UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

IDENTIFICATION AND CONCENTRATION OF SELECTED PESTICIDE RESIDUES IN GHANAIAN COCOA BEANS

BY

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DECLARATION

This is to certify that this thesis is the result of research undertaken by Sefakor Adzo Fialor towards the award of MPhil Food Science Degree in the Department of Nutrition and Food Science, University of Ghana under the supervision of Professor Emmanuel Ohene Afoakwa and Professor Firibu Kwesi Saalia. All references made to other people's work have been duly acknowledged.

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DEDICATION

I dedicate this research to the Lord God Almighty for granting me the grace and favour to complete this project.

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LIST OF ABBREVIATIONS

AI	Active Ingredient
CSSVD	Cocoa Swollen Shoot Virus Disease
ECD	Electron Capture Detector
GC	Gas Chromatography
ICCO	International Cocoa Organization
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
mg/kg	Milligram per kilogram
MRL	Maximum Residue Levels/Limits
MS	Mass Spectrometer
ND	Not Detected
PCA	Principal Component Analysis
PC1	Principal Component One
PC2	Principal Component Two
QCCL-Cocobod	Quality Control Company Limited of Ghana Cocobod
QqQ-LC/MS	Triple Quadrupole Liquid Chromatograph-Mass Spectrometer
rpm	Revolutions per minute
WHO	World Health Organization

ABSTRACT

Pest and disease have been the major causes of low cocoa production worldwide and the use of chemicals in the form of pesticides is one of the main ways of mitigating their undesirable outcome. However, inappropriate application of pesticides does not only affect the quality of cocoa bean products and the wellbeing of consumers of such products but can also damage the natural flora and fauna in the environment. Hence the reason why evaluating the concentrations of pesticide residues is necessary in establishing the quality of a cocoa. The goal of this study was therefore to determine the concentrations and distribution of specific pesticide residues in cocoa beans from the six cocoa growing regions of Ghana.

Three classes of pesticides were tested for in cocoa beans obtained from seventeen (17) districts in the Brong Ahafo, Eastern, Central, Western North, Ashanti and Western South cocoa growing regions of Ghana. These were the neonicotinoids (Thiamethoxam, Clothianidin, Imidacloprid and Acetamiprid); the synthetic pyrethroids (Cypermethrin, Deltamethrin, Fenvalerate, Lambda Cyhalothrin and Permethrin) and the organophosphorous compound Chlorpyrifos. Pesticide residue analyses were done separately on the whole unshelled beans, the nibs and the shells using a GC/ECD for the synthetic pyrethroids and organophosphorous compound and a QqQ-LC/MS for the neonicotinoids.

The results obtained showed that the mean concentrations of the neonicotinoids in all the three matrices ranged from <0.001 to 0.018 mg/kg in the shells, <0.001 to 0.0025 mg/kg in the nibs and <0.001 to 0.005 mg/kg in the whole beans with Imidacloprid being the predominant one. Ashanti Region had the highest concentration of Imidacloprid in all the three matrices whilst Eastern Region recorded the least concentration of Imidacloprid in the shells (0.009 mg/kg) and whole unshelled beans (0.002 mg/kg). In relations to the synthetic pyrethroids tested for, the results obtained indicated that out of the three matrices,

it was only the shells that had recordable concentrations of pyrethroids being present and these were Cypermethrin (0.013 mg/kg) and Permethrin (0.012 mg/kg). Regional analysis also showed Permethrin to be present in bean shells from both Ashanti and Western South Region at concentrations of 0.01 mg/kg and 0.02 mg/kg respectively. Cypermethrin on the other hand was present in bean shells from four regions ranging in concentrations of 0.011 mg/kg to 0.020 mg/kg.

Chlorpyrifos which was the only organophosphorous compound tested for was found to be present in about 42% of the cocoa shell samples analysed at concentrations ranging from 0.0108 to 0.0396 mg/kg with an average concentration of 0.0184 mg/kg being recorded. Eastern Region had the highest concentration of Chlorpyrifos in the shells (0.026 mg/kg), while Western North Region recorded the highest concentration in the whole beans (0.027 mg/kg) and Central Region recorded the highest concentration in the nibs (0.015 mg/kg). On the whole, all the pesticide residue concentrations measured were below the established EU and Japan MRLs.

Principal Component Analysis (PCA) used to assess the associations amongst the various pesticides and between the pesticides and the regions revealed that Imidacloprid/Thiamethoxam; Thiamethoxam/Cypermethrin and Chlorpyrifos/Imidacloprid were in comparable proportions and prevalence in the cocoa nibs. For the shells however, Imidacloprid/Cypermethrin and Deltamethrin/Permethrin/Thiamethoxam were of similar proportions. PCA of the whole cocoa beans also indicated a close association between Cypermethrin/Thiamethoxam and Permethrin/Clothianidin. Out of all the regions, Central Region had the highest association with all the 10 pesticides in the shells and with Imidacloprid in the whole beans whilst Western South Region showed an association with Chlorpyrifos in the nibs.

CHAPTER ONE

INTRODUCTION

1.1 Background

At the end of the 2015/2016 cocoa crop year which was from 1st October 2015 to 30th September 2016, about 3,965,000 tonnes of cocoa had been produced worldwide and Africa alone had produced about 2,911,000 tonnes out of this gross world amount. During this 2015/2016 crop year, Ghana was one of the major cocoa producers, having a gross production of about seven hundred thousand metric tonnes (700,000 MT) which translated to about 20% of the world's total cocoa production (ICCO, 2017). Although these world production figures are massive, there is still the potential to produce much more.

Declining cocoa production in West Africa in recent years has been attributed to factors such as planting of low yielding varieties, old cocoa trees, reduced soil fertility, and most especially pest and disease infestation (Kongor *et al.*, 2016). Currently, plants are protected from pest and disease infestation through the use of clean planting materials, appropriate crop and farm hygiene, biological agents, proper nutrition and use of chemicals. However, in most cocoa producing countries, it is the use of synthetic chemicals to control pest and disease infestation that is most prevalent. This is not only done at the farm level but also at various stages along the supply chain especially in post-harvest management practices (Crozier, 2013; European Parliament & Council, 2005).

Pests and causative agents of diseases in cocoa range from fungi, viruses, algae, weeds, rodents, insects (both crawling and flying), birds, snails, mites, and to any other animal that has undesirable effects on cocoa (Adams *et.al.*, 2005; Afrane & Ntiamoah, 2007). Diseases such as Witches broom, black pod, cocoa swollen shoot virus disease (CSSVD), vascular streak die back and moniliasis pod rot can cause more than a 40% loss in cocoa yield (Afoakwa, 2014). In Ghana, the most commonly reported diseases and pests of cocoa are *Phytophthora megakarya* (black pod local name: *akate*), Swollen shoot virus (local name: *cocoa sasabro*), frosty pod rot and attack from mirids of the *Sahlbergella* and *Distantiella* spp (Afrane & Ntiamoah, 2007). During storage, cocoa beans are also subject to pests such as beetles (*Cryptolestes ferrugineus*), warehouse moths (*Ephestia elutella*), brown capsids (*Sahlbergella singularis*), black capsids (*Distantiella Theobroma*) and rodents (Ayenor *et al.*, 2004).

Pesticides which are used in the control of pest and disease infestations are chemical substances that either destroy, repel, mitigate or prevent the effect of any pest or disease. Pesticides can be classified depending on either the type of pest they control, the stage during production at which they are applied (such as; planting, harvesting or storage), their mode of action, the manner in which they are applied or their chemical structure. With classification based on the chemical structure, there are the organochlorines, organophosphates, carbamates, triazines and phenoxys, synthetic pyrethroids, neonicotinoids and many others and these exist in many forms including granules, pellets, aerosols, sprayable concentrates, solutions, soluble powders and fine powders similar to dust (Adams *et al.*, 2005; Asogwa & Dongo, 2009).

Studies carried out in Ghana indicate, that the majority of pesticides identified in relation to cocoa and cocoa producing regions are under five classes out of the WHO established 15 classes of pesticides and these are: the organochlorines, organophosphorous compounds, pyrethroids, neonicotinoids and carbamates (Crentsil *et al.*, 2011; Dankyi *et al.*, 2014; Frimpong *et al.*, 2012a; Frimpong *et al.*, 2013; Frimpong *et. al.*, 2012b; Okoffo *et al.*, 2016). This study focused on the identification and quantification of pesticides in three of these five classes: organophosphorous compounds, synthetic pyrethroids and neonicotinoids.

1.2 Rationale for the Study

Pesticide residue concentration is one of the major food safety and quality parameters of cocoa and with the rising food safety concerns, it is the desire of stakeholders in the food industry to produce food that is free of these harmful contaminants. Pesticide residue in cocoa is undesirable for many reasons. Aside health implications such as cancer, neurotoxicity, birth defects, improper functioning of glands among others, some pesticides especially insecticides are absorbed by the fat component of the cocoa beans which can result in an undesirable change of the flavour of the final products known as tainting (Afoakwa, 2014; Afrane & Ntiamoah, 2007; NPASP, 2012).

Currently, there is more research and publications worldwide on the pesticide residue situation of food commodities such as vegetables and fruits than there is on cocoa. Since 1990, there have been relatively fewer studies on pesticides in cocoa and amongst cocoa farmers than any other food commodity (Bateman, 2015; Zainudin *et al.*, 2015). In Ghana, cocoa produced in the country is considered to be of premium quality on the world market and one of the major foreign income earners of the country (Crentsil *et al.*, 2011; Dankyi *et al.*, 2015; Frimpong *et al.*, 2012a). Therefore, investigating the concentrations and distribution of pesticide residues in our cocoa beans as it pertains to all the cocoa growing regions of the country will contribute to developing a well established reputation for quality Ghanaian cocoa which is an advantage in this current market of heightened food safety awareness (ICCO, 2013). Moreover, the assessment of the levels of pesticide residues in the cocoa regions can also provide information for risk assessment in the future (Crentsil *et al.*, 2011).

1.3 Main Objective

The main objective of this study was to determine the concentration and distribution of some pesticide residues present in fermented dried cocoa beans from the cocoa growing regions of Ghana.

1.3.1 Specific objectives

i. To determine the levels and regional distributions of the neonicotinoids: Acetamiprid, Clothianidin, Imidacloprid and Thiamethoxam, present in the shells, nibs and whole beans of fermented cocoa beans.

ii. To determine the concentration and regional distributions of synthetic pyrethroids: Lambda Cyhalothrin, Deltamethrin, Fenvalerate, Cypermethrin and Permethrin present in the shells, nibs and whole beans of fermented cocoa beans.

iii. To determine the presence of the organophosphorous compound Chlorpyrifos present in the shells, nibs and whole beans of fermented cocoa beans and its regional distribution.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Species of Cocoa

The cocoa tree is mostly grown in West Africa, Central America, Southeast Asia and South America with countries such as Ghana, Ivory Coast, Ecuador, Malaysia, Nigeria, Indonesia, Cameroon and Brazil being the major producers (Afoakwa, 2014). In Ghana, there are two cocoa seasons; the main crop season which has bigger beans and is usually exported and the light crop season which has smaller beans and is usually sold domestically (Quarmine *et al.*, 2012). The main crop season is usually from October of one year to May of the next year whilst the light crop season runs from about July to September of the same year.

The cocoa tree (*Theobroma cacao*) is usually a short tree of between 4 to 8 meters but when it is grown in the shade of large forest trees it can grow up to a height of even 10 meters (Afoakwa, 2014). This crop is however very sensitive to moisture stress (lack of water) than other tropical plants (Kongor *et al.*, 2016). A typical cocoa fruit comes in the form of pods which are about 15-25 cm long and contain a minimum of thirty (30) seeds (Afoakwa, 2014). It is the dried fermented seeds of the cocoa pod that are referred to as the cocoa bean and are processed into intermediate products such as cocoa cake, cocoa butter, cocoa liquor and cocoa powder (Afoakwa *et al.*, 2011a). These intermediate products are then used in the production of subsequent cocoa products ranging from food commodities such as chocolates, beverages, biscuits, food coatings etc. to cosmetics such as body creams and lotions, bathing soaps among many others.

There are four major varieties of cocoa grown worldwide - Forastero, Trinitario, Criollo and Nacional varieties. These varieties differ in the way their pods look, the number of beans per pod per season and the flavour characteristics of the dried fermented beans (Afoakwa *et al.*, 2011b). Out of all the varieties, it is the Forastero variety that is usually grown in West Africa and it is more resistant to pest and diseases such as swollen shoot, yellow mosaic, witches broom, black pod, capsids and cocoa pod borer (*Conopomorpha cramerella*) than the other varieties. Pods from the Forastero tree when ripe are short, yellow and smooth. The beans are flat and usually deep or pale purple in colour due to the high concentration of anthocyanins present. The beans of this variety also have a very strong chocolate flavour and it is because of this desirable flavour attribute that it is usually used in chocolate production. In Ghana, three main hybrid species of the Forastero variety of cocoa are usually grown – Amelonado, Amazonica and 'Akokora bedi' with the last being the most dominant species (Afoakwa, 2014; Kongor *et al.*, 2016).

The Criollo variety on the hand produces beans that are considered to be the highest quality worldwide. However the tree and fruit of this variety is highly susceptible to insect and disease attack and as such it is only cultivated in some few countries in Asia and Central America. Fruit pods of the Criollo variety are long and yellow or red when ripe with white or faint purple seeds. The beans are usually less bitter when compared to the other varieties (Afoakwa, 2014; Kongor *et al.*, 2016).

The Trinitario variety is a hybrid of the Forastero and Criollo varieties and was initially developed in Trinidad. It produces higher quality beans and the fruit and tree are more resistant to pests and diseases than the other varieties. The Nacional variety on the other hand is one of the least cultivated and least known variety of cocoa. It is originally from Ecuador and has distinct flavour attributes (Afoakwa, 2014).

2.2 Cocoa Shells

Cocoa shells which are obtained either before or after cocoa beans are roasted are considered to be one of the under-utilized components of the cocoa fruit (Okiyama *et al.*, 2017). Currently, it is mainly used as mulch or as fuel for industrial boilers. The cocoa shell has a lipid profile that is closely identical to that of cocoa butter and contains a high amount of phenolic compounds and fibers. Because of its fibre content, it is currently being considered as a source of dietary fibre and bulking agent in a number of food formulations (Okiyama *et al.*, 2017). Also the phenolic compounds such as catechins, epicatechins and procyanidins that are present in the shells confer good antioxidant properties to the shells.

Beside its uses in agriculture, Fioresi *et al.* (2017) suggested its use as an adsorbent to entrap environmental pollutants including gases, heavy metals and even industrial dye. In recent years, major companies in the food industry such as Barry-Callebaut and Kraft Foods have also proposed uses of cocoa shells in food formulations. However there is minimal information on the wholesomeness of cocoa shells for food formulations in terms of its pesticide residue concentrations.

2.3 The Cocoa Matrix and its Complexity

Food crops that usually undergo pesticide residue analysis have been grouped into four major matrices based on the composition of the food commodities. These matrices are: high water content matrices, high oil content matrices, high acid content matrices and dry matrices. The cocoa bean is considered to be a high oil content matrix and this significantly affects the processes required to prepare the sample for analytical analysis and even the kind of analytical analysis that can be done (Villaverde *et al.*, 2016). When such high oil content food commodities are not properly 'cleaned', the presence of even the tiniest amount of fat can cause significant damage to the ion sources, the detectors and even the columns of the analytical instruments used (Zainudin *et al.*, 2015). Also it has been found in pesticide residue analysis that food matrices with high fat content can interfere significantly with the effective identification and quantification of the active ingredient of interest (Zainudin *et al.*, 2015).

2.4 Diseases and Pests of Cocoa

Pests are used to refer to a wide range of fungi, weeds, rodents, insects, birds, snails and any animals or crops that cause harm to another animal or crop. Pests and diseases are the major causes of low cocoa yield in West African countries where most cocoa farms are relatively small with insufficient pest and disease control being implemented (Wessel & Quist-Wessel, 2015). Frost pod rot and witches' broom diseases for example caused the Americas which was producing about 36% of the world's cocoa in 1980 to produce only 12% in 2000. Likewise the cocoa pod borer has over the years caused a significant drop in cocoa production from the Asian and Oceanic regions of the world. Diseases that affect the cocoa pod such as the black pod disease (*Phytophthora megakarya*) which is common to West Africa and the frosty pod disease (*Moniliophthora roreri*) which is common to Latin America cocoa have the capacity of causing more than 80% loss in yields in just one season (Bateman, 2015). The most common diseases and pests that affect cocoa are highlighted in the following subsections.

2.4.1 Cocoa Swollen Shoot Virus Disease

One of the major diseases that affects Ghanaian cocoa is the Cocoa Swollen Shoot Virus Disease (CSSVD). Over the years, the effect of this disease has been mitigated by planting resistant hybrid species; however there are still situations of outbreaks in newly created cocoa farms (Wessel & Quist-Wessel, 2015). CSSVD which is spread by the mealy bug causes infected pods to be smaller in size and more roundish in shape. It is common in other major cocoa producing countries such as Nigeria, Sri Lanka, Ivory Coast and Togo. This disease not only affects the pods but also damages the trees thus drastically reducing the volume of subsequent yields (Afoakwa, 2014).

Different strains of the cocoa swollen shoot virus have different effects on the cocoa tree and fruit. Generally, this virus can result in stunting, root and stem swelling, mottling of young leaves and chlorosis in the mature leaves and pods. So far the only effective way of totally controlling cocoa swollen shoot viral disease aside the use of pesticides is by cutting down infected trees and even seemly healthy trees that are next to the infected ones (Andres *et al.*, 2017).

