LEVELS OF SELECTED PESTICIDE RESIDUES IN COCOA BEANS FROM ASHANTI AND BRONG AHAFO REGIONS OF GHANA

BY

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the university, except where due acknowledgement has been made in the text.

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DEDICATION

This dissertation is dedicated to my parents, Mr and Mrs Boakye for their encouragement and support throughout my life.

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ABSTRACT

The monitoring of pesticide residues in food has become a priority objective worldwide in order to get an extensive evaluation of food quality and to avoid harm to human health. This study presents results of analysis conducted on twenty one pesticide residues in cocoa beans sampled from the Brong Ahafo and Ashanti regions of Ghana. The pesticides were extracted from cocoa beans using liquid liquid extraction followed by clean up with solid-phase extraction cartridges. Final extracts were dissolved in ethyl acetate and analysis carried out by Gas Chromatography with Electron Capture Detector. Pesticides were identified by their retention times and quantification using an external calibration method. Twenty pesticide residues were detected in the cocoa bean samples. Aldrin was below detection limit in the cocoa beans sampled from the two regions. Chloropyrifos exhibited the highest concentrations of 10.55 mg/kg for samples from Mim in the Brong Ahafo region and 9.81 mg/kg for samples from Offinso in the Ashanti region. The lowest pesticide concentration of 0.01 mg/kg was recorded for Endosulfan I for samples from Sankore in the Ashanti region and Delta HCH which had a value of 0.01 mg/kg for samples from Offinso, Apagya, Juaso and New Edubiase respectively. From the results it could be deduced that 50% of the pesticides residues detected in the Brong Ahafo region were above the EU allowable limit whereas 45% was recorded for Ashanti region.

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attack pests that feed on the cocoa beans. These pesticides contribute may to high concentrations of residues in the cocoa beans . The bags into which cocoa beans are packed may also be contaminated with pesticides that can result in pesticide residues in the cocoa beans.

Alpha and Delta HCH residues were detected in all the cocoa beans sampled in the different locations in the Brong Ahafo region. However, beta and gamma isomers were present in 80 to 90% of the samples analysed. Among the HCH isomers there was an increasing trend for the concentrations of this class of pesticides as follows delta < beta< gamma< alpha. The concentration of Delta HCH ranged from 0.01 mg/kg for cocoa beans from Sankore to 0.05 mg/kg for cocoa beans from Nyinase A (Table 4.2).

ABBREVIATIONS

POPs	Persistant Organic Pollutants
OPs	Organophosphate pesticides
OCPs	Organochlorine Pesticides
MRL	Maximum residue limits
CDCP	Centers for Disease Control and Prevention
EU	European Union
FAO	Food and Agricultural Organization
UNEP	United Nations Educational Programme
WHO	World Health Organisation

ICCO	International Cocoa Organization
AAB	Acetic Acid Bacteria
NIP	National Implementation Plan
GAP	Good Agricultural Practices
PIC	Prior Informed Consent
LOD	Limit of Determination
LOQ	Limit of Quantification
JMPR	Joint Meeting on Pesticide Residues
WPPR	Working Party on Pesticide Residue
USGS	United States Geological Survey

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CHAPTER ONE

1. INTRODUCTION

The main cash crop in Ghana is cocoa and the country currently has annual production of around 850,000 metric tonnes (Ghana web, 2013). Cocoa represents around 30% of Ghana's total export earnings and it is the second most important export commodity after gold (ICCO, 2006). Ghana is the second largest cocoa producer after Côte d'Ivoire (ICCO, 2006). A report screened by Reuters Television indicates that Ghana produces fourteen percent (14%) of the total production of the world's cocoa (Reuters, 2009). Cocoa serves as the major source of revenue for the provision of socio-economic infrastructure in the country (Anon, 1995). In terms of employment, the industry employs about 60% of the national agricultural labour force in the country (Appiah, 2004). For these farmers, cocoa contributes about 70-100% of their annual household incomes (COCOBOD, 1998). In terms of quality, Ghana is recognized as the world leader in premium quality cocoa beans production and the quality of Ghana's cocoa has been a benchmark for assessing cocoa from other countries (Osei, 2008).

Cocoa is affected by a range of pests and diseases, with some estimates putting losses as high as 30% to 40% of global production (ICCO, 2010). In Ghana, cocoa mirids have been recognized as a serious pest since 1908 due to their devastating effect (Dungeon, 1910). The most common species of mirids in Ghana and West African countries are *Distantiella theobroma* and *Sahlbergella singularis*. Mirids are the major insects that affect cocoa worldwide. Mirid damage alone, if left unattended to for three years, can reduce yields by as much as 75% (ICCO, 2010).

Pesticides have been used on cocoa for more than 50 years, with notable early research carried out independently in Ghana, Nigeria, Brazil, Cameroon, Costa Rica, Côte d'Ivoire, Indonesia, Malaysia and Togo. By the early 1970s, a number of effective control techniques had become established, and there was little incentive for change until environmental awareness of pesticides increased in the 1990s (ICCO, 2010). Most notable amongst these were concerns over the use of lindane for the control of cocoa insect pests. The use of lindane was eventually phased out because of its persistence and toxicity- but in some countries, not until the early 21st century (Bateman, 2008). Endosulfan was restricted for cotton pest control but is now misused for the control of capsids on cocoa (Obayashie *et al*, 2009).

Since 1950, the use of pesticides in the world has increased 50 folds and 2.5 million tons of industrial pesticides are now used annually (Farag *et al*., 2011). In 1970, 2.7 x10^5 L of insecticides were used in Ghana (Foeline, 2000). Twenty years later the use of insecticides has been increased to 1.9 x 10^6 L, in addition to 360,900 L of herbicides and 150,000 kg of fungicides (Foeline, 2000). Results of field surveys conducted show that chemical pesticides appear to be the most important agents for controlling pests (Gerken *et al.* 2001). In Ghana, the majority of pesticides used in agriculture are employed in the forest zones located in the Ashanti, Brong Ahafo, Western and Eastern Regions of the country (Amoah *et al.*, 2006). The Ashanti and Brong Ahafo regions were chosen as sampling areas because the majority of Ghana's cocoa is produced from these areas. More large-scale farmers (85% of the farmers in that group) than small-scale farmers (74%) used chemical pesticides. A study by Childs (1999) indicates as well that pesticides are used by more than two thirds of farmers.

The use of organochlorine insecticide has proved to be effective in Ghana. The current recommended insecticides for cocoa are Actellic/Talstar (Pirimiphos methyl), Akate Master (Bifenthrin), Actara (Thiamethoxam), Promecarb and Confidor (Imidacloprid) (ICCO, 2010). Organochlorine pesticides (OCPs) are a large class of multipurpose chlorinated hydrocarbons that have been extensively applied in recent decades against pests and have a long history of use in the United States and around the world. Most organochlorine insecticides are very stable solids with limited vapour pressure and very low water solubility. The hazardous nature of organochlorines is a result of their toxicity in combination with high chemical and biological stability and a high degree of lipophilicity (Biziuk, 1996). These aforementioned characteristics make the OCPs prone to bioaccumulation along the food chain involving a wide range of trophic levels (Fildago, 2003). Foetuses and children may be exposed to pesticides *in utero* as well as through breast milk (Jurewicz and Hanke, 2008).

It has been reported that, in 1978, data submitted to the Food and Agricultural Organization / World Health Organization (FAO/WHO) indicated that the levels of lindane in cocoa beans from Ghana were between 0.0 and 0.3 mg kg^{-1} and between 0.051-0.10 mg kg^{-1} in cocoa butter. The residue in cocoa mass was 0.038 mg kg^{-} which were below the established FAO/WHO maximum residue limit of 1.0 mg kg^{-1} **(**FAO/ WHO, 1978**).** It has been reported that the persistence of pesticides depends on such factors as, formulation in which the chemical is applied, the mode of application, the type of pesticide applied, the geographical location, temperature, moisture, soil organic matter, soil pH, clay content, the type of soil and microbial activity (Hill *et al.,* 1967, Iwata *et al.,* 1973, Ohisa *et al.,* 1982). Most of organochlorine pesticides have been banned because they are highly persistent insecticides, and their residues still appear as

pollutants in food as well as in the environment (Abou-Arab, 1999). Ghana's primary legislation regulating the use of pesticides is the Environmental Protection Act of 1994 while the two main bodies responsible for pesticides surveillance and monitoring are the EPA – whose Chemicals Control and Management Centre is directed by the Pesticides Registrar – and the Plant Protection and Regulatory Services Directorate (PPRSD). The Customs, Excise and Preventive Service (Management) Law of 1993 regulates all imports into Ghana including chemicals (Northern Presbyterian Agricultural Services and Partners, 2012).Some of these chemicals, despite the current ban and their restrictive use in the developed world, have found increasing use in developing countries because of their lower cost and effectiveness. As also described by Danso *et al.* (2002), farmers mix cocktails of various pesticides to increase their potency.

Many benefits have been achieved from the use of synthetic pesticides in agriculture, but in spite of the obvious advantages, the potential adverse impact on food consumers health must be considered. Although pesticides are manufactured under very strict regulation processes to function with logical certainty and minimal impact on human health and the environment, serious concerns have been raised about health risks resulting from residues in food (Damalas and Eleftherohorinos, 2011; Eskenazi *et al*., 2008). Research data available have indicated the presence of pesticide residues in dairy products, meat (Darko and Acquaah, 2007, 2008) fish, water, sediments (Darko *et al.*, 2008), human blood and breast milk (Ntow, 2001), fruits and vegetables (Hanson *et al*. 2007; Hussain *et al*., 2002; El-Nahhal, 2004). Many studies have linked organochlorine pesticides exposure with consumption of contaminated food and product mostly meat, diary, fish and marine animals (Fitzgerald *et al.*, 2001, Hagmar *et al.*, 2001, Mwevura *et al.*, 2002, Bradman *et al.*, 2007). OCPs accumulate in the environment and adipose

tissues after consumption of food including cocoa products, posing problems to human health (Ejobi *et al.*, 1996). According to the Centers for Disease Control and Prevention (CDCP) in the USA, most people have organochlorine pesticides present in their bodies (CDCP, 2005). Exposure to low concentrations of organochlorine chemicals over a long period may eventually lead to a substantial body burden of the chemicals (Quintana *et al*., 2004). OCPs have a wide range of both acute and chronic health effects, including cancer, neurological damage and birth defects. Many OCPs are also suspected as endocrine disruptors (Walorczik, 2008).

Organophosphate pesticides (OPs) are synthetic in origin and are normally esters, amides or thiol derivatives of phosphoric acids. Currently, more than 30% of the registered pesticides on the world market (Hill, 2003) and about 45% of those registered with USEPA (Roger and Dagnac, 2006) are organophosphates. OPs, because of their high toxicity, fast biodegradation, low bioaccumulation and broad target spectrum are extensively used in the agricultural and veterinary practices. Their ability to degrade made them an attractive alternative to the persistent OCPs. However, their intensive and indiscriminate application, as well as their high acute toxicity generated risks to human and the environment (Stoytcheva *et al*, 2002). Some OPs such as diazinon have significant lipid solubility allowing fat storage with delayed toxicity (Gallow and Lawryk, 1991). OPs irreversibly inactivate acetylcholinesterase, which is essential to nerve function in insects and mammals. Even at relatively low levels, organophosphates may be hazardous to the brain development of fetuses and young children.

In the Federal Republic of Germany, maximum permissible quantities of authorised OPs in food range from 0.01- 0.05 mg/kg for food products of animal origin and between

0.02 and 3 mg/kg for food products of plant origin (Höchstmengen *et al.,* 1999). For the general population, intake of OPs occurs mainly via residues on or in food; daily OPs intake by the general population has been estimated to be 5-10 µg in the USA (Yess 1991, Gunderson, 1995), 7 µg in Finland (Penttila and Siivenen, 1996) and 67 µg in Italy (Leoni *et al* 1995). The results of residue analyses in Germany suggest a situation comparable to that in the USA (Angerer and Hardt 1997). In 1999 the US Environmental Protection Agency (USEPA) initiated a complete review of all pesticides. The first class to be evaluated was the OPs because of their threat to human and all mammalian life (EPA, 2003). As a result of the evaluation, the EPA eliminated OPs production in the US and began a program to phase OPs out of use (EPA, 1999). In the absence of OPs, synthetic pyrethroid pesticides, which were derived from the chrysanthemum in the 1970's, are quickly increasing in popularity because of their high efficiency against insects, greater stability in the environment and a greater safety to human and wildlife (Al-Makkawy *et al.* 1999).

Pyrethroids are as effective as chlorpyrifos, the most commonly used organophosphate, which puts them in a place to be a realistic and competitive replacement to organophosphates (Tang *et al.* 1996). Synthetic pyrethroid pesticides were derived in the early 1970's but have not been widely used until recently due to the recent reduction in OPs use (Drenner *et al.* 1993). The widespread use of pyrethroids began in the 1980s after the development of photostable pyrethroids like permethrin and fenvalerate. Although pyrethroids are generally safer for the environment than OPs, it is more difficult to determine if they are causing problems in the environment than OPs because they are toxic at such small concentrations (Almakkawy *et al.,* 1999).

Since cocoa is affected by a range of pests and diseases that reduce yields of global cocoa production as high as 75%, it becomes necessary to apply pesticides to control, prevent and destroy these organisms. After application, pesticides are degraded by chemical and physical processes in the environment. The rate of break-down depends on many factors, including the stability of the pesticide in question, but factors such as temperature, pH, moisture, aeration and mode of application are extremely important. However these factors may be unfavourable and the time between the application and harvest may be limited thereby preventing the conversion of harmful components of pesticides into their less toxic derivatives prior to exportation. Allowing sufficient time to elapse between application and harvest enables any residue to degrade to acceptable levels (*i.e.* the MRL). Reducing the dosage reduces the time to which acceptable levels are reached, but pest control may be impaired.

Recently, there have been some complaints about high levels of chemical residues in cocoa beans in general (Osei, 2005). With the signing of the Stockholm Convention on Persistent Organic Pollutants and the development of global monitoring programs, there is an increased need for laboratories in developing countries to determine pesticides by establishing food safety agencies that will regulate the pesticide residual concentrations in food commodities (Muir and Sverko, 2006). Standards and regulations for the export markets have increased dramatically over the last decade in number and stringency in response to food safety scares and a rise in concern for health, environmental, and social aspects of food. This has led to the establishment of maximum residue limits by the national or international food safety authorities which are playing an increasingly decisive role in deciding market access and are becoming, in a sense, a defacto mandatory requirement for the agriculture exporter. Statutory maximum

residue levels (MRLs) for pesticides in food and water have been defined in most countries and residue analysis has become a requirement to support the enforcement of legislation (Pico, 2003). Various organizations such as United States- Environmental Protection Agency (US-EPA), United States Food Drug Administration (US-FDA), European Union (EU) , Food and Agricultural Organization /World Health Organization (FAO/WHO) have issued directives aimed at specifying maximum permissible levels which are to be incorporated in the national regulations of the member states within a specified period. The MRL for most pesticide residues in cocoa beans imported into Japan and EU should not exceed 0.1 mg/kg.

The Maximum Residue Level (MRL) is the maximum amount of the pesticide residue which if found in food substances will not cause any health hazard (Gerken *et al*., 2001). MRLs encourage food safety by restricting the concentration of a residue permitted on a commodity, and by limiting the type of commodity in which it is allowed (European Communities, 2005; FAO/UNEP/WHO, 1991; FDA, 2005). Monitoring programs have therefore been established to control pesticide residues in food of plant origin in order to ensure compliance with national and international law, and to reassure consumers that food crops are healthy. Since Ghana is one of the leading cocoa exporters worldwide, it is therefore necessary to monitor the levels of pesticide residues in cocoa beans to determine whether the cocoa beans produced in Ghana conforms to international standards.

