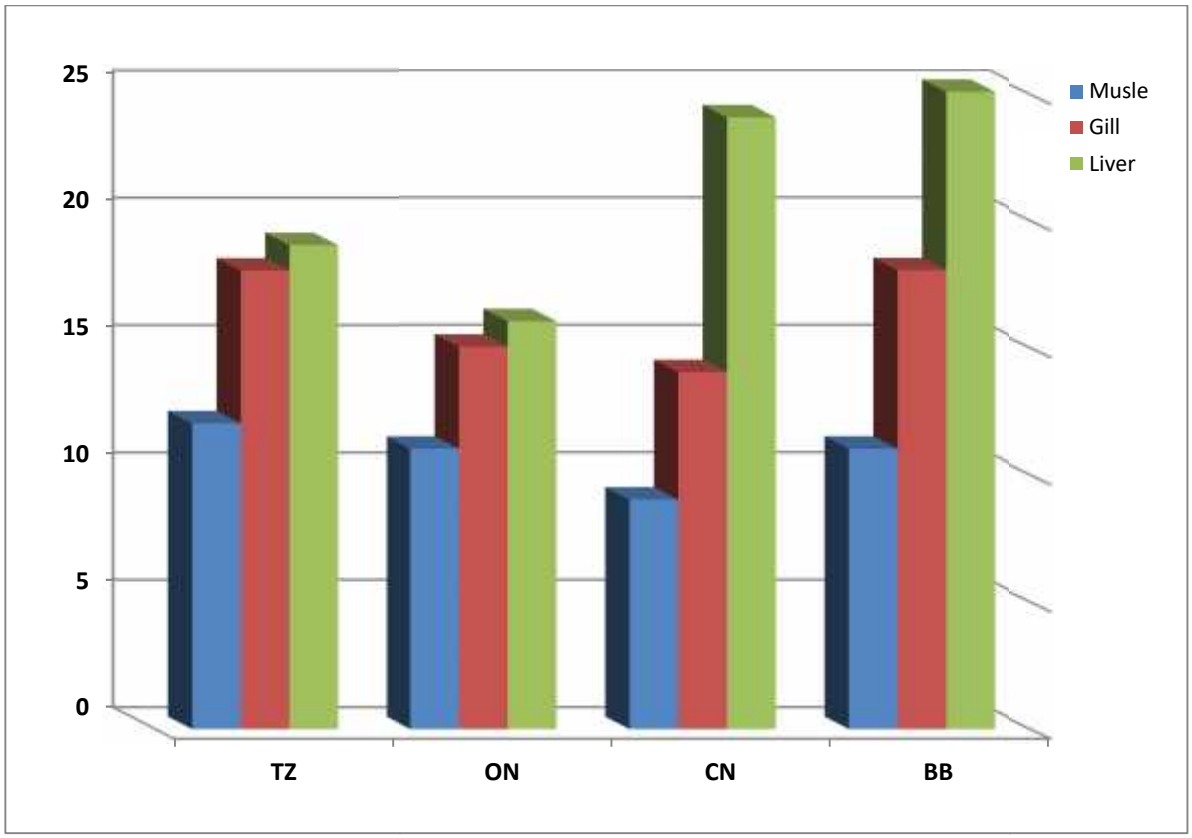


Fig.7.1: Total number of detected pesticides in various tissues of four fish species

TZ: *Tilapia zilli* ON: *Oreochromis niloticus*
CN: *Chrysichthys nigrodigitatus* BB: *Bagrus bayad*

7.3.2 Organochlorine pesticide residues in fish tissues

Tables 7.2 - 7.5 presents the residue levels of all the pesticides in tissues of the four fish species analysed. The results of organochlorine pesticide (OCP) residues are presented in this section. Only three OCP residues (β-lindane, methoxychlor and aldrin) were detected in the muscle tissues of the four fish species analysed. Beta Lindane and methoxychlor were detected in the muscle of all the 4 species while *Tilapia zilli* registered the presence of aldrin as the third residue. The level of methoxychlor was higher than of β-lindane in all the fish species, exception in *Oreochromis niloticus*. The mean concentration range of detected OCPs in muscle tissues of the fishes was 0.78µg/kg – 94.0 µg/kg. Both values were recorded in *Bagrus bayad;* the highest for methoxychlor and the lowest for β-lindane. The ranking order of decreasing mean concentration of OCP in muscles of the 4 species follows: *B. bayad* > *C. nigrodigitatus* > *T. zilli* > *O. niloticus* (Tables 7.2 - 7.5).

Gills of the fish species registered the presence of seven OCP residues, mainly β-lindane, heptachlor, p,p'-DDD, α-endosulfan, β-endosulfan, endosulfan sulphate and methoxychlor. Beta-lindane, heptachlor, α-endosulfan, endosulfan sulphate and methoxychlor were detected in the gill tissues of *T. zilli*. Beta-lindane, p,p'-DDD, α-endosulfan and endosulfan sulphate were detected in *O. niloticus*; Beta-lindane, α-endosulfan, β-endosulfan and methoxychlor in *C. nigrodigitatus* and β-lindane, heptachlor, α-endosulfan and methoxychlor in *B. bayad*. The mean concentration range of OCP residues detected in gills of the 4 fish species was 0.90 µg/kg – 18.3 µg/kg. The highest concentration of 18.83 µg/kg was recorded for heptachlor and in *T.zilli*. The ranking order of decreasing mean concentration in the gills was *C. nigrodigitatus* > *O. niloticus* >*B. bayad* > *T. zilli* (Tables 7.2 - 7.5).

In the liver of the four fish species, a total of 10 OCP residues were detected. These are beta, gamma and delta-lindane, heptachlor, α and β-endosulfan, endosulfan sulphate, p,p'-DDE, endrin and methoxychlor. All of the above, with the exception of delta-lindane was detected in B. bayad. Similarly, with the exception of endosulfan sulphate, all the listed OCP residues were recorded in the liver of *C. nigrodigitatus*. T. zilli and O. niloticus registered the least number of detected pesticides (4) in the liver tissue. The mean concentration range of OCP residues in the liver of the fish species was 11.0 µg/kg – 4671.0 µg/kg. The three highest values of 4671.0 µg/kg, 1771.0 µg/kg and 750.60 µg/kg were all recorded in *C. nigrodigitatus* for methoxychlor, heptachlor and endrin respectively. The order of decreasing mean concentration of OCP residues in liver of the fishes was *C. nigrodigitatus* > *B. bayad* > *O. niloticus* > *T. zilli* (Tables 7.2 - 7.5).

Table 7.2: Mean concentration (µg/kg) and distribution of pesticides in body tissues of *Tilapia zilli*

	Muscle			Gill			Liver		
Organochhlorines	**Conc. ± SD**	**range**	**Freq (%)**	**Conc. ± SD**	**Range**	**Freq (%)**	**Conc. ± SD**	**range**	**Freq (%)**
β-HCH	1.22 ± 0.7	0.50 – 1.83	20	4.66 ± 2.3	1.53 – 7.20	100	11.0 ± 9.4	2.38 – 25.16	60
Aldrin	16.33	16.33	10	18.83 ± 0.9	18.14 – 19.52	20	12.6 ± 0.9	11.91 – 13.21	20
α-endosulfan	-			15.0 ± 7.4	2.58 – 22.90	60	35.2 ± 1.4	34.14 – 36.21	20
Endosulfan sulfate	-			4.17	4.17	10	-		
Methoxychlor	3.73 ± 1.4	2.19 – 5.44	50	2.5 ± 0.8	1.18 – 3.29	60	26.4 ± 4.4	22.44 - 31.11	30
	Load=21.28			**Load=45.16**			**Load=85.20**		
Pyrethroids	-			-			-		
Allethrin	-			-			12.8 ± 1.6	11.61 – 13.96	20
Fenpropathrin	-			-			27.3 ± 5.2	23.65 – 31.01	20
Permethrin	31.0 ± 7.1	22.60 – 35.91	30	11.5 ±7.0	5.00 – 23.84	80	415.5 ±35.3	83.2 – 952.10	50
Cyfluthrin	6.15	6.15	10	17.3 ± 10.8	4.26 – 34.65	80	88.7 ± 47.6	7.45 – 131.50	50
Cypermethrin	1.1	1.09	10	2.0	2.01	10	45.8 ± 17.5	30.00 – 62.84	40
Fenvalerate	1.4	1.41	10	2.3	2.24	10	11.0 ± 8.5	3.23 – 25.30	50
Deltamethrin	2.2 ± 1.5	0.67 – 4.43	50	5.11 ± 0.4	4.61 – 5.52	30	-		
	Load=41.85			**Load=38.21**			**Load=601.20**		
Organophosphate									
Methamidophos	8.6 ± 0.3	8.50 – 9.00	30	20.0 ± 1.5	18.56 – 21.51	30	28.1 ± 5.6	19.44 – 351.20	50
Ethoprophos	-			18.5 ± 9.1	3.65 – 33.40	20	20.3 ± 15.9	8.96 – 31.52	20
Diazinon	-			-			8.1	8.10	10
Dimethoate	-			-			35.8 ± 2..0	14.9 5 – 53.10	40
Pirimiphos-methyl	-			11.63 ± 8.1	1.05 – 22.20	20	14.1	14.06	10
Chlorpyrifos	9.16 ± 4.3	4.27 – 12.21	30	9.24 ± 6.6	2.68 – 15.88	30	9.2 ± 1.1	8.47 – 10.01	20
Malathion	4.35	4.35	10	8.49	8.49	10	-		
Fenitrothion	-			9.1 ± 4.4	4.18 – 12.95	20	28.1 ± 4.8	24.67 – 31.5	20
Parathion	-			-			2.1	2.08	10
Chlorfenvinphos	-			2.69	2.68	10	-		
Profenofos	3.79 ± 0.5	3.46 – 4.12	20	17.55 ± 2.2	14.96 – 18.59	30	5.17	5.17	10
	Load=25.9			**Load=97.20**			**Load=150.93**		

Load = Total of the means of all residues in a pesticide group

Table 7.3: Mean Concentration (µg/kg) and distribution of pesticides in body tissues of *Oreochromis niloticus*