2.4.2 Black Pod Disease

Another disease that commonly affects cocoa in Ghana is the black pod disease (Phytophthora pod rot). This disease is caused by the causative organisms *Phythophthora palmivora* and *Phythophthora megakarya* and is common in other cocoa producing countries such as Nigeria, Togo and Cameroon (Wessel & Quist-Wessel, 2015; Afoakwa, 2014). The disease affects every part of the cocoa tree and is most seen in the browning, blackening and rotting of the pods. The pods can also shrivel up and become mummified looking. If the fungi spreads beyond the external walls of the pod to the interior walls, it can significantly reduce the pulp content and quality around the beans because the fungi feeds on the free sugars of the pulp. This reduction in pulp content can cause the beans to ferment un-uniformly because the affected pods are similar in nature to that of unripe pods which don’t ferment properly (Afoakwa, 2014). Although, removing infected pods and reducing the thickness of the tree canopies over the cocoa plants reduces the spread of this disease, the most effect means of control is by the application of pesticides (Wessel & Quist-Wessel, 2015).

2.4.3 Mirid

Mirid attack is also a common pest infestation that affects Ghanaian cocoa yields. Caused by *Distantiella theobroma* and *Sahlbergella singularis*, this pest infestation can lead to the death of the affected tree (Wessel & Quist-Wessel, 2015). Mirids attack the stems, shoots and pods of the cocoa tree and are prevalent in trees that are less than 30 years old (Adu-Acheampong *et al.*, 2014). Mirids pierce these sections of the plant with their suckers and suck out the juice present. As they suck, they inject salivary secretions into the plant tissue and this leads to the breakdown of the cells there which is shown by necrosis and the appearance of lesions (Aikpokpodion *et. al.*, 2012).

2.4.4 Capsids

Capsids can reduce yield by more than 25% in a single crop year. Capsids have the potential to incapacitate seedlings thus preventing them from maturing to become full trees. The capsids can also cause a significant delay in the ability of the cocoa plant to bear fruit. These insects destroy the soft tissues and the young shoot of the cocoa plant by injecting poisonous saliva through their mouth parts when they puncture the surface of the plant during feeding (Afoakwa, 2014).

2.5 Pesticides

Pesticides are chemicals that are used to reduce the effects of pests. They usually have a trade name, a common name and a chemical name and can be made up of one or more active ingredients (Adams *et al.*, 2005). Trade names are the most prominent names on the label of the pesticide and most would usually be in all capital letters or only the first letter would be in capital letters. The common name on the other hand which is not usually boldly written and may not be clearly seen at the first glance refers exclusively to the active ingredients (AI) present in that pesticide formulation. The common name is sometimes written beside the word 'Guarantee'. The chemical name is also used to represent the active ingredient but does not usually appear on the label of the pesticide (Adams *et al.*, 2005).

Pesticides cause damage to pests by various routes including: direct contact (whereby the pesticides kill the pests once it touches it); secondary contact (the pest passes over a leaf or part of the plant that has been sprayed already) and ingestion (whereby the pests feed on a part of the plant that has been sprayed with pesticides. Some pesticides do not kill the intended pest but rather act as repellents which drive the pests away from the crop (Bateman, 2015).

The control of diseases and pest with the use of chemicals is not only done at the farm level but at various stages along the supply chain especially in post-harvest management (Crozier, 2013). Studies have shown that dried fermented cocoa beans can be contaminated with pesticides through practices such as pre-harvest pesticide treatment of the pods which contain the beans and post-harvest treatment of the dried beans. Post-harvest treatment of the beans with pesticides is usually done through the treatment of the storage facility where the beans are kept either before the beans are placed there or after the beans are placed there (Dong, 2012). When the crop is treated with pesticides before harvest, the pesticide can seep through the cocoa pod and infect the beans thus causing the final dried fermented beans to have pesticide residue. Aside the direct application of pesticides to the beans, indirect pesticide application such as pesticides from previous planting seasons present in the soil or drift from neighbouring farms or the atmosphere can also result in significant contamination of cocoa beans (Frimpong *et al.*, 2012c).

In Ghana, a wide range of pesticides have been approved for use in food crops including cocoa. Lambda Cyhalothrin which is a synthetic pyrethroid has been approved for the control of insects that attack cowpea, vegetables and soya bean. Imidacloprid (a neonicotinoid) is for the control of cocoa pests whilst Deltamethrin (a pyrethroid) has been approved as an insecticide for vegetables, public health purposes and for dry cocoa beans in storage. Cypermethrin which is also a pyrethroid is for general insect control and insect infestation of vegetables and cotton and also for the control of aphids, worms and borers. Fenvalerate is for the control of insects in stored produce such as cereals and grains excluding dried cocoa beans. Chlorpyrifos (an organophosphorous compound) has been approved mainly for the treatment of insects in cereals, vegetables, ornamental plants and for public health purposes whereas Thiamethoxam (a neonicotinoid) is for the control of sucking and chewing insects in vegetables (SAL Consult, 2014).

2.5.1 Classes of Pesticides

There are different classifications of pesticides and one of them is by the World Health Organization (WHO). The organization's classification is based on the chemical composition of the active ingredients in the pesticide formulation and based on these, pesticides are classified as bipyridylium derivatives, carbamates, arsenic compounds, coumarin derivatives, copper compounds, nitrophenol derivatives, mercury compounds, organotin compounds, organochlorines, organophosphorous compounds, triazine derivatives, pyrazoles, phenoxyacetic acid derivatives, synthetic pyrethroids, thiocarbamates and neonicotinoids (IPCS, 2009).

This study focused on three out of these classes which were the synthetic pyrethroids (Deltamethrin, Cypermethrin, Permethrin, Lambda Cyhalothrin and Fenvalerate), the neonicotinoids (Clothianidin, Acetamiprid, Thiamethoxam and Imidacloprid) and the organophosphorous compound Chlorpyrifos.

2.5.1.1 Pyrethroids

Pyrethroids are synthetic analogs of pyrethrins. These pyrethrins are present in pyrethrum extracts of the chrysanthemum flower which is usually found in tropical climates. It is because pyrethrins are easily degraded in the presence of sunlight, water and oxygen and are very expensive to use in the control of insects that their synthetic counterparts - the pyrethroids were developed (BASF, 2013; Frimpong *et al.,* 2012b). Pyrethroids unlike their natural counterparts are environmentally stable and very effective in the control of beetles, aphids, mites, mealy bugs, white flies and caterpillar infestations in food crops. When used in cocoa production, pyrethroids are mostly used against insects such as mirids and the cocoa pod borer (Bateman, 2015). Although pyrethroids are not usually soluble in water they have a strong affinity for soil particles and easily adhere to them (BASF, 2013). These synthetic pyrethroids are also more toxic to bees and aquatic organisms such as fish than they are to humans. Also, because of the high toxicity of pyrethroids, it is usually recommended that only about 10 to 40g of pyrethroids should be used per a whole hectare of land (Frimpong *et al.,* 2012b).

Over the years, synthetic pyrethroids have been linked to health complications such as the disruption of the endocrine system of mammals which can negatively affect the development of the sexual reproductive organs and appropriate immune system functioning (Afful *et al.,* 2013). They have also been found to cause nausea, irritation, tingling, burning or numbness in sensitive parts of the body such as the face and skin, dizziness, itching, vomiting, muscle spasms, convulsions and diarrhoea. Extreme exposure can even result in paralysis or death via respiratory complications.

2.5.1.1.1 Mode of action

Pyrethroids are neurotoxins that work by altering the functioning of the sodium channels of the nerve cells of organisms. These channels are critical in the transmission of electrical signals (nerve impulses) along the cells. Synthetic pyrethroids causes the sodium channels to stay open longer than they should and this disrupts the efficient movement of nerve impulse thus resulting in repetitive firing of electrical signals along the nerve cells which interferes with the functioning of the nervous system of the insects (BASF, 2013).

2.5.1.1.2 Physical form and chemical structure

2.5.1.1.2.1 Deltamethrin

This active ingredient in its pure form is a colourless or white crystal which boils at 300ºC. Deltamethrin is more toxic to insects than mammals due to the vast difference in body size and body temperature between the two species (Johnson *et. al.*, 2010). In soil, its half-life has been found to be between 5.7 to about 209 days whilst in plants it has a half-life of about 5.9 days to 17 days. It is considered to be the most toxic of all existing pyrethroids so far (Gilbert & Becker, 2014). The World Health Organization classifies Deltamethrin as a moderately hazardous (Class II) technical grade active ingredient (IPCS, 2009). Figure 2.1 shows the chemical structure of Deltamethrin.

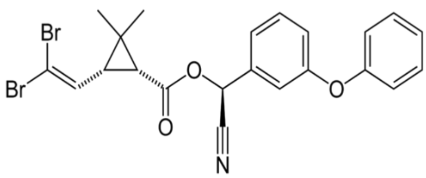

Figure 2.1: Chemical Structure of Deltamethrin ($C_{22}H_{19}Br_2NO_3$)

2.5.1.1.2.2 Fenvalerate

Fenvalerate as a synthetic pyrethroid occurs as a yellow-brown viscous liquid. Although it has an insecticidal action similar to that of Permethrin (another synthetic pyrethroid) it is more environmentally degradable than Permethrin. It has a half-life of 35 to

60 days in soil (Gilbert, 2014). Fenvalerate is more stable in acidic solutions than in alkaline solutions and pesticide formulations containing Fenvalerate usually have it as a mixture of four isomers. Figure 2.2 is the chemical structure of Fenvalerate.

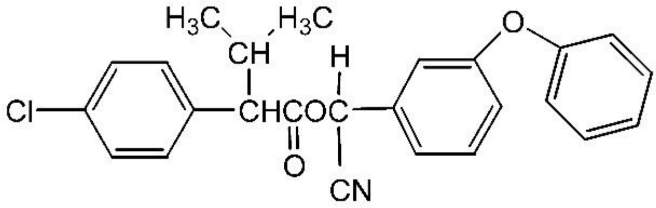

Figure 2.2: Chemical structure of Fenvalerate (C_{25} H_{22} $ClNO_3$)

2.5.1.1.2.3 Permethrin

Permethrin is a viscous liquid that can be either yellow, light orange or brown in colour (PubChem, 2017a). It has a broad spectrum of applications ranging from the treatment of insect infestation in food crops, livestock and even clothing. It is also used in insecticide formulations that are to be topically applied in humans such as mosquito repellants (Toynton *et. al.*, 2009). The chemical structure of Permethrin is shown in Figure 2.3.

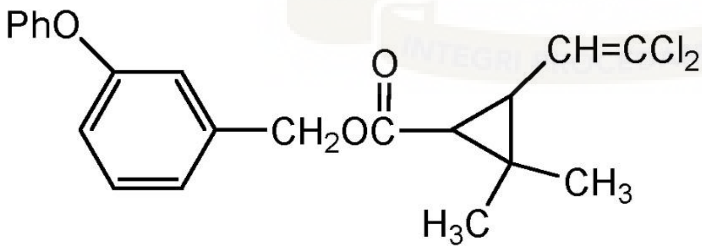

Figure 2.3: Chemical Structure of Permethrin ($C_{21}H_{20}Cl_2O_3$)

2.5.1.1.2.4 Lambda Cyhalothrin

This is one of the most dominant isomers of cyhalothrin and has a molecular weight of 449.9g/mol. It has a strong affinity for soil and exists as either beige or colourless in its pure form and has been shown to turn yellow when in solution. It has a half-life of about 4 to 12 weeks (Thoreby & Mergel, 2010). The chemical structure of lambda cyhalothrin is shown in Figure 2.4.

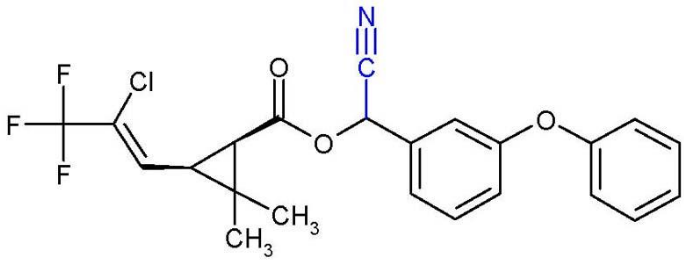

Figure 2.4: Chemical Structure of Lambda Cyhalothrin ($C_{23}H_{19}ClF_3NO_3$)

2.5.1.1.2.5 Cypermethrin

Cypermethrin in its pure form exists as a yellow viscous semi-solid substance with a molecular weight of 416.298g/mol (PubChem, 2017b). The mode of action of Cypermethrin is by obstructing the appropriate functioning of the peripheral and central nervous system of insects. Figure 2.5 shows the chemical structure of Cypermethrin.

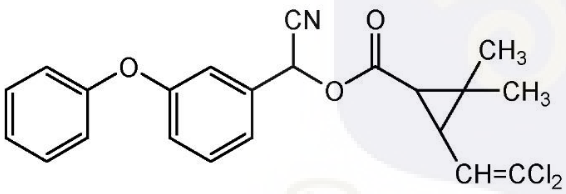

Figure 2.5: Chemical Structure of Cypermethrin ($C_{22}H_{19}Cl_2NO_3$)

2.5.1.2 Organophosphorous Compounds

This group of active ingredients have a high acute toxicity but they do not persist in the environment as much as the organochlorines (Akoto *et. al.*, 2013). One of the main advantages of using organophosphorous compounds for pest control is that they have acceptable residual characteristics and a broad spectrum of the kinds of pests that they can control (BASF, 2013).

2.5.1.2.1 Mode of action

Organophosphates are acetlycholinesterase inhibitors and as such they obstruct the action of acetlycholineterase. This enzyme's primary function is to break down acetylcholine after it has transmitted the nerve message across the gap between two nerve cells known as the synapse. When organophosphorous compounds inhibit the functioning of acetlycholinesterase, acetylcholine is not broken down and as such the postsynaptic nerve cell (the receiving nerve cell) is continuously stimulated or activated to receive a message from a presynaptic nerve cell (the giving nerve). This continuous activation of the nerve cells results in overstimulation of the whole nervous system which results in the death of the pest (BASF, 2013).

Organophosphates are considered to be one of the most dangerous insecticides to humans. Exposure to organophosphates can result in acute poisoning within minutes with symptoms such as dizziness, nausea, excessive sweating or salivation and in extreme cases respiratory failure which leads to death. Although organophosphorous compounds break down in the environment, some of them are highly hydrophilic and can easily leak into ground water (BASF, 2013).

2.5.1.2.2 Physical Form and Chemical Structure

2.5.1.2.2.1 Chlorpyrifos

In its pure form, Chlorpyrifos is a white crystalline solid that is not soluble in water (PubChem, 2017c). The WHO classifies Chlorpyrifos as a moderately hazardous (Class II) active ingredient (IPCS, 2009) and it has been known to cause nausea, dizziness, confusion, respiratory complications and in severe cases death (EPA, 2017). Long term exposure to Chlorpyrifos can result in chronic diseases such as lung cancer (PubChem, 2017c). The chemical structure of Chlorpyrifos is shown in Figure 2.6.

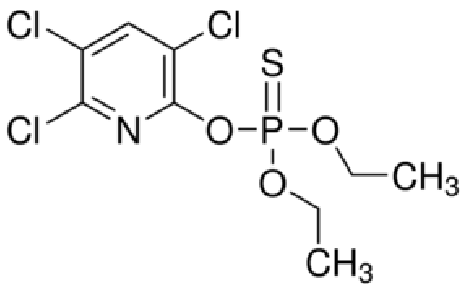

Figure 2.6: Chemical Structure of Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$)

2.5.1.3 Neonicotinoids

This class of pesticides are currently one of the most common active ingredients in insecticide formulations in Ghana. Pesticide formulations containing neonicotinoids are either given freely or applied freely to cocoa farms in Ghana by Cocobod. It is usually applied to the leaves and branches of the tree with either a back-pack sprayer or a motorized mist blower (Dankyi *et al.,* 2014).

In Ghana, Imidacloprid, Thiamethoxam and Thiacloprid are the neonicotinoids that are approved for the pre-harvest treatment of cocoa fruit and trees and they are usually applied during the months of August, September, October and December when mirid infestation is usually high (Dankyi *et al.,* 2015).

Neonicotinoids are the synthetic analogs of a compound found in tobacco called nicotine but are more stable in sunlight than nicotine. They are systemic in action which means they translocate very fast throughout the plant tissue be it the leaves, shoots, fruits or roots when applied. Neonicotinoids are highly hydrophilic and thus can easily leach into ground water. However they are very effective in the control of sucking and chewing insects such as aphids, whiteflies, beetles and weevils (BASF, 2013).

A couple of years ago in 2013, a moratorium was placed on the registration of Clothianidin, Imidacloprid and Thiamethoxam containing pesticide formulations for use in crop production in the European Union. Although this restriction is yet to be lifted, these neonicotinoids are still used in the control of insects in cocoa (Bateman, 2015).

2.5.1.3.1 Mode of action

Neonicotinoids mimic the action of acetylcholine (a neurotransmitter) and thus are able to bind to the acetylcholine receptors of cells. When neonicotinoids bind to the acetylcholine receptors, they desensitise the receptors such that the receptors cannot function properly. Neonicotinoids are more toxic to insects than to humans because they have a higher affinity for insect acetylcholine receptors than for human acetylcholine receptors (BASF, 2013).

2.5.1.3.2 Physical form and chemical structure

2.5.1.3.2.1 Imidacloprid

This is a neonicotinoid used mostly for the control of termites, sucking insects and other insects that are resident in soil. In its pure form it exists either as a colourless crystal or a beige coloured powder and is classified as a moderately hazardous (Class II) active ingredient by the WHO (IPCS, 2009; Gervais *et. al.*, 2010) . Figure 2.7 below is the chemical representation of Imidacloprid.

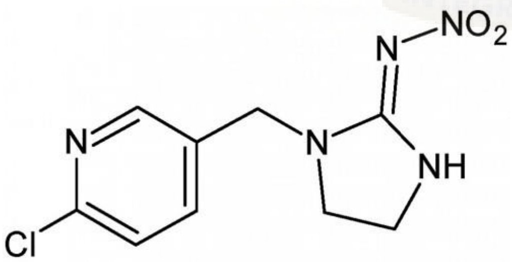

Figure 2.7: Chemical Structure of Imidacloprid ($C_9H_{10}ClN_5O_2$)

2.5.1.3.2.2 Acetamiprid

Acetamiprid in its pure form exists either as a white crystal or powder and is mainly used in the post-harvest treatment of cocoa. It has a molecular weight of 222.68 g/mol (PubChem, 2017d). The chemical structure of Acetamiprid is shown in Figure 2.8.