1.1 OBJECTIVES

The objectives of this study are to:

- Determine the levels of pesticides residues (DDT, DDD, aldrin, dieldrin, heptachlor, heptachlor epoxide, endosulfan 1 and 2. cypermetrhrin 1 and 2 technical cypermethrin, fenvelerate, bifenthrin, profenefos, alpha, beta, gamma and delta HCH, chloropyrifos, permethrin 1 and2) in cocoa beans from Ashanti and Brong Ahafo regions of Ghana using Gas Chromatography with Electron Capture Detection (GC- ECD).
- Compare the levels with Maximum Residual Levels established by various organizations to determine their suitability for consumption.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 History of cocoa

Columbus, on his fourth voyage to America, reportedly discovered a canoe off the Yucatan Peninsula laden with fruit and cocoa beans. But it was only years later at the beginning of the 16th century that Cortez confirmed the remarkable value assigned to the cocoa beans (ADM, 2006). He found that the Aztecs, Olmec and the Mayas valued them so much that they used them both as means of payment and as the source of a beverage drunk at court and religious ceremonies. Because of the Aztecs' belief that Cortez was the reincarnation of their God Quetzalcoatl, he was showered with gifts and honours, including cocoa beans. The cocoa beans were then consumed primarily in the form of a drink known as *xocolatl*, the Aztec name for the bitter stimulant.

The Spanish refined the recipes adding sugar and heating the ingredients to improve the taste. By 1828, the cocoa press was developed, allowing the extraction of cocoa butter Although its exact origins are not known, the cacao tree was then exclusive to the Americas. The closest estimates put the area of origin in and around the valleys of the Amazon and Orinoco Rivers. Evidence suggests that the tree has been cultivated for more than 3,000 years (ADM, 2006). In November 2007, archaeologists reported finding evidence of the oldest known cultivation and use of cacao at a site in Puerto Escondido, Honduras, dating from about 1100 to 1400 BC (Penn, 2007).

2.2 Production of cocoa in Ghana

Cocoa was introduced to West Africa in the nineteenth century. It was brought to Ghana from Fernando Po in 1879 and from Sao Tome in the 1880's. History attributes the

commercial cultivation of cocoa in Ghana to Tettey Quarshie, a native who had travelled to Fernando Po and returned with Amelanodo cocoa pods (Canatus and Aikins, 2009). The first documented shipment of beans from Ghana was in 1891, when 2 bags were sent from Accra to Hamburg, and since then, cocoa has been the main export crop and a major source of foreign exchange and domestic income earner (Canatus and Aikins 2009). Until 1977, Ghana was the world's leading producer of cocoa with the market shares ranging from 30-40%. Records indicate that production increased from a level of 36.3 metric tons in 1891 to an all time peak of about 557,000 metric tons in 1965 giving Ghana a global output share of about 33% and the leading cocoa producer. Thereafter, production continued to drop and reached the lowest level of 158,956.00 metric tons in 1984, which constituted about 9% of world's production (Joy, 2010). Consequently, Ghana lost her position as the world's number one producer.

As part of efforts to arrest the decline in cocoa production, the Government of Ghana through Cocoa Board initiated a National Cocoa Diseases and Pest Control (CODAPEC) programme, popularly known as "Mass Spraying" to assist all cocoa farmers in the country to combat the capsid/mirid and the Black Pod disease. Under this programme, cocoa farms across the country were sprayed with insecticides and fungicides at no cost to the farmers. Also introduced is the Hi Tech programme. These programmes have resulted in tremendous increases in cocoa bean production from 340,562 metric tons in the 2002 season to 496,846 metric tons in 2003 and 736,000 metric tons in the 2004 seasons, respectively (Appiah, 2004; ICCO, 2004). The percentage of locally processed beans has also jumped from 20% to 35% with further re-capitalization and expansion programs underway to reach a target of 50% in the near future. It has therefore been the intention of government, which is committed to reaping the maximum benefit from the

cocoa sector, to ensure that the country increases its cocoa production and also processes more of the beans into downstream products for both the local and export markets (Awuah, 2002). However, along with the positive effects of the CODAPEC programme, some negative impacts on the environment have also been caused. For instance the use of pesticides on the farms can lead to the destruction of part of the soil flora and fauna through both physical and chemical deterioration (Cowell and Clift, 1997). While the Government of Ghana stresses the need for diversifying the nation's economic structure, it also emphasizes the important role of the cocoa sector and has set the target of achieving one million metric tons of cocoa output by 2010 (NDPC, 2006; IITA, 2007). For the 2010-2011 cocoa season, Ghana attained an unprecedented level of more than one million tonnes of cocoa production (Ghana web, 2012)

The trend is towards increasing the value added in the country, with the share of processed cocoa beans and cocoa intermediate products increasing. This will increase the need for external and internal laboratory tests in the cocoa sector. The Ghana Cocoa Board has developed, through its specialized Quality Control Company Limited (QCCL), a highly recognized expertise and internationally trusted reputation in maintaining consistently high quality of exported cocoa beans. Through intensive training of small holder farmers in disease control, pruning of trees and the observance of the required best practices in the fermentation of beans, and through the enforcement of strict grading procedures, QCCL has been able over the years to maintain Ghana's cocoa quality that meets strict specifications in commodity exchanges with price quotations for future delivery, and consistently fetches premiums on world market prices. (CFC and ICCO, 2007)

2.3 Classification of Cocoa (Theobroma cacao)

Cacao tree belong to the family of sterculiaceae and the genus *Theobroma*. Its natural habitat is the lower storey of the evergreen rainforest. There are over twenty species in the genus but *Theobroma cacao* is the only one cultivated widely (Ojo and Sadiq, 2010). Since its discovery in the 18th century at the Amazon basin, its cultivation has spread to other tropical areas of south and central America, and indeed West Africa, which became the major producer from the mid 1960s. Recently, with the application of molecular marker, cocoa was re-classified to belong to the family Malvaceae (Alvenson *et al*., 1999).

Traditionally, two main genetic groups, "Criollo" and "Forastero", have been defined within cacao based on morphological traits and geographical origins (Cheesman, 1944). A third group, "Trinitario", has been recognized and consists of "Criollo - Forastero" hybrids (Cheesman., 1944). For other authors, "Criollo" and "Trinitario" should be considered as traditional cultivars rather than genetic groups (Motamayor *et al*., 2002). Two other traditional cultivars have been described: Nacional and Amelonado (Motamayor *et al*., 2002). Nonetheless, a sound classification of *Theobroma cacao* L. populations, based on genetic data, is lacking for the breeding and management of its genetic resources. Genetic improvement of cocoa through breeding has focused on increasing yield and disease resistance. To increase yield, breeders have capitalized on heterosis that occurs in crosses between trees from different genetic groups (Warren, 1992).

2.4 Factors that affect cocoa yield

The successful cultivation of cocoa requires a special climate that is mostly found within the area bounded by the Tropics of Cancer and Capricorn. The majority of the world's crop is now grown within 10° North and South of the equator. It will grow from sea level up to a maximum of some 1,000 meters, although most of the world's crop grows at an altitude of less than 300 meters (ADM, 2006).

Daily, seasonal, or annual variations in the values of the climatic element are of greater importance in determining the efficiency of crop growth (Ayoade, 2004). A number of factors have an interrelated impact on the growth of cocoa plant. These factors range from the weather element of rainfall, temperature, sunlight and humidity to others such as, pest and diseases as well as soil nutrient status (Ojo and Sadiq, 2010). Cocoa is highly sensitive to changes in climate, particularly to temperature due to its effects on evapo-transpiration (Anim-Kwapong and Frimpong, 2005). The mean minimum temperature is between 20 ^{o}C to 22 ^{o}C while the mean maximum during the dry and wet season varies from 31^{o}C to 32 ^{o}C and 27 ^{o}C to 29 ^{o}C, respectively (Wood and Lass., 1987). It is observed that the higher the temperature (Maximum of 32 ^{0}C), the higher the yield, while the lower the relative humidity, the better the yield. Total annual rainfall of the cocoa growing areas of these countries ranges from 1200 mm to 3000 mm. Cocoa is known to produce well with minimal but sustained water availability throughout the year (Obatolu *et al*., 2003). Rainfall must be well distributed across the year, with a minimum of 1,000 mm. Meanwhile, yearly variation in the yield of cocoa is affected more by rainfall than any other climatic factors. Cocoa prefers calm conditions and persistent moderate wind can cause a severe damage to yield.

2.5 Harvesting and fermentation of cocoa beans

During the last decade knowledge about the cocoa fermentation process has been increasing (Adhana and Fleet 2003, Camu *et al* 2007, Lagunes *et al*, 2007, Nielson *et al* 2005, Nielson *et al*, 2007, Schwan and Wheals 2004). While a vast amount of research has been undertaken to speed up the cocoa bean fermentation process, there has been little success. Clearly, the different stages of fermentation are essential in the creation of the complex organic components essential to the final taste and enjoyment of cocoa.

In the last 5 years, the microbiology and biochemistry of Ghanaian cocoa bean heap fermentation processes have been studied in detail (Camu *et al*, 2007, Jespersen *et al* 2005, Nelsen *et al* 2005, Nielsen *et al* 2007). The micro biota involved in natural cocoa bean fermentation process reflects the environmental factors pH, oxygen tension and temperature, and the metabolism of substrates of the cocoa bean pulp .This results in production of significant amounts of ethanol, lactic acid and acetic acid representing a succession of yeasts, lactic acid bacteria and acetic acid bacteria in the cocoa bean fermentation course (Ardhana and Fleet 2003, Schwan 1998, Schwan and Wheals, 2004).

During early and mid-time spontaneous fermentation of freshly harvested pulp and cocoa beans piled into a heap, yeasts produce ethanol under anaerobic conditions causing depectinization of the pulp enabling the pulp to flow away and air ingress (Schwan *et al*, 2004). Citrate and pulp sugars are converted to acetic acid, lactic acid and mannitol by yeasts enabling a slight increase in pH of the pulp (Camu *et al*, 2007).

During the aerobic phase, *Acetobacter pasteurianus* is the main AAB species involved in spontaneous cocoa bean fermentation; additionally, AAB oxidize the ethanol of the yeasts into acetic acid. Also changes in nature and concentration of amino acids, pyrazines, peptides, polyphenols and alkaloids during cocoa bean fermentation, mainly

through endogenous enzymatic activities, microbial consumption and conversion, or physical diffusion (Camu *et al* 2007, Hansen *et al.*, 1998, Hashim *et al.*, 1998, Jinap *et al.*, 2003, Jinap and Zeslinda 1995, Jinap *et al.*, 1995, Yoigt *et al.*, 1994).

Lactic acid bacteria and pectins are broken down by pectinases, which results in liquefaction of the pulp and starts the second aerobic phase of fermentation. Acetic acid produced by acetic acid bacteria is key metabolite of the cocoa bean fermentation process. The lactic acid bacteria plays a role in fermentation of cocoa as acidification inhibits the growth of spoilage agents. The volatile short chain fatty acid diffuses into the bean and this combination together with the heat produced by the exothermic conversion of the ethanol into acetic acid, causes the death of the seed embryo, the disruption of the internal cellular structure of the beans and the end of fermentation (Camu *et al.*, 2007, Nielsen *et al.*, 2007). In turn, biochemical changes in the beans are initiated, leading to the enzymatic formation of precursor molecules that are necessary for the development of the characteristic aroma, flavour, and colour of the beans (de Brito *et al.*, 2000, Hansen *et al.*, 1998, Holm *et al.*, 1993). These properties are developed further during drying, roasting, and final processing of well-fermented cocoa beans (Thompson *et al.*, 2001).

2.6 Drying of cocoa

Fermentation is followed by sun drying for 7 to 8 days. Cocoa beans should preferably be sun-dried to a moisture content of 7.5%. When artificial drying is used, the process should mimic sun drying, using low temperature/ambient air for the initial drying with higher temperatures only for the final stage. Because of the high rainfall and cloud cover in Brazil and Malaysia, the beans are dried using mechanical rotary driers (ADM, 2006).

2.7 Pest of cocoa beans

Ghana bags about 850,000 tonnes of cocoa in a year and stores it at warehouses but there are a lot of constraints or losses due to its storage. One of the main constraints is insect pest infestation. Literature shows that the development of relatively cheap and very effective methods as pesticides and fumigants being the most preferred choice in insect management (Registration Requirements for Adjuvant Products, 1993).

Currently several fumigants are used against stored product insect pest. For fumigants with phosphine, solid metal phosphine are used in cocoa beans (Aluminum phosphide) and warehouse (Magnesium phosphide). Phosphine generators mix the metal phosphide with moisture to rapidly produce gaseous phosphine which can be delivered to the site in cylinder and released directly to the cocoa beans (Regnault, 2005). Other fumigant includes hydrogen cyanide, ethyl formate. suphuryl fluoride, hydrogen cyanide, carbon disulphide, and ethyl formate (Regnault, 2005).

Pesticides are applied either to cocoa beans either directly or crevices in warehouses . The most common contact insecticide used are: (1)Organophosphates (Malathion, Dichlorvos, fernitriothion, Pirimiophos methyl, Chlorpyrifos methyl, Chlorpyrifos with deltamethrin); (2) Pyrethrins (bioresmethrin, permethrin and deltamethrin) (3) Synergizer (Saleem, 1996).These pesticides are sprayed during the storage of cocoa beans to attack pests that feed on the cocoa beans. These pesticides contribute may to high concentrations of residues in the cocoa beans . The bags into which cocoa beans are packed may also be contaminated with pesticides that can result in pesticide residues in the cocoa beans.

2.8 Pesticides

Pesticides are a group of chemicals made for the purpose of killing or otherwise deterring "pest" species. The word *pesticide* may refer to insecticides, herbicides, fungicides, or other pest control formulations. Pesticides are inherently toxic and often associated with adverse health effects in non-target organisms (US EPA, 2009). Pesticides can be classified by target organism, chemical structure and physical state (AMA, 1997). It can also be classed as inorganic, synthetic or biologicals (AMA, 1997). examples of synthetic pesticides are organochlorine, carbamate, pyrethroid and organophosphate

FAO has defined the term of pesticide as: any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit and substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport (FAO, 2002). Pesticides have been used in the public health sector for disease vector control and in agriculture to control and eradicate crop pests for the past several decades in Ghana (Clarke *et al.,* 1997). There has been a rapid rise in the quantity of pesticides used in agriculture over the past ten years (Hogson, 2003). Until the early 1980s, many chlorinated insecticides, mainly; aldrin, dieldrin, DDT, and lindane have been used in controlling pests of crops, vectors of some diseases and other aspects of public health in

Ghana (UNEP, 2002). Some of these pesticides are still widely used by farmers because of their effectiveness and their broad-spectrum activity (Amoah *et al.*, 2006).

Persistent Organic Pollutants (POPs) are organic chemical substances that are carbon-based. Persistent Organic Pollutants (POPs) are compounds that resist photochemical, biological and chemical degradation (Kanan *et al*, 1994, Parimi *et al*, 2006). POPs possess a particular combination of physical and chemical properties such that, once released into the environment, they remain intact for exceptionally long periods of time; become widely distributed throughout the environment as a result of natural processes involving soil, water and, most notably, air; accumulate in the fatty tissue of living organisms including humans, and are found at higher concentrations at higher levels in the food chain; and are toxic to both humans and wildlife (Stockholm Convention, 2004).