	Muscle			Gill			Liver		
Organochhlorines	**Conc.±SD**	**Range**	**Freq (%)**	**Conc.±SD**	**Range**	**Freq (%)**	**Conc.±SD**	**Range**	**Freq (%)**
β-HCH	3.25 **± 3.5**	0.50 - 8.00	80	8.8 **± 7.2**	1.00 – 20.00	80	12.9 **± 10.8**	1.13 – 36.40	80
Heptachlor	-			-			203.0**± 91.5**	105.12 – 311.60	40
α-endosulfan	-			17.2 **± 11.2**	1.47 - 54.20	60	63.3 **± 42.5**	23.30 – 122.50	40
p,p'-DDD	-			15.7 **± 6.9**	7.33 – 24.30	40	-		
Endosulfan sulfate	-			2.9 **± 1.9**	1.32 - 6.13	**60**	**23.3 ± 7.5**	16.40 – 31.40	**30**
Methoxychlor	1.2 **± 0.9**	0.50 – 2.36	40	-			-		
	Load=4.45			**Load=44.6**			**Load=302.5**		
Pyrethroids									
Allethrin	-			-			58.0 **± 35.2**	32.30 – 111.20	60
Bifenthrin	16.3 **± 7.4**	11.00 – 32.50	20	3.2 **± 1.4**	2.21 – 4.26	20	-		
Fenpropathrin	-			14.1 **±11.7**	6.67 – 35.1	60	-		
Permethrin	22.0 **± 16.0**	5.00 - 41.60	70	54.2 **± 24**	14.17 – 81.50	80	379.0 **± 54.7**	325.00 – 455.10	40
Cyfluthrin	10.0 **± 2.5**	8.00 – 11.60	20	46.0 **± 13.2**	30.11 – 81.50	40	28.6 **± 12.5**	15.11 – 44.20	40
Cypermethrin	11.0 **±8.9**	1.00 - 25.10	60	5.6 **± 2.5**	2.11 – 11.00	80	43.4**± 31.9**	11.66 – 78.11	40
Fenvalerate	1.6 **± 1.4**	0.60 – 2.61	20	7.6 **± 7.1**	2.11 – 13.10	20	53.4 **± 45.0**	22.11 - 133.10	60
Deltamethrin	7.5 **± 2.1**	6.00 - 9.00	20	7.2 **± 3.2**	1.28 – 8.80	40	9.0 **± 3.0**	6.78 – 11.09	20
	Load=68.40			**Load=137.9**			**Load=571.38**		
Organophosphate									
Methamidophos	6.7 **± 1.2**	4.70 – 8.96	80	22.2 **± 9.0**	18.52 – 32.50	30	30.7 **± 13.4**	21.20 – 40.00	20
Phorate	4.1 **± 0.8**	3.26- 5.24	40	-			2.6 **± 1.4**	1.49 – 3.61	20
Chlorpyrifos	3.3 **± 1.1**	2.15 – 4.73	40	20.0 **± 14.4**	2.74 – 33.69	50	3.73 **± 2.3**	2.05 – 5.40	20
Malathion	-			10.0**± 2.2**	8.39 – 11.52	20	-		
Fenitrothion	-			-			39.5 **± 19.0**	6.18 – 86.60	40
Chlorfenvinphos	-			-			4.4	4.39	10
Profenofos	-			16.0 **± 4.8**	12.58 – 21.50	30	25.1	25.11	10
	Load=14.10			**Load=68.20**			**Load=106.02**		

Load = Total of the means of all residues in a pesticide group

Table 7.4: Mean Concentration (µg/kg) and distribution of pesticides in body tissues of *Chrysichthys nigrodigitatus*

	Muscle			Gill			Liver		
Organochhlorines	**Conc. ± SD**	**Range**	**Freq (%)**	**Conc. ± SD**	**Range**	**Freq (%)**	**Conc. ± SD**	**Range**	**Freq** (%)
β-HCH	2.7 **± 1.6**	0.50 – 8.28	30	2.7 **± 1.6**	0.50 - 5.12	80	21.0 ± 13.1	1.48 - 71.86	80
γ-HCH	-			-			65.0	65.00	10
δ-HCH	-			-			100.0 ± 48.8	15.60 – 185.20	30
Heptachlor	-			-			1771.0 ± 103	65.10 - 246.51	40
α-endosulfan	-			10.16	10.16	10	555.0 ± 91.3	2.21 – 2607.50	70
p,p'-DDE	-						207.6 ± 109	8.52 - 635.10	40
Endrin	-						750.6	750.61	10
Beta endosulfan	-			0.9 **± 0.4**	0.50 - 1.20	30	294 ± 143	4.29 - 730.14	40
Methoxychlor	31.0 **± 14.8**	8.00 - 45.97	50	1.93 **± 1.0**	0.50 - 3.15	50	4671.0 ± 139	1320.0- 8112.0	30
	Load=33.70			**Load=15.69**			**Load=8435.16**		
Pyrethroids									
Allethrin	-			-			112.1 ± 89	18.21 – 255.00	**50**
Bifenthrin	-			-			115.0 ± 84	27.45 - 221.20	30
Fenpropathrin	-			12.5	12.49	10	199.1	199.10	10
λ-cyhalothrin	-			58.6	58.60	10	1064.0	1064.10	10
Permethrin	41.0 **± 25.4**	2.14 - 180.89	100	15.3 **± 8.5**	1.11 - 23.20	80	861.4 ± 102	46.40 – 2887.0	80
Cyfluthrin	7.8 **± 4.1**	5.20 - 15.16	60	22.5 **± 14.2**	4.48 - 42.16	60	234.6 ± 164	5.86 – 485.61	70
Cypermethrin	6.44 **± 2.3**	4.75 - 8.13	20	23.7 **± 11.3**	6.94 - 34.20	50	376.7 ± 140	16.01 - 782.50	70
Fenvalerate	4.15 **± 1.7**	1.24 - 5.31	50	5.2 **± 4.0**	1.73 - 12.62	70	483.0 ± 84	14.26 – 1661.00	80
Deltamethrin				14.2 **±10.3**	5.12 - 25.48	30	233.3 ± 85	177.20 - 380.60	50
	Load=59.40			**Load=151.97**			**Load=3679.20**		
Organophosphate									
Methamidophos	-			-			19.3 ± 9.7	12.41 – 26 .30	20
Dimethoate	6.57	6.57	10				29.5 ± 8.5	23.41 - 35.48	20
Pirimiphos-methyl	4.1	4.05	10	47.8 **± 3.1**	46.00 - 51.36	30	21.0 ± 0.8	19.96 – 21.14	20
Chlorpyrifos	1.6	1.61	10	2.34 **± 0.2**	2.19 - 2.51	30	4.35	4.35	10
Malathion	-			7.69 **± 2.3**	6.10 - 9.28	20	-		
Parathion	-			-			8.1 ± 2.7	6.17 - 10.06	20
Chlorfenvinphos	-			-			13.9 ± 3.4	11.52 - 16.31	20
	Load=12.27			**Load=57.84**			**Load=96.18**		

Load= Total of the means of all residues in a pesticide group

Table 7.5: Mean Concentration (µg/kg) and distribution of pesticides in body tissues of *Bagrus bayad*

	Muscle			Gill			Liver		
Organochhlorines	**Conc. ± SD**	**Range**	**Freq**	**Conc. ± SD**	**Range**	**Freq**	**Conc. ± SD**	**Range**	**Freq**
β-HCH	0.78 **± 0.1**	0.50 - 1.513	40	3.14 **± 2.6**	0.658 - 9.187	80	20.0 **± 20.1**	1.23 - 57.21	90
γ-HCH							57.3 **± 27.7**	4.50 -162.21	60
Heptachlor				4.0 **± 0.7**	3.26 - 4.80	30	591.0 **± 150.4**	68.14 - 1156.40	60
α-endosulfan				17.5 **± 0.0**	17.51	20	200.0 **± 123**	9.55 - 582.61	70
p,p'-DDE							75.8 **± 18**	62.84 - 88.73	20
Endrin							38.9 **± 3.3**	36.59 - 41.23	20
Beta endosulfan							627.3 **± 0.2**	627.00	20
Endosulfan sulfate							**150.0 ± 92**	45.3 - 223.1	**30**
Methoxychlor	94.0 **± 30**	56.20 - 152.60	40	4.1 **± 2.7**	1.25 - 8.51	80	289.7 **± 12**	36.47 - 508.10	70
	Load=94.78			**Load=28.76**			**Load=2050.00**		
Pyrethroids									
Allethrin				4.1 **± 1.1**	2.87 -5.22	30	90.0 **± 85**	5.48 - 173.55	40
Bifenthrin	8.2 **± 5.1**	6.25 - 10.11	20						
Fenpropathrin				6.84	6.84	10	1.69	1.69	10
Lamda-cyhalothrin							12.4 **± 8.2**	2.96 - 21.52	40
Permethrin	49.8**± 31.3**	3.25 - 105.00	90	36.1 **± 24**	14.67 - 88.50	90	2190.0 **± 211**	103.3 – 5378.4	80
Cyfluthrin	12.2 **± 6.9**	5.98 - 16.52	40	41.2 **± 23.2**	8.35 - 71.36	80	122.3 **± 182**	75.11 – 463.8	30
Cypermethrin	13.3 **± 11.8**	4.21 - 22.31	20	11.6 **± 9.8**	2.35 - 22.20	30	76.7 **± 38**	20.40 - 274.31	80
Fenvalerate				4.1 **± 1.8**	2.56 - 6.62	40	104 **± 68**	32.2 - 216.90	80
Deltamethrin	1.3	1.29	10				31.0 **±20**	12.50 - 53.31	30
	Load=84.8			**Load=103.92**			**Load=328.51**		
Organophosphate									
Methamidophos				8.76 **± 2.3**	7.11 - 10.40	20	37.7 **± 6.2**	33.32 – 42.13	20
Ethoprophos	11.9 **± 9.8**	4.95 - 18.91	20	3.0 **± 1.1**	2.10 - 4.51	40	36.1 **± 5.6**	32.10 - 40.14	20
Fonofos							19.1**± 1.5**	18.01 - 20.11	20
Dimethoate				33.3 **± 0.0**	33.30	20	36.5 **± 18**	18.82 - 52.35	40
Chlorpyrifos	6.2 **± 0.7**	1.19 - 11.21	20	25.6 **± 17.5**	4.14 - 42.91	50			
Malathion				6.48	6.48	10	32.8 **± 0.5**	32.41 - 33.12	20
Fenitrothion				8.7 **± 2.3**	6.33 - 11.03	30	193.0 **± 79**	108.0 - 300.0	40
Chlorfenvinphos	6.3 **± 5.2**	2.63 – 10.0	20	5.4 **± 3.1**	2.94 - 8.88	30	13.7 **± 6.2**	4.41 - 18.18	40
Profenofos	17.0 **± 7.8**	5.01 - 26.14	50	31.6 **± 20**	12.41 - 52.33	30	35.7 **± 6.2**	31.30 - 40.00	20
	Load=41.40			**Load=122.84**			**Load=404.60**		

7.3.3 Synthetic pyrethroid pesticide residues in fish tissues

Six of the nine targeted synthetic pyrethroid (SPs) residues were recorded in the muscle tissues of the fishes and these are bifenthrin, permethrin, cyfluthrin, cypermethrin, fenvalerate and deltamethrin. All the six were detected in the muscle of *O. niloticus*. All, with the exception of bifenthrin, was detected in *T. zilli* while fenvalerate was the only non-detect of the six in *B. bayad*. *Chrysichthys nigrodigitatus* also recorded all with the exception of bifenthrin and deltamethrin (Tables 7.2 - 7.5). The concentration range of SPs in muscle of the fishes was 0.60 µg/kg – 180.89 µg/kg. The highest concentration of 180.89 µg/kg was recorded for permethrin in the muscle of *C. nigrodigitatus.* Mean concentration of SPs in muscle tissues of the four fish species was highest for *B. bayad* (16.96 µg/kg), followed by *C. nigrodigitatus* (14.85 µg/kg), O. *niloticus* (11.40 µg/kg) and *T. zilli* (8.37 µg/kg).