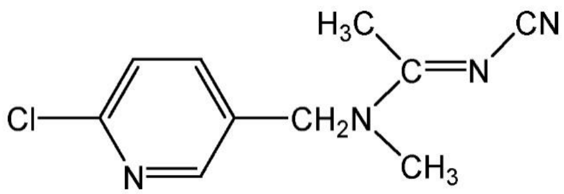

Figure 2.8: Chemical Structure of Acetamiprid ($C_{10}H_{11}ClN_4$)

2.5.1.3.2.3 Thiamethoxam

It has a molecular weight of 291.71 g/mol and is soluble in water. It can exist either as light brown granules or a crystalline powder (PubChem, 2017e).

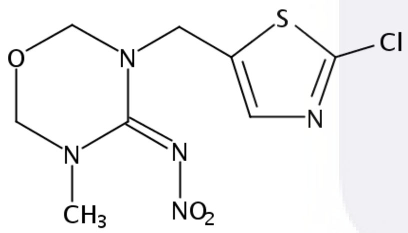

Figure 2.9: Chemical Structure of Thiamethoxam ($C_8H_{10}ClN_5O_3S$)

2.5.1.3.2.4 Clothianidin

Clothianidin is a metabolite of Thiamethoxam and is formed in plants and insects. It has a molecular weight of 249.67 g/mol and is usually a colourless powder in appearance (PubChem, 2017f)

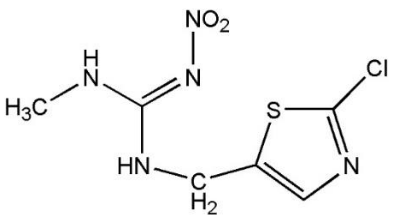

Figure 2.10: Chemical Structure of Clothianidin ($C_6H_8ClN_5O_2S$)

2.5.2 Maximum Residue Limits (MRLs)

The amount of pesticide residues that may be present in food commodities is influenced by factors such as the amount of pesticide applied before harvesting, the time between pesticide application and the harvesting of the food commodity, the mode of pesticide application and the conditions to which the food commodity is subjected to after harvesting and during storage (Agyekum *et al.*, 2015).

Maximum Residue Limits are defined as "the maximum concentration of pesticide residue likely to occur in or on food and feed after the use of pesticides according to good agricultural practices". It is expressed as milligrams of residue per kilogram of food/animal feed (mg/kg) and for pesticides that no MRL has been established, 0.01 mg/kg is the default MRL value applied (Afoakwa, 2014). In other words, the MRL of a pesticide is the legal maximum amount of the pesticide that can be present in a food commodity after the commodity has been treated with the pesticide which is based on good agricultural practices and protects consumers from health hazards (Akoto *et al.*, 2013).

MRLs are usually set for raw, unwashed and unpeeled food commodities and for those set by the European Union they are usually in the range of 0.01 to 10 mg/kg (Dong, 2012; Villaverde *et al.*, 2016). The regulation of pesticide use through the establishment of MRLs is necessary because it reduces exposure of consumers to harmful or unnecessary intake of pesticides; it also controls over use of products intended for plant protection and it also permits the free circulation of food products across international borders once the residue content on or in them conforms to established MRLs (Lee, 2003).

Table 2.1: EU and Japan MRLs of the ten pesticides analysed in this research

Active Ingredient	EU MRL (mg/kg)	Japan MRL (mg/kg)
Deltamethrin	0.05	0.05
*Fenvalerate	None	None
*Permethrin	None	None
Lambda Cyhalothrin	0.05	0.01
Cypermethrin	0.1	None
Chlorpyrifos	0.1	0.05
Imidacloprid	0.05	0.05[a]
Acetamiprid	0.1	0.01
Thiamethoxam	0.05	0.02[a]
Clothianidin	0.05	0.02[a]

*These pesticides are classified as not to be used in cocoa at all and as such no MRLs have been set for them.

[a] In Japan this MRL is based on the nibs (de-shelled beans) only

Source: Bateman, (2015)

2.6 Pesticides and Cocoa Farming

Studies on pesticide use amongst cocoa farmers in four countries: Ghana, Ivory Coast, Nigeria and Cameroon revealed that although there are various means of pest control, about 76-97% of farmers solely use chemicals in the management of pests and diseases. It was also identified that there are over a hundred chemical products and over thirty different active ingredients used in pest and disease management (Crozier, 2013). Studies have shown that farmers may at times use pesticides for purposes and in ways which are different from the purposes for which they were manufactured and this can have adverse effects on both the environments and humans (Asogwa & Dongo, 2009).

Due to the adverse effects that extreme usage of pesticides can have on the environment and on human and animal health, a number of pesticides have been banned for use in cocoa production. In Ghana, these pesticides include DDT, endosulfan, lindane, aldrin, dieldrin and endrin (NPASP, 2012). These pesticides however may still be in use because modern pesticides that have been introduced as replacements may be expensive and thus because of the increased cost of treatment involved in using these pesticides, the farmers would prefer to use previously banned pesticides or rely solely on the mass spraying which is done by Cocobod (ICCO, 2013).

2.7 Effects of Pesticide use on the Environment and Humans

Problems associated with inappropriate pesticide use can be grouped into three major categories: Food safety, technical issues and the impact on the environment in terms of sustainability. The main concern in terms of food safety is the negative effect the pesticides have not only on the farmer but on the consumer as well. In recent years, it has been shown that people are more likely to be exposed to pesticide residues through what they eat than through other routes of exposure such as drinking water and air (Bempah *et. al.*, 2015) and this has contributed to the heightened demand among the populace for food commodities that are safe from pesticide residues.

Beside food safety, one of the next major problems associated with uncontrolled and unapproved pesticide usage are the technical issues that arise. Technical issues refers to the pests developing resistance to the pesticides thereby causing the farmers to increase the amount of pesticide applied in order to achieve the desired results. This ultimately leads to relatively high residue concentrations in the final product which is undesirable.

Lastly the environmental effect of pesticide use is undesirable mainly because of how it affects the sustainability of the environment. Inappropriate pesticide application can pollute naturally occurring water bodies and even ground water which would be detrimental to the health of beneficial organisms such as bees, fish and birds (Bateman, 2015). Pesticide use also contaminates the soil and results in the death of beneficial natural flora and fauna.

Excessive and uncontrolled use of pesticides not only affects the environment and crops but it also affects humans. Short term exposure to pesticides can cause acute poisoning which will present in the form of severe headaches, respiratory complications, nausea, vomiting, irritation of the skin and eye. Long term exposure can also result in chronic health problems like cancers, infertility and neurological disorders (SAL Consult, 2014).

2.8 Method of Analysis of Pesticide Residue in Cocoa

Gas or liquid chromatographs used in tandem with detectors such as mass spectrometers or electron capture have become the gold standard in the identification and quantification of pesticide residues in food commodities such as cocoa beans. This is mainly because these method of analyses not only offers simultaneous identification and quantification of a large number of pesticides but also allows analysis to be done at a very fast rate with excellent separation of the analytes involved (Bempah *et al.*, 2015; Villaverde *et al.*, 2016). Due to the high cost involved in pesticide residue analysis and also because of the time, reagents and equipment that are need, multi-residue methods that are able to analyse more than one active ingredient (pesticide) in a single analytical run are most desirable in this present day and age (Grimalt & Dehouck, 2016).

Over the years, it has been identified that for the pesticide residue concentration in a food sample to be effectively identified and quantified, the food commodity should undergo six major processing stages. All protocols that are developed for pesticide residue analysis of food commodities are centered around these six processing steps (Tan & Chai, 2011).

These stages are independent of the type of chromatographic instrument that would be used in the analysis. The six major stages are as follows:

1. The food sample is firstly homogenised to obtain a uniform matrix.
2. The pesticide residues are then extracted using the appropriate solvents.
3. This is followed by a clean-up step which is done to remove components in the food matrix such as fat cells and flavour compounds that may interfere with the chromatographic column of the instrument to be used.
4. The clean-up step is followed by elution or fractionation of the extracted analytes.
5. The eluent is then concentrated and re-constituted in a solvent which is compatible with the specific chromatography instrument to be used.
6. The above solution is then introduced into the GC or LC and run (Tan & Chai, 2011).

Due to the complex nature of cocoa beans, one of the critical steps in processing the beans prior to analysis on a gas or liquid chromatography column is the clean-up stage. The complexity and number of clean-up stages required is largely dependent on the amount of materials in the beans that are co-extracted with the pesticide residues. If the co-extracted materials such as fat, flavour compounds, phenolic compounds, tocopherol etc. are less, then the clean-up procedure would be less complicated (Chung & Chen, 2011). Studies have shown that florisil, alumina and aminopropyl (NH_2) columns are very efficient when handling high fat matrices such as cocoa beans because they remove quite a number of interfering substances and also ensure recoveries higher than 70% (Chung & Chen, 2011). In this study, aminopropyl NH_2 columns with a graphitized carbon black section were used for samples that were to be run on either the gas or liquid chromatography instruments.

2.8.1 Gas Chromatograph with Electron Capture Detector

For decades now, gas chromatography has been one of the most suitable methods for detecting pesticide residue level in cocoa beans. And even in this day and age, it is an essential tool because pesticides such as organophosphorous compounds which have poor ionisation and as such cannot be effectively identified when using the LC can be efficiently analysed with the GC (Villaverde *et al.,* 2016).

Gas chromatography is mostly used in the separation of compounds whose individual components are volatile and stable in the presence of heat (Chauhan *et al.*, 2014). The commonest detectors that are usually used in tandem with GC include mass spectrometry (MS), electron capture detector (ECD) and flame ionization detectors (FID) (Fisher & Scott, 1997). The electron capture detector is one of the most sensitive detectors and as such is suitable in the analysis of pesticide residue content of food commodities such as cocoa.

2.8.2 Triple Quadrupole Liquid Chromatograph - Mass Spectrometer (QqQ-LC/MS)

Compared to gas chromatography, liquid chromatography is a more recent analytical technique that is used in the analysis of the pesticide residue content of cocoa beans. This chromatographic equipment is usually preferred in analysing compounds that are polar and thermally sensitive (Lee, 2003). LC coupled to MS is gradually becoming the instrument of choice in the routine analysis of polar pesticides because it can detect these pesticides even at trace levels and usually requires a simpler clean-up procedure as compared to gas chromatography (Lee, 2003).

In recent years, a new form of the LC/MS was developed which is the Triple Quadrupole Liquid Chromatograph-Mass Spectrometer (QqQ-LC/MS) also known as LC-

MS/MS. The use of the QqQ-LC/MS is preferred mainly because of its high sensitivity, precision and specificity. Also, due to the multiple stages of separation that the chemical components of the analyte undergo, it ensures a significant reduction in the amount of interferences that are present on the final chromatogram that is seen. The heightened sensitivity of this equipment also makes it very beneficial in the analysis of residue concentrations at trace levels because it can measure concentrations in the magnitude of parts per billion (ppb) or parts per trillion (ppt). However, to prolong the shelf life of the instrument, it is recommended that the final residue extract that is to be analysed be diluted. This method of diluting the final extract reduces the matrix load in the chromatographic instrument and thus contributes to promoting the shelf life of the equipment (Villaverde *et al.*, 2016).

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

The LOD is referred to as the minimum concentration of the pesticide of interest that can be detected by the machine. It is different from the LOQ in the sense that the LOQ is the minimum amount of the pesticide of interest that can be quantified with acceptable precision. The LOQ is set by the experimenter after a series of validations or experiments and is usually about two or three times the magnitude of the LOD. The LOQ is dependent on the model and age of the equipment in question and is usually considered to be a more accurate representation of the pesticide residue situation of a food commodity than the LOD (Afoakwa, 2014; Reynolds, 2003). Usually in routine pesticide residue analysis, any value obtained which is lower than the LOD is recorded as not detected (ND) whilst any value which is higher than the LOD but lower than the LOQ is recorded as (<LOQ).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection

There are six main cocoa growing regions in Ghana and about seventy-three (73) cocoa growing districts. In this study, cocoa beans were obtained from a total of seventeen (17) cocoa districts representing the six cocoa regions. Random sampling without replacement was used in the selection of the districts and about three districts were considered for each region except for the Western North Region where only two (2) districts were considered.

For the Central Region, the districts used were: Agona Swedru, Breman Asikuma and Twifo Praso. For the Ashanti Region the districts were: Dadieso, Obuasi and Juaso. For the Eastern Region, the districts were Akim Oda, Suhum and Koforidua. The districts that were used from the Brong Ahafo Region were Goaso, Kasapem and Sunyani. For the Western South Region the districts considered were Enchi, Dunkwa and Bogoso whilst for the Western North Region the districts considered were Bonsu Nkwanta and Sewfi Wiaso.

About one kilogram of beans from each of the aforementioned districts that were to be used for the analyses were taken during the 2016/2017 cocoa growing season. The beans were obtained from the Quality Control Company Limited of the Ghana Cocoa Board (QCCL-Cocobod) and the warehouses of Cocobod located in the Tema Municipality of the Greater Accra Region of Ghana. Since cocoa beans from the various districts are brought to the warehouses in truckloads with a truckload having between four hundred (400) to six hundred (600) bags of cocoa, sampling was done by using the sampling horn to collect beans from each bag in a truckload. The beans collected were then bulked together and thoroughly mixed. The bulked sample was then divided into four sections and one diagonal part made up of two sections was discarded and the remaining two diagonal sections were bulked again. This process was repeated until about 1kg of beans was obtained. The beans were then placed in transparent aseptic sample bags and transported to the research laboratory of QCCL-Cocobod where the analyses were done.

3.2 Sample Preparation

Multi-residue analysis method for agrochemicals by GC/ECD/MS and HPLC IPDAD as specified by the Agricultural Products Department of Food Safety, Ministry of Health, Labour and Welfare, Japan was used with modifications (Syoku-An, 2006) in the analysis of the pesticide residue content of the cocoa nibs, shells and whole beans.

Firstly, foreign objects in the form of sticks and stones in each 1kg of sample were removed manually by hand picking and then divided into two parts of 500g each. One part was stored at -40ºC for twenty-four (24) hours and then de-shelled whilst the second part was also packaged whole and stored at -40ºC for about forty-eight (48) hours before being milled.

3.2.1 De-Shelling

To enable efficient de-shelling of the raw cocoa beans, the whole beans when frozen for a minimum of 24 hours at -40ºC were manually crushed with a hammer. The shells were then carefully separated from the nibs and each was packaged separately. Thus, obtaining only shells and only nibs for each sample. The process of the de-shelling and the final outcome that was obtained are shown in Figures 3.1 to 3.3.

Figure 3.1: Manually crushed beans before separation

Figure 3.2: Nibs and Shells after separation

Figure 3.3: The separated nibs and shells of one district

3.2.2 Milling

After de-shelling, the nibs and shells were again frozen at -40ºC for about 24 hours before they were milled into a fine powder using a high speed blender (Model: Warring Xtreme Commercial Blender, U.S.A.) and then collected into airtight sample bags and labelled accordingly. The whole unshelled beans were also stored at -40ºC for a minimum of 48 hours before being milled into a fine powder. The freezing of the samples prior to being milled was to ensure that the powder to be used for analysis did not clump together.

To prevent cross contamination during the milling process, the blender was thoroughly cleaned between samples. Each sample was also given a unique code indicative of the region and type of cocoa matrix (nib, shell or whole bean) it was.

Figure 3.4: Samples of milled cocoa beans

3.3 Extraction

About 10 g of the milled shells, nibs or whole beans were then weighed into 250 ml Naglene jars and 20 ml of distilled water was added and left for 15 minutes. At the end of the set time, 100ml of Acetonitrile was added and the resulting mixture was then homogenised at 1500 rpm for 1 minute using a homogenizer (Polytron PT 3100 D Kinematica AG homogenizer, Luzern-Switzerland). Afterwards it was centrifuged at 2500 rpm for 5 minutes using a Centurion Scientific centrifuge (Wagtech International, Thatcham-United Kingdom). The supernatant was then filtered and collected in a 500 ml round bottom flask whilst to the residue was added 50 ml of acetonitrile and this was then homogenized, centrifuged and filtered again. The second supernatant obtained was then added to the previously obtained supernatant and this was concentrated to about 15 ml below 40ºC using a rotary evaporator (BUCHI Labortechnik, Flawil-Switzerland). The setup for the aforementioned filtration procedures is shown in Figure 3.5.

Figure 3.5: Filtration set up for pesticide residues extraction

The concentrate was then loaded onto a Chem Elut Diatomaceous earth column and left for 5 minutes after which 80 ml of an ethyl acetate: n-Hexane (1:4) solution was added and the eluent collected into a 250 ml round bottom flask. The eluent was then concentrated to dryness below 40ºC whilst 120ml of dichloromethane was added to the diatomaceous earth column and a second eluent collected. The first eluent was used for the identification of pesticides belonging to the synthetic pyrethroid and organophosphorous compound class whilst the second eluent was used for the identification of pesticides belonging to the neonicotinoid class.

3.4 Clean-Up

Two different stages of clean-up were carried out dependent on the pesticides that were to be analysed and the chromatograph that was to be employed. The organophosphorous compound (Chlorpyrifos) and the synthetic pyrethroids (Cypermethrin, Deltamethrin, Fenvalerate, Lambda Cyhalothrin and Permethrin) were analysed using a Gas Chromatograph with Electron Capture Detector (Shimadzu 2010 GC/ECD, Canby-U.S.A) whilst the neonicotinoids (Acetamiprid, Clothianidin, Imidacloprid and Thiamethoxam) were analysed using a Triple Quadrupole Liquid Chromatograph-Mass Spectrometer (Agilent 6460 Triple Quad LC/MS, Yishun-Singapore).

3.4.1 Sample Clean-Up for GC with ECD Run

The concentrated form of the first eluent obtained from the diatomaceous earth columns was dissolved in 25 ml of acetonitrile and sonnicated in an ultrasonic water bath (Bandelin Electronic, Germany) for about 1 minute. A 5 ml aliquot of this solution was then loaded on to a pre-conditioned BondElut GCB/NH_2 (500mg/500mg) column and eluted with 20 ml of acetonitrile. The eluent was collected in a 100 ml round bottom flask and concentrated below 40ºC using a rotary evaporator.