Pesticide residue is the residual amount of active components of a particular pesticide or group of pesticides found in a commodity after the pesticide has accomplished the primary purpose of its application; or the residual amount of a pesticide found in a product which has been in the area of the pesticide application (Essumang *et al.*, 2009). When a pesticide product is applied on the field, the chemical is gradually lost as a result of breakdown, leaching and evaporation and the residue is the amount that remains after application (Cox, 1995). While some pesticides have long residual activity and therefore persist in the environment, others have short residual activity, disappear from the environment or produce low residue concentration. Pesticide residues on crops are monitored with reference to Maximum Residue Limits and are based on analysis of quantity of a given Active Ingredient remaining on food product samples.

2.9 Organochlorine pesticides

OCPs are synthetic chlorinated hydrocarbons and include DDT and derivatives, hexachlorocyclohexane (HCH), aldrin, dieldrin, heptachlor and endosulfan as well as some commercial or industrial chemicals and by-products of combustion such as polychlorinated biphenyl (PCBs), hexachlorobenzene (HCB), polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (Osibanjo, 2003). The essential structural feature about organochlorine insecticides is the presence of carbon-chlorine bonds. The peculiar characteristics of these chemicals are that they have a relatively high chemical stability and persist in the environment for long periods. They are transboundary pollutants travelling long distances from their point of release in the environment and bioaccumulate in humans and other organisms through the food chain. OCPs are highly lipophilic and resistant to microbial degradation (UNEP, 2001; Kaushik and Kaushik, 2007). The lipophilic character increases with an increasing number of chlorine atoms in the molecule, thus contributing to the sorption of these pollutants onto organic particles. Human beings, like other mammals, have lipid-rich tissues that efficiently retain and accumulate lipophilic contaminants (Travis *et al.,* 1988) and they transfer much of the contaminant loads to offspring during nursing, resulting in trans-generational transfer of contaminants (Kanja *et al.,* 1992; Waliszewski *et al.,* 2001; Mun˜ oz-de-Toro *et al.,* 2006). It is of public health importance to evaluate OCPs in humans because these persistent organic pollutants, which have weakly estrogenic or antiestrogenic effects (Dalvie *et al.,* 2004), are considered endocrine disrupters. Organochlorines are among the chemicals found most often in the hundreds of tests of human body tissue that have been conducted around the world. Even those chemicals that have been banned for decades are showing up consistently in food samples tested by the U.S. Food and Drug Administration (Schafer *et al.,* 2001). These lipophlic OCPs, with high resistance to degradation and long half-lives in humans, have been confirmed

to bioaccumulate in blood, breast milk, and adipose tissues of humans through dietary intake (Smith and Gangolli, 2002).

The concentration of fat-soluble contaminants like the OCPs is expected to be considerably higher in breast milk than in whole blood because the blood flow to the breast is much more rapid than is the rate of milk secretion. The residues of OCPs in breast milk can reflect a maternal body burden and can be used to estimate the bioaccumulative dose in a nursing infant. Elevated levels of p,p DDT and p,p DDE in human milk have been found in most developing countries located in the tropics, including Mexico (Waliszewski *et al.*, 1999), Thailand (Stuetz *et al*., 2001), Guangzhou in China (Wong *et al*., 2002) and Vietnam (Minh *et al*., 2004) because of continuous human exposure to DDT used for malaria control until the end of 1990s.

In contrast, low p,p DDT and p,p DDE levels in human milk have been found in developed countries, such as Germany (Fu¨ rst *et al*., 1994), Sweden (Nore´n and Meironyte´, 2000), Japan (Konishi *et al*., 2001) and United Kingdom (Kalantzi *et al.*, 2004), due to the increasing duration of restrictions or bans on OCPs. Literature data show that the residues of these compounds have been found at every level of the food chain (Hayes and Laws, 1991).

2.10 Organophosphate pesticides

Organophosphates are organic esters of phosphoric acid, thiophosphoric acid and other phosphoric acids. The organophosphorus compounds, first developed by Gerhard Scharder in Germany, are widely employed over the world for their broad spectrum insecticidal and short persistence in the environment. Forty organophosphate pesticides are registered in the U.S, with at least 73 million pounds used in agricultural and residential settings (Latimes, 2010). Since the removal of OCPs from use, OPs have

become the most widely used insecticides. In developing countries OPs are widely used because they are cheaper than the newer alternatives.

Some organophosphates travel long distances and persist in cold climates. Researchers have detected OPs in Arctic and sub-Arctic environments. Ice core samples from Svalbard, Norway, revealed five organophosphate compounds: chlorpyrifos, terbufos, diazinon, methyl parathion, and fenitrothion. The Arctic Monitoring and Assessment Program 2009 reports the presence of chlorpyrifos in a number of locations: Surface water, ice, and fog from the Bering and Chukchi seas; Alaskan snow and fish from Alaskan parks; and Arctic and Subarctic Canadian lakes.

Organophosphate pesticides degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Many metabolites (or breakdown products) of organophosphates are more toxic than the primary chemicals themselves--making them acutely lethal to sensitive species like amphibians (USGS, 2007). Major metabolites of chlorpyrifos and malathion were 100 times more toxic than their parent compounds to frogs in California's Central Valley, where nearly 25% of the nation's organophosphate use is concentrated. Because organophosphates share a common toxicity mechanism, exposure to several OP insecticides and their breakdown products could intensify their toxic effects. OPs share a common mechanism of cholinesterase inhibition and poison insects and mammals by phosphorylation of AchE enzyme nerve endings.

OPs are regularly detected at low levels in a range of food items. Usually residue levels are below the statutory maximum residue levels. OP residues found in UK carrots has proved a recent exception. Ministry of Agriculture, Fisheries and Food figures for 1995

showed that 1-2% of carrots contain OP residues up to 25 times higher than expected. OPs implicated included chlorfenvinphos, quinalophos and triazophos. In the higher residue samples, the acceptable daily intake was exceeded by up to three times (Pesticide Safety Directorate, 1995).

During the early 2000, the EPA began phasing out residential uses of the two primary OPs, diazinon, and chlorpyrifos (CDPR 2008). EPA's decision to eliminate certain uses of the OP insecticides because of their potential for causing toxicity in people, especially children, has led to their gradual replacement with another class of insecticides, the pyrethroids (Oros and Werner, 2005)

2.11 Pyrethroids

Pyrethroids are synthesized derivatives of naturally occurring pyrethrins which are taken from pyrethrum, the oleo resin extract of dried chrysanthemum flowers. The 1st generation pyrethroids, developed in the 1960s, include bioallethrin, tetramethrin, resmethrin and bioresmethrin. They are more active than the natural pyrethrum, but are unstable in sunlight. By 1974, the Rothamsted team had discovered a 2nd generation of more persistent compounds notably: permethrin, cypermethrin and deltamethrin. Pyrethrins and pyrethroids are estimated at 23% of the insecticide world market, with more than 3500 registered formulations, and are widely used in agriculture, public health and food preparation. (USEPA, 2005; Casida and Quistad, 1998).

Synthetic pyrethroid insecticides have been used for more than twenty years to control insect pests in a variety of crops (Maund *et al*., 2001), but they have become increasingly popular following outright bans or limitations on the use of cholinesterase-inhibiting insecticides (Luo and Zhang, 2011; Feo *et al*., 2010). In 2001, EPA withdrew the residen-

tial registrations for two commonly applied OP pesticides, chlorpyrifos and diazinon (US EPA 2000; 2001), resulting in a significant increase in the market penetration of the pyrethroid products. Today, pyrethroids are used in agriculture, forestry, horticulture, public health and are active ingredients of many insect-control products intended for indoor home use (Feo *et al.,* 2010).

The insecticidal properties of pyrethrins are derived from keto alcoholic esters of chrysanthemic and pyrethroic acids. These acids are strongly lipophilic and rapidly penetrate many insects and paralyse their nervous system (Reigart *et al.,* 1999). The insecticidal activity of synthetic pyrethroids was enhanced further by the addition of a cyano group at the benzylic carbon atom to give alpha-cyano (type II) pyrethroids. They are substantially more resistant to degradation by light and air, thus making them suitable for use in agriculture. Pyrethroids are usually combined with piperonyl butoxide, a known inhibitor of key microsomal oxidase enzymes. This prevents these enzymes from clearing the pyrethroid from the body of the insect, and assures the pyrethroid will be lethal and not merely a paralyzing agent. The acute toxicity calculated by LD_{50} ranges from low to high, depending on the specific formulation. Low toxicity is attributed to two factors, limited absorption of some pyrethroids and rapid absorption and biodegradation in mammalian liver (Reigart *et al.,* 1999). Pyrethroids are popular for insecticides because exoskeletons of insects are sufficiently porous to pyrethroids.

Pyrethroids, the synthetic analogues of naturally occurring pesticides were developed to capture the effective insecticidal activity of this botanical insecticide, with increased stability in light, yielding longer residence times. Characteristics of pyrethroids insecticides include high efficiency, wide spectrum, low biodegradability (Pap *et al.*, 1996) , low mammalian and avian toxicity while also demonstrating strong selectivity for insects and invertebrates (Ecobichon, 1996; Fishel, 2005). The low vapor pressures and high octanol-water coefficients of pyrethroids indicate a low propensity to volatilize and a high affinity for organic matter, soils and clay (Oros and Werner, 2005). Sorption to particulates reduces bioavailability to non target organisms, and also makes it more likely that these insecticides will be retained at the application site.

Pyrethroid releases into the terrestrial environment occur largely via spray drift from both agricultural and non-agricultural applications, although accidental spills and direct application to soil surfaces can also be considered sources of release. However, because of their strong tendency to adsorb to soils and organic matter, these compounds are unlikely to undergo significant migration from areas of direct application, except on particulates that are carried by wind or water. Photolysis is likely a significant degradation pathway for pyrethroids in the soil, and is influenced by soil characteristics.

2.12 Routes of exposure to pesticides

Diet or food consumption is an important pathway of human exposure to POPs and ingestion represents the main route of exposure for POPs for the general population compared to other exposure routes such as inhalation and dermal contact (Liem, 1999; Sweetman *et al.*, 2000; Falandysz *et al.*, 2002). Some investigations have confirmed that more than 90% of human contaminants come from food (Fu¨ rst, *et al.*, 1990). Most

exposure to these chemicals occurs via occupation, dietary intake especially food of animal and plant origin, water, ambient and indoor air, dust, and soil (Cruz *et al*., 2003).

2.12 Toxicological effects of pesticides

WHO/FAO (1990) estimated an annual worldwide total of 3 million cases of acute and severe pesticide poisoning with some 220,000 deaths. The majority of these cases of poisoning and deaths occur in the developing countries, although far greater quantities of pesticides are used in the developed countries (Bhanti *et al*., 2004). Most pesticides show a high degree of toxicity because they are intended to kill certain organisms and thus create some risk of harm (Abdelgadirand and Adam, 2011; Zidan, 2009).

The toxicological effects are mostly due to the inhibition of acetylcholinesterase in the nervous system, resulting in respiratory, myocardial and neuromuscular transmission impairment (Goh *et al.*, 1990). The toxic effect of OCPs seems to be linked to a chain reaction of their gradual dechlorination in body fluids and the formation of free radicals interfering with subcellular structures. Chronic toxicity studies have shown carcinogenic properties in some chlorinated insecticides, benzenes and phenols (Hougton, 1991, Millikan, 1995, WHO, 1991). Because these chemicals are toxic to living organisms, increased accumulation in the food chain may pose serious health hazards to the general populace (Jayashree and Vasudevan, 2007). There is much evidence to show that OCPs interact with the endocrine system, resulting in numerous biological effects that may affect the health of humans and animals (Mun˜ozde- Toro *et al.,* 2006). Many organochlorines are known or suspected hormone disruptors, and recent studies show that extremely low levels of exposure in the womb can cause irreversible damage to the reproductive and immune systems of the developing foetus. Studies also revealed that

OCPs have strong potential to cross placental barriers even in minute concentration and cause serious neonatal damage. Exposure to OCPs, particularly to DDT and its metabolites has been associated with adverse health effects, including neuro developmental delay (Ribas-Fito´ *et al.*, 2003), reproductive effects (Dalvie *et al.*, 2004b), gestational-age babies (Lon gnecker *et al*., 2001), and immunotoxicity (Cooper *et al*., 2004). Similarly, epidemiological studies have suggested an etiological relationship between exposure to OCPs and Parkinson's diseases (Fleming *et al*., 1994).

2.13 Bans on the use of pesticides

According to Pesticide Action Network (PAN), the organophosphate parathion is one of the most dangerous pesticides (Kegley *et al*., 2010). The WHO, PAN and numerous environmental organisations propose a general and global ban (Northern Presbyterian Agricultural Services and Partners, 2012). It is banned in 23 countries and its import is illegal in a total of 50 countries. Its use was banned in the U.S. in 2000 and it has not been used since 2003. EPA banned residential uses of chlorpyrifos in 2001 but the pesticide is still widely used in fields and orchards across the US (CSN, 2010).

Most of these OCPs are agricultural pesticides or industrial compounds that also double as environmental pollutants. In response to their adverse effects seen in wildlife and humans, the production and use of these compounds were banned in industrialised countries during the 1970s (Jaraczewska *et al.*, 2006), or subjected to restrictions in use in many others. As at December 2008, the organochlorine pesticides; aldrin, chlordane, DDT, dieldrin, endrin, lindane (HCH), heptachlor were among banned pesticides by Environmental Protection Agency of Ghana. OCPs; aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, toxaphene, hexachlorobenzene constitute ten of the twelve

chemical substances currently defined under the Stockholm Convention on POPs (Afful *et al.*, 2010). While bans and phase-outs of these chemicals occurred during the 1970s and 1980s in most developed countries, they were not in place in many developing countries (Muir and Sverko, 2006). Article 7 of the Stockholm Convention requires National Implementation Plans (NIPs) to be developed by signatory countries (UNEP, 2001). For countries where OCPs are still in use or loosely regulated, the NIPs will eventually lead to the phase-out of OCPs for agricultural use and reduced use for disease vector control.

In developing countries, farmers face immense risks of exposure owing to the use of toxic chemicals that are banned or restricted in other countries (Nasr *et al*., 2007; Al-Eed *et al*., 2006; Adhikari, 2010). Although the use of almost all the POPs listed under the Stockholm and Rotterdam Conventions have been banned in Ghana since 1985, the intensive past applications of these chemicals for agricultural and public health purposes suggest that they may still be present in the environment (Ghana NIP, 2007). The NIP of Ghana which is the official blue print for the implementation of the Stockholm Convention on POPs indicates that almost 1 tonne of HCB and technical DDT were imported into Ghana in 2001 and 2002 (Ghana NIP, 2007). Anecdotal reports also show that technical DDT and lindane (which have been officially discontinued as restricted chemicals for use on cocoa since 1985 and 2002 respectively) have been misapplied on non-target food crops. The residual levels of technical DDT and lindane in foodstuffs and biota are, however, expected to be decreasing significantly in the face of the ban as well as the restrictions on their massive applications (Adu-Kumi *et al.*, 2010).

Prior Informed Consent is a process which identifies and shares government decisions to ban or severely restrict pesticides, and includes dissemination of decisions to importing countries where information may be difficult to obtain. Pesticides currently in the PIC Convention include: aldrin, captafol, chlorobenzilate, chlordane, chlordimeform, DDT, dieldrin, dinoseb, 1,2-dibromoethane, fluoroacetamide, lindane, heptachlor, hexachlorobenzene, mercury compounds, and certain formulations of parathion, methamidophos, monocrotophos, and phosphamidon (Bateman, 2008).