All the nine targeted SPs were detected in measurable quantities in the gills and liver tissues of the fish species being analysed. The number of detects in the gills were 5, 7, 7, and 6 for *T. zilli*, *O. niloticus*, *C. nigrodigitatus and B. bayad* respectively; while the corresponding values in liver tissues were 6, 7, 9 and 8 respectively. The respective mean concentration ranges of SPs in gills and liver tissues of the fish species were 1.11 µg/kg – 88.50 µg/kg and 1.69 *C. nigrodigitatus* – 5378.40 µg/kg(Tables 7.2 - 7.5). The highest concentration in the two tissues were recorded for permethrin in *B.bayad*. The mean concentration of SP residues in the gills follows the order *C. nigrodigitatus* > *O. niloticus* > *B.bayad* > *T. zilli*, while that for the liver tissues is *C. nigrodigitatus* > *B.bayad* > *T. zilli* > *O. niloticus* (Tables 7.2 - 7.5).

7.3.4 Organophosphorus pesticide residues in fish tissues

Out of the target 13 OP pesticides, 9 were encountered and quantified in muscle and gill tissues of the four fish species under study, while 12 were measured in their liver tissues. In the muscle tissues, only 4 (methamidophos, chlorpyrifos, malathion and profenofos) were detected in *T. zilli*; 3 (methamidophos, phorate and chlorpyrifos) in *O. niloticus*, 3 (Dimethoate,pirimiphos-methyl and chlorpyrifos) in *C. nigrodigitatus* and 4 (Ethoprophos, chlorpyrifos, chlorfenvinphos and profenofos) in *B.bayad*. The range of OP residue concentration in muscle of the fishes was 1.19 µg/kg – 26.14 µg/kg (Tables 7.2 - 7.5). The highest concentration was recorded for profenofos and in *B.bayad.* The average OP residue concentration in the muscle tissues of the fishes is ranked as follows *B. bayad* (9.10 µg/kg) > *T. zilli* (6.48 µg/kg) > *O.niloticus* (4.70 µg/kg) > *C. nigrodigitatus* (4.09 µg/kg) (Tables 7.2 - 7.5).

Three OPs were detected in the gills of *C. nigrodigitatus*, 4 in O. *niloticus*, 6 in *T.zilli* and 8 in *B. bayad.* Six residues were detected in liver of both *O. niloticus* and *C. nigrodigitatus;* eight in *B. Bayad* and nine in *T. zilli.* The concentration ranges in gills and liver respectively are 1.05 – 52.33 µg/kg and 1.49 – 351.20 µg/kg (Tables 7.2 - 7.5). Profenofos registered the highest concentration of 52.33 µg/kg in the gills and this was recorded in *B.* bayad while the highest concentration in the liver was recorded for methamidophos and in *T. zilli*. The order of decreasing mean concentration of OP residues measured in gills is *C. nigrodigitatus* > *B. bayad* > *O. niloticus* > *T. zilli*; and that in the liver is *B. bayad* > *O. niloticus* > *T. zilli* > *C. nigrodigitatus* (Tables 7.2 - 7.5).

7.3.5 Analysis of residues in tissue of fishes

Residues that were detected in all tissues in each fish species were identified. One-way analysis of variance was carried out to assess whether pesticide residues varied significantly between tissue samples in each species. Possibilities less than 0.05 ($p < 0.05$) were considered statistically significant. Results of the analysis for tissues of *T. zilli*, O. *niloticus*, *C. nigrodigitatus* and *B. bayad* are presented in Tables 7.6, 7.7, 7.8 and 7.9 respectively. Five pesticides were present and quantified in the three tissues under investigation in *Tilapia zilli*; four in *Oreochromis niloticus*, five in *Chrysichthys nigrodigitatus* and seven in *Bagrus bayad.* Generally, statistical differences exist between tissue concentrations in all the fish species. In *T. zill*i, chlorpyrifos concentration did not differ significantly among the tissues. In *O. niloticus*, there was no significant difference in deltamethrin concentration the tissues. Similarly, muscle and gill tissue did not differ significantly in fenvalerate concentration in *C. nigrodigitatus*. Even though generally residue concentrations in tissues follow the order Liver > Gill > muscle, significant differences did not exist between concentrations in gill and muscle tissues.

Table 7.6: Mean concentration of selected pesticide residues in different tissues of *Tilapia zilli*

	Concentration (µg/kg)			P-values
	Muscle	Gill	Liver	
β-lindane	$1.22^{a} \pm 0.7$	$4.66^{ab} \pm 2.3$	$11.0^{b} \pm 9.4$	0.047
Methoxychlor	$3.73^{a} \pm 1.4$	$2.50^{a} \pm 0.8$	$26.4^{b} \pm 4.4$	0.0001
Permethrin	$31.0^{a} \pm 7.1$	$11.5^{a} \pm 7.0$	$415.5^{b} \pm 35.3$	0.008
Methamidophos	$8.60^{a} \pm 0.3$	$20.0^{b} \pm 1.5$	$28.10^{b} \pm 5.6$	0.001
Chlorpyrifos	$9.16^{a} \pm 4.3$	$9.24^{a} \pm 6.6$	$9.20^{a} \pm 1.1$	1.000

Within rows, means with different superscript letters are statistically significant, $p<0.05$

Table 7.7: Mean concentration of selected pesticide residues in different tissues of *Oreochromis niloticus*

	Concentration (µg/kg)			P-values
	Muscle	Gill	Liver	
β-lindane	3.25^{a} ± 3.5	8.8^{a} ± 7.2	12.9^{a} ± 1.2	0.087
Permethrin	22.0^{a} ± 16	54.2^{a} ± 24.9	379.0^{b} ± 54.7	0.0001
cypermethrin	11.0^{a} ± 8.9	5.6^{a} ± 2.5	43.4^{b} ± 31.9	0.003
Deltamethrin	7.5^{a} ± 2.1	7.2^{a} ± 3.2	9.0^{a} ± 3.0	0.396

Within rows, means with different superscript letters are statistically significant, $p<0.05$

Table 7.8: Mean concentration of selected pesticide residues in different tissues of *Chrysichthys nigrodigitatus*

	Concentration (µg/kg)			P-values
	Muscle	Gill	Liver	
β-lindane	2.9^{a} ± 0.6	3.0^{a} ± 1.6	21.0^{a} ± 3.0	0.192
Methoxychlor	31.0^{a} ± 14.8	1.93^{b} ± 1.0	4671.0^{C} ± 339.0	0.003
Permethrin	41.0^{a} ± 5.4	15.3^{a} ± 8.5	861.4^{b} ± 120.2	0.008
Cyfluthrin	7.8^{a} ± 4.1	22.5^{a} ± 14.2	234.6^{b} ± 16.4	0.002
Fenvalerate	4.15^{a} ± 1.7	5.2^{a} ± 4.0	483.0^{b} ± 48.0	0.026

Within rows, means with different superscript letters are statistically significant, $p<0.05$

Table 7.9: Mean concentration of selected pesticide residues in different tissues of *Bagrus bayad*

	Concentration (µg/kg)			P-values
	Muscle	Gill	Liver	
β-lindane	1.2^{a} ± 1.1	3.14 ^{a} ± 2.6	20.0^{a} ± 4.1	0.011
Methoxychlor	150.0^{a} ± 13.1	4.1^{b} ± 2.7	289.6^{a} ± 2.2	0.004
Permethrin	49.8^{a} ± 13.3	36.1^{a} ± 24.6	2190.0^{b} ± 21.1	0.001
Cyfluthrin	12.2^{a} ± 1.9	41.2^{a} ± 23.3	342.3^{b} ± 32.1	0.0001
Chlorfenvinphos	6.3^{a} ± 5.3	5.4^{a} ± 3.1	13.7^{a} ± 6.2	0.158
Ethoprophos	11.9^{a} ± 9.8	3.0^{a} ± 1.1	36.1^{b} ± 5.6	0.002
profenofos	17.0^{a} ± 7.8	31.6^{a} ± 11.9	35.7^{a} ± 6.2	0.181

Within rows, means with different superscript letters are statistically significant, $p<0.05$

7.3.6 Health Risk analysis

Tables 7.10, 7.11, 7.12 and 7.13 present health risk estimate for systemic effects associated with pesticide residues in *T. zilli,* O. *niloticus, C. nigrodigitatus* and *B. bayad* respectively. Concentrations in only muscle and/or gill tissues were used for the health risk estimates because these constitute the edible parts (the liver is normally gutted out with the intestines and therefore not consumed, hence it was not involved in the risk analysis). Where pesticides were quantified in both muscle and gill tissues, the concentrations in the two tissues (muscle and gill) were pooled for the health estimate analysis. The tables comprise reference dose (Rfd) or acceptable daily intake (ADI), the computed dietary exposure dose and the corresponding health hazard indices for children between 0-1 year, 1-11 years and adults. Aldrin, alpha-endosulfan and ethoprophos hazard indices in *T. zilli* were greater than unity for under one year children and 1 or close to 1 for the age 1-11year old (Table 7.10). In *O. niloticus* and *C. nigrodigitatus* however, only alpha-endosulfan recorded Hazard index of more than 1 (Tables 7.11 and 7.12). *Bagrus bayad* on the other hand recorded two pesticides (alpha-endosulfan and ethoprophos) with Hazard index greater than 1 (Table 7.13).

Table 7.10: Health hazard indices of pesticides in *Tilapia zilli*

Substance	ADI(ug/kg)	Conc.(ug/kg)	Exposure dose(ug/kg/day)			Hazard index (HI)		
Organochhlorines			**0-1yr**	**1-11yrs**	**Adult**	**0-1yr**	**1-11yrs**	**Adult**
β-HCH	5.00	5.88	0.048	0.0161	0.007	0.010	0.003	0.001
Aldrin	0.10	35.16	0.288	0.096	0.041	2.88	1.0	0.411
α-endosulfan	0.05	15.00	0.123	0.041	0.018	2.46	0.819	0.351
Endosulfan sulfate	0.05	4.17	0.034	0.011	0.005	0.684	0.228	0.098
Methoxychlor	5.00	6.23	0.051	0.017	0.007	0.010	0.003	0.001
Pyrethroids								
Permethrin	50.00	42.50	0.349	0.116	0.050	0.007	0.002	0.001
Cyfluthrin	3.00	79.80	0.654	0.218	0.093	0.218	0.073	0.031
Cypermethrin	50.00	3.10	0.025	0.008	0.004	0.000	0.000	0.000
Fenvalerate	20.00	3.70	0.030	0.010	0.004	0.001	0.000	0.000
Deltamethrin	10.00	7.31	0.060	0.020	0.008	0.006	0.002	0.001
Organophosphate								
Methamidophos	1.00	28.60	0.234	0.078	0.033	0.234	0.078	0.033
Ethoprophos	0.10*	18.50	0.152	0.050	0.021	1.517	0.505	0.216
Pirimiphos-methyl	4.00	11.63	0.095	0.032	0.014	0.024	0.008	0.003
Chlorpyrifos	10.00	18.40	0.151	0.050	0.021	0.015	0.005	0.002
Malathion	30.00	12.84	0.105	0.035	0.015	0.004	0.001	0.000
Fenitrothion	5.00	9.10	0.075	0.025	0.011	0.015	0.005	0.002
Chlorfenvinphos	0.50	2.69	0.022	0.007	0.003	0.044	0.014	0.006
Profenofos	30.00	55.45	0.455	0.151	0.065	0.015	0.005	0.002

