HEAVY METALS AND PESTICIDE RESIDUES IN HONEY FROM THE MAJOR HONEY PRODUCING FOREST BELTS IN ASHANTI, BRONG AHAFO AND WESTERN REGIONS OF GHANA

By

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DECLARATION

I hereby declare that this submission is my own work towards the Master of Philosophy in Analytical Chemistry and that, to the best of my knowledge, it contains no material previously published by another person or materials 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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ABSTRACT

The purpose of this work was to determine the concentrations of heavy metals and pesticides residues in honey. In all forty five honey samples were purposively collected from Ashanti, Brong Ahafo and Western Regions of Ghana. QuEChERS method, a multiresidual method for analysis of pesticide residues in low –fat matrix was employed in the extraction procedure. Varian CP-3800 Gas Chromatograph with a CombiPAL Autosampler, equipped with electron capture detector for synthetic pyrethroids and Organochlorine pesticides while pulse flame photometric detector for organophosphate were used. Aldrin, γ-HCH, β-HCH, Σendosulfan, cyfluthrin, cypermethrin, deltamethrin, permethrin methoxychlor, ΣDDT, chloripyrifos, fenvelerate, malathion, dimethoate and diazinon were all detected at the concentration of 0.01 with the exception of cyfluthrin and permethrin which were detected at 0.02 and 0.04 mg/kg respectively, in each of the pesticides. All the pesticides residues detected were below the respective maximum residue limit. Hence honey samples analyzed do not pose any health risk to the consumer. For the heavy metal analysis using AAS, the concentrations were in the order as: Cd 0.02 <Zn 0.22 < Pb 0.84 < Fe 2.30 < Cu 3.23 mg/kg. Cadmium recorded the lowest concentration 0.03 mg/kg whilst Cu recorded the highest concentration of 3.23 mg/kg.

Keywords: honey, pesticides, residues, heavy metal, organochlorine, organophosphorus, synthetic pyrethroids

DEDICATION

This research is dedicated to my lovely wife, Mrs. Sandra Addai Mununkum who has been my constant source of inspiration and to my parents Rev. Canon Emmanuel Addai Mununkum and Mrs. Dora Addai Mununkum and finally, to my lovely daughter, Emmanuella Yaa Addai Mununkum.

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CHAPTER ONE

INTRODUCTION

Background

1.1.1 Honey

Honey is a sweet viscid fluid produced by honey bees from the nectar of flowers. It is composed largely of dextrose and laevulose (Fontana et al., 2010). Honey is a mixture of sugars and other compounds (Johnson & Jadon, 2010). Honey bees collect nectar from flowers and turn it into a product considered to be a delicious food and known to be a healthier nutritional choice than sugar (Bilandžić et al., 2012). It is widely used for both nutritional and medicinal purposes (Al-Waili et al., 2012). Honey matrix has different components such as sugars, organic acids and insoluble matter. It contains significant amounts of mineral matter, vitamins and enzymes (Tahboub et al., 2006).

1.1.2 Honey Production

Honey is produced by honey bees (*Apis mellifiera*) from pollen, plant nectars and honey dew (Slebioda & Namie, 2013). Honeybees are estimated to forage on plants growing over a relatively large area (more than 7 km^2) and when going from flower to flower they come into contact with air, water, soil, branches and leaves (Bilandžić et al., 2012). Honey bees perform the vital task of pollinating agricultural crops and native species and are important to the commercial production of honey and bees wax. The honeybees gather nectar, water and pollen from flowers in making the honey (Jia et al., 2008). Honey is composed of over 300 chemical substances belonging to different chemical compound groups. These are mainly carbohydrates, water, polysaccharides, fatty acids,

proteins, minerals, dyes, fragrances, enzymes, hormones and vitamins in amounts depending on the plant from which the honey was made (Slebioda & Namie, 2013). Bee products are natural food products; they are rich in minerals, antioxidants and simple sugars (Al-Waili et al., 2012).