The concentrate was then dissolved with 2 ml of diethyl ether/n-hexane (2:8) solution and sonnicated for about 30 seconds. This was then loaded on to a pre-conditioned HyperSep

SI column and eluted with 10 ml of the diethyl ether/n-hexane (2:8) solution. The eluent obtained from this stage was evaporated to dryness with the rotary evaporator and the concentrate obtained was dissolved in 1 ml n-hexane/acetone (1:1) solution and placed in 2 ml vials with the aid of Pasteur pipettes for analysis on the GC/ECD. A schematic representation of the extraction and clean-up procedure for the samples to be analysed on the GC/ECD is shown in Appendix 1.

On the GC/ECD, about 2 µL of the analyte was injected into a fused silica capillary column of internal diameter of 0.25 mm. The mobile phase used was nitrogen gas at a flow rate of 1ml/min and each sample run took about thirty-four minutes to complete.

3.4.2 Sample Clean-Up for QqQ-LC/MS Run

The concentrate of the second eluent obtained from the diatomaceous earth columns was dissolved in 25 ml of acetonitrile and sonnicated for about a minute. A 5 ml aliquot was then taken and loaded onto a pre-conditioned BondElut GCB/NH_2 (500mg/500mg) column and eluted with 30 ml of acetonitrile. The eluent was then evaporated to dryness using the rotary evaporator and the concentrate which was obtained was dissolved in 10 ml acetonitrile/water solution (2:8). About 2 ml of this resulting solution was filtered into a 2 ml glass vial prior to analysis on the QqQ-LC/MS. A schematic representation of the extraction and clean-up procedures prior to analyses on the QqQ-LC/MS is shown in Appendix 1.

On the QqQ-LC/MS, about 2 µL of the analyte was injected onto a column of pore size 2.7 µm. The mobile phase used on the QqQ-LC/MS was an acetonitrile/water (2:8) solution at a flow rate of 0.8ml/min and each sample run took about fifteen (15) minutes to complete.

3.5 Instrumental Analysis on the GC/ECD and QqQ-LC/MS

For both chromatographs, the external standard method of quantification using peak area was employed in determining the concentration of the pesticide residues of interest. This method involved running a standard mixture of known concentration (calibration standards) of the pesticides of interest on both the GC/ECD and the QqQ-LC/MS. The area of the standard was then compared to that of the corresponding peak of the sample. The peak areas whose retention times coincided (that is peak areas of the standard was at the same location as that of the sample) was then extrapolated onto the corresponding calibration curves to obtain the concentration of the pesticide in the sample (Agyekum *et al.,* 2015; Frimpong *et al.,* 2012b). The LOD and retention times of the various pesticides (Table 3.1 and 3.2) being analysed was determined using prepared fortified samples and calibration standards. Chromatograms also showing the respective peaks of the various pesticides are shown in Appendix 2 and 3.

Table 3.1: Retention times and LOD of organophosphorous compound and synthetic pyrethroids run on the GC/ECD

Pesticide	LOD (mg/kg)	Retention Time (minutes)
Chlorpyrifos	0.007	10.09
Lambda Cyhalothrin	0.007	19.71
Permethrin	0.007	22.39
Cypermethrin	0.007	25.45
Fenvalerate	0.003	28.85
Deltamethrin	0.007	31.77

Table 3.2: Retention times and LOD of neonicotinoids run on the QqQ-LC/MS

Pesticide	LOD (mg/kg)	Retention Time (minutes)
Thiamethoxam	0.001	9.07
Clothianidin	0.001	9.70
Imidacloprid	0.001	9.90
Acetamiprid	0.001	10.29

3.6 Preparation of Fortification and Calibration Standards

The fortification (spiking) and calibration standards that were used for analyses were prepared using pure standards of greater than 90% purity of the active ingredients (Appendix 4). Fortification standard mentioned here after refers to the standard used in determining the efficiency of the extraction procedures (Reynolds, 2003).

Due to the different chromatographic equipment used in the analyses, two sets of fortification and calibration standards were prepared. The calibration standards that were used for the GC/ECD were of concentrations 0.02, 0.04 and 0.08 µg/ml whilst the fortification standard for this same equipment was of concentration of 0.1 µg/ml and made up of pure forms of each of the synthetic pyrethroids (Cypermethrin, Permethrin, Fenvalerate, Deltamethrin and Lambda Cyhalothrin) and the organophosphorous compound (Chlorpyrifos).

On the other hand, the calibration standards that were used for the QqQ-LC/MS were of concentrations: 0.001, 0.002, 0.004, 0.01 and 0.02 µg/ml whilst the fortification standard used was of concentration 0.5 µg/ml. Since only the neonicotinoids were run on the QqQ-LC/MS, standards prepared contained pure forms of Thiamethoxam, Clothianidin, Imidacloprid and Acetamiprid.

The calibration and fortification standards were prepared from a stock solution of concentration of 1000 µg/ml. This stock solution was prepared by dissolving 10 mg of the pure standard of the pesticides in an appropriate solution (acetone for the synthetic pyrethroids and organophosphorous compound and acetonitrile for the neonicotinoids).

3.7 Quality Control and Assurance

To ensure accuracy of results, all glassware were first washed with methanol and then rinsed with distilled water and sonnicated in an ultrasonic bath for 15 minutes before being rinsed with distilled water followed by acetone. The glassware were then dried at 65ºC for about 12 hours and stored in a dust free environment prior to use. Also, all samples were analysed in duplicate and fortification standards and reagent blanks were incorporated in each batch of samples analysed. This made a total of about 140 analytical runs that were conducted. All reagents used were of analytical grade. Prior to the run of sample on the QqQ-LC/MS or the GC/ECD, the system was tested with the prepared calibration standards. Also to account for any possible error, procedural internal standards which are internal standards that are added at the beginning of an analytical procedure were used in each batch of analysis that was run (Syoku-An, 2006).

3.8 Statistical Analysis

Minitab 17 and Excel 2013 for windows were the statistical software tools used in the analysis of the data obtained. Multivariate analysis in the form of Principal Component Analysis was used in the determination of the associations between the levels of residues measured in relation to the cocoa regions.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Neonicotinoids Levels Present in Dried Fermented Ghanaian Cocoa Beans

4.1.1 Distribution of Neonicotinoids in the Three Cocoa Matrices

The pesticide residue analysis done on the nibs, shells and whole cocoa beans showed very low concentrations of the neonicotinoids in all the cocoa matrices analysed. The concentration of the neonicotinoids ranged from <0.001 to 0.018 mg/kg in the shells, <0.001 to 0.0025 mg/kg in the nibs and <0.001 to 0.005 mg/kg in the whole beans. Acetamiprid and Clothianidin were not identified in the nibs of any of the samples analysed whilst Thiamethoxam although present in the nibs was in concentrations below the set limit of detection of the QqQ-LC/MS instrument. Acetamiprid and Clothianidin had the least mean concentration in relation to the shells of the beans as can be seen in Figure 4.1.

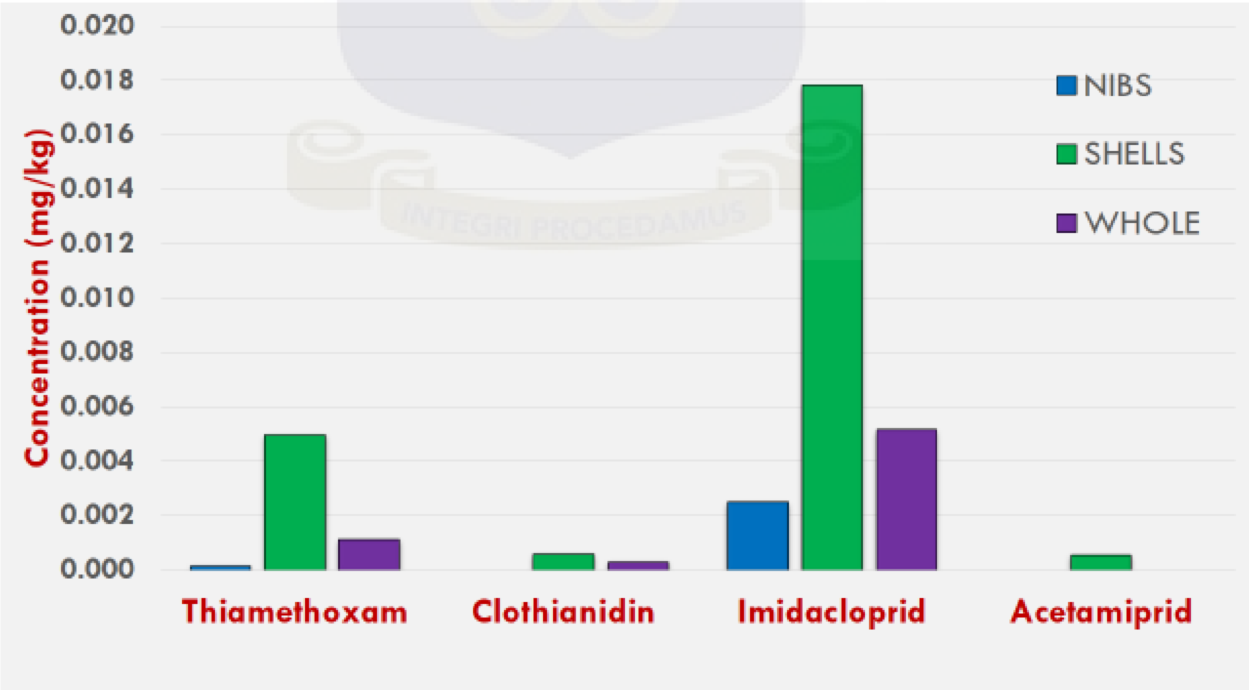

Figure 4.1: Distribution of the neonicotinoids in the three cocoa matrices

Imidacloprid on the other hand which was first introduced in 1991 and is considered to be one of the earliest and commonest neonicotinoid in use today (Bass *et.al.,* 2015) showed the highest concentration in all the three matrices: 0.003 mg/kg in the cocoa nibs, 0.018 mg/kg in the cocoa shells and 0.005 mg/kg in the whole unshelled beans. This is similar to work done by Dankyi *et. al.* (2015) where they identified Imidacloprid to be the most prevalent insecticide in the nibs and shells of Ghanaian cocoa beans.

Imidacloprid is supplied by Cocobod to cocoa farmers under its mass spraying exercise (Adu-Acheampong *et al.,* 2014; Ayenor *et al.,* 2004) and is used in the pre-harvest treatment of cocoa especially in the treatment of sucking and biting insects such as whiteflies, beetles and aphids that attack the cocoa plant. The comparatively higher concentrations of Imidacloprid recorded as compared to the other neonicotinoids can be an indication of the relatively short time between application of the pesticide and harvesting of the beans. Nevertheless, the concentrations of Imidacloprid measured in this study were significantly lower than that of Dankyi *et al.* (2015) who recorded concentrations of Imidacloprid present in the cocoa nibs to be in the range of 0.011 mg/kg to 0.035 mg/kg.

Thiamethoxam, which was present in the shells of the cocoa beans at a concentration of about 0.005 mg/kg is supplied by Cocobod and is also used in the pre-harvest treatment of cocoa trees and pods. It has been shown to be the precursor for Clothianidin which is formed during the metabolism of Thiamethoxam in insects and plants (Nauen *et. al.,* 2003). The concentrations of Clothianidin recorded during this study are therefore as a result of the breakdown of Thiamethoxam that was applied during the pre-harvest treatment of the cocoa pod and plant.

Acetamiprid is also supplied by Cocobod for the post-harvest treatment of dried fermented cocoa beans whereby it is used to fumigate the warehouse where the beans are kept before transportation. Fumigation of the warehouses of the Licensed Buying Companies that are located in the various cocoa districts and regions is usually done by the Extension Services Department of Cocobod. As such, the very low acetamiprid concentrations recorded in the shells and its absence in the nibs can be an indication of appropriate application of this pesticide.

Neonicotinoids are generally hydrophilic in nature and would be expected to be of higher concentrations in the shells than in the nibs of the beans and this is what was observed. Similar results have also been obtained in studies conducted which showed that neonicotinoids tend to accumulate in the shells of cocoa beans than in the nibs (Dankyi *et al.*, 2015). However, it is important to note that the time between application of the pesticides and when the bean pods were harvested or the time of application of the pesticides prior to analysis could also be a reason for the greater concentrations recorded in the shells as compared to the nibs (Agyekum *et al.*, 2015).

The low concentrations of the neonicotinoids that were recorded in all the three matrices can be due to the fact that these groups of insecticides have a very low potential to bio-accumulate. Field studies for neonicotinoids such as Thiamethoxam have also indicated that it degrades fast in the environment (Maienfisch *et al.*, 2001).

4.1.2 Regional Concentrations of Neonicotinoids in Cocoa Nibs

All the six cocoa growing regions studied had the neonicotinoid Imidacloprid being present in varying concentrations with Ashanti Region recording the highest concentration of 0.0035 mg/kg which is still relatively lower than the Japan MRL of 0.050 mg/kg being allowed in cocoa nibs. This was closely followed by Brong Ahafo Region with a concentration of 0.003 mg/kg and Eastern Region recorded the least concentration of 0.001 mg/kg. The concentrations of all the other neonicotinoids recorded including Thiamethoxam were below the LOD of the QqQ-LC/MS used and as such cannot be reported as the residue levels present in the nibs. Figure 4.2 is a bar chart depicting the regional distributions of these pesticides.

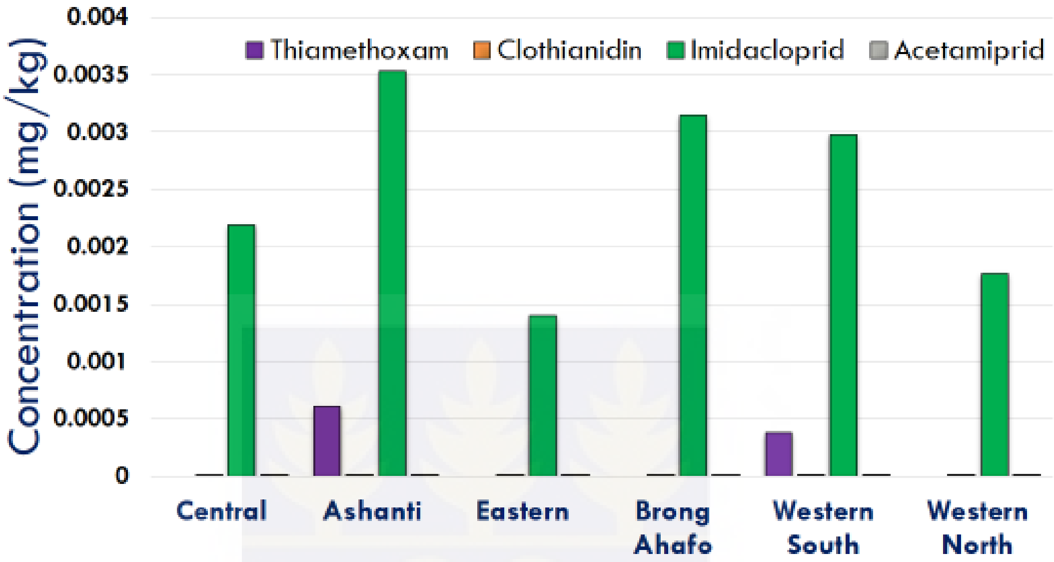

Figure 4.2: Regional concentrations of neonicotinoids in the cocoa nibs

4.1.3 Regional Concentrations of Neonicotinoids in Cocoa Bean Shells

The predominant neonicotinoid identified in the shells of the cocoa beans sampled was Imidacloprid and again, amongst the regions it was Ashanti Region which had the highest concentration of about 0.035 mg/kg and the second highest region was Brong Ahafo Region which recorded concentrations of 0.031 mg/kg. The Eastern Region recorded the least concentration of Imidacloprid of 0.009 mg/kg. As at now MRLs have not been fully established for the acceptable residue concentrations of neonicotinoids in the shells of cocoa beans. Nevertheless, these concentrations are considerably lower than that which has been set for whole cocoa beans which ranges from 0.050 mg/kg to 0.1 mg/kg for the European Union and 0.01 mg/kg to 0.05 mg/kg for Japan (Bateman, 2015). The low concentrations of Imidacloprid recorded in the cocoa shells of the various regions are dissimilar to that recorded by Dankyi *et al.* (2015) who recorded 0.2 mg/kg as the maximum Imidacloprid concentration in cocoa shells.

Acetamiprid was identified in districts of three regions: Ashanti, Eastern and Western South Region in concentrations ranging from <0.001 mg/kg to 0.003 mg/kg. Whilst Thiamethoxam was present in the districts from all the six regions in concentrations ranging from <0.001 mg/kg to 0.035 mg/kg (Appendix 6). Clothianidin was also present in four out of the six regions (Central, Ashanti, Eastern and Western South) at a maximum concentration of 0.001 mg/kg which is borderline with the LOD of the QqQ-LC/MS equipment used.

The significantly low concentrations of the neonicotinoids recorded in the shells of the beans can be attributed to the fact that cocoa farmers are putting into practice the training they receive on pesticide application and as such levels of pesticides being applied on the farms are in appropriate quantities unlike as was stated for vegetable farmers in Ashanti and Western regions of Ghana (Afari-Sefa *et. al.*, 2015). Figure 4:3 shows the regional distribution of the four neonicotinoids that were tested for.