NB: highlighted cells show HI with possible health implications

Table 7.11: Health hazard indices of pesticides in *Oreochromis niloticus*

Substance	ADI(ug/kg)	Conc.(ug/kg)	Exposure dose(ug/kg/day)			Hazard index		
Organochhlorines			**0-1yr**	**1-11yrs**	**Adult**	**0-1yr**	**1-11yrs**	**Adult**
β-HCH	5.00	12.05	0.099	0.033	0.014	0.020	0.007	0.003
α-endosulfan	0.05	17.20	0.141	0.047	0.020	2.821	0.939	0.403
p,p'-DDD	-	15.70	-	-	-	-	-	-
Endosulfan sulfate	0.05	2.90	0.024	0.008	0.003	0.475	0.158	0.068
Methoxychlor	5.00	1.20	0.010	0.003	0.001	0.002	0.001	0.000
Pyrethroids								
Bifenthrin	15.00	19.50	0.160	0.053	0.023	0.011	0.004	0.002
Fenpropathrin	30.00	14.10	0.116	0.038	0.016	0.004	0.001	0.000
Permethrin	50.00	76.20	0.625	0.208	0.089	0.012	0.004	0.002
Cyfluthrin	3.00	56.00	0.459	0.153	0.066	0.153	0.051	0.022
Cypermethrin	50.00	16.60	0.136	0.045	0.019	0.003	0.001	0.000
Fenvalerate	20.00	9.20	0.075	0.025	0.011	0.004	0.001	0.000
Deltamethrin	10.00	14.70	0.120	0.040	0.017	0.012	0.004	0.002
Organophosphate								
Methamidophos	1.00	28.90	0.237	0.079	0.034	0.237	0.079	0.034
Phorate	0.10	4.10	0.034	0.011	0.005	0.336	0.112	0.048
Chlorpyrifos	10.00	23.30	0.191	0.064	0.027	0.019	0.006	0.003
Malathion	30.00	10.00	0.082	0.027	0.012	0.003	0.001	0.000
Profenofos	30.00	16.00	0.131	0.044	0.019	0.004	0.002	0.001

NB: highlighted cells show HI with possible health implications

Table 7.12: Health hazard indices of pesticides in *Chrysichthys nigrodigitatus*

Substance	ADI(ug/kg)	Conc.(ug/kg)	Exposure dose(ug/kg/day)			Hazard index		
Organochhlorines			**0-1yr**	**1-11yrs**	**Adult**	**0-1yr**	**1-11yrs**	**Adult**
β-HCH	5.00	5.40	0.044	0.015	0.006	0.008	0.003	0.001
α-endosulfan	0.05	10.60	0.087	0.029	0.012	1.738	0.579	0.248
Beta endosulfan	0.05	0.90	0.007	0.002	0.001	0.148	0.049	0.021
Methoxychlor	5.00	32.93	0.270	0.090	0.038	0.054	0.018	0.008
Pyrethroids								
Fenpropathrin	30.00	12.50	0.103	0.034	0.015	0.003	0.001	0.000
Lamda-cyhalothrin	5.00	58.60	0.480	0.160	0.068	0.096	0.032	0.014
Permethrin	50.00	56.30	0.462	0.154	0.066	0.009	0.003	0.001
Cyfluthrin	3.00	30.30	0.248	0.083	0.035	0.083	0.028	0.012
Cypermethrin	50.00	30.14	0.247	0.082	0.035	0.005	0.002	0.001
Fenvalerate	20.00	9.35	0.077	0.025	0.011	0.004	0.001	0.000
Deltamethrin	10.00	14.20	0.116	0.039	0.017	0.012	0.004	0.002
Organophosphate								
Dimethoate	1.00	6.57	0.054	0.018	0.008	0.054	0.018	0.008
Pirimiphos-methyl	4.00	51.90	0.426	0.142	0.061	0.106	0.035	0.015
Chlorpyrifos	10.00	3.94	0.032	0.011	0.004	0.003	0.001	0.000
Malathion	30.00	7.69	0.063	0.021	0.009	0.002	0.001	0.000

NB: highlighted cells show HI with possible health implications

Table 7.13: Health hazard indices of pesticides in *Bagrus bayad*

Substance	ADI(ug/kg)	Conc.(ug/kg)	Exposure dose(ug/kg/day)			Hazard index (HI)		
Organochhlorines			0-1yr	1-11yrs	Adult	0-1yr	1-11yrs	Adult
β-HCH	5.00	3.92	0.032	0.011	0.005	0.006	0.002	0.001
Heptachlor	0.10	4.00	0.033	0.011	0.005	0.328	0.109	0.047
α-endosulfan	0.05	17.50	0.143	0.048	0.021	2.870	0.956	0.410
Methoxychlor	5.00	98.10	0.804	0.268	0.115	0.161	0.054	0.023
Pyrethroids								
Allethrin	8.00	4.10	0.034	0.011	0.005	0.004	0.001	0.001
Bifenthrin	15.00	8.20	0.067	0.022	0.010	0.004	0.002	0.001
Fenpropathrin	30.00	6.84	0.056	0.019	0.008	0.002	0.001	0.000
Permethrin	50.00	85.90	0.704	0.235	0.101	0.014	0.005	0.002
Cyfluthrin	3.00	53.40	0.438	0.146	0.063	0.146	0.049	0.021
Cypermethrin	50.00	24.90	0.204	0.068	0.029	0.004	0.001	0.001
Fenvalerate	20.00	4.10	0.034	0.011	0.005	0.002	0.001	0.000
Organophosphate								
Methamidophos	1.00	37.60	0.308	0.103	0.044	0.308	0.103	0.044
Ethoprophos	0.10	14.90	0.122	0.041	0.017	1.222	0.407	0.174
Dimethoate	1.00	33.30	0.273	0.091	0.039	0.273	0.091	0.039
Chlorpyrifos	10.00	26.80	0.220	0.073	0.031	0.022	0.007	0.003
Malathion	30.00	6.48	0.053	0.018	0.008	0.002	0.001	0.000
Fenitrothion	5.00	8.70	0.071	0.024	0.010	0.014	0.005	0.002
Chlorfenvinphos	0.50	11.70	0.096	0.032	0.014	0.192	0.064	0.027
Profenofos	30.00	48.60	0.398	0.133	0.057	0.013	0.004	0.002

NB: highlighted cells show HI of 1 or greater (with possible health implications)

7.3.7 Pesticide residues and bioconcentration in *Ceratophyllum demersum* and *Nymphaea lotus*

Tables 7.14 and 7.15 present pesticides detected in *Ceratophyllum demersum* and *Nymphaea lotus* and the extent to which the pesticides are bioconcentrated by these aquatic plants. The presence of 3 organochlorines, 4 synthetic pyrethroids and 7 organophosphorus pesticide residues were recorded in *C. demersum* (Table 7.14). Of the detected organochlorine pesticides, endrin recorded the highest mean concentration of 5.90 µg/kg whilst β-lindane and p,p'-DDE were detected in low concentrations of 0.50 µg/kg and 0.53 µg/kg respectively. They all recorded high incidence rate between 80% – 100% (Table 7.14). The levels of synthetic pyrethroids were generally low, with mean concentration range of 0.62-2.17 µg/kg. Cypermethrin registered the highest mean residue of 2.17 µg/kg among the pyrethroids. The organophosphorus residues recorded the highest levels in *C. demersum* with a mean concentration range of 2.86 – 129.0 µg/kg. Methamidophos registered the highest organophosphorus mean concentration (129.0 µg/kg) while malathion recorded the lowest (2.86 µg/kg). From the values in Table 7.14, organophosphorus pesticides had the highest load (241.66 µg/kg) followed by the OCPs (6.93 µg/kg) and SPs (5.21 µg/kg) in *Ceratophyllum demersum*.

Nymphaea lotus on the other hand registered the presence of more pesticide residues (Table 7.15). The presence of 4 organochlorines pesticides, 6 synthetic pyrethroids and 8 organophosphorus pesticide residues were detected. The highest mean residue was recorded for γ-lindane (35.90 µg/kg), followed by methamidophos (8.66 µg/kg) (Table 7.15). Unlike *Ceratophyllum demersum*, *Nymphaea lotus* bioconcentrated OCPs most, followed by OPs and then SPs (Tables 7.14 and 7.15).

Examination of the results reveals that all the detected pesticides in the aquatic plants were bioconcentrated, with the exception of endrin in *Nymphaea lotus* and β-HCH (in *Ceratophyllum demersum*) since their concentrations in the aqueous medium were more than their corresponding concentrations in the plant tissues (Tables 7.14 and 7.15). Both plants have higher bioconcentration of organophosphorus pesticides than the OCPs and SPs and follow the order: OPs > SPs > OCPs. It is also observed that *Ceratophyllum demersum* generally have higher bioconcentration of OPs than *Nymphaea lotus*. For instance whereas the bioconcentration factors for methamidophos, diazinon and chlorpyrifos in *C. demersum* respectively were 330.8, 4470.0 and 2305, the corresponding figures for the same pesticides in *N. lotus* were 22.2, 800.0 and 305.0 (Tables 7.14 and 7.15).

Table 7.14: Pesticides in *Ceratophyllum demersum* and bioconcentration factor

	Mean Conc. in C. *demersum* (µg/kg)	Conc. range (µg/kg)	Freq. (%)	Ambient conc.in water (µg/L)	Bioconcentration Factor (BCF)
Organochlorines					
β-HCH	0.50 ± 0.23	0.50-0.61	100	3.800	0.13
p,p'-DDE	0.53 ± 0.00	0.50-0.61	80	0.050	1.06
Endrin	5.90 ± 0.50	5.30-6.40	80	2.400	2.45
Load	**6.93**				
Pyrethroids					
Lamda-cyhalothrin	1.42 ± 0.59	1.10-2.10	60	0.068	20.90
Cyfluthrin	1.00 ± 0.14	0.80-10.00	40	0.011	91.00
Cypermethrin	2.17 ± 0.50	1.81-2.74	60	0.720	3.00
Fenvalerate	0.62 ± 0.47	0.50-1.30	80	0.010	62.00
Load	**5.21**				
Organophosphates					
Methamidophos	129.00 ± 21.80	113.60-154.30	60	0.390	330.80
Diazinon	44.70 ± 1.2	3.10-5.30	60	0.010	4470.00
Chlorpyrifos	46.10 ± 6.4	38.70-50.00	60	0.020	2305.00
Malathion	2.86	2.86	20	0.010	286.00
Fenitrothion	11.00 ± 3.50	8.50-13.40	40	0.004	2750.00
Parathion	4.70 ± 0.32	4.50-5.10	60	nd	-
Profenofos	3.30 ± 1.10	2.50-4.10	40	0.010	330.00
Load	**241.66**				