2.14 Maximum Residue Limits

The levels of pesticide residues on crops are monitored with reference to maximum residue limits (MRLs), which are established by each country and sometimes cause conflicts because residue levels acceptable in one country could be unacceptable in another (Torres *et al.*, 1996). The required rates of application may vary, under different agricultural and climatic conditions, from country to country, and between regions of the same country (Torres *et al.*, 1997). An MRL is the maximum concentration of a pesticide (active ingredient) residue likely to occur in or on food or animal feed after use of the pesticide according to Good Agricultural Practice (GAP) (1CCO, 2008). The MRL is expressed in mg of residue per kg food. For many pesticides, however, this is set at the Limit of Determination (LOD). LOD can be considered a measure of presence/absence, but true residues may not be quantifiable at very low levels. For this LOQ is often quoted in preference (usually approximately 2X the LOD).

The MRL is usually determined by measurement, during a number (in the order of 10) of field trials, where the crop has been treated according to GAP and an appropriate pre-harvest interval has elapsed. Field trials are conducted to establish how a particular

pesticide should be used under GAP (Bateman, 2008). MRLs are adopted by bodies like the EU as legal limits to residues on crop products. MRLs for pesticides in various crop products imported into the EU, including cocoa beans, are set out in Annexes to regulation 396/2005/EC (USDA, 2009). For MRLs are often mistaken for toxicological safety limits. If an MRL is exceeded it takes an additional calculation to establish whether the toxicological limit is exceeded. However MRLs must be toxicologically acceptable. However, in many cases they are much lower than the toxicological limit simply because no more is necessary to achieve adequate control of the pest (EC, 2004).

2.15 Organizations that have established MRL's in cocoa beans

Standards and regulations for the export markets have increased over the last decade dramatically in number and stringency in response to food safety scares and a rise in concern for health, environmental, and social aspects of food. In view of increasing consumer awareness of food safety issues, traceability is becoming an important agenda for the global cocoa market. Markets now require minimum residue levels of pesticides, mycotoxins, PAH, heavy metals etc, in cocoa beans. Pesticide residue monitoring programs are the only tool to control the quantity of pesticides on food and to enforce tolerances. These programs are mainly conducted by official laboratories and place emphasis in raw agricultural products. The data collected under these regulatory monitoring is extensive (European Commission, 2001; FAO/UNEP/WHO, 1991; FDA, 2005; JMPR, 1999; MAFF, 2000; The Netherlands Food Inspection Service, 2002).

The Joint FAO/WHO Food Standards Programme and the *Codex Alimentarius* Commission were set-up to protect the health of consumers and ensure fair practices in the food trade. It was initially believed that, if all countries harmonized their food laws and adopted internationally agreed standards, such issues would be dealt with naturally.

Through harmonization, the founders envisaged fewer barriers to trade and more freedom of movement among countries. One scientific committee is the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) established in 1963 following a decision by FAO Conference that the *Codex Alimentarius Commission* should recommend maximum residue limits for pesticide and environmental contaminants in specific food products to ensure the safety of foods containing residues. It was also decided that JMPR should recommend methods of sampling and analysis. However, problems arise from the fact that cocoa importing countries use different methodologies to establish MRLs and different analytical methods to determine pesticide residues in cocoa beans (ICCO, 2011). Different MRL levels in different countries and different measurement technologies constitute complications for international trade in cocoa. These issues call for harmonization in legislation and its implementation. In the EU and USA, samples of cocoa beans are first de-husked before residue analysis takes place, whereas at the time of writing, whole beans are analysed in Japan and Australia (ICCO, 2011).

The EU Regulation is designed to safeguard the health of consumers of cocoa products, by highlighting the need to use pesticides appropriately so that residue levels are not exceeded in cocoa destined for Europe. Recent changes to legislation in the EU and Japan have concentrated minds over crop protection practices in cocoa and other commodity crops (Bateman, 2008). The crop protection activities of farmers and middlemen will therefore be of great concern to all in the cocoa trade, some of whom may have a limited working knowledge of pesticide science. Technologies are presently available for the safe use of pesticides in cocoa and awareness on their correct and proper use needs to be stimulated. At the same time, consumers seek cocoa of high quality, containing minimal pesticide residues (ICCO, 2011). Hence the introduction of

maximum residue limits, which restrict the allowable pesticide content in cocoa and cocoa products to protect consumers. From the 1st September 2008, assessment of the quality of cocoa imported into the EU will include measurement of traces of substances that have been used upstream in the supply chain, including pesticides used on farms or in storage (Bateman, 2008). International trade in foodstuffs and raw materials originating from plants requires compliance with these national and international maximum limit value regulations.

In Japan, the Food Sanitation Law was modified on 29 May 2006, with analysis of cocoa included on a "positive list" published by the Ministry of Health, Labour and Welfare. This is a system that prohibits distribution of foods that contain agricultural chemicals above a certain maximum residue limit that has not been implemented. Such agricultural chemicals include over 600 pesticides, veterinary drugs, and feed additives (Standards and Evaluation Division, 2006). Some samples were found to have excessive residue levels and shipments were rejected. During the period January to October 2009 alone, there was approximately 4,800 metric tons of cocoa beans banned from importation to Japan (ICA, 2009). In the USA, the EPA established the Food Quality Protection Act (FQPA) of 1996 and was considered approximately equivalent to 91/414/EEC but regulates the amount of pesticide residues permitted on food for consumption (ICCO, 2010). International Standard ISO 22000 on Food Safety Management Systems provides information, plans, principles and recommendations to work towards greater food safety in the cocoa supply chain (Bateman, 2010)

2.16 Analytical methods for the determination of pesticides

Analytical methods for the analysis of OCPs are widely available and are the result of a vast amount of environmental analytical method development and research on persistent organic pollutants (POPs) over the past 30–40 years. Access to modern capillary gas chromatography (GC) equipment with either electron capture or low-resolution mass spectrometry (MS) detection to separate and quantify OCPs is essential. However, screening of samples, especially in areas of known use of OCPs could be accomplished with bioanalytical methods such as specific commercially available enzyme-linked immuno absorbent assays (Muir and Sverko, 2006). New analytical techniques such two-dimensional GC (2D-GC) and "fast GC" using GC–ECD may be well-suited for broader use in routine OCP analysis in the near future given their relatively low costs and ability to provide high-resolution separations of OCPs. At present, more than 60% of registered pesticides and their metabolites can be analyzed by using gas chromatography.

GC ECD is the most widely used technique especially for the determination of OCPs in different matrices (Santos and Galceran, 2004; Bruner, 1993). For the isolation of target compounds from matrices various, extraction and clean-up procedures have been employed. Soxhlet extraction is considered to be the standard method used for the extraction of OCPs from soils. The soxhlet and shaking flask extractions are time consuming and require large volume of organic solvents (Bøwadt *et al.*, 1995; Hartonen *et al.*, 1997; Schantz *et al.*, 1998). Therefore, in order to reduce the extraction time, amount of solvent required, as well as sample amount, new extraction procedures, i.e., supercritical fluid extraction (SFE) (Bøwadt and Hawthorne, 1995), microwave assisted extraction (MAE) (Eskilsson and Björklund, 2000) and accelerated solvent extraction (ASE) (Björklund *et al.*, 2000) etc., have been developed as alternative techniques.

More recent procedures, i.e., SFE, MAE and ASE, gave shorter extraction time and reduced solvent consumption because these extraction procedures are working at high temperatures above the boiling point of the solvent. Except for SFE, preconcentration and clean-up steps have to be performed for MAE and ASE procedures. On the other hand, time and cost needed for SFE are quite high as well as for ASE (Berset *et al.*, 1999).

Trace analysis of OCPs in environmental samples is usually performed by GC combined with a previous extraction or a pre-concentration step including traditional liquid–liquid extraction (LLE) (Barcelo´, 1993, Fatoki and Awofolu, 2003; Tahboub *et al*., 2005), solid phase extraction (SPE) (Aguilar *et al*., 1996; 1997), solid phase microextraction (SPME) (Page and Lacroix, 1997; Aguilar *et al*., 1999; Tomkins and Barnard, 2002; Li *et al*., 2003; Dong *et al*., 2005) and the more recently developed liquid phase microextraction under different names i.e., dispersive liquid–liquid microextraction (DLLME) (Cortada *et al*., 2009a; Leong and Huang, 2009; Tsai and Huang, 2009), liquid-phase microextraction (LPME) (Huang and Huang, 2007; Farahani *et al*., 2008), single-drop microextraction (SDME) (Cortada *et al*., 2009b), polymer-coated hollow fiber microextraction (PC-HFME) (Basheer *et al*., 2004), stir bar sorptive extraction (SBSE) (Leo´n *et al*., 2003; Pe´rez-Carrera *et al*., 2007), ultrasound assisted emulsification-microextraction (USAEME) (Ozcan *et al*., 2009a), vortex assisted liquid-liquid microextraction (VALLME) (Ozcan, 2010). Anastassiades *et al*., developed a dispersive SPE method which involves the use of bulk SPE material as opposed to packed tubes to facilitate sample cleanup of the liquid extract prior to GC-MS analysis (Lebotey 2004, Anastassiades *et al*., 2003). LLE is probably the most widely used method for the extraction of OCPs (Barcelo´, 1993, Fatoki and Awofolu, 2003;

Tahboub *et al*., 2005). In the SPE method, analytes may be adsorbed, and complex matrices can cause settling in cartridges (Leong and Huang, 2009; Ozcan *et al*., 2009a; Quayle *et al*., 1997).

USAEME procedure combines micro-extraction system and ultrasonic radiation in one step. Ultrasonic radiation is a powerful means for acceleration of various steps in analytical procedure for both solid and liquid samples (Priego-Lo´pez and Luque de Castro, 2003; Aydin *et al*., 2006; Tor *et al*., 2006a; 2006b; Ozcan *et al*., 2009a; 2009b; 2009c; 2010). USAEME technique leads to an increment in the extraction efficiency in a minimum amount of time (Luque de Castro and Priego-Capote, 2006; 2007). Some other advantages of USAEME are viable, simple, rapid, low cost, and it needs less amount of sample and extraction solvent (Ozcan *et al*., 2009a; Saleh *et al*., 2009; Luque de Castro and Priego-Capote, 2007). However, the disadvantage of this method is that excessive ultrasound energy may degrade the analytes in water and may cause irreversible damages to the properties of analytes (Luque de Castro and Priego-Capote, 2007; Sanchez-Prado *et al*., 2008).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Sampling

Dry cocoa beans were sampled from the main warehouse (Abuakwa and Kaase) where cocoa beans from different districts of the Ashanti (fig.3.1) and Brong Ahafo (fig.3.2). Regions of Ghana are received. The consignment was divided into smaller lots of about 30 bags and the split wire was applied as widely as possible to detect foreign matter. The split wire is made of copper metal of dimensions 50 mm long x 30 mm wide

A sampling horn made of aluminium metal was used to draw samples from all sides of each cocoa bag and bulked into a container The dimensions of the sampling horn was 100mm long x 15 mm internal diameter. The bulked sample was thoroughly mixed and quartered. Two quarters of the opposite sides were rejected. The process was repeated until a final sample of 300 beans was counted. One of the bags was sent to the laboratory for analysis (Quality Control Company Division, 1994).

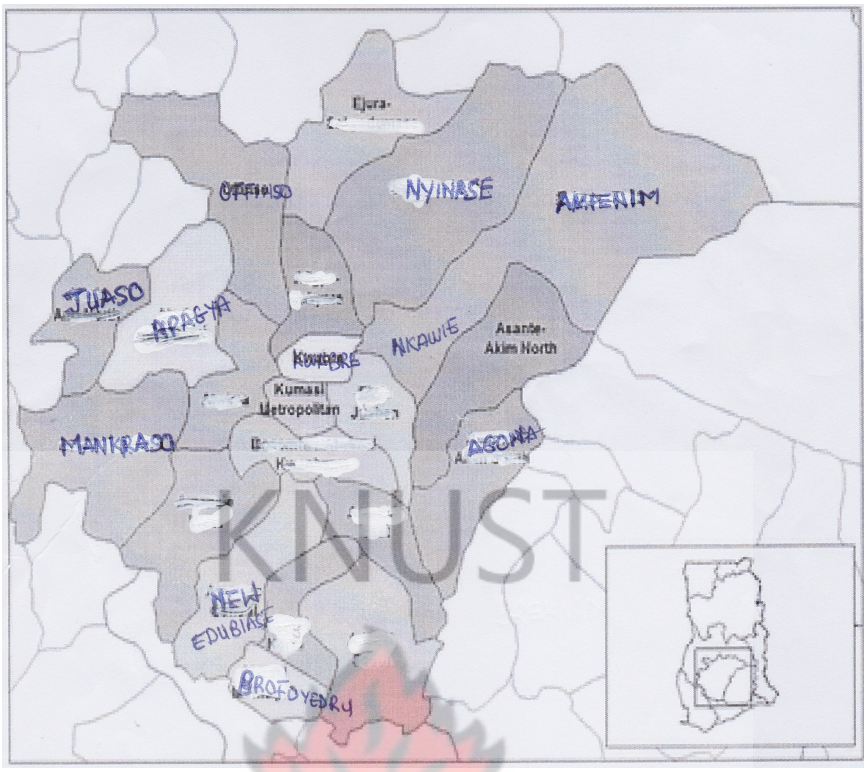


Figure 3.1: A map of Ashanti region showing the sampling areas

Figure 3.2: A map of Brong Ahafo region showing the sampling areas

3.2 REAGENTS AND MATERIALS

TABLE 3.1 Identification of reagents and materials

REAGENT	GRADE	SOURCE
Acetonitrile	Pesticides	Sigma Aldrich company, USA
Toluene	Pesticides	SMM chemicals Pty Ltd
Acetone	Pesticides	Labort Fine PVT Ltd, India
Hexane	Pesticides	BDH Laboratory Supplies, England
Sodium sulphate (anhydrous)	AnalaR	Labort Fine PVT Ltd, India
Sodium chloride	AnalaR	Avondale laboratory, England
Sodium hydroxide	AnalaR	BDH Laboratory Supplies, England
Dipotasium hydrogen phosphate	AnalaR	PX PARK Scientific Ltd, UK
Potassium dihydrogen phosphate	AnalaR	BDH Laboratory Supplies, England
Ethyl Acetate	AnalaR	SMM chemicals Pty Limited
Bond elute C18 SPE cartridge, 1g/6ml	-	Supelco USA (Analytical)
ENVI-Car/LC-NH_2,500mg/500mg,6ml		Supelco USA (Analytical)
Filter Paper, No. 4		Whatman International Ltd England

3.3 INSTRUMENTATION AND APPARATUS

TABLE 3.2 – EQUIPMENT IDENTIFICATION

Equipment	Type
Gas Chromatograph	Shimadzu GC – 2010 with AOC 20i Autoinjector and AOC 20S Autosampler and Electron Capture Detector
Analytical Column	30 m x 0.25 mm internal diameter fused silica capillary column coated with VF-5ms (0.25µm

	film).
Centrifuge	Sanyo (MSE) Harrier 18 / 80
Vacuum manifold	
Macerator	IKA Ultra Turrrax Homogenizer
General laboratory glassware	Round bottomed flasks, volumetric flasks, centrifuge tubes, separating funnels, funnels, measuring cylinders
Rotary film evaporator	Buchi Rotary evaporator (India)
Recirculating chiller	Buchi company (India)
Ultrasonic bath	Sanyo Company

3.4 ANALYSIS

3.4.1 Experimental Procedures

Before analysing the samples (before experiment) all glasswares were acid washed and cleaned with distilled water before they were dried in the oven at 200 ^{o}C for about four hours.

3.4.2 Preparation of standard solutions

All the pesticides standard stock solutions were prepared in ethyl acetate with the aid of an ultrasonic bath, by dissolving a weight of the pesticides which when corrected for purity will be equivalent to 1000 µg/ml (e.g. 10mg in 10ml). This was labelled as a Parent Standard Reference for each Pesticides standard e.g Aldrin, ALD 1.