1.1.3 Honey Composition

Honey is made up of about 70% monosaccharides (glucose and fructose) and 10% oligosaccharides. The minor constituents are composed of several units (from two to six) of glucose and fructose with the glycosidic bond in different positions and configuration (Sanz, Sanz, & Martínez-Castro, 2004). With respect to carbohydrates, honey is mainly fructose (about 38.5%) and glucose (about 31.0%) (Blasco et al., 2011) making it similar to the synthetically produced inverted sugar syrup which is approximately 48% fructose, 47% glucose, and 5% sucrose. Honey's remaining carbohydrates include maltose, sucrose, and other complex carbohydrates (Johnson & Jadon, 2010).

Fructose and glucose are ketose and aldose sugars respectively with chemical structural differences, and consequently different patterns of metabolism, despite both being monosaccharides with quick burning tendency. It is important to note that glucose is rapidly metabolized for absorption into the blood system for energy provision. The fructose absorption is slow, and will continue to sustain the individual with energy (Ajibola et al., 2012). Honey contains about 18.0 % water, while protein content is low and mineral content in honey ranges from about 0.04 % in pale honeys to 0.2 % in dark Honeys. Honey is known to be rich in both enzymatic and non-enzymatic antioxidants (Al-Waili et al., 2012). However, the specific composition of any batch of honey, as well

as the contaminants presents in it depends on the crops surrounding the beehive (Blasco et al., 2011).

1.1.4 Uses of Honey

Honey has various health advantages; it is extensively consumed by children, old and sick people as both food and medicine worldwide. Hence, honey must be free from any chemical contaminants and be safe for human consumption (Tahboub et al., 2006). Honey products have received renewed interest as an essential natural resource that can be employed in new therapies free from side effects that are often encountered with the use of synthetic chemical medicines. It is known that feeding infants with honey helps to improve their memory and growth, reduce anxiety and enhance the children's performance as they grow in life (Ajibola et al., 2012).

Medicinally, honey has been used to treat coughs and sore throats, ulcers, ear aches, measles and eye diseases. Honey has potential therapeutic properties in infections; wound healing and cancer medicines (Al-Waili et al., 2012). The therapeutic property of honey has called for its resurgence interest presently. Honey can also prevent deteriorative oxidation reactions in foods, such as the browning of fruit and vegetables and lipid oxidation in meat, as well as inhibit the growth of food borne pathogens and microorganisms that cause food spoilage (Al-Waili et al., 2012).

Honey has been used as a source of carbohydrate and sweetener (Feás et al., 2013). Honey is a natural and wholesome product consumed by many people around the world. It is being used as a natural sweetener in food manufacturing practices, cosmetics and also as a pharmaceutical in the treatment of various human infections (Erdo, 2007).

Honey can be used as instant energizer as it contains sugars which are quickly absorbed by our digestive system and converted into energy (Johnson & Jadon, 2010).

Modern science has made it possible to stress on medicinal significance of honey as it contains bactericidal and bacteriostatic, nematicidal, antiviral, antifungal, antioxidant and antitumoral substance. Thus, this bee product has been demonstrated to be a suitable alternative for healing wounds, burns and various skin conditions (Feás et al., 2013). When honey is used with other herbal preparations it enhances the medicinal qualities of those preparations and also helps them to reach the deeper tissues. Honey is also used as a medicine because of its antioxidant and antibacterial properties (Johnson & Jadon, 2010). Honey is subjected to numerous clinical and laboratory investigations, in order to demonstrate its beneficial medicinal effects, such as, antiseptic, wound-healing properties when applied topically or inhibiting growth of Methicillin-resistant Staphylococcus aureus strains among other bacteria (Kujawski & Pinteaux, 2012).

In some cases, the consumption of relatively large amounts of natural honey (between 70 to about 95 g) can produce a mild laxative effect on the digestive system of individuals. The consumption of honey provides calcium, which is readily absorbable and strengthen bone mass development and hence reduction in the risk of osteoporosis or low bone mass that is causative agent of fractures in adults and also helps to promote oral health and wellness. That honey with high level of antibacterial activity has the potential to reduce the risk of dental caries (Ajibola et al., 2012).

1.1.5 Pesticides

The honey benefits can be suppressed by pesticides introduced to honey during its processing and arising from both agricultural and beekeeping practices (Blasco et al., 2011). Honey like other foods is not completely safe; it is prone to various types of contaminations and adulterations from the environment and hence the presence of pesticides in honey products decreases its quality (Al-Waili et al., 2012).