Load = Total of the means of all residues in each pesticide group

Table 7.15: Pesticides in *Nymphaea lotus* and bioconcentration factor

	Mean Conc. in *N. lotus*(µg/kg)	Conc. range (µg/kg)	Freq. (%)	Ambient conc. In water (µg/L)	Bioconcentration Factor (BCF)
Organochlorines					
β-HCH	3.90 ± 2.80	1.30-8.00	80	3.800	1.27
γ-HCH	35.90 ± 4.90	29.60-39.90	100	0.100	359.00
p,p'-DDE	0.66 ± 0.63	0.50-1.10	40	0.050	13.20
Endrin	0.70 ± 0.27	0.50-0.90	40	2.400	0.30
Load	**41.16**				
Pyrethroids					
Bifenthrin	2.33 ± 0.71	1.31-2.93	80	0.049	47.00
Lamda-cyhalothrin	2.20 ± 0.99	1.00-3.00	80	0.068	32.40
Permethrin	0.80 ± 0.31	0.50-1.00	40	0.032	25.00
Cyfluthrin	6.20 ± 0.89	5.60-7.50	80	0.011	563.60
Cypermethrin	1.90 ± 0.45	1.57-2.20	40	0.720	2.60
Deltamethrin	0.52 ± 0.00	0.50-0.54	40	0.330	1.58
Load	**13.62**				
Organophosphates					
Methamidophos	8.66 ± 4.95	2.24-14.30	80	0.390	22.20
Ethoprophos	5.10 ± 0.57	4.70-5.50	40	0.030	170.00
Phorate	2.80 ± 0.46	2.40-3.10	40	0.006	466.70
Diazinon	4.00	4.00	20	0.01	800.00
Pirimiphos-methyl	2.14	2.14	20	0.01	214.00
Chlorpyrifos	6.10 ± 0.36	5.80-6.50	60	0.020	305.00
Parathion	4.70 ± 0.46	4.36-5.00	40	nd	-
Chlorfenvinphos	2.93	2.90	20	0.080	36.60
Load	**32.8**				

Load = Total of the means of all residues in each pesticide group

7.4 Discussions

7.4.1 Organochlorine pesticides concentrations and composition in tissues of fishes

The determinations were done on wet-weight basis of the tissue samples. In all, eleven Organochlorine pesticides-OCPs (β-HCH, γ-HCH, δ-HCH, heptachlor, α-endosulfan, β-endosulfan, endosulfan sulfate, *p,p'*-DDD, *p,p'*-DDE, endrin and methoxychlor) were quantified in muscle, gill and liver tissues of the four fish species investigated (*Tilapia zilli*, *Oreochromis niloticus*, *Chrysicththys nigrodigitatus* and *Bagrus bayad*). The mean concentration range recorded in the tissues of all the fishes was 0.78 µg/kg – 4671 µg/kg. The highest of 4671 µg/kg was recorded for methoxychlor in the liver of *Chrysicththys nigrodigitatus* (Table 7.4) while β-HCH recorded the lowest concentration in the muscle tissue of *Barus bayad* (Table 7.5). Three OCPs (β-HCH, heptachlor and methoxychlor) were detected in the muscle of the fishes in the mean concentration range of 0.78 -94 µg/kg. The highest and the lowest were both recorded in *B. bayad* for methoxychlor and β-HCH respectively. Methoxychlor and β-HCH were the most frequently encountered OCPs in muscle of the fishes. From Tables 7.2 – 7.5, it can be established that OCP residue concentrations in the tissues of the fishes follow the order liver > gill > muscle. So also imputed values from the data gathered established that on the average, 57.5%, 85.0% and 76.0% of muscle, gill and liver tissue samples respectively registered the presence of OCP residues in the fish species. The European Union (EU) default Maximum Residue Level (MRL) of any pesticide in fish tissue is 0.01mg/kg (10 µg/kg). In line with this, only methoxychlor and heptachlor exceeded MRL in the fish muscle tissues. Heptachlor (16.33 µg/kg) exceeded MRL in muscle of *T. zilli*, while methoxychlor exceeded in muscle tissues of both *C. nigrodigitatus* (31.00 µg/kg) and *B. bayad* (94.00 µg/kg). Thus in all, 13% of the OCPs residues in muscle tissues of the fish species exceeded MRL.

Table 7.16 compares results of the present study with some from other regions of the world as well as with those from some local studies. Comparison of the mean OCP residue ranges in muscle tissues from the present study with results from other local studies show that values from this study are higher (Table 7.16). Whereas the highest local OCP concentration range in the muscle of fish (0.3- 7.13 µg/kg) was quoted by Afful *et al*., (2010) for fish species from the Densu River, that in this study was 0.78 – 94.00 µg/kg. Compared however to the ranges quoted by some researchers from other regions of the world, the concentration range in this study is lower. For instance Pazou *et al*., (2006) reported a mean concentration range less than limit of detection – 134.00 µg/kg for fish species from Oueme river basin in Benin; Said and Hamed, (2005) also recorded a range of 1.217 - 3759 µg/kg for species from El-Temsah and Bitter Lakes of Suez Canal, Egypt while Adeyemi *et al*., (2008) registered astronomical mean concentration range 10.00 - 8920 µg/kg for OCPs in muscle tissues of three species from Lagos Lagoon in Nigeria (Table 7.16). Fishes in Ghanaian aquatic ecosystems can therefore generally be considered relatively less contaminated.

Seven OCPs were detected in gill tissues of the fishes, namely β-HCH, heptachlor, α-endosulfan, β-endosulfan, endosulfan sulfate, *p,p'*-DDD and methoxychlor. β-HCH had the highest incident rate of 100% in *T. zilli*, 80% in *O. niloticus* and *B. bayad*, and 90% in *C. nigrodigitatus*. The range of mean concentration in all the three species was 0.90 – 18.33 µg/kg. The least concentration of 0.90 µg/kg was detected for β-endosulfan in *C. nigrodigitatus* and the highest (18.83 µg/kg) for heptachlor in *T. zilli*. This range, as compared to that of Gbeddy *et al*., (2012) was much lower. The liver tissues recorded the highest number of OCP residues (10) namely β, γ, λ-HCH, heptachlor, endrin, α-endosulfan, β-endosulfan, *p,p'*-DDE, *p,p'*-DDD and

methoxychlor with a mean concentration range of 11.00 - 4671 µg/kg. Four of the ten quantified OCPs were recorded in the liver of *T. zilli,* with a mean range of 11.00 – 35.2 µg/kg (Table 7.2) and in *O. niloticus* 12.90 – 63.30 µg/kg (Table 7.3). *Bagrus*. *bayad* and *C. nigrodigitatus* each recorded the presence of 9 OCPs and with respective mean concentration ranges of 20.00 – 627.30 µg/kg and 21.0 -4671.0 µg/kg. Concentrations in the liver were not only significantly higher but also several ten folds higher, in most cases than corresponding concentrations in the other tissues (gill and muscle).

Table 7.16 Mean concentrations of pesticide residues in fish tissues from local and international studies compared to the present study.

Reference	No. of species	Fish tissue	Contaminants detected	Range (µg/kg)	Location
Local studies					
Darko *et al*., (2008)	1 (Tilapia)	Muscle	4 OPs	0.018 – 5.232	Lake Bosomtwi
Essuman *et al*.,(2009)	NS	Muscle	14 OCPs	0.10 – 0.30	Lagoons in Central Region
Afful *et al*., (2010)	6	Muscle	14 OCPs	0.3 – 71.30	Densu basin
Adu-Kumi et al., (2010)	2	Muscle	17 OCPs	Nd –290.00	Weija, Bosomtwi and Volta
Fianko *et al*., (2011)	NS	Muscle	14 OCPs	0.51 – 7.99	Densu River basin
Gbeddy *et al.*, (2012)	2	Muscle	15 OCPs	0.10 – 17.35	Kpando (Volta Lake)
		Gill	″	0.56 – 37.75	″
Present study	4	Muscle	3 OCPs	0.78 – 94.00	Afram Lake arm of Volta
		Gill	7 OCPs	0.90 – 18.83	″
		Liver	11 OCPs	11.0 –4671.0	″
		Muscle	7 Pyrethroids	1.10 – 49.80	″
		Gill	8 Pyrethroids	2.00 – 58.60	″
		Liver	9 Pyrethroids	9.0 –2190.00	″
		Muscle	8 OPs	1.60 – 17.00	″
		Gill	9 OPs	2.34 – 47.80	″
		Liver	13 OPs	2.1 – 193.00	″
Studies from other regions					
Nemr & Abd-Allah (2004)	4	Muscle	12 OCPs	20.0 –211.00	Egyptian markets
Said & Hamed (2005)	3	Muscle	11 OCPs	1.217 –3757.00	Suez Canal-Egypt
Pazou et al., (2006)	5	Muscle	9 OCPs	Nd –1364.00	Oueme River basin–Benin
Fenandez-Bringas *et al*., (2008)	1	Muscle	16 OCPs	0.08 – 6.97	Mexico
Adeyemi *et al*., (2008)	3	Muscle	9 OCPs	10.0 –8920.0	Lagos Lagoon – Nigeria
Akan *et al.*, (2013)	4	Muscle	11 OCPs	0.12 - 4.81	Alau Dam, Borno State – Nigeria
		Gill	11 OCPs	0.22 – 7.45	
		Liver	11 OCPs	0.35 – 8.98	″

NS – Not stated; Nd – Not detected; OCPs – Organochlorine pesticides;
OPs – Organophosphorus pesticides

7.4.2 Synthetic pyrethroid concentrations and composition in tissues of fishes

The targeted nine synthetic pyrethroids (SPs) were all detected in the fish tissue samples in a mean concentration range of 1.00 - 2190 µg/kg. Seven of them (bifenthrin, permethrin, cyfluthrin cypermethrin, fenvalerate and deltamethrin) were detected in muscle of the three fish species in a mean concentration range of 1.10 – 49.8 µg/kg. The least concentration (1.10 µg/kg) was recorded for cypermethrin in *Tilapia zilli* while the highest concentration (49.8 µg/kg) was recorded for permethrin in *Bagrus bayad*. On the average, 77.5%, 87.5% and 70.0% of muscle, gill and liver tissue samples respectively recorded the presence of SPs residues in the four fish species. Forty-four percent (44.4%) of the SPs (bifenhtrin, permethrin, cyfluthrin and cypermethrin) exceeded the MRL in the muscle tissues of the fishes. Permethrin exceeded MRL in all the fishes and also recorded the highest incidence rate in all fish musle samples; 100% in *Chrysicththys nigrodigitatus, 90% in Bagrus bayad* and 70% in *Oreeochromis niloticus*. Eight pyrethroids were detected in the gills of all the fishes in a mean concentration range of 2.00 – 58.60 µg/kg. These are allethrin, bifenthrin, permethrin, cyfluthrin, fenvalerate, fenpropathrin and deltamethrin, and 9 SPs, with a mean concentration range of 1.69 – 2190.00 µg/kg were quantified in the liver of the fishes. The lowest mean concentration of 1.69 µg/kg was recorded for fenpropathrin in *B. bayad* while the highest value (2190.00 µg/kg) was detected for permethrin in the same *B. bayad*. Pyrethroid load in the liver tissues follow the order C. *nigrodigitatus* (3679.20 µg/kg) > *B. bayad* (2628.10 µg/kg) > *T. zilli* (601.20 µg/kg) > *O. niloticus* (571.38 µg/kg).