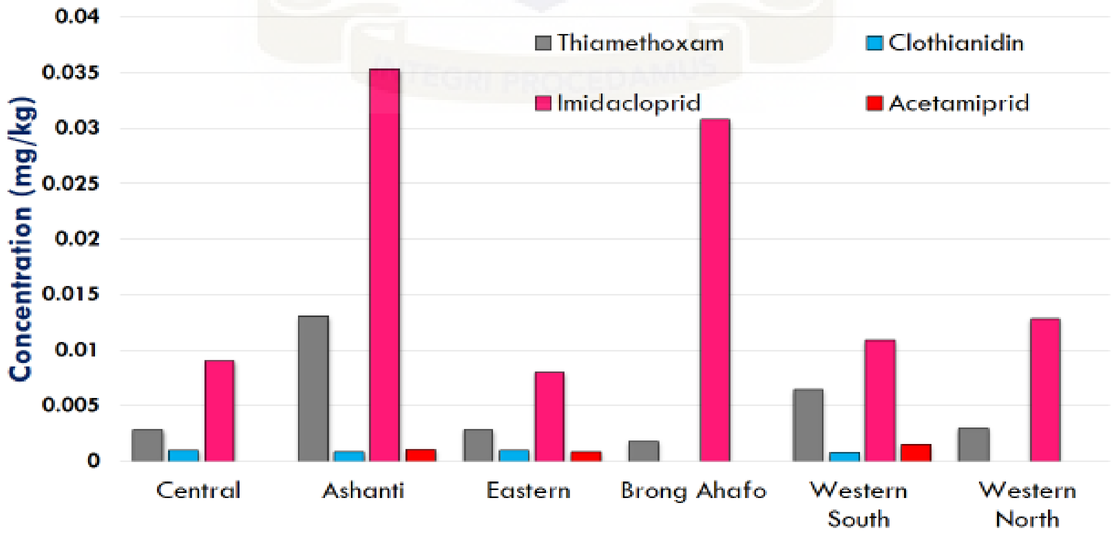

Figure 4.3: Concentration of neonicotinoids in the shells of cocoa beans from the regions

As is shown in the chart of Figure 4.3, Acetamiprid and Clothianidin were present in concentrations just around the LOD with Brong Ahafo and Western North Regions not recording any Clothianidin present in the shells. Also, Ashanti Region had the highest concentration of Thiamethoxam (0.013 mg/kg) in the cocoa shells.

4.1.4 Regional Concentrations of Neonicotinoids in Whole Cocoa Beans

Majority of the regulations established for the appropriate concentrations of pesticides in cocoa beans are based on the whole unshelled beans. In the pesticide analysis of neonicotinoids in the whole dried fermented cocoa beans from the various regions, Ashanti Region, Eastern Region and Western South Region had three out of the four neonicotinoids being present. The most prevalent neonicotinoid was Imidacloprid, followed by Thiamethoxam and finally Clothianidin. Here again Ashanti Region recorded the highest concentration of Imidacloprid (0.009 mg/kg) whilst Eastern Region had the least Imidacloprid concentration of 0.002 mg/kg. Clothianidin was present in Eastern Region cocoa beans at a concentration of about 0.001 mg/kg whilst its concentration in the beans from the Western South and Ashanti Regions were below the LOD. It was not detected in the whole beans from the other regions.

Acetamiprid was not identified in any of the whole bean samples from any of the regions. Although it was identified in very low concentrations in the shells (approximately 0.001 mg/kg), its absence in the whole beans is not an unusual phenomenon. This is because although the whole beans used in the residue analysis includes the shells and the nibs, the concentration of Acetamiprid in the shells is so low that it would easily be 'lost' during the extraction and clean-up processes when the whole beans were analysed. This is not only possible for Acetamiprid but also for the other neonicotinoids that were present in very low concentrations in the shells of the beans but were not detected when the whole beans were analysed. Figure 4.4 shows the regional distribution of the neonicotinoids in the whole unshelled cocoa beans.

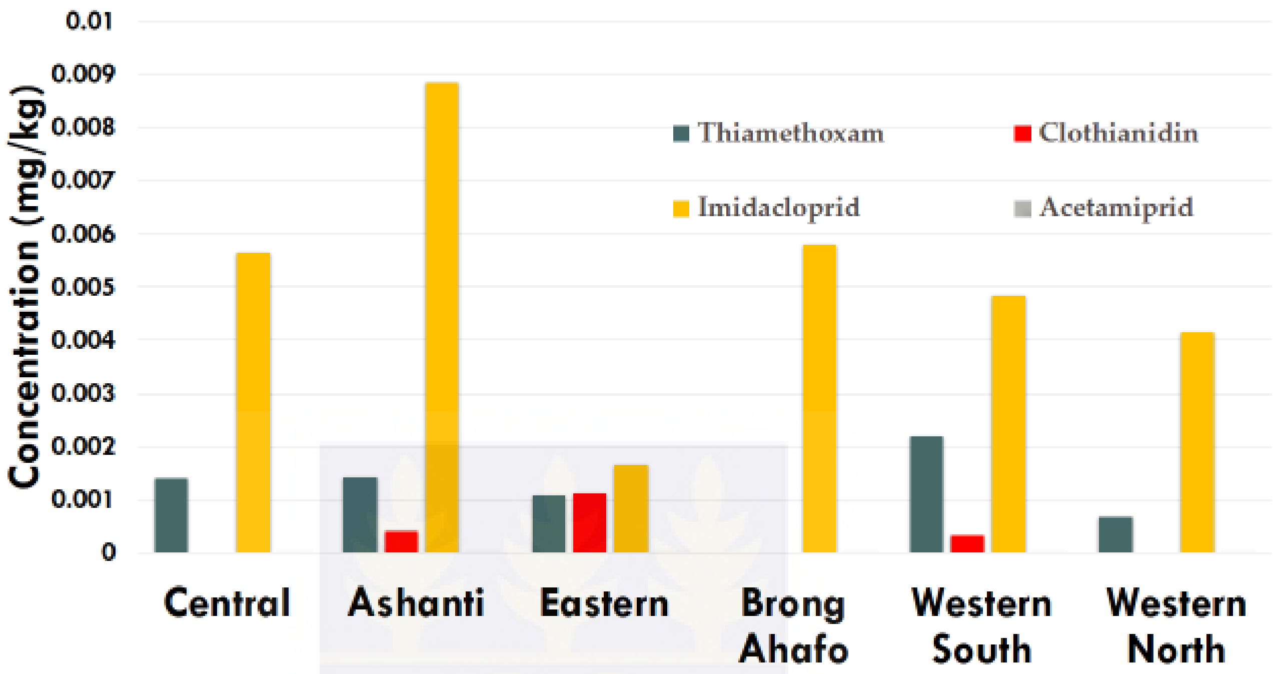

Figure 4.4: Regional Concentrations of Neonicotinoids in Whole Beans

4.2 Synthetic Pyrethroid Levels Present in Dried Fermented Ghanaian Cocoa Beans

4.2.1 Concentrations of Pyrethroids in the Three Cocoa Matrices

Pesticide residue analysis of the pyrethroid levels in the shells, nibs and whole cocoa beans revealed the shells as having the greatest number of the pyrethroids being present. All of the pyrethroids studied were present in the shells of the cocoa beans in varying concentrations with the highest being Cypermethrin at a concentration of 0.013 mg/kg in the shells of the beans analysed. This was followed by Permethrin which was also present in the shells at a concentration of 0.012 mg/kg (Fig 4.5). The mean concentrations of all the other pyrethroids recorded for the other matrices were below the limit of detection of the GC/ECD equipment used.

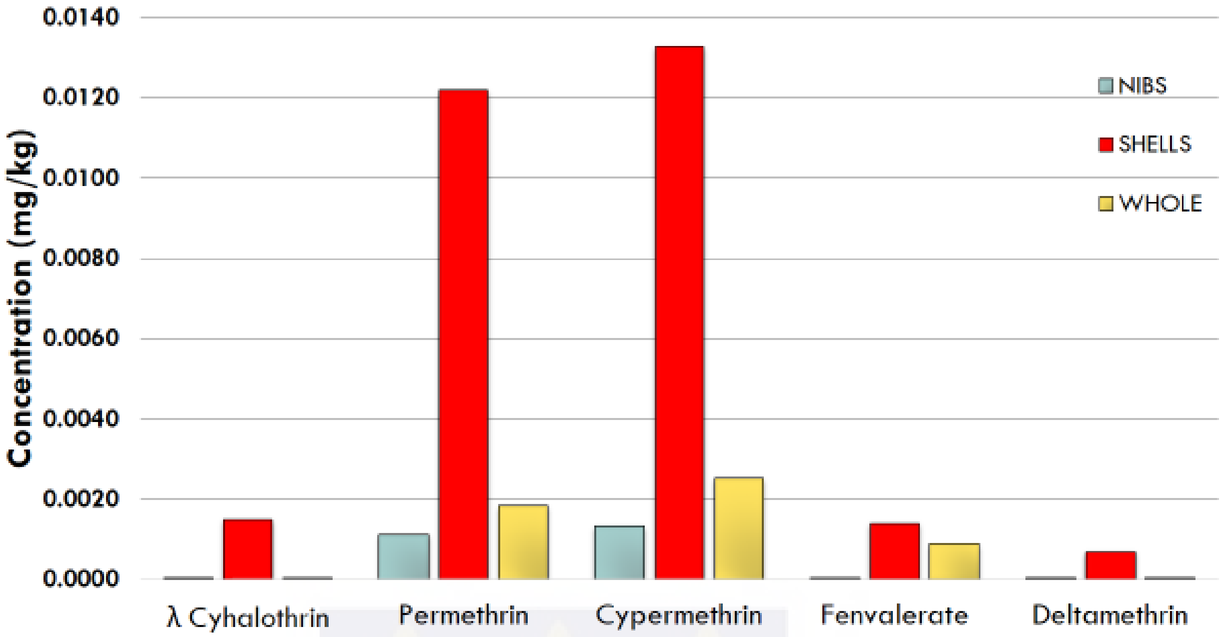

Figure 4.5: Distribution of Synthetic Pyrethroids in the three cocoa matrices

4.2.2 Concentration of Pyrethroids in Cocoa Shells from the Cocoa Regions

In terms of the regions, it was only Kasapem district in the Brong Ahafo Region that recorded the presence of the pesticide Lambda Cyhalothrin in its beans. This was at a concentration of about 0.009 mg/kg which is very close to the set LOD of the chromatographic equipment of 0.007 mg/kg. Permethrin was found to be present in bean shells from both Ashanti and Western South Region at concentrations of 0.01 mg/kg and 0.02 mg/kg respectively.

Cypermethrin on the other hand was present in four out of the six regions ranging in concentration from 0.011 mg/kg in shells of beans from the Brong Ahafo Region to 0.020 mg/kg in the Central Region. The concentrations recorded for Brong Ahafo is similar to a study done in that region to determine the Cypermethrin levels present in the soils of cocoa farms. That study reported a Cypermethrin concentration ranging from <0.01 to 0.04 mg/kg (Fosu-Mensah *et. al.*, 2016).

The mean concentrations of Fenvalerate and Deltamethrin recorded for any of the regions were very low and below the LOD. However, for the individual districts, Fenvalerate was identified in three out of the seventeen districts at a maximum concentration of 0.02 mg/kg (Kasapem district of the Brong Ahafo Region) whilst Deltamethrin was present in one district at a concentration of 0.01 mg/kg (Dadieso district of the Brong Ahafo Region) and can be seen in Appendix 6. The various concentrations of the identified pyrethroids for the different regions are shown in Figure 4.6.

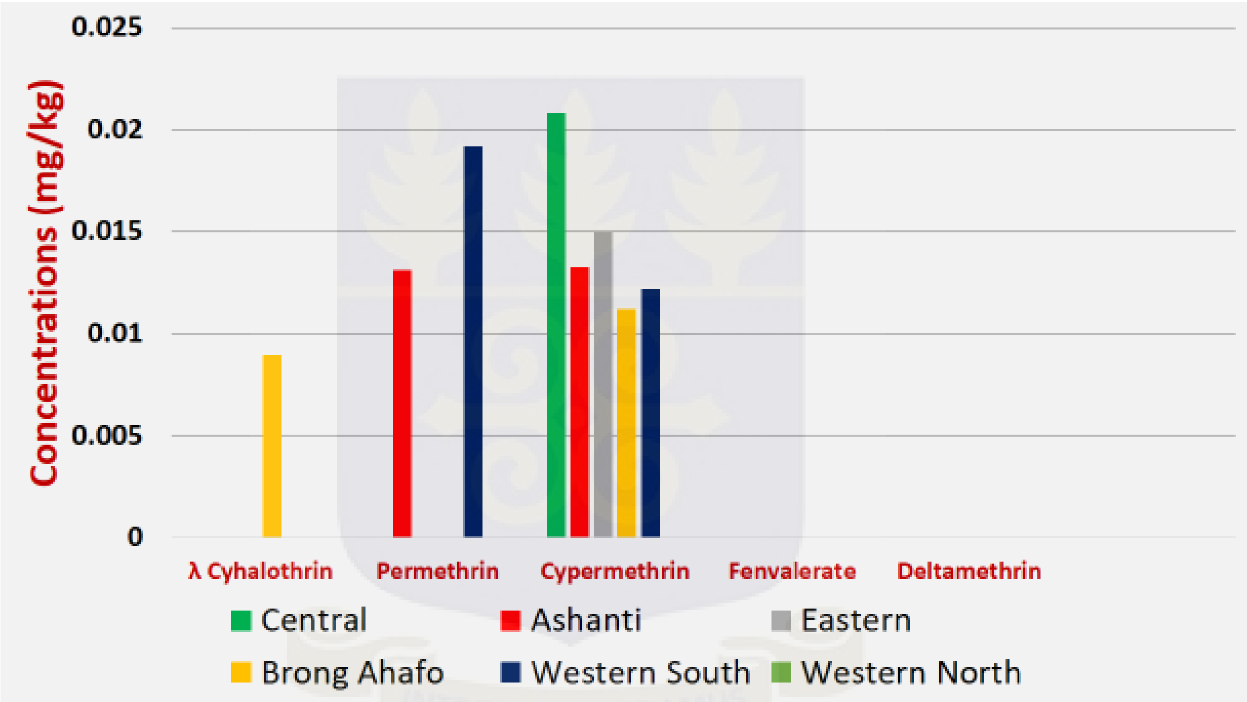

Figure 4.6: Concentrations of synthetic pyrethroids in shells from the six cocoa regions

4.2.3 Concentrations of Pyrethroids in the Nibs and Whole Beans from the Cocoa Regions

Apart from Permethrin and Cypermethrin, none of the pyrethroid concentrations measured in the cocoa nibs for the regions were above the LOD of the GC/ECD equipment used. Permethrin was found to be present in the nibs of cocoa from the Western North Region at a concentration of about 0.007 mg/kg.

Permethrin was recorded only in the Sewfi Wiaso district of the Western North Region at a concentration of 0.02 mg/kg which is higher than what was reported in a study done on cowpea and maize cereals in the Ashanti Region (Akoto *et al.*, 2013). Cypermethrin was also present in the nibs of beans from three districts but it was Juaso in the Ashanti Region that recorded the highest concentration of 0.008 mg/kg. These concentrations are significantly lower than the established EU MRL of 0.1 mg/kg in whole beans. Also, the concentrations of Cypermethrin recorded in the whole bean samples analysed were significantly lower than that reported by Frimpong *et al.* (2012b) who recorded mean concentrations of Cypermethrin in whole beans to be from 0.02 mg/kg to 0.04 mg/kg.

4.3 Concentration of Chlorpyrifos in Ghanaian Cocoa Beans

4.3.1 Levels of Chlorpyrifos in the Shells, Nibs and Whole Cocoa Beans

Chlorpyrifos was found to be present in greater concentrations in the shells of the beans than in any of the other matrices analysed. About 42% of the cocoa shell samples analysed showed the presence of Chlorpyrifos at concentrations ranging from 0.0108 to 0.0396 mg/kg with an average concentration of 0.0184 ± 0.007 mg/kg being recorded. The greater concentrations in the shells than any of the other matrices is not uncommon as Chlorpyrifos has been found to be in greater concentrations in the peels than in the pulp of other food commodities such as tomato (Agyekum *et al.*, 2015). It was present in the nibs of the cocoa beans at concentrations between <0.007 mg/kg and 0.017 mg/kg.

Even though, it would be expected that Chlorpyrifos which has mainly been approved for the pre-harvest control of insects in food commodities such as cereals and vegetables (SAL Consult, 2014) and is lipophilic in nature would be present to a greater proportion in the nibs of the beans than in the shells, this was not the case. The greater concentrations of Chlorpyrifos in the shells relative to the nibs can be an indication that the said pesticide had been applied close to the time of harvest of the beans such that it had not had sufficient time to translocate from the shells to the nibs of the beans.

Concerning the whole beans analysed, the Chlorpyrifos concentrations that were measured did not exceed 0.04 mg/kg which is below the European Union and Japan MRLs of 0.1 mg/kg and 0.05 mg/kg respectively (Bateman, 2015). This value is also within the range recorded in other studies where Chlorpyrifos levels in whole beans ranged from 0.001 to 0.2 mg/kg (Zainudin *et al.*, 2015). Koforidua cocoa district in the Eastern Region recorded the highest Chlorpyrifos concentration of 0.037 mg/kg in the whole beans samples analysed.

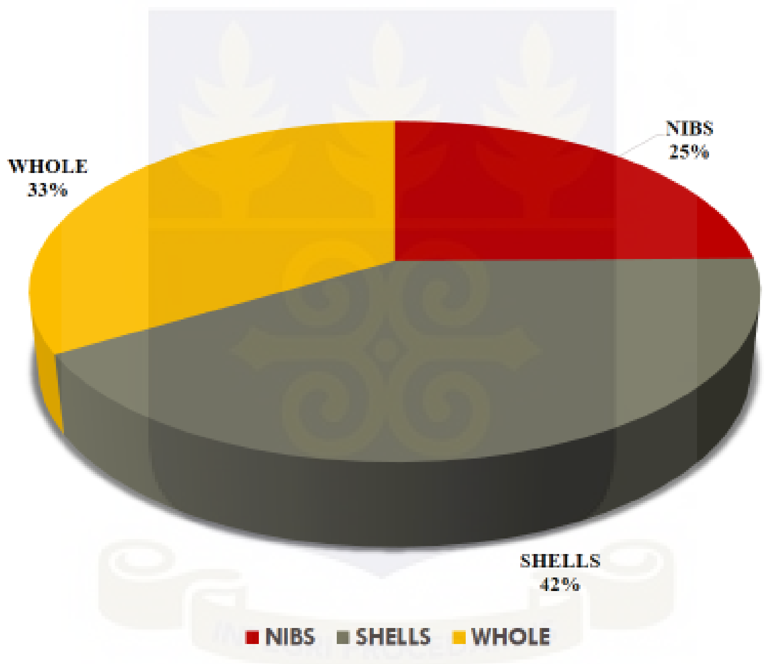

Figure 4.7: Percentages of samples containing chlorpyrifos as it pertains to the shells, nibs and whole beans

4.3.2 Regional Distribution of Chlorpyrifos in Cocoa Nibs, Shells and Whole Beans

Figure 4.8 shows the mean concentrations of Chlorpyrifos measured for the various regions as it pertains to the nibs, shells and whole beans analysed. Eastern Region had the highest concentration of Chlorpyrifos in the shells (0.026 mg/kg), whilst Western North Region recorded the highest concentration in the whole beans (0.027 mg/kg) and Central Region recorded the highest concentration in the nibs (0.015 mg/kg). Ashanti and Central

Region had similar levels of Chlorpyrifos being present in the shells of the beans whilst Eastern and Ashanti Region had similar levels of Chlorpyrifos being present in the whole beans.