Honey bees are greatly affected by pesticides and they transport these pesticides into the colony as contaminated nectar which ends as a contaminated honey. Therefore, pesticide residues monitoring is essential to improve the quality and the safety of honey (Tahboub et al., 2006). The monitoring has become important since pesticides have always been threats to human health due to their inherent toxicity (Jia et al., 2008). Consumers of honey are always at risk because they are exposed to pesticides, usually in minute quantities in honey and the monitoring pesticide residues in honey helps to assess the potential risk of this product to consumer's health. Providing information on the pesticides which have been used in the field crops, surrounding the hives is very important (Rissato et al., 2007).

The use of pesticides to protect crops against plagues and insects is one of the most important ways to ascertain agricultural quality and to increase productivity (Flores et al., 2010). Pesticides have both advantages and disadvantages. Pesticides are used to attract and repel pests as well as those used to regulate plant growth. Pesticides may cause damage or injury to non-target species. Bee products, such as honey are widely consumed as food and medicine and their contamination may carry serious health hazards.

Honey and other bee products are polluted by pesticides, heavy metals, bacteria and radioactive materials.

1.1.6 Health Implication of Pesticides in Honey

Pesticide residues in honey cause genetic mutations and cellular degradation and presence of antibiotics might increase resistant human or animal's pathogens (Al-Waili et al., 2012). Exposure to the hazardous pesticides is a concern for the general population. Pesticide residues have also been reported in patients with acute drug toxicity as a result of accidental or suicide intended consumption. The biological monitoring is to determine pesticides or their metabolites directly in biological fluids to measure exposure to these contaminants (Jia et al., 2008).

Pesticides can be categorized into four based on their functional groups namely; Organonitrogen, Organochlorine, Organophosphorus and Pyrethroid which is regarded as the fourth major generation of synthetic organic insecticides developed (Li et al., 2013). Pesticides in biological systems are potentially carcinogenic and may cause alterations in endocrine, reproductive and nervous systems. The chemicals also reduce the activity of neurotransmitters and hence cause irreversible effects on the nervous system. For these reasons, most countries have restricted or banned the use of PCBs and organochlorine pesticides (OCPs). However, the environmental persistence of POPs, along with the large volume usage of these compounds in the past (for PCBs and OCPs) and in the present (for PBDEs), suggests that they could remain a serious environmental problem for a number of years (Erdo, 2007).

The accumulated pesticide causes a potential risk for human health, because of their sub acute and chronic toxicity. There has been increasing attention of the public to the quality of honey. The control of pesticides in honey is a vital issue for primary health in the world. The presence of heavy metals and pesticide residues in honey has compelled the need for analysis (Basavarajappa & Raghunandan, 2013).

1.1.7 Heavy Metals in Honey

Honey is one of the most important human foods. It has medicinal, nutritive and disease preventive capabilities due to its chemical composition (Kohler & Mwangi, 2012). In order to have a beneficial effect, honey must be free of any contaminating agents (Ruschioni et al., 2013).

Honey contains many trace minerals that are essential to health: phosphorus, iron, aluminium, magnesium, copper, manganese, silica, chlorine, calcium, potassium and sodium. All the above components are elements of the earth in which plants grow. Plants absorb elements and deliver them to the nectar, which is a major resource used by bees to make honey. Therefore, honey will vary in mineral content not only according to the type of soil but also according to the kind of plants from which the bees took the nectar from (Matei et al., 2004).

Honey bees are exposed to numerous pollutants during their foraging activities, their body hair can easily retain atmospheric residues and they can be contaminated via food resources when gathering pollen and nectar from flowers, or through water (Ruschioni et al., 2013). The metals are picked up by the roots and distributed throughout the entire plant including the nectar and pollen. The amount of metal in the plant increases with the

amount of metal in the soil. Also, in regions where there is a significant amount of lead in the air, it may land on the plant and adhere to the sticky surfaces of pollen. Bees collect both the contaminated pollen and nectar and transport it back to the hive. It is, therefore, desirable to perform the analysis of honey to ensure that it is free of pollutants such as heavy metals (Kohler & Mwangi, 2012).