Pesticides in general have been found to have adverse hispathological effects in fishes, including shrinkage of liver cells, changes of cell nuclei, and atrophy of some cells (Murthy *et al*., 2013). The high occurrence rate of SPs in fish tissues is therefore a cause for concern. Pyrethroids in

particular are known to be toxic to fishes. When exposed, fishes experience body spasms, hyperactive swimming, reduced reaction to stimuli and eventual death in some instances (Palmquist *et al*., 2012). In addition, they affect reproductive viability in fish and also alter embryonic and early life stage development. According to Reigart and Roberts (2013), pyrethroids are lipophilic, do not readily volatilize and strongly adsorb to organic matter. They therefore easily bioaccumulate in tissues of fishes, causing contamination in the food chain. When exposed to humans through diet, persons, especially children can show signs of poisoning such as headache, nausea, convulsion, numbness and tremors among others (Fishel, 2014).Therefore even though pyrethroids emerged as a response to environmentally persistent organochlorines as well as inorganic pesticides, there is the need to exercise restrained in their use in order to avert attendant health hazards related to their abuse.

7.4.3 Organphosphorus pesticides concentrations and composition in tissues of fishes

Thirteen of the OPs were quantified in the tissues of the fishes. Liver tissues recorded the presence of 12; gill and muscle each recorded the presence of 9. The 9 recorded for the muscle tissue were methamidiphos, chlorpyrifos, malathion, profenofos, phorate, dimethoate, pirimiphos-methyl, ethoprophos and chloefenvinphos, all within the mean concentration range of 1.60 – 17.00 µg/kg. Four OPs, namely chlorpyrifos, chlorfenvinphos, ethoprophos and profenofos exceeded the MRL in the muscle of the fishes. In terms of percentage, 30.8% of the OPs exceeded MRL. Analysis of incidence rate of OPs in the various organs of the fish show that on the average, 52.5% each of muscle, gill and liver samples recorded their presence. It was observed that in each fish species, OP load was highest in liver and least in muscle tissues, hence following the trend liver > gill > muscle. Overall, 27% of all pesticides analysed exceeded MRL in muscle tissues of the three fish species and the following banned pesticides were identified in

the various fish tissues: β-lindane, γ-lindane, δ-lindane, heptachlor, aldrin, endrin, DDT, methamidophos and parathion.

There is generally dearth of research on levels of synthetic pyrethroids and organophosphorus pesticide in aquatic biota even though these are pesticides that are mostly in use in agriculture including cultivation of horticultural crops along water bodies. It is therefore difficult to compare results from the present study to work done by scientists from Ghana and other countries around the world. However, Essuman *et al*., (2009) investigated levels of four OPs (dichlorvos, diazinon, chlorpyrifos and fenitrothion) in muscle tissues of fish species from two lagoons in the Central Region of Ghana and reported mean concentration range of 0.1 – 0.3 µg/kg. This is far lower than the range of 1.1 – 17.0 µg/kg reported in this study.

There is the need to conduct more research into levels of pesticides in our food commodities, particularly the organophosphates since they have higher mammalian toxicity than the other groups of pesticides (Kazemi *et al*., 2012a). Studies on the effect of OPs on fishes by Shoaib *et al.,* (2012) suggest that reduction of protein levels in tissues of fish occurs when exposed to OPs. According to them, reduction of tissue protein is as a result of impaired protein synthesis resulting from hormonal imbalance, impaired cellular metabolism and DNA damage or necrosis of cells. Moreover, organophosphorus pesticide poisoning through dietary exposure in humans can be acute and exhibit early symptoms such as muscle twitching, sweating and salivation, weakness and headache (Kazemi *et al*., 2012a). Thus apart from considerable effect on fish nutritive quality due to tissue protein reduction, long-term exposure of organisms to pesticides may pose a high risk of health hazard to the general public through consumption of pesticide contaminated fishes. Serious monitoring and regulatory mechanisms must therefore be put in place by responsible agents to safeguard life.

7.4.4 Tissue-specific bioaccumulation of pesticides in fish species

In all the four species of fish, the order of decreasing concentrations of pesticides in the various organs was liver > gill > flesh. Significant difference generally however existed only between liver and the other tissues. Thus liver was the predominant organ for organochlorine, organophosphorus and synthetic pyrethroid pesticides bioaccumulation; while muscle and gill were found to contain concentrations at relatively lower levels (Tables 7.6 – 7.9). This demonstrates higher bioaccumulation potency in hepatobiliary system than the extrahepatic tissues (Zhao *et al.,* 2014). Works done by Akan *et al*., (2013b) and Zhao *et al*., (2014) also demonstrated the same trend of OCPs, and OPs levels in liver, gill and muscle tissues of various fishes, including those in this study. The relative lower residual levels found in muscle of the fishes indicate muscle tissues are not the target organ for accumulation of lipophillic substances (Ballesteros *et al*., 2011). Since bioaccumulation of toxic chemicals in fishes occurs mainly through both absorption by external membrane and by dietary uptake (Yang *et al*., 2007), it was expected that gill, which is the primary tissue of filter-feeding fishes, and medium for pollutant accumulation through the gill-water transfer (Yang *et al*., 2007) will experience higher bioaccumulation of contaminants (as experienced by Zhao *et al*., 2013 in another study). Higher concentrations in this study were rather found in liver of all the fish species studied. This indicates that food uptake could be the main exposure route for accumulation of pesticides from the environment (Ballesteros *et al*., 2011). The pollutants, once they are absorbed by dietary uptake are metabolized and biotransformed, as they are transported and distributed to various body organs, including the liver, which receives large volume of toxin-laden blood for deamination (Zhao *et al.,* 2014). It is therefore not surprising that liver tissues in this study not only registered very high contaminant concentration, but also a high variety of pesticides; both parent and metabolite compounds.

7.4.5 Health Risk Estimates

Tables 7.10, 7.11, 7.12 and 7.13 the health risks for systemic effects associated with pesticide residues that were quantified in muscle and gill tissues of *Tilapia zilli, Oreochromis niloticus, Chrysicththys nigrodigitatus* and *Bagrus bayad* respectively. Each table comprises computed dietary exposure to pesticides and the associated hazard indices for age groups up to 1 year; > 1– 11 years and above 11 years (adults or grown-ups). Acceptable daily intakes (ADIs) or References doses (RfD), which is a level of intake of a chemical that can be ingested daily over a lifetime without any appreciable risk involved (WHO, 2001) for each pesticide is also indicated.. The ministry of Food and Agriculture of Ghana (MOFA, 2011) quotes the per capita consumption of fish in Ghana as being 0.82 kg/person/day.

Examination of analysed data presented in Tables 7.10 – 7.13 reveal that aldrin, α-endosulfan, ethoprophos and to some extent endosulfan sulfate levels in various fishes present health hazard to children between the ages of 0 – 11 years. In children of 0 – 1 years, hazard indices (HIs) of 2.88, 2.46 and 1.52 were computed for aldrin, α-endosulfan and ethoprophos respectively in T. *zilli;* 2.82 and 1.738 for , α-endosulfan in *O. niloticus* and C**.** *nigrodigitatus* respectively; and 2.87 and 1.22 for , α-endosulfan and ethoprophos respectively in B. *bayad*. Since the HIs for the pesticides in each case were greater than 1, they pose direct health hazard to human health, particularly to children between the ages of 0 – 1 year.

For children of ages between 1 – 11 years, , α-endosulfan in all the four fish species pose health risk since it recorded HIs close to one. Also, the calculated exposure doses for α-endosulfan in each case were either higher or close to the ADI of α-endosulfan (0.05 µg/person/day). Hazard indices for adults were all below 1 and their calculated dietary exposure doses as well were all

below the corresponding ADIs of the pesticides (Tables 7.10 – 7.13) and so no health risk can directly be imputed or associated with adult consumption of these fishes. The differences of risk associated to children and adults basically stems from differences in their body weights. This therefore confirms the fact that children are basically the most vulnerable subgroup of the population. The younger and/or smaller the body weight, the higher the associated risk. Fianko *et al*., (2011) also reported associated health hazard with consumption of tilapia fish from Densu River by children of age group 1 – 11 years. Their health risk analysis indicated systemic toxicity for this age group came from concentration levels of α-endosulfan, heptachlor, dieldrin and endosulfan sulfate. Gbeddy *et al*., (2012) in a similar study however reported risk levels of no health concern associated with low OCPs concentrations in *T. zilli* and *C. nigrodigitatus* from the Volta Lake at Kpando Torkor of Ghana. In the present study, *Tilapia zilli* recorded four pesticides with HI > 1; *Bagrus bayad* recorded 2 and *Oreochhromis niloticus* and *Chrysicththys nigrodigitatus* each registered 1 pesticide with HI > 1. Hence, health risk associated with the consumption of these fishes in decreasing ranking order follows: *Tilapia zilli* > *Bagrus bayad* > *Oreochhromis niloticus* = *Chrysicththys nigrodigitatus*. Also, it is observed that α-endosulfan presents the highest risk of systemic effect since it recorded hazard indices greater than unity in all the fish species. It is followed by ethoprophos, with HIs greater than 1 in two fish species. It should also be emphasized that although HIs of most pesticides were below 1, there was no zero risk because pesticides were present in all fish tissue samples. Alpha-endosulfan, heptachlor and endosulfan sulfate for instance are generally persistent, lipophilic and bioaccumulative in the environment as well as at trophic levels of the food chain (Sun *et al.*, 2006; Fianko *et al*., 2011). These pesticides can therefore, through biomagnifications, reach high concentration in tissues of organisms higher in the food chain, including man, and therefore present food safety challenges.

7.4.6 Bioconcentration of pesticides in aquatic flora

Information on bioconcentration of pesticides in two aquatic macrophytes: *Ceratophyllum sp*.and *Nymphaea sp*. is presented in Tables 7.14 and 7.15 respectively. Ambient concentration in water samples from where the two aquatic flora were harvested were used in computing the bioconcentration factors. A bioconcentration test is conducted to obtain information concerning the ability of an aquatic species to accumulate a test material directly from water. Bioconcentration factor (BCF) is the ratio of contaminant concentrations in biota and the surrounding water (Nowell *et al*., 1999) and gives an indication of the ability of an aquatic plant to concentrate chemicals into its system from the surrounding water.