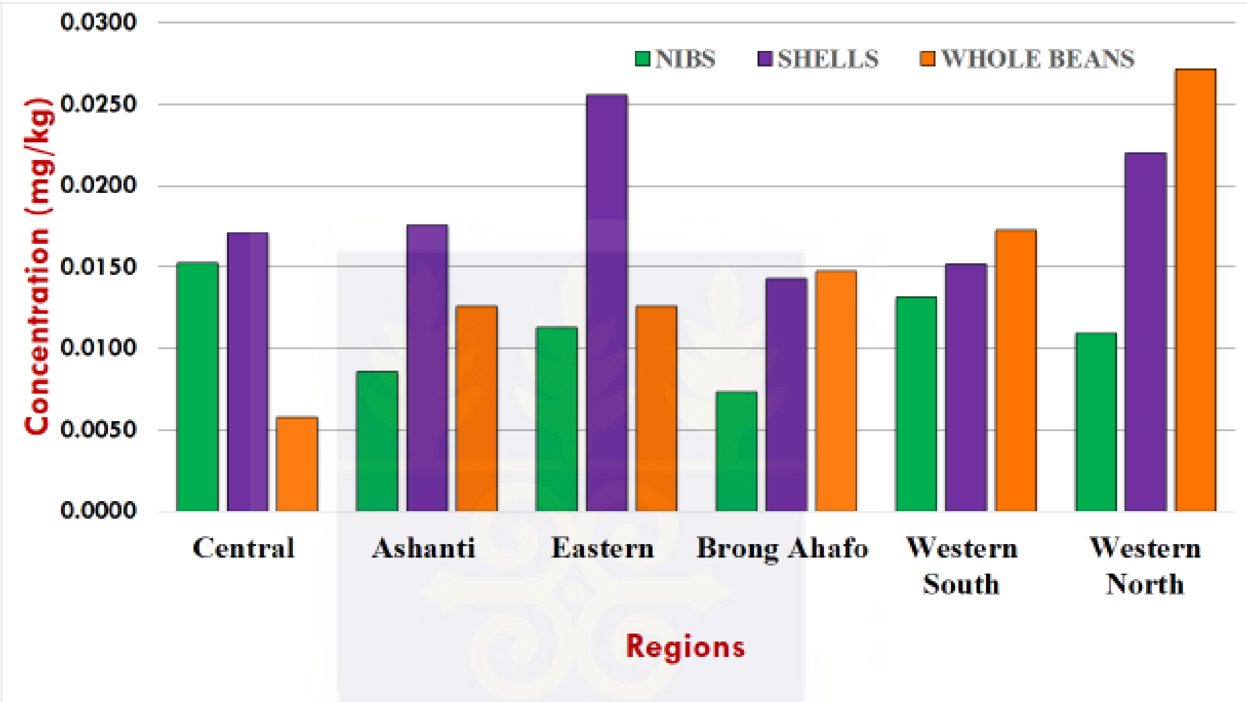

Figure 4.8: Regional distribution of Chlorpyrifos levels in the cocoa nibs, shells and whole beans

Chlorpyrifos which was found in the nibs of fifteen out of the seventeen districts analysed ranged in concentrations of <0.007 to 0.0166 mg/kg with an average concentration of 0.011 ± 0.005 mg/kg being recorded. Juaso and Breman Asikuma districts of the Ashanti and Central Region respectively had the highest Chlorpyrifos concentrations.

4.4 Comparison of the Whole Bean Concentrations of all the 10 Pesticides for the Various Regions

Comparison of the pesticide levels in the whole beans across all the regions shows that Chlorpyrifos was the predominant one in concentrations greater than the other pesticides. Although Chlorpyrifos is mainly for cereals and vegetables, its higher concentrations compared to the other pesticides tested for corresponds to findings reported by Adu-Acheampong *et al.* (2014) that cocoa farmers at times apply or convince the spraying gangs to mix approved and non-approved insecticides so as to increase the overall efficacy of the pesticides. This is also evidenced by the presence of Permethrin (a pesticide not approved for use on cocoa) in the beans from some of the regions.

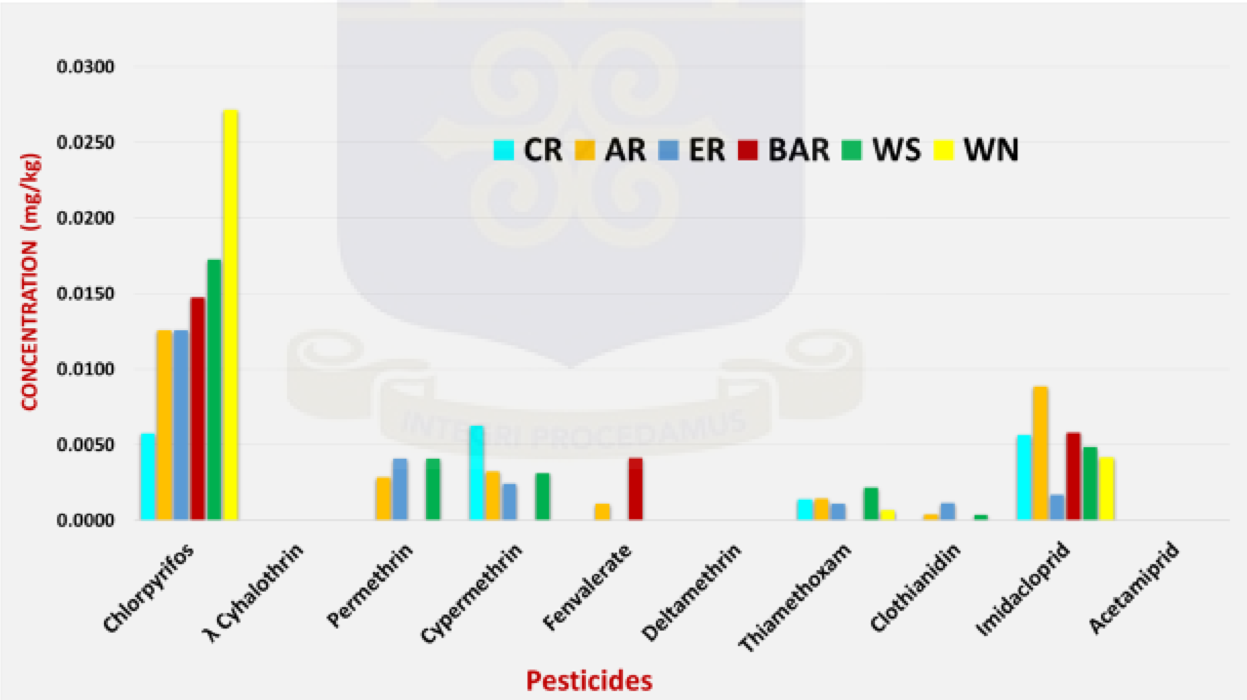

Figure 4.9: Mean concentrations of the pesticides as it pertains to the various regions

The generally low concentrations recorded for all the pesticides in all the matrices could be attributed to a host of factors some of which are appropriate pesticide application by farmers and spray gangs and rapid volatilization, degradation or metabolism of the pesticides during the storage of the cocoa beans. Aside these factors, drying of food commodities such as fruits has been shown to reduce the pesticide concentration present and this may translate to cocoa beans as well (Dong, 2012). Also, the loss of some pesticides during the process of sample preparation prior to the sample being run on the chromatographic equipment such as the extraction, evaporation or clean-up steps cannot be avoided (Chung & Chen, 2011). However it should be noted that exposure to even low doses of pesticides over a long period of time can lead to chronic toxicity (Akoto *et al.*, 2013).

4.5 Associations within the Pesticides Residues and between the Residues and the Regions

Multivariate analysis in the form of Principal Component Analysis (PCA) was used to determine the associations amongst the pesticides measured and between the pesticides and the various regions and not necessarily the differences in the concentrations of the pesticides. In the determination of the Principal Component (PC) values, the concentrations of the pesticides measured for the individual districts were considered as replicates of the regions they belong to.

The PCA values that accounted for the highest variation in the data which was mostly PC1 and PC2 were used in developing a biplot which graphically depicted the associations between the pesticides and the regions. There are three main things that can be gleaned from each biplot: the dispersion of the data; the similarities and differences between the proportions of the pesticides which can be deduced from the acuteness or obtuseness of the angles between them and finally the relationships between the prevalence of the pesticides and the regions.

The numbers in the quadrangle of the biplot are representative of the regions considered and they are designated as:

Central Region is 1

Ashanti Region is 2

Eastern Region is 3

Brong Ahafo Region is 4

Western South Region is 5

Western North Region is 6

4.5.1 Principal Component Analysis of Cocoa Nibs

In relation to the nibs, the proportion of variance values showed that the Principal Component 1 (PC1) accounted for about 0.4579 (45.79%) of the variation in the data obtained. This was followed by Principal Component 2 (PC2) which accounted for 0.2407 (24.07%) of the variation in the data obtained.

For the nibs of the six regions analysed, the eigenvectors of PC1 show that the active ingredient Thiamethoxam accounted for about 0.5662 (56.62%) of the variation in the data obtained and this was followed by Imidacloprid at 0.5219 (52.19%), Permethrin at 0.4771 (47.71%), Chlorpyrifos at 0.3130 (31.30%) and finally Cypermethrin at 0.2852 (0.2852%) as is shown in Table 4.1.

Table 4.1: Principal Components Analysis (Eigenvectors & Eigenvalues) of Cocoa Nibs

VARIABLES	PC1	PC2	PC3	PC4	PC5
Chlorpyrifos	-0.3130	0.6445	-0.3469	0.5545	-0.2426
Permethrin	0.4771	-0.1572	0.5033	0.6323	-0.3074
Cypermethrin	-0.2852	-0.7048	-0.4494	0.1892	-0.4291
Thiamethoxam	-0.5662	-0.2292	0.3184	0.4081	0.5991
Imidacloprid	-0.5219	0.1036	0.5683	-0.3006	-0.5509
Eigenvalues	2.2893	1.2033	0.9208	0.5254	0.0612
PROPORTION OF VARIANCE	**0.4579**	**0.2407**	**0.1842**	**0.1051**	**0.0122**

4.5.1.1 Biplot of Cocoa Nibs

Since PC1 and PC2 accounted for majority of variation (over 50%) in the data obtained, they were used in developing the biplot. Only five pesticides were found to be present in the nibs of the beans analysed and these were: Chlorpyrifos, Imidacloprid, Thiamethoxam, Cypermethrin and Permethrin.

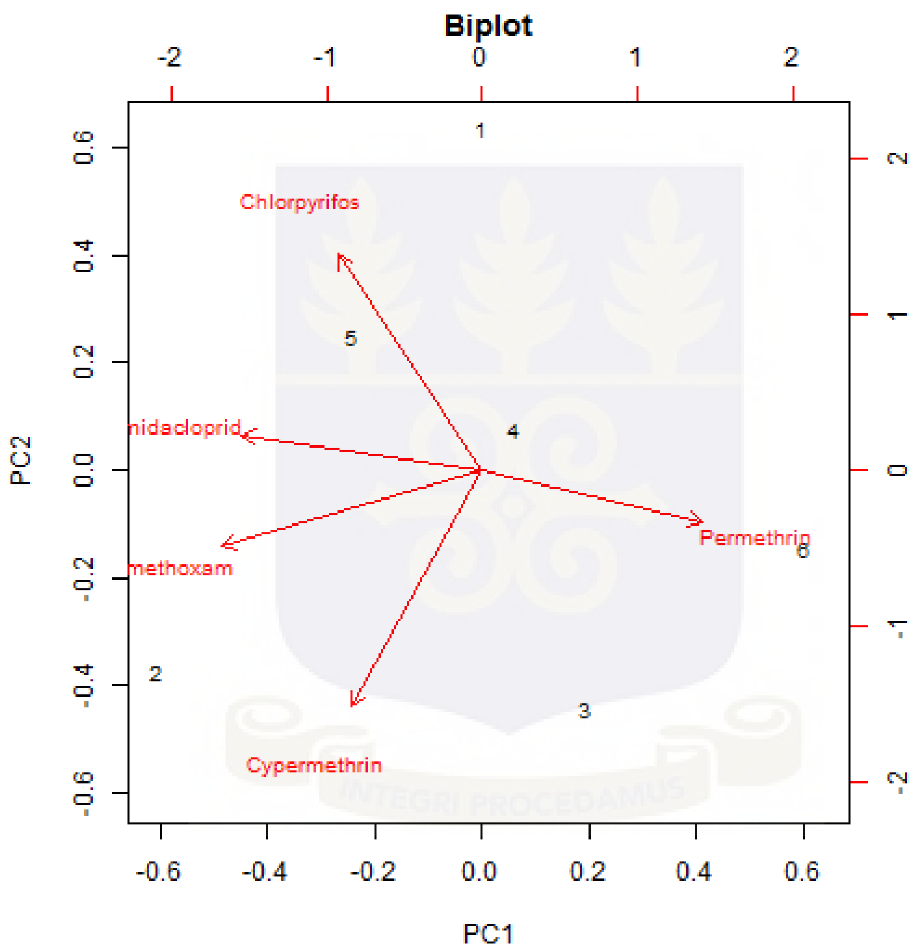

Figure 4.10: Biplot of cocoa nibs showing the associations between the regions and the pesticides

4.5.1.2 Relationships between pesticides

Figure 4.10 shows the dispersion of the five (5) pesticides identified in the cocoa nibs. The moderate spread of the pesticides within the quadrangle is an indication that the pesticides were not of the same proportions but varied. A closer look at the biplot also shows an association between Imidacloprid and Thiamethoxam; Thiamethoxam and Cypermethrin; Chlorpyrifos and Imidacloprid and this is shown by the acuteness of the angles between these pesticides. However, the wide angle between Permethrin and any of the other pesticides identified shows that there is no association or relationship between the concentration and prevalence of Permethrin and any of the other pesticides.

These results show that in terms of the nibs, Imidacloprid and Thiamethoxam are the most associated or the most similar in relation to both their concentrations and occurrence. Therefore, in the development of marker pesticides in nibs either Imidacloprid or Thiamethoxam can serve as marker pesticides. This also means that in the routine analyses of pesticides, instead of measuring both Imidacloprid and Thiamethoxam, the researcher or investigator can measure only Imidacloprid or only Thiamethoxam and this would give an indication of the concentration and presence of the other one. This is also true for Chlorpyrifos and Imidacloprid, Thiamethoxam and Cypermethrin but most especially true for Imidacloprid and Thiamethoxam because it is these last two pesticides that have the smallest angle between them.

4.5.1.3 Relationships between pesticides and cocoa regions

In relation to the regions, the biplot of Figure 4.10 shows that out of the five pesticides identified Permethrin was the most prevalent one in the Western North cocoa region of Ghana. Brong Ahafo Region had all the five pesticides present in comparable proportions whilst Western South Region on the other hand was more closely associated with Chlorpyrifos than any of the other pesticide. The biplot also shows that Central Region, Ashanti Region and Eastern Region were not closely associated with any of the pesticides identified in the nibs.

4.5.2 Principal Component Analysis of Cocoa Shells

Table 4.2 shows that PC1 accounted for 0.3706 (37.06%) of the variation in the data whilst PC2 accounted for 0.2937 (29.37%) of the variation thus PC1 and PC2 accounted for over 50% of the variation in the data obtained. As is shown in the table, Lambda Cyhalothrin accounted for 0.4256 (42.56%) variation in the data which was followed by Fenvalerate 0.4227 (42.27%) and then Thiamethoxam 0.4198 (41.98%) as determined by PC1. Each of the other pesticides accounted for less than 40% of the variation in the data.

Table 4.2: Principal Component Analysis (Eigenvectors & Eigenvalues) Of Cocoa Shells

VARIABLES	PC1	PC2	PC3	PC4	PC5	PC6
Chlorpyrifos	-0.1021	-0.4056	0.0918	0.6278	0.0466	0.3576
Lambda Cyhalothrin	0.4256	0.2512	0.2279	-0.1014	0.2790	0.1659
Permethrin	-0.2816	0.2424	-0.4218	-0.3489	-0.4257	0.3585
Cypermethrin	-0.0300	0.3786	-0.4829	0.4348	0.1030	-0.5755
Fenvalerate	0.4227	0.2618	-0.2589	-0.0834	0.2037	0.3676
Deltamethrin	-0.3367	0.3896	0.1663	0.2702	0.1013	0.3691
Thiamethoxam	-0.4198	0.2656	0.2539	0.0574	-0.2406	-0.0103
Clothianidin	-0.3422	-0.0893	-0.4233	-0.0266	0.6294	0.2301
Imidacloprid	0.0127	0.5163	0.3503	0.1343	0.1388	0.0010
Acetamiprid	-0.3777	-0.0694	0.2599	-0.4276	0.4506	-0.2459
Eigenvalues	3.7056	2.9369	1.4822	1.1778	0.6979	0
PROPORTION OF VARIANCE	**0.3706**	**0.2937**	**0.1482**	**0.1178**	**0.0698**	**0**

4.5.2.1 Biplot of Cocoa Shells

The biplot shows that the data are clustered together and minimally spread. This is an indication of strong associations between the pesticides analysed and the regions they were present in. All ten of the pesticides tested for were found to be present in the shells of the cocoa beans albeit some were in considerably low concentrations.