These Parent Standard References were diluted for use as fortification standards in the procedural recovery process, and as calibration standards in instrument calibration. A

summary of the dilutions that were carried out for the fortification and calibration are presented in table 3.3 and table 3.4.

TABLE 3.3 Preparation of fortification standards and calibration standards.

References of standard solution used	Concentration (µg/ml)	Volume taken (ml)	Final Volume (ml)	Equivalent concentration (µg/ml)	References of standard solution produced
Each standard	1000	1.0	50	20	MIX 1
MIX 1	20	2.5	25	2.0	MIX 2
MIX 2	2.0	2.5	25	0.2	MIX 3
MIX 1	20	5	50	2.0	MIX 4

3.4.3 Fortification standard preparation

1 ml aliquot of each 1000 µg/ml pesticide standard prepared was measured into a 50 ml volumetric flask and adjusted to volume with ethyl acetate giving the resultant mixed standard concentration of 20 µg/ml MIX 1.

Further dilution was carried out by measuring 2.5 ml of MIX 1 into a 25 ml volumetric flask to give a resultant concentration of 2.0 µg/ml MIX 2. Another dilution was carried out measuring 2.5 ml aliquot of MIX 2 into 25 ml volumetric flasks with a resultant concentration of 0.2 µg/ml MIX 3.

3.4.4 Recovery

1 ml of MIX 1 was added to 20 g of an aliquot of a prepared matrix (1ml = 20 µg of each analyte). This is equivalent to a fortification level of 1 mg/kg. Extraction and clean – up procedures as in the methodology were carried out before its injection into the GC. Same

chromatographic conditions were used. This was repeated for fortification levels of 0.5 mg/kg and 0.2 mg/kg.

TABLE 3.4 Preparation of standard calibration standards

References of standard solution used	Concentration (µg/ml)	Volume taken (ml)	Final Volume (ml)	Equivalent concentration (µg/ml	References of standard solution produced
MIX 1	20	5	50	2.0	MIX 4
MIX 4	2	7.5	10	1.5	MIX 5
MIX 4	2	5	10	1.0	MIX 6
MIX 4	2	2.5	10	0.5	MIX 7
MIX 6	1.0	2.0	10	0.2	MIX 8

3.4.5 Instrument calibration standard preparation

MIX 1 was diluted by measuring 5.0 ml of its aliquot into a 50 ml volumetric flask. It was adjusted to the mark with ethyl acetate giving a resultant concentration of 2.0 µg/ml MIX 4.

MIX 4 was diluted by measuring 7.5 ml of its aliquot into a 10 ml volumetric flask. It was adjusted to the mark with ethyl acetate giving a resultant concentration of 1.5 µg/ml MIX 5

MIX 4 was further diluted with 5 ml of its aliquot into a 10 ml volumetric flask and adjusted to the mark with ethyl acetate giving a resultant concentration of 1.0µg/ml

MIX 6

MIX 4 again was diluted with 2.5 ml of its aliquot into 10 ml volumetric flask and adjusted to the mark with ethyl acetate giving a resultant concentration of 0.5µg/ml

MIX 7

MIX 6 was diluted by measuring 2.0 ml of aliquot into 10 ml volumetric flask and adjusted to the mark with ethyl acetate giving a resultant concentration of 0.2µg/ml

MIX 8

3.4.6 Preparation of 0.5 mol/l phosphate buffer (pH 7.0)

52.7 g of dipotassium hydrogen phosphate and 30.2 g of potassium dihydrogen phosphate were weighed in a 250 ml beaker. Distilled water was used for dissolution and the pH adjusted to 7 with 1 mol/l NaOH and 1 mol/l HCl solution and transferred into a 1.0 L volumetric flask.

3.4.7 Sample preparation

Sample preparation, extraction, cleanup and analysis were carried out according to the procedure described in multi-residue method for agricultural chemicals (Syoku-An , 2006).

The sample was milled using a Hammer Mill through inn sieve. 10 g was weighed into a 250 ml Nalgene jar. Distilled water (20 ml) was added and left for 15 min. An amount 40 ml of acetonitrile was added and homogenised for 2 min. It was then centrifuged at 3000 rpm for 3 min and filtered through flutted (No. 4) filter paper into a 100 ml volumetric flask. To the residue in the Nalgene jar and 20 ml acetonitrile, homogenised for 2 min and the dispersing element rinsed with 5 ml acetonitrile into the jar. The suspension was centrifuged at 3000 rpm for 3 min and was filtered into the 100 ml volumetric flask. The residue was rinsed with 15 ml acetonitrile, the filtrate made up to 100 ml with acetonitrile in the volumetric flask. An amount of 20 ml of the filtrate pipetted into a 250 ml separating funnel and 10 g of NaCl and 20 ml of 0.5 mol/l phosphate buffer (pH 7.0) was added, shaken for 20 min on an end to end shaker left to stand for 10min for longer sep-

aration and the aqueous layer was carefully discarded and the acetonitrile layer taken for clean - up.

3.4.6 Clean – up step 1 using bond elute C – 18 cartridges

Bond Elute C-18, 1g/6ml cartridge (Supelco, USA) was conditioned with 10 ml acetonitrile. A receiving flask was placed under the cartridge to collect elute. Approximately 20 ml of the sample extract was loaded into the cartridge and eluted with 2 ml acetonitrile. An amount of 5 g Na_2SO_4 was placed on a filter paper in a funnel and the extract dried over it. The container was rinsed with acetonitrile and passed through the Na_2SO_4. The sample solution was transferred into 50 ml round bottom flask and evaporated at 40 ^oC to dryness on a Buchi rotary evaporator. Residue was dissolved in 2 ml of 1:3 (toluene: acetonitrile)

3.4.7 Clean – up step 2 using ENVI – Carb/LC – NH_2

ENVI – Carb/LC – NH_2, (500mg/500mg)/6ml cartridge from Supelco (USA) was conditioned with 10 ml of 1:3 toluene: acetonitrile. The 2 ml extract from the previous clean – up step was loaded into the cartridge and eluted. The cartridge was eluted with 20 ml of 1:3 (toluene: acetonitrile). The sample solution was transferred into a 50 ml round bottom flask and evaporated at 40 ^oC to approximately 1 ml by rotary evaporator. An amount 10 ml of acetone was added to the flask and was concentrated near to dryness. It was then re – dissolved in 2 ml ethyl acetate, prior to analysis by GC – ECD. Extracts was then stored in a refrigerator until analysis.

TABLE 3.5 Summary of chromatographic conditions for pesticides

APPARATUS:	
Instrument	**Description**
Gas Chromatograph	Shimadzu GC – 2010 with AOC 20I Autoinjector and AOC

	20S Autosampler and Electron Capture Detector
Analytical column	30 m x 0.25 mm internal diameter fused silica capillary column coated with VF-5ms (0.25 µm film).
Temperatures:	
Item	**Conditions**
Injector	Splitless mode, temperature 225 ° C
Oven	60 ºC / 2 min 25 ºC/1min 180 ºC / 1min 5 ºC /1 min 300 ºC
Detector – ECD	300 ºC
Gases;	
Gas	**Flow rate**
Nitrogen (carrier)	1 ml/min constant flow

3.5 Data Analysis

The formula below was used for the calculation of various concentrations of pesticide residues in µg/ml to mg/kg

$$\frac{\left\{\left[\frac{C}{F}\right] x\,5\right\}}{M}$$

Where C=concentration in µg/ml

F= Concentration Factor (10)

M = mass in grams

Recovery

The equation below is used to calculate the percentage of the pesticides that were recovered during the analysis

The percentage recovery was calculated as:

$$\%\ \text{Recovery} = \frac{\text{Amount of analyte recovered}}{\text{Amount of analyte spiked}} \times 100$$

CHAPTER FOUR

4. RESULTS AND DISCUSSION

Pesticide residues were determined in cocoa beans from the Ashanti and Brong Ahafo regions of Ghana using Gas Chromatography with Electron Capture Detection. Validation of the method was achieved using spiking process at fortification levels of 2.0 1.5 and 1.0 µg/ml. The percentage recovery ranged between 67% to 130%. Concentrations of the pesticide residues in each cocoa bean samples were calculated (in mg/kg). A total of 20 pesticide residues were detected in cocoa bean samples, corresponding to percentage positive of 95.24%. Pesticide residues identified in the cocoa beans were HCH isomers, DDT and its metabolites, heptachlor and its metabolite, Dieldrin, endosulfan I and II, pyrethroids namely Bifenthrin, cypermethrin and its isomers, permethrin I and II, profenefos, fenvalerate and chloropyrifos. Individual standard solutions were prepared from stock standard solution and their individual retention times obtained injection. The individual retention times which were used to identify the detected pesticides in the cocoa beans are presented in Table 4.1

TABLE 4.1 SUMMARY OF RETENTION TIMES FOR PESTICIDES

STANDARDS	RETENTION TIME (min)
Beta HCH	13.736
Gamma HCH	13.867
Alpha HCH	13.953
Delta HCH	14.596
Chloropyrifos	16.924
Heptachlor Epoxide	18.574
Endosulfan I	19.621
Profenefos	20.107
Dieldrin	20.791
Aldrin	17.563
Endosulfan II	21.535
DDD	21.990
DDT	23.348
Fenvalerate	24.002
Bifenthrin	24.995
Heptachlor	25.292
Permethrin I	28.524
Permethrin II	28.772
Cypermethrin II	30.200
Cypermethrin I	30.566
Cypermethrin T	30.690

For the Multiresidue analysis, mixed standard solutions in ethyl acetate were prepared from each pesticide standard by serial dilutions of the stock standard. The chromatogram of standard mix is presented in the appendix

Alpha and Delta HCH residues were detected in all the cocoa beans sampled in the different locations in the Brong Ahafo region. However, beta and gamma isomers were present in 80 to 90% of the samples analysed. Among the HCH isomers there was an increasing trend for the concentrations of this class of pesticides as follows delta < beta< gamma< alpha. The concentration of Delta HCH ranged from 0.01 mg/kg for cocoa beans from Sankore to 0.05 mg/kg for cocoa beans from Nyinase A (Table 4.2). Most of the cocoa beans sampled from the Brong Ahafo had concentrations below the MRL value of 0.02 mg/kg established by EU with the exception of cocoa beans from Brekum and Nyinase A as depicted in Table 4.2 that were above the MRL. Cocoa beans sampled from Brong Ahafo region had concentrations of beta HCH which were below detection limit (0.0001 mg/kg) for samples from Sankore and Nyinase A (Table 4.2).

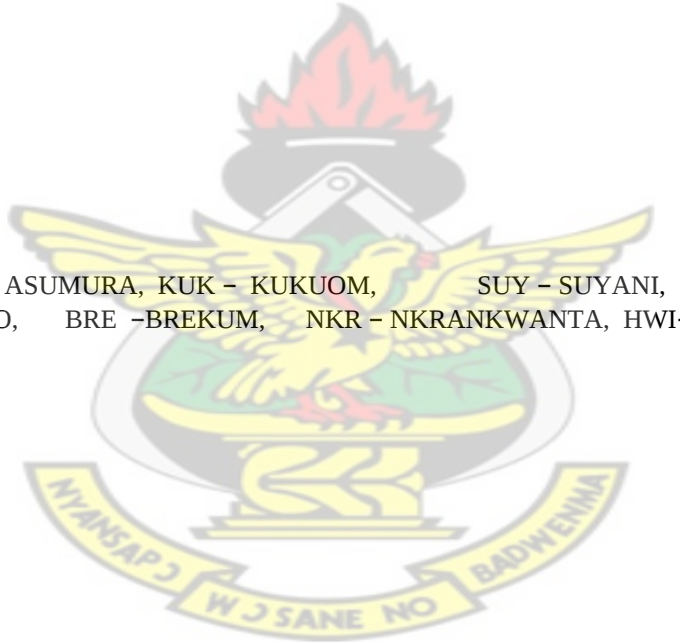
The highest concentration of 0.28 mg/kg was recorded for cocoa beans from Brekum. All the samples had concentrations above the EU MRL of 0.02 mg/kg with the exception of cocoa beans from Suyani and Kukuom. Levels of gamma HCH (lindane) in cocoa beans were below detection limits for samples from Brekum (Table 4.2). The highest concentration of 0.31 mg/kg was recorded for samples from Nyinase A (Table 4.2). All samples from Brong Ahafo recorded levels below the EU permissible levels for gamma HCH. Out of the total number of samples, 90% of the cocoa beans analysed had concentrations of lindane above Japanese MRL of 0.10 mg/kg except cocoa beans from Brekum. In the Brong Ahafo region concentrations of the alpha HCH ranged from

0.40 mg/kg for cocoa beans from Sankore to 0.85 mg/kg for cocoa beans from Nyinase A (Table 4.2). All the cocoa beans had concentrations above the MRL established by EU, Japan and Codex Alimentarius. From literature it is not common to find high levels

of alpha HCH residues in food samples especially in the tropics because the higher temperature conditions favour its photochemical degradation as compared with the temperate region (Stockholm Convention, 2007). Due to its persistence alpha-HCH can still be detected at low levels in the environment. In a study conducted in 14 districts of Haryana in India 140 bovine milk samples collected between 1998 and 1999 were analysed for organochlorine pesticide residues. Four percent (4%) of the samples exceeded the MRL of 0.05 mg/kg as recommended by WHO for alpha-HCH (Sharma *et al*., 2006). A monitoring study of 192 samples of cow's milk from Mexico revealed 0.001 - 0.201 mg/kg alpha-HCH (ATDSR, 2005).