Bioconcentration factors (BCFs) are used to relate pollutant residues in aquatic organisms to the pollutant concentration in ambient waters. And they give an indication of the kind of health hazard associated with the consumption of a specific aquatic organism. A high BCF from an aqueous medium with an ambient low level of chemical concentration indicates the high ability of an aquatic organism to concentrate the specific chemical into its biological system. This becomes a concern, particularly if the organism occupies a lower trophic level in food chain in the ecosystem. *Ceratophyllum sp*. and *Nymphaea sp*. as reported by Lass, (2015), FAO, (2015) and Toowoomba (2000) constitute important sources of plant food for fish and a high level of chemicals in them means biomagnifications at higher trophic levels and ultimately in man who is at the apex of the food chain. According to the European Union regulation for registration of chemicals (2011), a substance fulfils the bioaccumulation criterion when the bioconcentration factor in aquatic species is higher than 2000; and “very bioaccumulative” criterion when the bioconcentration factor in aquatic species is higher than 5000. With this in view therefore, Ceratophyllum particularly in aquatic systems must be of much concern regarding the pollution

of the aquatic food chain since it has shown the ability of chemicals fulfiling both bioaccumulation (fenitrothion and chlorpyrifos) and "very bioaccumulative" criterion (diazinon) in it. *Nymphaea sp*. can be considered to be a relatively safe primary producer in an aquatic food chain since its bioconcentration ability is much lower. In spite of the high bioconcentrations of diazinon, fenitrothion and chlorpyrifos in *Ceratophyllum sp*., results of health risk analysis of pesticides in fish tissues show that they are not of much concern. While chlorpyrifos and fenitrithion occur in appreciable levels in fish tissues, diazinon in particular was detected only once and in a low concentration of 8 µg/kg in the liver of *Tilapia zilli* (Table 7.2) and was therefore not considered for health risk analysis. Reasons for this observation could be that even though these aquatic macrophytes could be sources of food for the fishes, the fish species might have effective depurative mechanism for eliminating diazinon from their body system or they could have alternative preferred sources of food. This however requires further investigation for conclusive results. However, the high bioconcentration factor of diazinon, fenitrotion and chlorpyrifos in *Ceratophyllum sp* should be a pointer to the fact that many more pesticides could bioconcentrate to alarming levels in the numerous aquatic weeds found in the Afram arm of the Volta Lake and which could serve as food for many aquatic fauna. This could have an implication for biomagnifications of pesticide contaminants along the aquatic food chain and ultimately affect man who is at the apex of most food chains. More research into this area therefore needs to be carried out to identify aquatic macrophytes that of major concern as food chains contaminants, by reason of their bioconcentration potential.

CHAPTER EIGHT

GENERAL CONCLUSION, SUMMARY AND RECOMMENDATIONS

8.1 GENERAL CONCLUSION

The findings from this study established the facts that banned pesticides are still used and residual load of pesticides on fallowed lands along the banks of the Afram arm of the Volta Lake are heavy enough to potentially contaminate new crops that will be cultivated on them, even without further application of insecticides. The banned pesticides detected in the soils from the fallowed lands were: aldrin, dieldrin, endrin, gamma-lindane, DDT, heptachlor, parathion and methamidophos. Furthermore, determination of pesticide content of watermelon, onion and pepper samples showed that while every sample contained quantifiable presence of pesticide, 67% of all the crop samples contained at least, one pesticide with level above the maximum residue level permitted by the European Union. Estimation of health risk associated with pesticides present in the food crops indicate that β-lindane, dieldrin and methamidophos have potential for systemic toxicity in children while heptachlor shows health risk in both children and adults. Similarly, estimation of health risk associated with consumption of four species of fish: *Tilapia zilli, Oreochromis niloticus, Bagrus bayad and Chrysichthys nigrodigitatus* indicates that levels of heptachlor, α-endosulfan and ethoprohos in them present health hazard in children up to 11 years, while no health risk can directly be imputed or associated with adult consumption of these fishes. The order of pesticide concentration in the tissues of the fishes was generally: Liver > Gill > Muscle and the levels of methoxychlor, aldrin, bifenthrin, permethrin, cyfluthrin, cypermethrin, chlorpyrifos, chlorfenvinphos, ethoprophos and profenofos in the muscle tissues of the fishes exceeded 0.01mg/kg default EU MRL. *Ceratophyllum demer*sum and *Nymphaea lotus* were found to bioconcentrate pesticides. *Ceratophyllum demer*sum in particular fulfilled

the “very bioaccumulative” criterion since it was able to have bioconcentration factor of approximately 5000 for diazinon. Comparison of pesticides content of the Afram River during flood and recess regimes show significantly high incidence rate and concentration levels of pesticides, as well as high number of pesticides exceeding the WHO MRL during the flood regime. Drawdown farming is identified as the cause of high concentrations and incidence level of pesticides in water during the flood regime. Determination of Afram River bed sediment toxicity identifies γ-lindane, *p,p’*-DDE and dieldrin as contaminants whose concentrations have reached toxicity levels and could therefore pose health risk to benthic organisms in particular.

It can therefore be concluded that whereas Afram bank farming has a lot of economic benefits to farmers, it has also resulted in significant contamination of food crops and the environment. There is the need for farmers to be educated on the importance of observing the Pre-harvest Interval (PHI). This must however be accompanied by efficient monitoring system to ensure strict observance. More agricultural extension officers must therefore be trained to carry out proper monitoring of agricultural activities along the Afram arm of the Volta Lake. Farming in the drawdown area must be critically evaluated and appropriate policy measures put in place (by EPA) to reduce pesticide contamination of the water bodies.

8.2 SUMMARY

The summary of the most important findings and recommendations from the study are presented:

1. Farmland soils temporary left fallow, for about three months, were found from this study to have high load of residual pesticides. About 88% of total samples analysed contained one or more pesticide residues; and approximately 97% of target pesticides analysed were detected and quantified. Twenty four percent of the target pesticides had levels exceeding the maximum residue limit (MRL) of the United States Environmental Protection Agency (USEPA) and these are *p,p'*-DDT, *p,p'*-DDD, fenvalerate, cypermethrin, permethrin, chlorpyrifos, dimethoate, chlorfenvinphos and methamidophos. The presence of banned pesticides was also established and these were: aldrin, dieldrin, endrin, gamma-lindane, DDT, heptachloer, parathion and methamidophos. Further analysis indicated that the presence of DDT, aldrin and endrin were due to historical use while gamma-lindane indicates possible current input.

2. Analysis of three food crops, water melon, onion and pepper for pesticide residue and associated health risk show that 92% of pesticides analysed were detected in 92.9 % of the total samples analysed. Hundred percent of target organophosphorus and pyrethroid and 80% of organochlorine pesticides were quantified in the samples. Seventy percent of the total samples recorded pesticide residues with concentration levels above the Eurpean Union (EU) Maximum Residue Limit (MRL). Of the target pesticides analysed, β-lindane, γ-lindane, δ-lindane, γ-chlordane, *p,p'*-DDE, dieldrin, endrin, methoxychlor, heptachlor, allethrin, methamidophos, ethoprophos, dimethoate and fenitrothion,

constituting 37.8% exceeded the MRL. Estimation of health risk associated with pesticide present in the food crops indicate that β-lindane, dieldrin and methamidophos have potential for systemic toxicity in children while heptachlor shows health risk in both children and adults. So even though fruits and vegetables are excellent sources of dietary fibre, vitamins, minerals and other natural substances that help protect the body from chronic diseases the high incidence of pesticides encountered in them in this study raises food safety concerns.

3. Seasonal rise of water level of the Afram Lake leads to occasional flooding of its immediate banks that are normally cultivated. Comparison of pesticides content of water during the flood and recess regimes show significant high incidence rate and concentration of pesticides, as well as high number of pesticides exceeding the WHO MRL during the flood regime. Consumption of lake water from the Afram during the flood regime is thus associated with higher health risk.

4. Toxicity risk assessment of surface sediment of Afram arm of the Volta Lake clearly identifies γ-lindane, *p,p'*-DDE and dieldrin as contaminants whose concentration levels in the sediment pose health risk to benthic organisms and therefore require remediation attention. Endrin, *p,p'*-DDT and *p,p'*-DDD are also potential risks to the aquatic ecosystem but require further investigation to clearly establish their status.

5. Four common fish species that are a delicacy and important source of protein to Ghanaians were investigated to ascertain pesticide residue levels in them as well as any

associated health risk. The fish species are: *Tilapia zilli*, *Oreochromis niloticus*, *Chrysichthys nigrodigitatus* and *Bagrus bayad*. The muscle tissues of the fishes were variously found to be contaminated with methoxychlor, aldrin, bifenthrin, permethrin, cyfluthrin, cypermethrin, chlorpyrifos, chlorfenvinphos, ethoprophos and profenofos whose levels all exceeded 0.01mg/kg which is the default EU MRL.

6. *Chrysichthys nigrodigitatus* and *Bagrus bayad* contained the highest load of pesticides. Analysis of muscle, gill and liver tissues show that pesticide content of liver is far higher than gill which is also greater than that in muscle. This trend is exhibited in all the fish species however statistical analysis did not showed diferences between residue concentrations in gill and muscle tissues.

7. The mean concentration range of organochlorine pesticide residue in the tissues of all the fishes was 0.78 – 4671 µg/kg. Methoxychlor and heptachlor, representing 13% of the organchlorine pesticide residues exceeded MRL in the fish muscle tissues. The mean concentration range of synthetic pyrethroids (SPs) in all tissues of the fish species was 1.10 – 49.8 µg/kg and the mean concentration range of organophosphate pesticides in all tissues was 1.60 – 17.00 µg/kg. About forty-four percent (44.4%) of the SPs exceeded the MRL in the muscle tissues of the fishes. So also 30.8% of the OPs exceeded MRL in the muscle tissues of the fishes and 27% of all pesticides analysed exceeded MRL in muscle tissues of the three fish species and the following banned pesticides were identified in the various fish tissues: β-lindane, γ-lindane, δ-lindane, heptachlor, aldrin, endrin, DDT, methamidophos and parathion.

8. Estimation of health risk indicates that levels of heptachlor, α-endosulfan and ethoprohos in the fish species present health hazards such as immune system dysfunctions, adverse pregnancy outcomes and perinatal mortality in children as well as mutagenic and teratogenic effects. Meanwhile no health risk can directly be imputed or associated with adult consumption of these fishes since hazard indices for adults were all below 1 and their calculated dietary exposure doses as well were all below the corresponding acceptable daily intakes or reference doses of the pesticides.

9. Various aquatic weeds, including *Ceratophyllum demer*sum and *Nymphaea lotus*, according to some authors, constitute food source to fishes. Bioconcentration of pesticides in these two particular aquatic macrophytes was investigated as an indicator to the possibility of aquatic weeds as source of contamination to fishes. The results identified *Ceratophyllum demer*sum in particular to have fulfilled the "very bioaccumulative" criterion since it was able to have bioconcentration factor of approximately 5000 for diazinon in the aquatic medium.

10. This investigation established that *Ceratophyllum demersum* as primary producer and a food that can be browsed by fish is source of serious pesticide contamination of the aquatic food chain. Since fish constitutes an important source of protein, and fresh water fish from the Volta Lake in particular constitutes a delicacy to the Ghanaian populace, *Ceratophyllum* sp. has an indirect effect of exposing humans to pesticide contamination and associated health risks.

8.3 Recommendations

1. Since Pre-Harvest Interval (PHI) has the effect of reducing incidence level and concentrations of pesticides in food commodities, there is the need for farmers to be educated on its importance. This must however be accompanied by efficient monitoring system to ensure strict observance.

2. In order to practice proper application of pesticides, there must be a good motivation and incentive to do so. One possible way is to provide ready market for all farmers whose produce meet food quality standard. This implies Ghana Standards Authority, Food and Drugs Authority, Ghana Environmental Protection Authority and all other stakeholder institutions must collaborate to ensure random testing of samples from farms as well as provision of the ready market.

3. More agricultural extension officers must be trained to carry out proper monitoring of agricultural activities along the Afram arm of the Volta Lake.