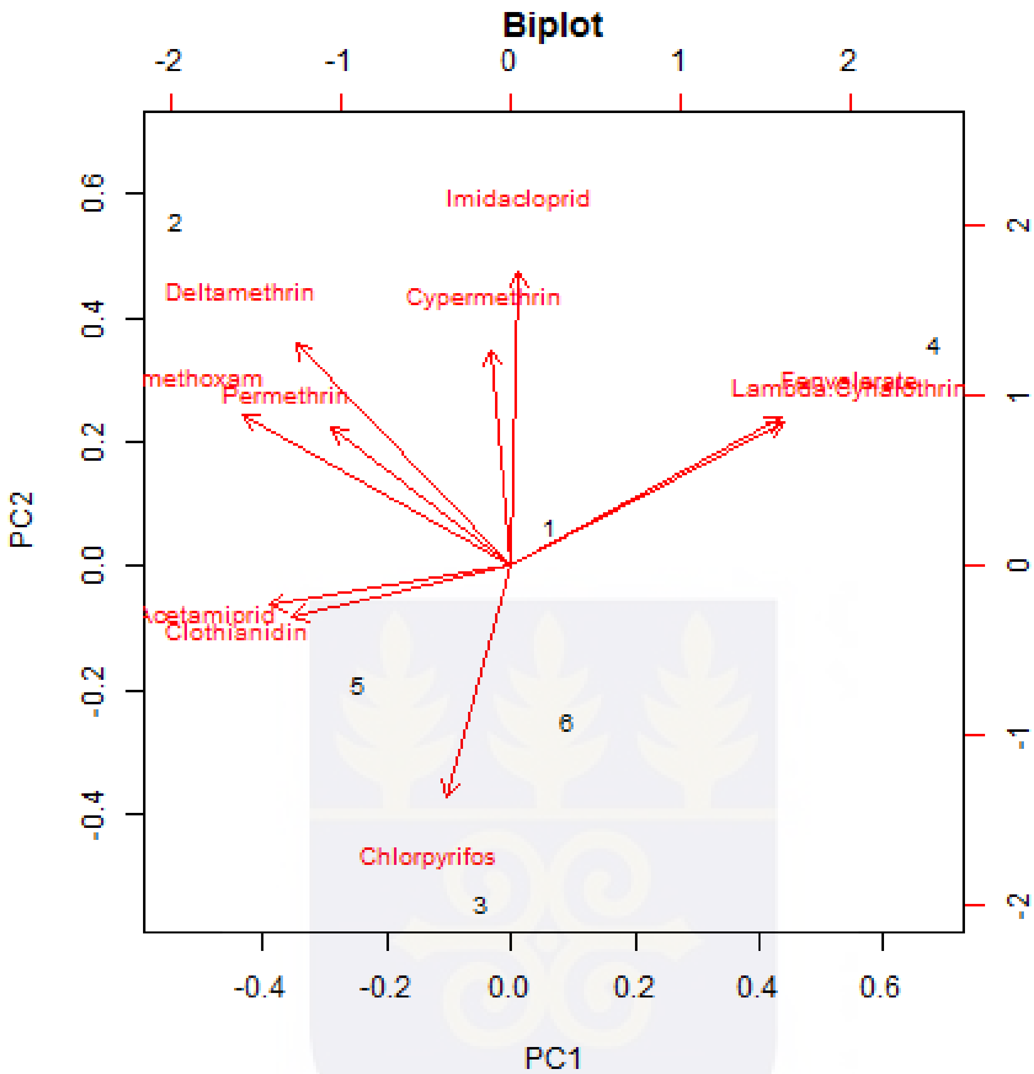

Figure 4.11: A biplot of cocoa shells showing the associations between the various pesticides and their regions

4.5.2.2 Relationships between the pesticides

The biplot showing the ten pesticides indicates that there was a strong association between Lambda Cyhalothrin and Fenvalerate (which may be outliers as they were both identified in a single district each); Acetamiprid and Clothianidin; Imidacloprid and Cypermethrin; Deltamethrin, Thiamethoxam and Permethrin. The close association between the Imidacloprid/Cypermethrin group, the Lambda Cyhalothrin/Fenvalerate group and the Acetamiprid/Clothianidin group can be indications of marker pesticides such that concentrations of one can be indicative of the concentrations and prevalence of the other.

Chlorpyrifos which was the only organophosphorous compound tested for did not show a close association with any of the other pesticides.

4.5.2.3 Relationships between the pesticides and the various regions

In considering the regions and the pesticide residues, the biplot indicates that Central Region had the most representation of the pesticides under study. Western South and Western North Regions on the other hand were equally associated with Chlorpyrifos. Ashanti Region had a slight association with Deltamethrin whilst Brong Ahafo Region was also slightly associated with Fenvalerate and Lambda Cyhalothrin. Eastern Region also showed a slight association with Chlorpyrifos.

4.5.3 Principal Component Analysis of Whole Cocoa Beans

The Principal Component 1 (PC1) accounted for over 50% (51.30%) of the variation in the data whereas the PC 2 accounted for about 27% of the variation in the data. Thus combined, PC1 and PC2 accounted for over 70% of the variation in the data. PC1 showed that Permethrin accounted for the highest variation in the data (44.6%), followed by Clothianidin (43.9%), Thiamethoxam (43.21%) and then Fenvalerate (41.2%). Imidacloprid accounted for the least variation (11.1%) in the data obtained.

Table 4.3: Principal Component Analysis (Eigenvectors & Eigenvalues) of Whole Cocoa Beans

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Chlorpyrifos	-0.2695	-0.5740	-0.0116	0.5950	-0.2932	-0.3797
Permethrin	-0.4456	-0.0094	-0.4591	-0.3080	0.4723	-0.5194
Cypermethrin	-0.4092	0.4058	0.0154	-0.2403	-0.7395	-0.1819
Fenvalerate	0.4119	0.0736	-0.5796	-0.0900	-0.2873	-0.1147
Thiamethoxam	-0.4321	0.2885	0.3884	0.0853	0.1904	0.0691
Clothianidin	-0.4388	-0.0682	-0.5158	0.1728	-0.0273	0.7104
Imidacloprid	0.1114	0.6423	-0.1896	0.6694	0.1566	-0.1743
Eigenvalues	3.59	1.90	1.09	0.29	0.13	0
Proportion of Variance	**0.5130**	**0.2708**	**0.1562**	**0.0418**	**0.0181**	**0**

4.5.3.1 Biplot of Whole Cocoa Beans

Seven out of the ten pesticides tested for were found to be present in the whole unshelled cocoa beans as is shown in Figure 4.12. The biplot of PC1 and PC2 also shows a moderately dispersed arrangement of the data.

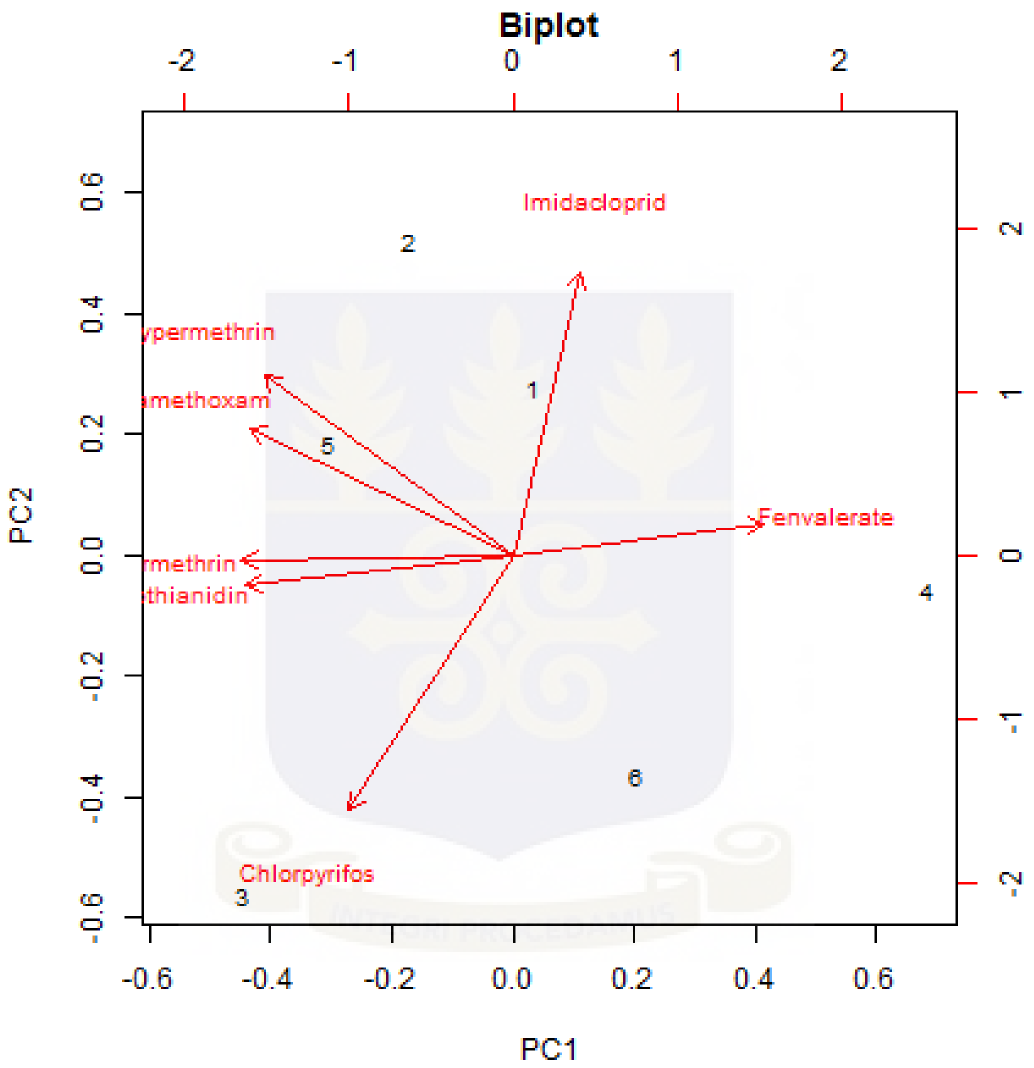

Figure 4.12: Biplot of whole beans depicting the associations between the pesticides and the regions

4.5.3.2 Relationships between pesticides

Two sets of pesticides occurred in similar proportion from the analyses of the whole beans and they were Cypermethrin/Thiamethoxam and Permethrin/Clothianidin. Therefore, in the routine identification of pesticide residue in whole cocoa beans, either Cypermethrin or Thiamethoxam can be measured whilst either Permethrin or Clothianidin can be measured too. There was no strong association between Chlorpyrifos and any of the other pesticides analysed. Imidacloprid and Fenvalerate however showed a close association but this is not significant enough to conclude on the use of either one of them as marker pesticides during routine pesticide analysis.

4.5.3.3 Association between the pesticides and the cocoa regions

The strong correlation identified between Cypermethrin and Thiamethoxam was most prominent in the Western South Region as is shown in the biplot of Figure 4.12. This close association of Cypermethrin and Thiamethoxam indicates that in the Western South Region, Cypermethrin and Thiamethoxam occur in similar or comparable proportions and thus in the analysis of cocoa beans from the Western South Region either of them can be measured which would give an indication of the presence of the other.

The biplot of PC1 and PC2 also showed that Imidacloprid was closely associated with Central Region and can be considered to be the predominant pesticide in this region. Whilst Fenvalerate and Chlorpyrifos were slightly associated with Brong Ahafo and Eastern Region respectively. On the other hand, Western North did not show any association with any of the pesticides identified in the whole cocoa beans.

4.6 Comparison of the Concentrations of the Pesticides Analysed with Established Regulations

Most of the established maximum residue limits for cocoa beans under the European Union are based on the whole unshelled beans. For the ten pesticides analysed in this work their established EU MRLs are all based on whole unshelled beans. Under Japan MRL regulations, accepted residue levels are set for either the whole beans or the nibs of the beans depending on the pesticide in question. Imidacloprid, Thiamethoxam and Clothianidin Japan MRLs are based on only the nibs. Two of the pesticides analysed in this work (Fenvalerate and Permethrin) are not to be present in cocoa beans under both the European Union and Japan regulations and as such there are no established MRLs for them (Bateman, 2015). An interesting observation made in this study was that none of the mean concentrations of the cocoa beans or nibs analysed exceeded the established EU and Japan MRLs as is shown in Table 4.4.

Table 4.4: Comparison of the mean concentrations of the pesticides with MRLs of EU and Japan

Pesticide	This Work (mg/kg)	EU MRL (mg/kg)	Japan MRL (mg/kg)
Deltamethrin	ND	0.050	0.050
Fenvalerate	0.001	No MRL set	No MRL set
Permethrin	0.002	No MRL set	No MRL set
Lambda Cyhalothrin	ND	0.050	0.010
Cypermethrin	0.003	0.100	No MRL set
Chlorpyrifos	0.015	0.100	0.050
Imidacloprid	0.005 (0.003[a])	0.050	0.050*
Acetamiprid	ND	0.100	0.010
Thiamethoxam	0.001 (ND[a])	0.050	0.020*
Clothianidin	ND (ND[a])	0.050	0.020*

*The Japan MRLs for these pesticides is based on only the nibs

[a] These values are nib concentrations

4. 7 Percentage Recovery and Interferences

No significant interferences were observed during the chromatographic runs of the samples. Interferences in this situation refers to responses measured on the detector that is as a result of compounds in the cocoa sample other than the pesticides of interest which influence (increase or decrease) the area and shape of the peak of the pesticide of interest analysed (Reynolds, 2003). Percentage recovery ranging between 70-110% was obtained during the laboratory analysis and was computed using the formula:

$$\% \text{ Recovery} = \frac{\text{(Concentration of spiked sample - Concentration of unknown sample)}}{\text{(Expected concentration of spiked sample)}}$$

The unknown sample in this case refers to a sample of either the nibs, shells or whole beans whose original pesticide residue concentration was less than the LOD and to which was added one (1ml) of the fortification standard to obtain the spiked sample.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

A total of ten (10) Active ingredients were analysed, and out of these 10, five (5) were identified in the nibs, all ten (10) were found in the shells and seven (7) in the whole beans. All the pesticides analysed were of considerable low concentrations in all the three matrices studied and none of them exceeded the established European and Japan Maximum Residue Limits. Therefore, in terms of food safety as it relates to pesticide residue concentrations, Ghanaian cocoa beans can be considered to be of good quality.

1. The neonicotinoids tested for in this research work showed Imidacloprid to have the highest concentration in the nibs, shells and whole cocoa beans. The regional comparisons of the various pesticide concentrations also showed that Ashanti Region had the highest concentration of Imidacloprid in all three matrices when compared with the other regions. The regional concentrations of the neonicotinoids were lower than established regulations.

2. Analysis of the synthetic pyrethroids in the shells, nibs and whole cocoa beans revealed the concentrations of Permethrin and Cypermethrin to be relatively higher than the other pyrethroids measured. In terms of the residue concentration in the shells of the beans, Western South and Central Regions had the highest concentration of Permethrin and Cypermethrin respectively. Also, regional analysis of the synthetic pyrethroid did not reveal the presence of Fenvalerate and Deltamethrin in the shells of the cocoa beans. Most of the pyrethroids tested for in the nibs of the

cocoa beans were below the set limit of detection of the chromatographic equipment used.

3. Chlorpyrifos which is mainly used in the pre-harvest treatment of cereals and vegetables was found to be present in the shells, nibs and whole beans than most of the pesticides analysed and it was also the only pesticide identified in all the regions studied. This may be an indication of farmers mixing approved and non-approved cocoa pesticides.
4. Principal Component Analysis when used to determine the associations amongst the various pesticides and between the pesticides and the regions showed that for the nibs, Imidacloprid & Thiamethoxam; Thiamethoxam & Cypermethrin; Chlorpyrifos & Imidacloprid were of comparable proportions and in routine analysis, either one in a set can be considered. Brong Ahafo and Western South Region also showed a strong association with Chlorpyrifos thus Chlorpyrifos can be considered to be the most predominant pesticide residue in nibs from these regions.
5. PCA of the shells also showed that Imidacloprid & Cypermethrin; Deltamethrin & Permethrin & Thiamethoxam; were of similar proportions and prevalence and as such either one in each set can be used in the routine analysis of pesticides. Out of all the regions, it was Central Region that was most associated with all the 10 pesticides.
6. The PCA of the whole cocoa beans from the various regions also showed that Cypermethrin &Thiamethoxam and Permethrin & Clothianidin were closely associated. For the regions, Imidacloprid was seen to be closely associated with Central Region whilst Western South Region was closely associated with Cypermethrin and Thiamethoxam.

5.2 Recommendations

Based on the findings from this study, the following recommendations are being made for further studies and policy implementation.

1. Further studies should be conducted to establish which pesticides are prevalent in which regions and this can be used in the development of protocols to measure marker pesticides during routine pesticide residue analysis.
2. Further studies should be conducted to determine the relationship between the geographical location of the cocoa regions and the pesticide residues present in cocoa beans.
3. It is recommended that because of the varying concentrations of the pesticide residues amongst the three cocoa matrices, future regulations such as the MRLs should consider the three matrices and not only the whole unshelled beans.
4. It is also recommended that the pesticide residue levels of other active ingredients in pesticide formulations that were not analysed in this research can be determined for the nibs, shells and whole unshelled beans of cocoa beans from the various cocoa regions of Ghana.