Table 4.2 Pesticide residues levels in cocoa beans from Brong Ahafo region

CONCENTRATION OF PESTICIDE RESIDUES (mg/kg)												
PESTICIDES	**SAN**	**ASU**	**KUK**	**SUY**	**NYI**	**GOA**	**BRE**	**NKR**	**HWI**	**MIM**	**EU**	**JAPAN**
Chloropyrifos	6.86	9.26	7.16	9.05	8.52	1.88	8.96	7.67	8.96	10.55	0.10	0.05
Endosulfan I	0.01	0.05	0.04	0.04	0.04	0.04	0.20	0.06	0.20	0.06	0.10	0.10
Profenefos	0.09	0.14	0.11	0.12	0.10	0.11	0.11	0.12	0.11	0.07	0.10	
Endosulfan II	0.04	0.09	0.08	0.08	0.07	0.11	0.07	0.08	0.09	0.09	0.10	0.10
Fenvalerate	0.40	0.71	0.38	0.04	0.05	0.29	0.18	0.30	0.32	0.34	0.50	
Bifenthrin	0.03	0.08	0.05	0.05	0.07	0.11	0.05	0.07	0.07	0.06	0.10	0.10
Permethrin I	0.36	0.60	0.19	0.22	0.37	0.29	0.19	0.22	0.39	0.26	0.10	0.05
Permethrin II	1.10	0.27	0.71	0.18	0.18	0.13	0.18	0.11	1.90	1.22	0.10	0.05
Cypermethrin I	0.03	0.05	0.04	0.04	0.04	0.04	0.20	0.04	0.07	0.06	0.10	0.03
Cypermethrin II	0.14	0.17	0.13	0.13	0.13	0.13	0.05	0.15	0.34	0.21	0.10	0.03
Cypermethrin T	0.33	0.59	0.05	0.08	0.10	0.06	0.13	0.10	0.23	0.12	0.10	0.03
Beta HCH	ND	0.03	0.02	0.02	ND	0.05	0.28	0.03	0.05	0.05	0.02	
Delta HCH	0.02	ND	0.02	0.02	0.05	0.01	0.04	ND	0.02	0.02	0.02	
Gamma HCH	0.17	0.15	0.18	0.19	0.31	0.16	0.00	0.12	0.21	0.21	1.00	0.10
Alpha HCH	0.40	0.52	0.47	0.48	0.85	0.46	0.56	0.49	0.57	0.62	0.02	
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	0.10
Hepta-exo epoxide	ND	ND	ND	0.03	0.37	ND	0.05	ND	0.03	0.02	0.02	
Dieldrin	0.03	0.06	0.03	0.05	0.15	0.04	0.03	0.03	0.05	0.06	0.02	
4, 4 – DDD	0.03	0.03	0.04	0.03	0.06	0.03	0.04	0.03	0.02	0.03	0.50	
4, 4 – DDT	0.15	0.46	0.24	0.08	0.08	0.17	0.11	0.19	0.20	0.22	0.50	0.10
Heptachlor	2.67	2.79	2.51	1.90	0.13	2.86	0.14	2.63	3.74	3.55	0.02	



SAN –SANKORE, ASU – ASUMURA, KUK – KUKUOM, SUY – SUYANI, NYI – NYINASE A ,
GOA – GOASO, BRE –BREKUM, NKR – NKRANKWANTA, HWI– HWIDIEM

On the other hand the trend for the HCH isomers for cocoa beans sampled from the Ashanti region was delta<gamma<beta<alpha. The concentration for delta HCH ranged from 0.01 mg/kg to 0.08 mg/kg. The lowest concentration detected in cocoa beans was from Offinso, Apagya, Juaso and New Edubiase (Table 4.3) respectively and the highest concentration was found in cocoa beans from Ampenim (Table 4.3). The concentration of delta HCH residues were within the EU and Japanese allowable limits in cocoa beans with the exception of the samples from Nkawie, Agona, Brofoyedru and Nyinahini which exhibited higher concentrations than the MRL values. Gamma HCH had concentrations ranging from 0.15 mg/kg to 0.24 mg/kg. The lowest concentration of gamma HCH was found in samples from New Edubiase (Table 4.3) and the highest concentration was recorded for samples from Agona (Table 4.3). All the cocoa beans from the various locations had delta HCH concentrations which were above the MRL value of 0.10 mg/kg established by Japanese and Codex Alimentarius. However the concentrations were below the EU permissible limits of 1.0 mg/kg. Levels of beta HCH in the cocoa beans ranged from 0.02 mg/kg for samples from Mankranso, Juaso and Offinso to 0.38 mg/kg for samples from Ampenim. Exactly 70% of the samples had concentrations higher than the EU, Japanese and Codex MRL values except (Tables 4.3).

Alpha HCH had concentrations in the range of 0.25 mg/kg for cocoa beans from Mankranso (Table 4.3) to 0.54 mg/kg for samples from Nkawie (Table 4.3). All the cocoa beans sampled from this region had concentrations higher than EU, Japanese and Codex Alimentarius. In both regions delta HCH recorded the lowest level of pesticide residue while the highest concentration was recorded for alpha HCH.

For DDT and its metabolites, DDT and DDD were detected in 100% of the analysed samples. The concentrations of DDT in all the samples from areas were higher than DDD. It could be

inferred from the trend that there is a continuous use of DDT in the area due to its lower cost and effectiveness as well as its broad spectrum activity despite its ban (Amoah *et al.*, 2006). The higher concentrations could also be attributed to the enormous past uses and due to its longer half life ie. slow degradation and persistence in the environment. For DDT, the lowest concentration was 0.08 mg/kg and was recorded for cocoa beans from Sunyani and Nyinase A and the highest concentration was 0.46 mg/kg which was recorded for cocoa beans from Asumura. Levels of DDD present in cocoa beans in Brong Ahafo region ranged from 0.03 ppm for cocoa beans from Sankore, Sunyani, Asumura, Goaso, Mim, Nyinase and Nkrankwanta. to 0.06 mg/kg for samples from Nyinase A. Although the food samples were contaminated with DDT and DDD, the maximum residue levels were below FAO's MRL and the standards set by some European countries and the concentrations of DDT found in this survey were lower than those recorded for fresh milk from some developing countries such as, Uganda 3.24 mg kg^{-1}, Nigeria 3.83 mg kg^{-1}, India 6.55 mg kg^{-1}, Kenya 6.99 mg kg^{-1}, South Africa 20.10 mg kg^{-1} and Ethiopia 7.75 mg kg^{-1} (FAO, 1986). During 1996, the UK Working Party of Pesticide Residues (WPPR) found residues of DDT in butter, milk, eggs, lamb, potatoes, deep water fish and shell fish. In a survey of non-indigenous deep water fish and shell fish, low levels of DDT residues and metabolites were found in 16 (50%) of the deep water samples and three (19%) of the 16 shell fish samples analysed

Table 4.3 Pesticide residues levels in cocoa beans from Ashanti region

CONCENTRATION OF PESTICIDE RESIDUE (mg/kg)												
PESTICIDES	**AMP**	**NYI**	**MAN**	**JUA**	**NEW**	**NKA**	**AGO**	**OFF**	**BRO**	**APA**	**JAPAN**	**EU**
Chloropyrifos	6.83	6.46	5.40	5.40	7.97	7.07	7.04	9.81	9.24	5.40	0.10	0.05
Endosulfan I	0.12	0.05	0.05	ND	0.06	0.04	0.04	0.10	0.20	ND	0.10	0.10
Profenefos	0.15	0.12	0.11	0.11	0.15	0.11	0.04	0.11	0.14	0.11	0.10	
Endosulfan II	0.16	0.09	0.07	0.07	0.05	0.08	0.06	0.07	0.08	0.07	0.10	
Fenvalerate	0.27	0.27	0.20	0.20	0.32	0.07	0.01	0.06	0.23	0.20	0.50	
Bifenthrin	0.08	0.08	0.06	0.06	0.08	0.07	0.06	0.06	0.05	0.06	0.10	0.10
Permethrin I	0.30	0.72	0.35	0.35	0.65	0.18	0.22	0.12	0.65	0.35	0.10	0.05
Permethrin II	0.93	1.12	0.11	0.11	1.10	0.20	0.29	0.18	1.19	0.11	0.10	0.05
Cypermethrin I	0.06	0.11	0.05	0.05	0.05	0.04	0.04	0.05	0.05	0.05	0.10	0.03
Cypermethrin II	0.54	0.16	0.14	0.14	0.15	0.14	0.12	0.16	0.51	0.14	0.10	
Cypermethrin T	0.40	0.43	0.38	0.38	0.57	0.11	0.09	0.51	0.43	0.38	0.10	
Beta HCH	0.38	0.07	0.02	0.02	0.04	0.05	0.03	0.02	0.04	0.25	0.02	
Delta HCH	ND	0.03	0,02	0.01	0.01	0.08	0.04	0.01	0.06	0.01	0.02	
Gamma HCH	ND	ND	ND	ND	0.15	0.21	0.24	0.17	0.20	ND	1.00	0.10
Alpha HCH	0.47	0.39	0.25	0.04	0.37	0.54	0.39	0.48	0.51	0.30	0.02	
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.05	0.10
Hea-exo-epoxide	0.07	0.03	0.03	0.04	0.02	0.04	0.19	0.01	0.01	0.01	0.02	
Dieldrin	0.05	0.07	0.04	0.03	0.49	0.04	0.04	0.06	0.07	0.03	0.05	
4, 4 – DDD	ND	ND	0.03	ND	0.03	0.02	0.04	0.04	0.05	0.03	0.50	
4,4 DDT	0.20	0.17	0.07	0.10	0.20	0.09	0.04	0.08	0.02	0.07	0.50	
Heptachlor	0.20	0.14	0.12	0.07	0.12	0.25	0.11	2.03	2.20	1.70	0.02	

NYI – NYINAHINI, MAN– MANKRANSO, JUA – JUASO, NEW – NEW EDUBIASE. NKA – NKAWIE,

OFF – OFFINSO, BRO – BROFOYEDRU, APA – APAGYA, AGO – AGONA AMP– AMPENIM

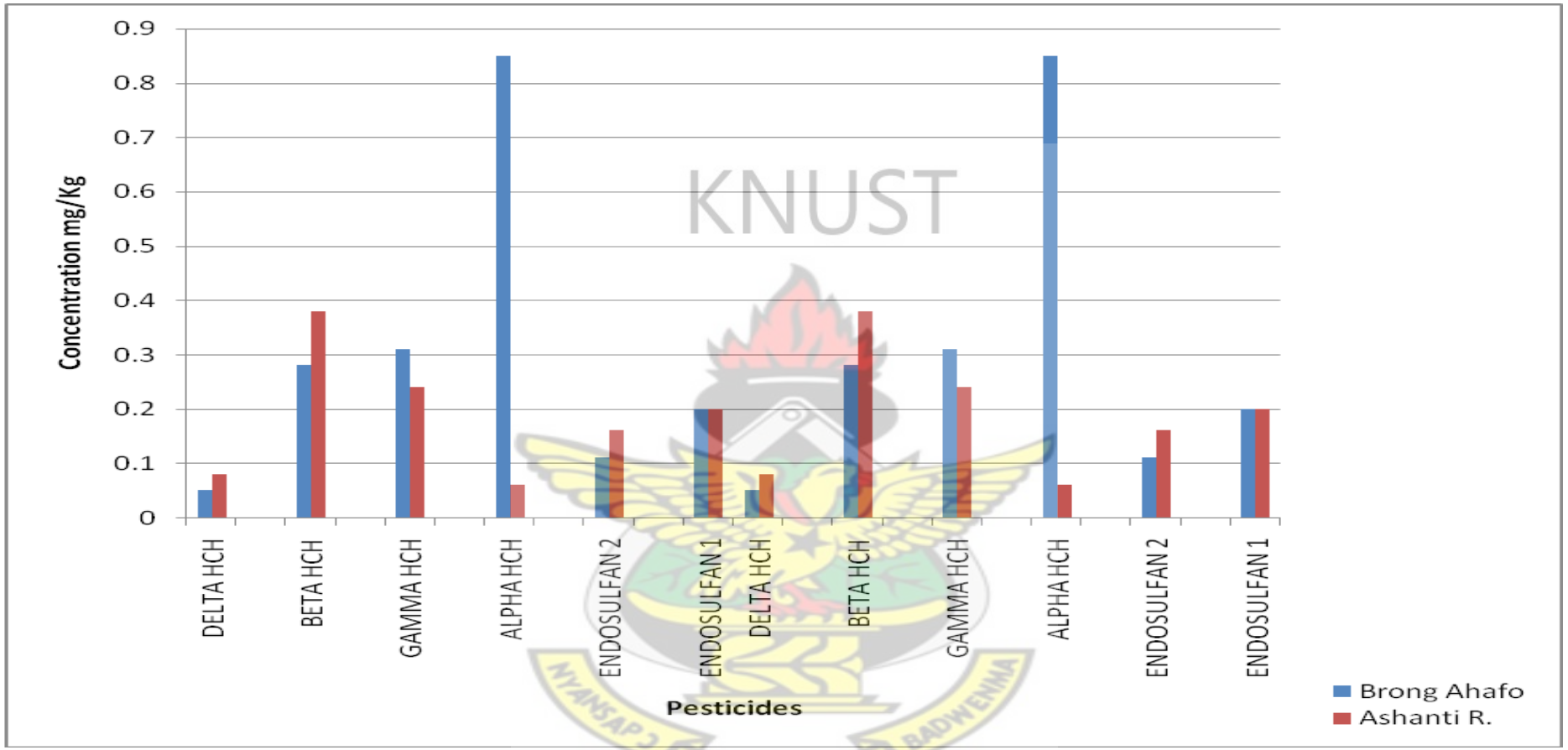


Figure 4.1: Concentration of pesticide residues in cocoa beans from Brong Ahafo and Ashanti regions.

Generally the concentrations of DDD were higher than the parent compound DDT. This is because there are no fresh inputs of DDT into the cocoa beans. In this study the presence of both DDT and its metabolite DDD in the samples may be due to the persistence and long range transport nature of DDT and its metabolite DDD (Ritter *et al.*, 1995) even though they have been banned since 1985 (EPA Ghana, 2008). The concentration of 4,4-DDT was lower than that of its metabolite p,p'-DDD. This may be that most of the DDT massively used in the past is in the metabolite state and fresh input of DDT in the environment is minimal. DDD residues were not detected in cocoa beans sampled from Ampenim, Nyinahini and Juaso. The highest concentration was 0.05 mg/kg for samples from Brofoyedru and the lowest concentration was 0.02 mg/kg for samples from Nkawie. All the samples were within the EU and Japanese MRL value of 0.50 mg/kg and 0.05 mg/kg respectively. DDT residues were ranged from 0.02 ppm for samples from Brofoyedru to 0.20 mg/kg for samples from Ampenim and New Edubiase. Out of the number of samples, 80% were above the Japanese MRL of 0.05 mg/kg except cocoa beans from Agona and Brofoyedru. Conversely all the samples analysed were below the EU permissible level of 0.50 mg/kg. In a work conducted in 1996 in the UK, eggs analysed as part of the Total Diet Survey were found to contain residues of DDT. In another Working Party on Pesticide Residues study in 1996 in the UK which monitored geese for the first time, residues of DDT as p,p'-DDE and p,p'-DDT were found in 25% of the samples, with a residue profile similar to that found in ducks in a 1995 survey in the UK (WPPR, 1996). A similar trend of the residual concentrations of DDT and DDD were observed for the two regions.

Heptachlor was present in all the cocoa beans whilst heptachlor exoepoxide was detected in 60%, out of the number of samples from the Brong Ahafo region. Concentrations of heptachlor were higher than its breakdown oxygenated product, the epoxide. This could be due to the incomplete breakdown of heptachlor which can be attributed to unfavourable degradation conditions and persistence in the environment though it is among the list of banned pesticides by Stockholm Convention for use on food (Afful *et al*, 2010). Concentrations of heptachlor ranged from 0.13 mg/kg for cocoa beans from Nyinase A to 3.74 mg/kg for cocoa beans from Hwidiem. All cocoa beans were above the EU MRL value of 0.02 mg/kg. For heptachlor exo epoxide, the lowest concentration was for 0.02 mg/kg for cocoa beans from Mim (Table 4.3) and the highest was 0.37 mg/kg for cocoa beans from Nyinase A (Table 4.2). Heptachlor epoxide was below detection limit for 50% of the cocoa beans sampled from the Brong Ahafo. All the cocoa beans sampled from this area had higher values than the EU MRL of 0.02 mg/kg except from Mim.

Heptachlor and its metabolite were detected in cocoa beans sampled from the Ashanti region of Ghana. Levels of heptachlor exo epoxide ranged from 0.01 mg/kg for Offinso, Brofoyedru and Apagya to 0.19 mg/kg for samples from Agona (Table 4.3). Out of the number of samples, 60% were above the EU MRL of 0.02 mg/kg except cocoa beans from New Edubiase, Offinso, Brofoyedru and Apagya. Concentration of heptachlor ranged from 0.07 mg/kg for samples from Juaso to 2.20 mg/kg for cocoa beans from Brofoyedru. All the samples analysed were above EU MRL of 0.02 mg/kg. It was observed that the concentration of heptachlor was higher than its metabolite.

Generally concentrations of Heptachlor and its metabolite from the Brong Ahafo region were higher than those of the Ashanti region.