4 In view of elevated pesticide content of water associated with flood regimes, as observed in this study, farming in the draw-down areas of the Afram arm of the Volta Lake must critically be evaluated by the EPA, Ghana and appropriate policy measures subsequently put in place to reduce contamination level during flood regimes. For instance it is possible, over a period of study, to identify the highest water mark during flood regimes and then prohibit farming activity below this point. If farming must be done in the drawdown at all, then it must be limited to those crops that do not necessarily require pesticide inputs. This action will have an added advantage of curbing rising incidence of siltation of the Volta Lake as a whole.

5. Other toxicants, mainly carbamates, fungicides and weedicides (such as glyphosate) whose use are also very high in the study area must be investigated in food commodities from the study area.

6. Finally, due to the constant exposure of these farmers to pesticides, the levels of various pesticides in body tissues of the farmers, as a matter of health concern must be investigated. Dissemination of health implications of such research results among the farmers could be a huge incentive for right attitude towards pesticide use.

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APPENDICES

Appendix I

Chemicals and Reagents

Acetonitrile, Ethyl acetate, Methylene chloride and acetone were of purest grade (pesticide grade) and were acquired from BDH laboratory Supplies, England. Certified organochlorine pesticide standards (Lindane; *α-lindane, β-lindane, γ-lindane, and δ-lindane, aldrin, heptachlor, γ-chlordane, α-endosulfan, p,p'DDE, dieldrin, endrin, β-endosulfan, p,p'-DDT, p,p'-DDD, Endosulfan-sulfate and Methoxychlor*); certified organophosphorus pesticide standards *(Methamidophos, Ethoprophos, Phorate, Diazinon, Fonofos, Dimethoate, Pirimiphos-methyl, Chlorpyrifos, Malathion, Fenitrothion, Parathion, Chlorfenvinphos, Profenofos*) and certified synthetic pyrethroids pesticide standards (*Allethrin, Bifenthrin, Fenpropathrin, Lamda-cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate, Deltamethrin*) with certified purity of at least 99% were obtained from Dr. Ehrenstofer GmBH (Augsburg, Germany).

Sorbent materials used for solid phase extraction were Supelclean Envi-Carb/LC-NH_2 SPE cartridges (500mg/500mg, 6ml size) from Supelco (Bellefonte, PA, USA); silica SPE cartridges (1000mg/6ml size) purchased from Phenomenex (Torrance, CA, USA) anhydrous magnesium sulphate and anhydrous sodium sulphate (from BDH laboratory Supplies, England).

Appendix II: Concentration of pesticides in water under flood regime

Pesticides	Mean (µg/L)	Range	Frequency (%)	WHO MRL (µg/L)
Organochlorines				
β-HCH	4.217	1.940-6.310	100	-
γ-HCH	0.065	0.01-0.158	100	2.00
δ-HCH	6.264	1.594-**16.788**	100	1.00
Heptachlor	0.011	0.01-0.016	80	0.03
Aldrin	1.031	0.627-1.588	100	0.03
α-endosulfan	0.020	0.01-0.071	75	0.01*
p,p'-DDE	0.040	0.01-0.078	90	2.00
Dieldrin	1.385	0.031-5.550	100	0.03
Endrin	1.900	0.216-3.970	100	0.60
Beta endosulfan	0.059	0.01-0.806	95	-
p,p'-DDT	0.066	0.044-0.092	15	2.00
p,p'-DDD	0.036	0.01-0.104	100	2.00
Endosulfan sulfate	0.023	0.01-0.111	100	0.01*
Methoxychlor	0.102	0.01-0.560	100	20.00
	Total=15.22	**0.01-16.788**		
Pyrethroids				
Allethrin	0.056	0.027-0.091	100	0.05*
Bifenthrin	0.051	0.015-0.112	100	0.05*
Fenpropathrin	0.341	0.045-0.680	85	0.05*
Lamda-cyhalothrin	0.067	0.023-0.234	90	20.00
Permethrin	0.066	0.017-0.233	100	0.05*
Cyfluthrin	0.010	0.010-0.021	60	0.05*
Cypermethrin	0.637	0.034-**2.135**	75	0.05*
Fenvalerate	0.010	0.010-0.011	55	0.05*
Deltamethrin	0.234	0.010-0.634	100	0.05*
	Total=1.47	**0.01-2.135**		
Organophosphate				
Methamidophos	0.751	0.040-1.120	45	
Phorate	0.010		5	-
Diazinon	0.012		5	0.05*
Fonofos	0.010		20	-
Dimethoate	0.044	0.020-0.064	15	0.05*
Pirimiphos-methyl	0.010		15	6.00
Chlorpyrifos	0.016	0.010-0.027	65	0.05*
Malathion	0.010	0.010-0.010	15	0.05*
Fenitrothion	0.015	0.010-0.026	35	30.00
Parathion	0.010	0.010	10	-
Chlorfenvinphos	0.010		5	0.05*
Profenofos	0.010		5	0.05*
	Total=0.91	**0.01-1.12**		

MRL = Maximum residue limit of pesticides allowed by WHO in drinking water.

* = MRL adopted by Ghana Standards Authority where that of WHO is not established.

Avg = Average of the mean concentrations of all pesticides in each pesticide group

Appendix III: Concentration of pesticides in water during recess regime

Pesticides	Mean (µg/L)	Range	Frequency (%)	WHO MRL (µg/L)
Organochlorines				
γ-HCH	0.187	0.010-0.269	35	2.00
δ-HCH	0.010		25	1.00
α-endosulfan	0.010		5	0.01*
p,p'-DDE	0.060		5	2.00
Dieldrin	0.084		5	0.03
Endrin	0.033		5	0.60
p,p'-DDT	0.035	0.010 -0.260	75	2.00
p,p'-DDD	0.010		5	2.00
Endosulfan sulfate	0.057	0.010-0.112	10	0.01*
Methoxychlor	0.011		5	20.00
	Total=0.497	**0.010 -0.269**		
Pyrethroids				
Fenpropathrin	0.036		5	-
Permethrin	0.015		5	0.05*
Cyfluthrin	0.013	0.011-0.016	15	0.05*
Cypermethrin	0.063	0.010-0.370	70	0.05*
	Total=0.127	**0.010-0.370**		
Organophosphate				
Methamidophos	0.077	0.055-0.115	55	-
Ethoprophos	0.028	0.020-0.036	30	0.05*
Phorate	0.010		10	-
Diazinon	0.013	0.010-0.017	40	0.05*
Dimethoate	0.091	0.012-**0.158**	25	0.05*
Pirimiphos-methyl	0.010	0.010-0.007	15	6.00
Chlorpyrifos	0.012	0.010-0.014	50	0.05*
Malathion	0.025	0.014-0.037	10	0.05*
Fenitrothion	0.016	0.010-0.020	20	30.00
Chlorfenvinphos	0.064	0.021-0.140	25	0.05*
Profenofos	0.036	0.010-0.068	60	0.05*
	Total=0.382	**0.010-0.158**		

MRL = Maximum residue limit of pesticides allowed by WHO in drinking water.

* = MRL adopted by Ghana Standards Authority where that of WHO is not established.

Avg = Average of the mean concentrations of all pesticides in each pesticide group

Appendix IV

One-way ANOVA to compare pesticides concentrations in muscle, gill and liver tissues of ***Tilapia zilli***

		Sum of Squares	df	Mean Square	F	Sig.
β-HCH	Between Groups	230.987	2	115.493	3.718	.047
	Within Groups	497.068	16	31.067		
	Total	728.055	18			
Methoxychlor	Between Groups	1289.913	2	644.957	141.426	.000
	Within Groups	50.164	11	4.560		
	Total	1340.077	13			
Permethrin	Between Groups	547437.026	2	273718.513	7.119	.008
	Within Groups	499858.144	13	38450.626		
	Total	1047295.170	15			
Metamedophos	Between Groups	710.793	2	355.396	21.531	.001
	Within Groups	132.047	8	16.506		
	Total	842.840	10			
Chlorpyriphos	Between Groups	.012	2	.006	.000	1.000
	Within Groups	124.893	5	24.979		
	Total	124.905	7			

All analyses done at 0.05 confidence interval

Appendix V

One-way ANOVA comparing pesticides concentrations in muscle, gill and liver of ***Oreochromis niloticus***

		Sum of Squares	df	Mean Square	F	Sig.
BHCH	Between Groups	377.943	2	188.971	2.744	.087
	Within Groups	1446.348	21	68.874		
	Total	1824.291	23			
Permethrin	Between Groups	366849.403	2	183424.702	197.058	.000
	Within Groups	14893.022	16	930.814		
	Total	381742.425	18			
Cypermethrin	Between Groups	4040.317	2	2020.158	8.648	.003
	Within Groups	3504.137	15	233.609		
	Total	7544.453	17			
Deltamethrin	Between Groups	20.102	2	10.051	1.120	.396
	Within Groups	44.872	5	8.974		
	Total	64.975	7			

All analyses done at 0.05 confidence interval

Appendix VI

One-way ANOVA comparing pesticides concentrations in muscle, gill and liver of ***Chrysichthys nigrodigitatus***

		Sum of Squares	df	Mean Square	F	Sig.
β-HCH	Between Groups	1501.262	2	750.631	1.832	.192
	Within Groups	6555.901	16	409.744		
	Total	8057.163	18			
Methoxychlor	Between Groups	5.000E7	2	2.500E7	10.833	.003
	Within Groups	2.308E7	10	2307866.624		
	Total	7.308E7	12			
Permethrin	Between Groups	3835606.917	2	1917803.459	5.989	.008
	Within Groups	7365418.834	23	320235.601		
	Total	1.120E7	25			
Cyfluthrin	Between Groups	213166.141	2	106583.071	9.319	.002
	Within Groups	182993.985	16	11437.124		
	Total	396160.126	18			
Fenvalerate	Between Groups	1097395.605	2	548697.802	4.566	.026
	Within Groups	2042762.395	17	120162.494		
	Total	3140158.000	19			

All analyses done at 0.05 confidence interval

Appendix VII

One-way ANOVA comparing pesticides concentrations in muscle, gill and liver of ***Bagrus bayad***

		Sum of Squares	df	Mean Square	F	Sig.
BHCH	Between Groups	2000.597	2	1000.298	5.795	.011
	Within Groups	3107.021	18	172.612		
	Total	5107.618	20			
Methoxychlor	Between Groups	304850.486	2	152425.243	7.678	.004
	Within Groups	337473.371	17	19851.375		
	Total	642323.857	19			
Permethrin	Between Groups	2.551E7	2	1.275E7	9.729	.001
	Within Groups	3.146E7	24	1310784.088		
	Total	5.696E7	26			
Cyfluthrin	Between Groups	294141.639	2	147070.820	19.582	.000
	Within Groups	105145.181	14	7510.370		
	Total	399286.820	16			
Chlorfenvinphos	Between Groups	140.188	2	70.094	2.554	.158
	Within Groups	164.668	6	27.445		
	Total	304.856	8			
Ethoprophos	Between Groups	1467.481	2	733.740	27.530	.002
	Within Groups	133.261	5	26.652		
	Total	1600.742	7			
Profenofos	Between Groups	680.329	2	340.165	2.200	.181
	Within Groups	1082.540	7	154.649		
	Total	1762.869	9			

All analyses done at 0.05 confidence interval