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APPENDICES

Appendix 1: Extraction and Clean-Up Procedure for Pesticides in Cocoa

Appendix 1a: Extraction and Clean-Up Procedure of Neonicotinoids for QqQ-LC/MS Run

- Weigh 10g of cocoa shells/nibs/beans
- Add distilled water (20ml) and leave for 15 min
- Add Acetonitrile (100ml)
- Homogenize (1500rpm for 1min)
- Centrifuge at 2500rpm for 5 min
- Filter

Supernatant 1

- Add Acetonitrile (50ml) to residue
- Homogenize (1500rpm for 1 min)
- Centrifuge (2500rpm for 5min)
- Filter

Supernatant 2

Discard Residue

Concentrate to about 15ml (below 40°C)

- Load residue onto Diatomaceous earth column and leave for 5 minutes
- Wash with n-Hexane (80ml)
- Elute with Dichloromethane (120ml)
- Concentrate to dryness (below 40°C)
- Dissolve residue with Acetonitrile (25ml)
- Condition GCB/NH_2 column with Acetonitrile (5ml)
- Load 5ml of residue dissolved in Acetonitrile
- Elute with Acetonitrile (30ml) & evaporate eluent to dryness (below 40°C)
- Dissolve concentrate in (10ml) acetonitrile/water (2:8)
- Filter (0.45µm pore size) into 2ml vials
- Run on QqQ-LC/MS

Appendix 1b: Extraction and Clean Up Procedure of Pyrethroids and Chlorpyrifos for GC/ECD Run

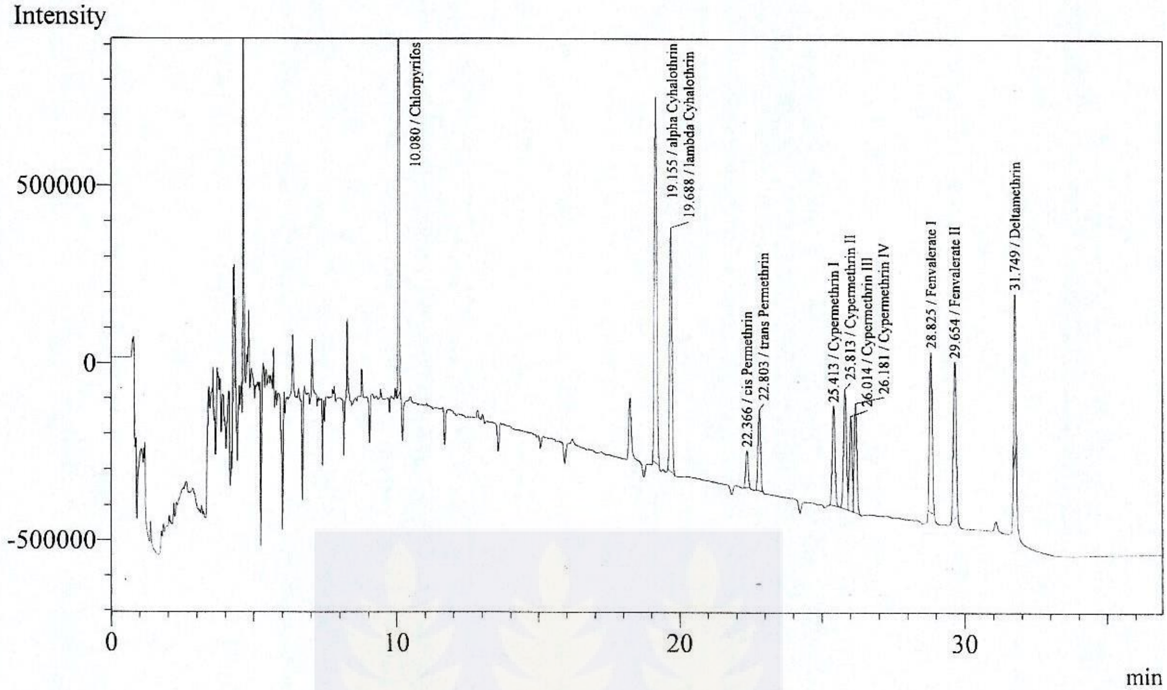
- Weigh 10g of cocoa shells/nibs/beans
- Add distilled water (20ml) and leave for 15 min
- Add Acetonitrile (100ml)
- Homogenize (1500rpm for 1min)
- Centrifuge at 2500rpm for 5 min
- Filter
  - Add Acetonitrile (50ml) to residue
  - Homogenize (1500rpm for 1 min)
  - Centrifuge (2500rpm for 5min)
  - Filter
    - Supernatant 2
    - Discard Residue

Supernatant 1

Concentrate to about 15ml (below 40℃)

- Load residue onto Diatomaceous earth column and leave for 5 minutes
- Add 80 ml Ethyl acetate/ n-Hexane (1:4)
- Concentrate to dryness (below 40℃)
- Dissolve residue with Acetonitrile (25ml)
- Condition GCB/NH_2 column with Acetonitrile (5ml)
- Load 5ml of residue dissolved in Acetonitrile
- Elute with Acetonitrile (20ml) & evaporate eluent to dryness (below 40℃)
- Dissolve concentrate in 2ml Diethyl ether/n-hexane (2:8)
- Condition SI (1000mg) column with 10ml n-hexane
- Load dissolved concentrate
- Elute with 10ml Diethyl ether/n-hexane (2:8) & evaporate eluent to dryness (below 40℃)
- Dissolve in 1ml n-hexane/acetone (1:1) and fill into 2ml vials
- Run on GC/ECD

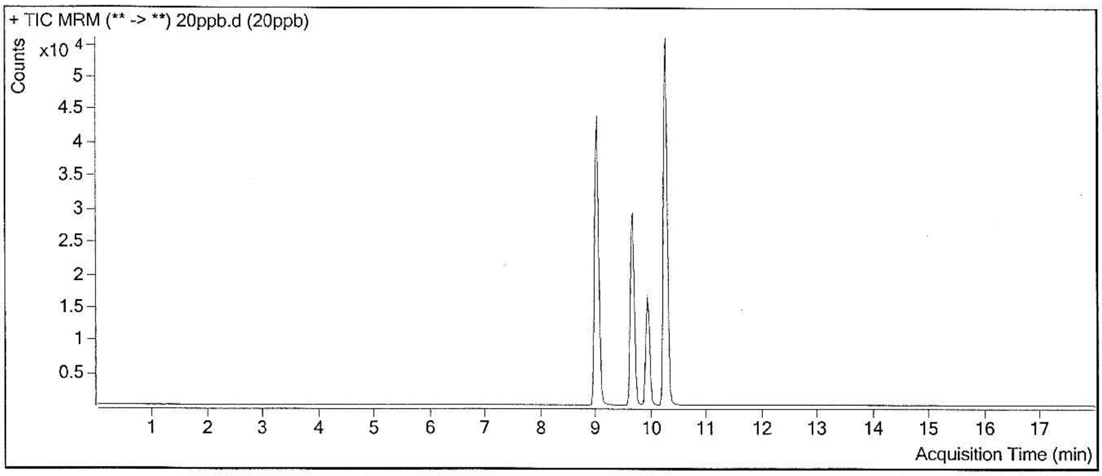
Appendix 2: Chromatogram of Organophosphorous Compound and Synthetic Pyrethroids from the GC/ECD



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	10.080	5282651	1608482	0.074	ug/m		1	Chlorpyrifos
2	19.155	6165000	1035072	0.079	ug/m		2	alpha Cyhalothrin
3	19.688	4066753	687555	0.080	ug/m		3	lambda Cyhalothrin
4	22.366	623080	105252	0.080	ug/m		4	cis Permethrin
5	22.803	1443939	229405	0.081	ug/m		5	trans Permethrin
6	25.413	1927179	280955	0.082	ppm		6	Cypermethrin I
7	25.813	2377997	338790	0.082	ppm		7	Cypermethrin II
8	26.014	1723885	267615	0.082	ppm	V	8	Cypermethrin III
9	26.181	2062866	309003	0.082	ppm	V	9	Cypermethrin IV
10	28.825	3085014	452975	0.081	ppm		10	Fenvalerate I
11	29.654	3095493	442421	0.082	ppm		11	Fenvalerate II
12	31.749	1855325	460461	0.080	ppm		12	Deltamethrin
Total		33709182	6217986					

Appendix 3: Chromatogram of Neonicotinoids from the QqQ-LC/MS

Sample Chromatogram



Quantitation Results

Compound	RT	Response	Conc(ng/mL)	Accuracy
Thiamethoxam	9.017	169648	20.2586	101.29
Clothianidin	9.682	101064	20.2247	101.12
Imidacloprid	9.947	46714	20.5546	102.77
Acetamiprid	10.287	211822	19.9196	99.60

Appendix 4: List of Standards, their Purities and Source

Active Ingredient	Purity (%)	Manufacturer
Chlorpyrifos	99.5	Wako Pure Chemical Industries Limited, Japan
Cypermethrin	96.0	Wako Pure Chemical Industries Limited, Japan
Fenvalerate	99.0	Wako Pure Chemical Industries Limited, Japan
Cyhalothrin (mixture of isomers)	98.0	Wako Pure Chemical Industries Limited, Japan
Deltamethrin	99.5	Wako Pure Chemical Industries Limited, Japan
Imidacloprid	98.0	Wako Pure Chemical Industries Limited, Japan
Acetamiprid	98.0	Kanto Chemical Company Limited, Japan
Thiamethoxam	99.8	Wako Pure Chemical Industries Limited, Japan
Clothianidin	99.7	Wako Pure Chemical Industries Limited, Japan

Appendix 5: Concentrations of the 10 Pesticides in the Cocoa Nibs as it Relates to the Districts and Regions Considered

Appendix 5a: Neonicotinoids

		Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid
Central Region	Agona Swedru	ND	ND	0.0027	ND
	Breman Asikuma	ND	ND	0.0019	ND
	Twifo Praso	ND	ND	0.0020	ND
Ashanti Region	Dadieso	ND	ND	0.0014	ND
	Obuasi	0.0018	ND	0.0042	ND
	Juaso	ND	ND	0.0049	ND
Eastern Region	Akim Oda	ND	ND	0.0018	ND
	Suhum	ND	ND	0.0013	ND
	Koforidua	ND	ND	0.0010	ND
Brong Ahafo Region	Goaso	ND	ND	0.0039	ND
	Kasapem	ND	ND	0.0026	ND
	Sunyani	ND	ND	0.0029	ND
Western South	Enchi	0.0011	ND	0.0028	ND
	Dunkwa	ND	ND	0.0033	ND
	Bogoso	ND	ND	0.0028	ND
Western North	Bonsu Nkwanta	ND	ND	0.0012	ND
	Sewfi Wiaso	ND	ND	0.0023	ND

*ND: Not Detected (<0.001mg/kg)

Appendix 5b: Synthetic Pyrethroids & Chlopryifos

		Chlorpyrifos	Lambda Cyhalothrin	Permethrin	Cypermethrin	Fenvalerate	Deltamethrin
Central Region	Agona Swedru	0.0155	ND	ND	ND	ND	ND
	Breman Asikuma	0.0166	ND	ND	ND	ND	ND
	Twifo Praso	0.0136	ND	ND	ND	ND	ND
Ashanti Region	Dadieso	0.0091	ND	ND	ND	ND	ND
	Obuasi	ND	ND	ND	ND	ND	ND
	Juaso	0.0166	ND	ND	0.0084	ND	ND
Eastern Region	Akim Oda	0.0107	ND	ND	0.0072	ND	ND
	Suhum	0.0129	ND	ND	0.0080	ND	ND
	Koforidua	0.0102	ND	ND	ND	ND	ND
Brong Ahafo Region	Goaso	ND	ND	ND	ND	ND	ND
	Kasapem	0.0127	ND	ND	ND	ND	ND
	Sunyani	0.0093	ND	ND	ND	ND	ND
Western South Region	Enchi	0.0133	ND	ND	ND	ND	ND
	Dunkwa	0.0130	ND	ND	ND	ND	ND
	Bogoso	0.0130	ND	ND	ND	ND	ND
Western North Region	Bonsu Nkwanta	0.0096	ND	ND	ND	ND	ND
	Sewfi Wiaso	0.0123	ND	0.0132	ND	ND	ND

ND: Not Detected (<0.007 mg/kg for chlorpyrifos, cypermethrin, permethrin, deltamethrin and lambda cyhalothrin; <0.003 mg/kg for fenvalerate)

Appendix 6: Concentrations of the 10 Pesticides in the Cocoa Shells as it Relates to the Districts and Regions Considered

Appendix 6a: Neonicotinoids

		Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid
Central Region	Agona Swedru	0.0033	0.0015	0.0054	Nd
	Breman Asikuma	0.0021	Nd	0.0077	Nd
	Twifo Praso	0.0030	0.0013	0.0140	Nd
Ashanti Region	Dadieso	0.0018	Nd	0.0041	Nd
	Obuasi	0.0350	0.0012	0.0758	0.0019
	Juaso	0.0023	0.0013	0.0259	0.0011
Eastern Region	Akim Oda	0.0021	0.0018	0.0059	0.0011
	Suhum	0.0050	0.0010	0.0062	0.0013
	Koforidua	0.0013	Nd	0.0120	Nd
Brong Ahafo Region	Goaso	0.0024	Nd	0.0148	Nd
	Kasapem	0.0011	Nd	0.0250	Nd
	Sunyani	0.0017	Nd	0.0525	Nd
Western South	Enchi	0.0127	0.0011	0.0158	Nd
	Dunkwa	0.0023	0.0010	0.0093	0.0033
	Bogoso	0.0043	Nd	0.0076	0.0011
Western North	Bonsu Nkwanta	Nd	Nd	0.0081	Nd
	Sewfi Wiaso	0.0059	Nd	0.0176	Nd

ND: Not Detected (<0.001 mg/kg)

Appendix 6b: Synthetic Pyrethroids and Chlopyrifos

		Chlorpyrifos	Lambda Cyhalothrin	Permethrin	Cypermethrin	Fenvalerate	Deltamethrin
Central Region	Agona Swedru	0.0174	ND	0.0207	0.0250	ND	ND
	Breman Asikuma	0.0190	ND	0.0144	0.0250	0.0096	ND
	Twifo Praso	0.0148	ND	0.0290	0.0123	ND	ND
Ashanti Region	Dadieso	0.0180	ND	0.0176	0.0247	ND	0.0122
	Obuasi	0.0148	ND	0.0218	ND	ND	ND
	Juaso	0.0200	ND	ND	0.0150	ND	ND
Eastern Region	Akim Oda	0.0180	ND	ND	0.0128	ND	ND
	Suhum	0.0189	ND	ND	0.0141	ND	ND
	Koforidua	0.0396	ND	0.0073	0.0180	ND	ND
Brong Ahafo Region	Goaso	0.0108	ND	ND	ND	ND	ND
	Kasapem	0.0162	0.0269	0.0099	0.0177	0.0155	ND
	Sunyani	0.0156	ND	ND	0.0159	ND	ND
Western South	Enchi	0.0160	ND	0.0215	0.0124	ND	ND
	Dunkwa	0.0166	ND	0.0185	0.0140	ND	ND
	Bogoso	0.0129	ND	0.0175	0.0102	ND	ND
Western North	Bonsu Nkwanta	0.0141	ND	0.0095	0.0146	ND	ND
	Sewfi Wiaso	0.0299	ND	0.0178	ND	ND	ND

ND: Not Detected (<0.007 mg/kg for chlorpyrifos, cypermethrin, permethrin, deltamethrin and lambda cyhalothrin; <0.003 mg/kg for fenvalerate)

Appendix 7: Concentrations of the 10 Pesticides in the Whole Cocoa Beans as it Relates to the Districts and Regions Considered

Appendix 7a: Neonicotinoids

		Thiamethoxam	Clothianidin	Imidacloprid	Acetamiprid
Central Region	Agona Swedru	0.0000	ND	0.0013	ND
	Breman Asikuma	0.0030	ND	0.0031	ND
	Twifo Praso	0.0012	ND	0.0125	ND
Ashanti Region	Dadieso	ND	ND	0.0029	ND
	Obuasi	0.0023	ND	0.0037	ND
	Juaso	0.0020	0.0012	0.0200	ND
Eastern Region	Akim Oda	0.0011	0.0012	0.0032	ND
	Suhum	0.0022	0.0022	0.0018	ND
	Koforidua	ND	ND	ND	ND
Brong Ahafo Region	Goaso	ND	ND	0.0069	ND
	Kasapem	ND	ND	0.0079	ND
	Sunyani	ND	ND	0.0026	ND
Western South	Enchi	0.0028	ND	0.0063	ND
	Dunkwa	0.0021	0.0010	0.0046	ND
	Bogoso	0.0017	ND	0.0036	ND
Western North	Bonsu Nkwanta	ND	ND	0.0032	ND
	Sewfi Wiaso	0.0014	ND	0.0051	ND

ND: Not Detected (<0.001 mg/kg)

Appendix 7b: Synthetic Pyrethroids and Chlopyrifos

		Chlorpyrifos	λ Cyhalothrin	Permethrin	Cypermethrin	Fenvalerate	Deltamethrin
Central Region	Agona Swedru	ND	ND	ND	0.0103	ND	ND
	Breman Asikuma	ND	ND	ND	0.0084	ND	ND
	Twifo Praso	0.0173	ND	ND	ND	ND	ND
Ashanti Region	Dadieso	0.0189	ND	0.0085	ND	ND	ND
	Obuasi	0.0189	ND	ND	ND	ND	ND
	Juaso	ND	ND	ND	0.0097	0.0033	ND
Eastern Region	Akim Oda	ND	ND	ND	0.0072	ND	ND
	Suhum	ND	ND	0.0122	ND	ND	ND
	Koforidua	0.0377	ND	ND	ND	ND	ND
Brong Ahafo Region	Goaso	0.0161	ND	ND	ND	ND	ND
	Kasapem	0.0113	ND	ND	ND	0.0123	ND
	Sunyani	0.0168	ND	ND	ND	ND	ND
Western South	Enchi	0.0219	ND	0.0122	0.0094	ND	ND
	Dunkwa	0.0161	ND	ND	ND	ND	ND
	Bogoso	0.0138	ND	ND	ND	ND	ND
Western North	Bonsu Nkwanta	0.0202	ND	ND	ND	ND	ND
	Sewfi Wiaso	0.0342	ND	ND	ND	ND	ND

ND: Not Detected (<0.007 mg/kg for chlorpyrifos, cypermethrin, permethrin, deltamethrin and lambda cyhalothrin; <0.003 mg/kg for fenvalerate)