Alpha and Beta endosulfan were present in all the cocoa beans sampled from the different locations in the Brong Ahafo region. Concentrations of the beta isomer were lower than the alpha. Levels of alpha endosulfan in cocoa beans sampled from Brong Ahafo region ranged from 0.01 mg/kg for samples from Sankore to 0.20 mg/kg for cocoa beans from Brekum and Hwedim. All the cocoa beans sampled from most of the locations in the Brong Ahafo region had concentrations lower than EU and Japan MRL values of 0.10 mg/kg with the exception of samples from Brekum and Hwedim. Alpha-endosulfan residues have also been recorded in crops and in fish in Ghana (Ntow, 2001; Osafo and Frimpong, 1998). An appreciable concentration of alpha endosulfan was measured in the breast milk samples of human beings and this might be due to the fact that it was considered for restrictive use in Ghana since 2008.

Levels of cocoa beans analysed for beta endosulfan in the Brong Ahafo region were between 0.04 mg/kg for samples from Sankore (Table 4.2) to 0.11 mg/kg for samples from Goaso (Table 4.2). All samples were within the EU and Japanese MRL values of 0.10 mg/kg with the exception of cocoa beans from Goaso which had concentration of 0.11 mg/kg, which is slightly above the set limit.

In the Ashanti region, the levels of alpha endosulfan in cocoa beans ranged from 0.04 mg/kg for samples from Nkawie and Agona to 0.20 mg/kg for samples from Brofoyedru. The concentrations of alpha endosulfan were below the EU, Japanese and Codex Alimentarius allowable levels of 0.10 mg/kg except cocoa beans from Brofoyedru. There were non detectable levels of alpha endosulfan in cocoa beans from Juaso and Apagya. Beta endosulfan in cocoa beans ranged from 0.05 mg/kg for samples from New Edubiase to 0.16 mg/kg for samples from Ampenim. All the samples were below the EU, Japanese and Codex MRL value of 0.10 mg/kg except cocoa beans from Ampenim. Generally the concentrations of alpha isomer

were higher than beta endosulfan. A similar trend was observed in both regions and the levels of beta endosulfan in the BA region were lower than samples from the Ashanti region.

Aldrin was below detection limit in all the cocoa beans sampled from the Brong Ahafo region. However its breakdown product, dieldrin, was found in all the samples analysed. Aldrin is one of the OCPs that is banned under the Stockholm Convention for POPs. Its absence could therefore be due to the fact that its use has been discontinued (UNEP, 2001). Aldrin is more volatile and readily degrades to dieldrin in the environment. However its metabolite was still present in cocoa beans because of its stability, lipophilicity and bioaccumulation in fats. More than 56% of the original weight of aldrin converts to dieldrin and about 19% of the original aldrin weight disappears (Osibanjo,1999). Levels of dieldrin in cocoa beans ranged from 0.03 mg/kg for samples from Brekum, Nkrankwanta, Sankore and Kukuom to 0.06 mg/kg for samples from Asumura. Concentrations of dieldrin in samples were within the EU allowable level in cocoa beans All the samples had levels below the Japanese MRL value of 0.10 mg/kg. A study conducted in 1999 by Adeyeye and Osibanjo revealed levels of aldrin in kolanut below detection limit.

Aldrin was below detection limit in all the cocoa beans analysed from the Ashanti region but its metabolite was present and the concentration ranged from 0.03 ppm for samples from Apagya and Juaso to 0.49 mg/kg for samples from New Edubiase. 60% of the samples analysed were within the EU MRL of 0.05 mg/kg except cocoa beans from Offinso, Brofoyedru, Nyinahini and New Edubiase. For the Japanese allowable levels in cocoa beans 90% of the samples were below the set value of 0.10 mg/kg except samples from New Edubiase. A study in 1999 by the United States Geological Survey involving dieldrin and 15 other organochlorines in fish, it was found that fish were more highly contaminated than soil samples adjacent

to the water where the fishes were sampled. Dieldrin was detected in fish at 50% of the sites sampled; 30% of the sites had values that exceeded human health consumption levels. In another research, dieldrin was detected in the liver and fat of arctic ground squirrels trapped near three lakes located at the foothills of the Brooks Range, Alaska between 1991 and 1993 (Allen-Gil *et al.* 1997). The mean concentrations of dieldrin in squirrel liver from Elusive Lake (seven samples), Feniak Lake (seven samples), and Schrader Lake (seven samples) were 10.91, 1.53, and 14.42 µg/g wet weight, respectively. Mean concentrations of dieldrin in snapping turtle eggs collected at four sites along the St. Lawrence River in the Mohawk territory of Akwesasne during June, 1998 ranged from 4 to 280 ng/g wet weight with an overall mean concentration of 38.13 ng/g wet weight (de Solla *et al.* 2001). Aldrin and dieldrin were detected in the plasma of juvenile alligators from three lakes in central Florida (Guillette *et al.* 1999). In 1985, fish samples taken from the lower Savannah River in Georgia and South Carolina were found to occasionally contain dieldrin but at concentrations of < 0.01 µg/g (10 ppb) (Winger *et al*., 1990);

A similar trend was observed for the two regions. However the levels of dieldrin in cocoa samples from the BA region were lower than the Ashanti region.

The general trend observed for the organochlorines in the BA region was Heptachlor >alpha HCH>DDT>Heptachlor exo epoxide>Gamma HCH>Beta HCH>Alpha endosulfan>Beta endosulfan>DDD = Dieldrin >delta HCH

Concentrations of chloropyrifos in cocoa beans from the Brong Ahafo region ranged from 1.88 ppm for samples from Goaso to 10.55ppm for samples from Mim. All the cocoa beans sampled in the BA region had concentrations above MRL values established by EU (0.10ppm), Japan (0.05ppm) and Codex Alimentarius. A study conducted by Kotey *et al* , (2006*)* revealed high levels of pesticide residues on vegetables cultivated in Ghana. Residue

analysis detected the presence of chlorpyrifos, DDT and cypermethrin, in shallots, with levels of chlorpyrifos exceeding the Codex MRL in most samples. The results regarding pesticide residues indicated that several pesticides, especially chlorpyrifos, are widely used by cocoa farmers in Ghana. This is similar with other studies (Okorley and Kwarteng, 2002). Residue violations for chlorpyrifos have also been observed on 10 samples of tomatoes grown in the Upper East Region of Ghana (Biney, 2001) and on seven samples of cabbage grown in the Greater Accra Region of Ghana (Odhiambo, 2005). In 2006, a study conducted by Amoah *et al*., in Ghana revealed that 47 lettuce samples with detectable chloropyrifos contamination exceeded the recommended residue level of 0.05 mg kg–1.

In the Ashanti region, concentration for chloropyrifos ranged from 5.40 mg/kg for cocoa beans from Juaso, Mankranso and Apagya (Table 4.3) to 9.81 mg/kg for samples from Offinso (Table 4.3). It was observed that none of the samples were below the EU, Japanese and Codex MRL of 0.10 mg/kg and 0.05 mg/kg. Generally, the concentrations of chloropyrifos in cocoa beans sampled from the Brong Ahafo were higher than in the Ashanti region. Profenefos, fenvalerate, bifenthrin, Permethrin I and II, Cypermethrin (alpha, beta and technical) were detected in all the cocoa beans from the Brong Ahafo region. Levels of profenefos in the cocoa beans sampled from the Brong Ahafo region ranged between 0.07 mg/kg for samples from Mim to 0.14 mg/kg for samples from Asumura. Samples from Asumura, Kukuom, Sunyani, Goaso, Brekum, Nkrankwanta and Hwedim (Table 4.2) were above the EU MRL values except samples from Mim which was 0.07 mg/kg.

Conversely the levels of profenefos in cocoa beans ranged from 0.04 mg/kg for samples from Agona to 0.15 mg/kg for samples from Ampenim and New Edubiase. Out of the total number of cocoa beans analysed, 90% were above the MRL set by EU except samples from Agona. In a study conducted, the levels of profenefos in water from four vegetable farms in

Patumtanee Province had concentrations above the European Commission threshold value of 0.05 mg/kg (Tethgatuk *et al*, 2001). Concentrations of profenefos in cocoa samples from the BA region was found to be lower than in samples from the Ashanti region.

All of the cocoa beans analysed indicated the presence of fenvalerate. Levels of fenvalerate in cocoa beans were within the range of 0.04 mg/kg to 0.71 mg/kg. Cocoa beans sampled within the Brong Ahafo region had levels above the EU MRL value of 0.05 mg/kg except Sunyani samples which had concentration of 0.04 mg/kg. Concentrations of fenvalerate from cocoa beans from the Ashanti region ranged from 0.01 mg/kg for samples from Agona to 0.32 mg/kg for cocoa beans from New Edubiase. About 90% of the samples recorded levels above the EU permissible limits with the exception of cocoa beans from Agona (Table 4.3). Ripley *et al.*, (2000) reported pesticide residues levels in cabbage and fruits in Canada; however, the levels were far below the MRLs. Significant levels of fenvalerate have been detected in the analysis of pesticide residues in fruits and vegetables in Sweden (Pihlstrom *et al*., 2007). The trend observed in the Ashanti region was found to be similar to the Brong Ahafo region. Generally the concentrations of fenvalerate was found to be higher in the Brong Ahafo region than in Ashanti region.

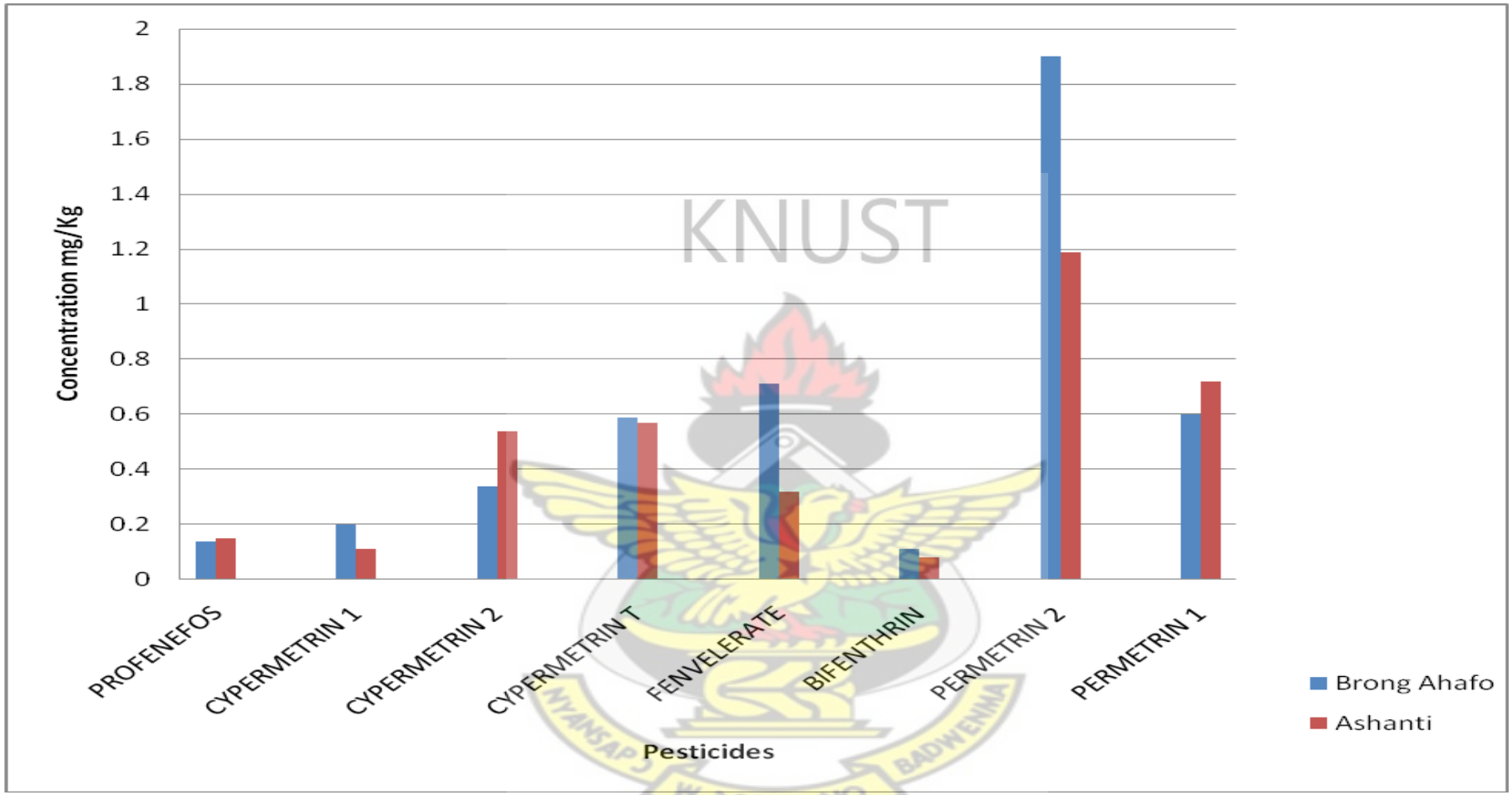


Figure 4.2 Concentrations of pesticide residues in cocoa beans from Brong Ahafo and Ashanti regions

Concentrations of bifenthrin in cocoa beans fell within the range of 0.03 ppm for samples from Sankore (Table 4.2) to 0.11 mg/kg for samples from Goaso (Table 4.2), All samples from the Brong Ahafo region were within the EU permissible level of 0.10 mg/kg except cocoa beans from Goaso which had a concentration slightly above the MRL value. On the other hand concentrations of bifenthrin in cocoa samples from the Ashanti region were within the EU and Japanese MRL value of 0.10 mg/kg. The highest concentration was recorded for Ampenim, Nyinahini and New Edubiase (0.08 mg/kg) (Table 4.3) while the lowest concentration was recorded for Brofoyedru (0.05 mg/kg) (Table 4.3). The concentrations of bifenthrin in cocoa beans sampled from the Ashanti region were lower than samples from Brong Ahafo region. All of the samples analysed from the Ashanti region had levels below EU and Japanese MRL values whereas 90% of the samples from Brong Ahafo region were below EU and Japanese MRL.

Concentrations of permethrin I in cocoa beans from the Brong Ahafo region ranged from 0.19 mg/kg to 0.60 mg/kg. All the samples had levels above the Japanese and EU permissible limit of 0.05 mg/kg and 0.10 mg/kg respectively for cocoa beans. Cocoa beans from the Brong Ahafo region had concentrations of permethrin II ranging from 0.11 mg/kg for samples from Nkrankwanta to 1.10 mg/kg for samples from Sankore. All the locations in the region had concentrations above the MRL value established by EU. Permethrin II had higher concentrations of residues in cocoa beans than permethrin I.

The concentration for permethrin I ranged from 0.12 mg/kg for cocoa beans from Offinso to 0.72 mg/kg for samples from Nyinahini. All the samples recorded levels above the Japanese and EU MRL of 0.05 and 0.10 mg/kg respectively. For permethrin II the lowest concentration was 0.11 mg/kg for cocoa beans from Mankranso and Juaso while the highest concentration was 1.19 mg/kg for samples from Brofoyedru. The concentrations of

permethrin II were higher than permethrin I. A similar trend was observed for the two regions. However the concentrations of permethrin II in the Brong Ahafo region was lower than in the Ashanti region. On the other hand concentrations of permethrin I recorded for the Brong Ahafo region were lower than the samples from Ashanti region. A research conducted in Ghana (Ato-Armah, 2011) reported higher levels of permethrin residues in cabbage from Ghana. In an FDA's Total Diet Study of 2003, Permethrin residues were detected in only 3% of the 1039 food samples tested. The range of permethrin levels found was 0.0008 - 4.7130 mg/kg (Food and Drug Administration Pesticide Program Residue Monitoring, 2003). In the 2006 United States Department of Agriculture (USDA) Pesticide Data Program (PDP) report on pesticide residues in food crops, total permethrin levels were measured in 1726 food samples including bananas, collard greens, summer squash, and watermelon. A total of 0.5% of these samples had detectable residues of permethrin ranging from 0.048-4.900 ppm. None of the detected levels exceeded their corresponding permethrin tolerances. When testing for the cis- and trans-isomers separately, the USDA examined several additional crops, including broccoli, cranberries, peaches, and spinach. Of the 8948 samples tested, 6.7% had detectable residues ranging from 0.004 to 5.30 ppm. Spinach samples alone accounted for 97% of the detected levels of permethrin (Pesticide Data Program Annual Summary, 2006).

Levels of alpha cypermethrin in cocoa beans sampled from the Brong Ahafo region ranged from 0.03 mg/kg for samples from Sankore to 0.20 mg/kg for samples from Brekum. All Samples were below the MRL value of 0.10 mg/kg for EU, Japanese and Codex Alimentarius with the exception of cocoa beans from Brekum. The concentrations of alpha cypermethrin in cocoa beans from Ashanti region ranged from 0.04 mg/kg for samples from Nkawie and Agona to 0.11 mg/kg for samples from Nyinahini. About 90% of the cocoa beans from this region were below the EU, Japanese and Codex Alimentarius permissible limits with the

exception of samples from Nyinahini that was slightly above the set limit. It can be deduced that the concentrations of the alpha cypermethrin in cocoa beans from the Brong Ahafo region were lower than the cocoa beans from the Ashanti region.

Cocoa beans from the Brong Ahafo region had concentrations of the beta isomer ranging from 0.05 ppm for samples from Brekum and 0.34 mg/kg for samples from Hwiediem. All the locations in the region had concentrations in the samples above the MRL values established by EU of 0.10 mg/kg except Brekum samples and limits established by Japanese with MRL of 0.03 mg/kg except Sankore samples.

For the Ashanti region the levels of the beta isomer ranged from 0.12 mg/kg for cocoa beans from Agona to 0.54 mg/kg for samples from Ampenim. None of the samples were below the EU, Japanese and Codex MRL. Genenrally, the cocncentrations of beta cypermethrin are higher for cocoa beans from Ashanti region than samples from the Brong Ahafo region. A research conducted on pesticides residues in cabbage from Cape Coast revealed levels of cypermethrin residue above the respective MRL values (Ato -Armah, 2011).

Concentrations of cypermethrin T in samples from the Brong Ahafo region ranged from 0.05 mg/kg in cocoa beans from Kukuom to 0.59 mg/kg in cocoa beans from Asumura All the samples were above the EU and Japanese permissible limits of 0.10 and 0.03 mg/kg with the exception of samples from Kukuom. For the Ashanti region samples had concentrations of cypermethrin T ranging from 0.09 mg/kg for Agona to 0.57 mg/kg recorded for New Edubiase. About 90% of the samples from this region were above the EU MRL of 0.10

mg/kg, Conversely, none of the samples were below the Japanese MRL of 0.03 mg/kg. For the two regions it could be inferred from the results that the concentrations of technical cypermethrin in cocoa beans from the Brong Ahafo region were lower than samples from Ashanti region. It was found that concentrations of cypermethrin T > beta cypermethrin >alpha cypermethrin. The general trend observed for the pyrethroids was Permethrin II >Fenvalerate >Permethrin I > Cypermethrin T >Beta cypermethrin,Alpha cypermethrin > Profenefos> Bifenthrin.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

From this study it can be concluded that 50% of the pesticides residues analysed in the BA region were above the EU permissible levels for cocoa beans whereas 45% of was recorded for Ashanti region.

Among the pesticides detected it was found that chloropyrifos recorded the highest residual concentrations of 10.55 mg/kg for samples from Mim in the Brong Ahafo region and 9.81 mg/kg for samples from Offinso in the Ashanti region. The lowestpesticide residual concentration was recorded for Endosulfan I (0.01 mg/kg) for Sankore in the Ashanti region and Delta HCH which had a value of 0.01 mg/kg for samples from Offinso , Apagya, Juaso and New Edubiase respectively.

5.2 RECOMMENDATION

Future work should concentrate on the whole and deshelled beans in order to compare levels of pesticide residues.

Work should be done on different methodologies for extraction of pesticide residues to compare the recovery efficiencies.

Work should be done on fresh and fermented beans

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APPENDIX

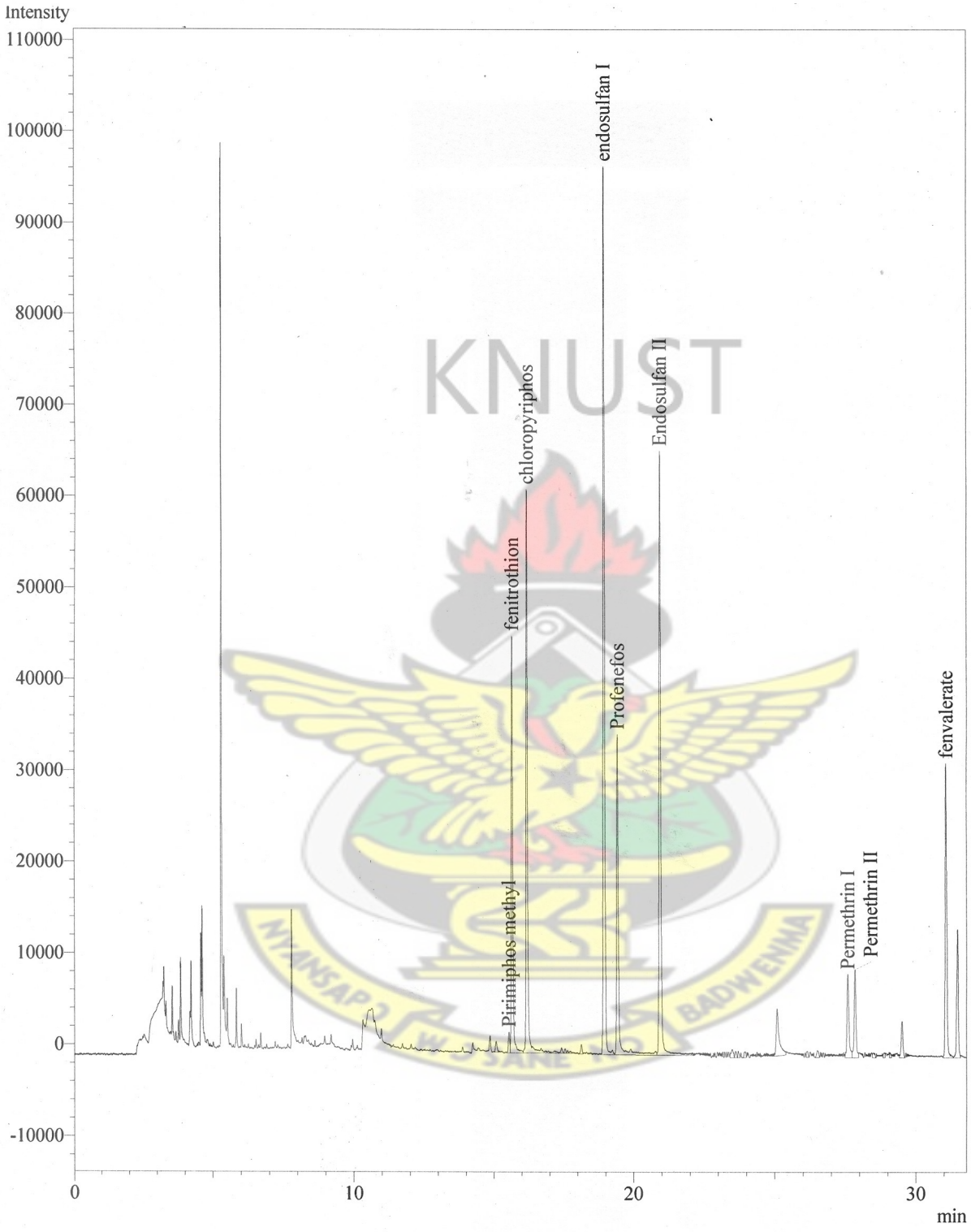

Figure 4.3: Chromatogram for standard mix 1

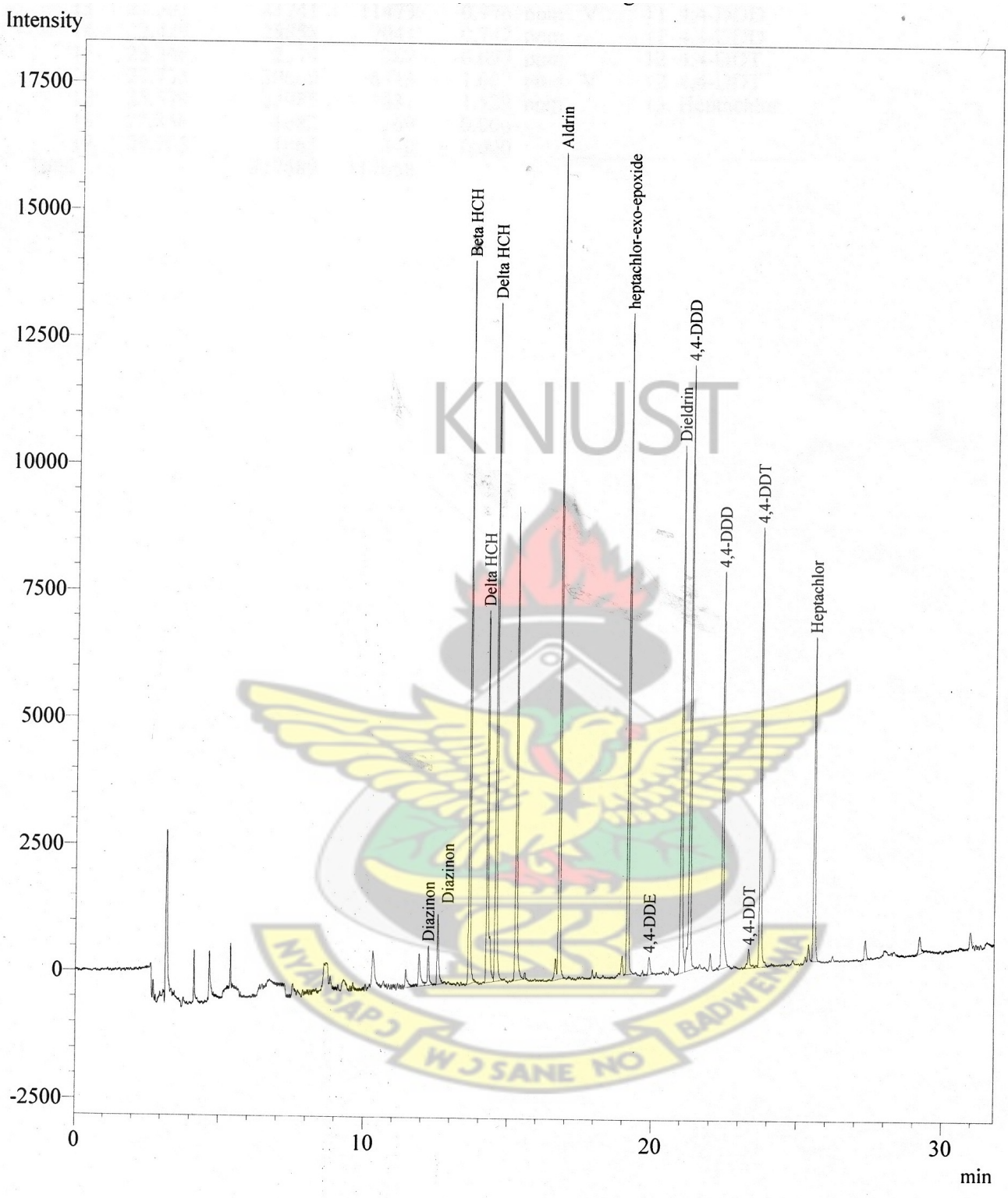

Figure 4.4: **Chromatogram for standard mix 2**

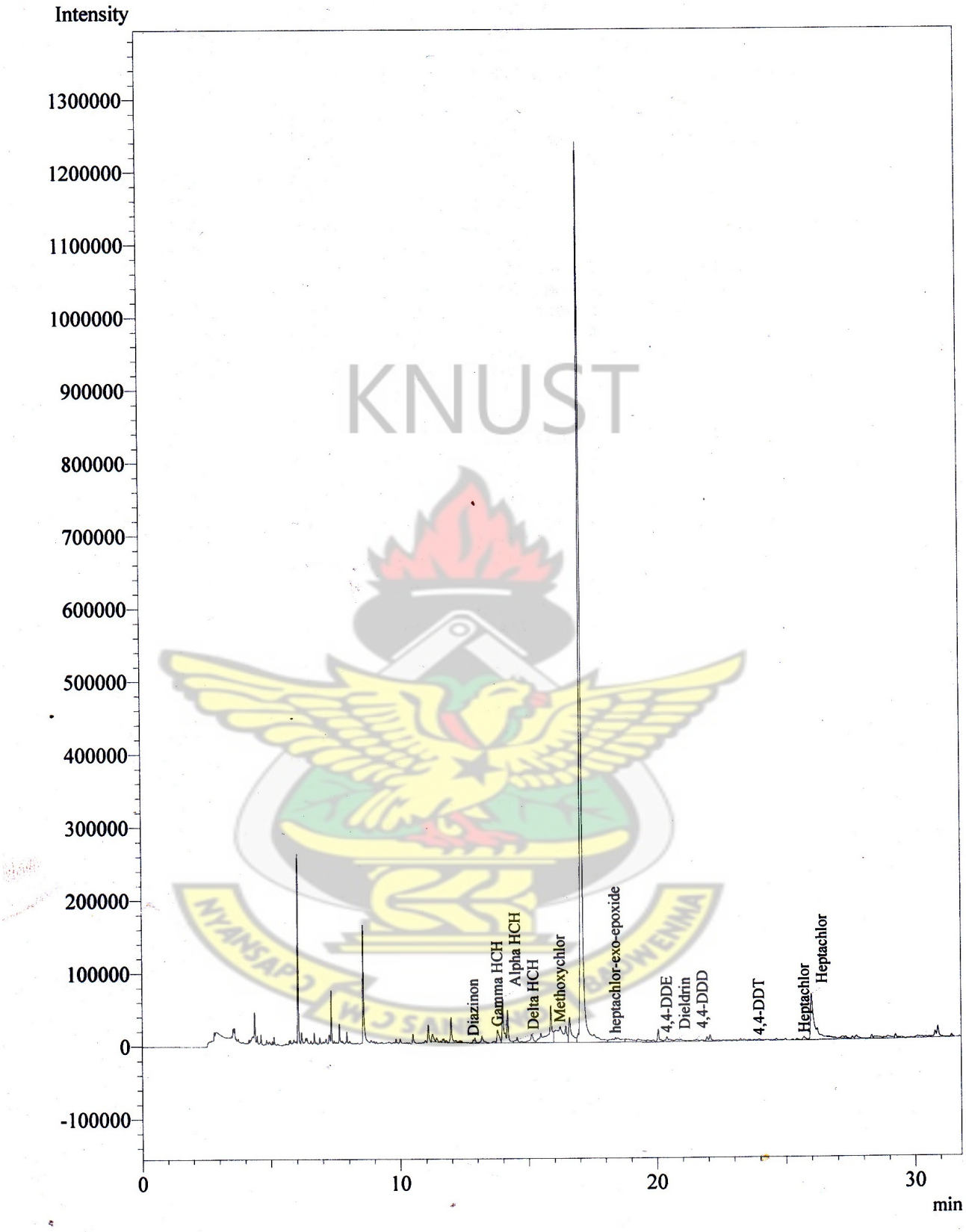

Figure 4.5 Chromatogram for pesticide residues in cocoa bean samples