Heavy metals are important in daily diets because of their essential nutritious value. Metals like iron, copper, zinc and manganese are essential metals since they play important roles in biological systems; whereas lead and cadmium, etc. are non-essential metals which can be toxic even in trace amounts. The essential metals can also have harmful effects when their intakes exceed the recommended quantities significantly (Tuzen & Soylak, 2005).

Since honey is a nutritional resource that depends on biotic and abiotic factors around the beehives, the presence of heavy metals could be related to its geographical and botanical origin. As the consumption of honey is increasing because of its multiple health promoting effects, the metal contaminants in the honey need to be evaluated (Singh et al., 2014).

Heavy metals present in honey above the admitted levels constitute a serious threat to human health through its possible toxic effects on the body. Even though a lot of research focuses on pollution in general, not a lot of work has been related to pollution with heavy metals (Iuliana & Cecilia, 2005). The concentration of different metals has been widely documented in honey from many countries. Determination of heavy metals in honey is of high interest mainly for quality control and nutritional aspect (Singh et al., 2014).

Cadmium (Cd) is a relatively rare element that occurs naturally in ores together with zinc, lead and copper or is emitted into the air through the process of volcanic eruption. Occupational exposure to cadmium, such as working with cadmium containing pigments, plastic, glass, metal alloys and electrode material in nickel - cadmium batteries, and non occupational exposure, such as food, water and cigarette smoke induces uptake of Cd from the environment into the body through pulmonary and enteral pathways. Cadmium absorbed and accumulates mainly in the kidney and liver, then it is bound to the apoprotein metallothionein (Abdel-moneim & Ghafeer, 2007).

Lead (Pb) and cadmium (Cd) are considered the principle toxic heavy metals and are thus most frequently studied. Lead, contained in the air and originating mainly from motor traffic can contaminate air and then directly nectar and honey dew. Generally, Pb is not transported by plants and Pb contamination is expected to diminish, due to the increased world-wide use of car-engine catalysts. On the other hand, Cd originating from metal industry and incinerators, is transported from the soil to plants and can then contaminate nectar and honeydew (Ogdanov, 2006).

1.1.8 Nutritional Importance of Heavy Metals

Heavy metals are important in daily diets because of their essential nutritious value. Copper as an essential element may influence growth, skin pigmentation, bone mineralisation, gastrointestinal and heart function (Bilandžić et al., 2012). It has been observed that composition of mineral materials in honey is identical to human blood. Microelements are very biologically active. They ensure the natural development of physiological reactions, take part in metabolism and impact general metabolism,

germination, circulatory systems and influence the reproduction of organism as catalysts of various biochemical reactions (Matusevicius et al., 2010). Microelements are the constitutive parts of the structures of different active biocompounds, such as zinc, copper and manganese in ferments, cobalt in vitamins, iodine, and cobalt in hormone, copper and iron in the respiratory ferments. Apart from microelements that are indispensable for human organisms, there is a group of microelements that are harmful – lead, cadmium, mercury, aluminum (Matusevicius et al., 2010).

1.2 Statement of the Problem

Honey is a natural, nutritious, healthy and popular food produced by honey bees from nectar of plants. Honey is often consumed by children, old and sick people. Many people consume honey as food without thinking about the safety of what they are consuming. Others use honey for medicinal purposes in treating coughs and sore throats, ulcers, earaches, measles and eye diseases. Honey has potential therapeutic properties in infections, wound healing and cancer medicines.

Consuming contaminated honeys may introduce foreign materials into the bodies of consumers. Therefore, in recent years, honey has received renewed interest as an essential natural resource that can be employed in new therapies free from side effects that are often encountered with the use of synthetic chemical medicines (Al-Waili et al., 2012).

Pesticides determination in honey is necessary to monitor and guarantee the consumers' health which will help to reduce the effect of pesticides residues in human (Blasco et al., 2004). As *Apis mellifera* bees move from nectar to nectar of different plants, the pesticide

residues may (bio)magnify in honey. Also heavy metals and pesticides in honey may further worsen the health status of people who take honey for medicinal purposes. Therefore, honey must be free from any chemical contamination and be safe for human consumption. The safety and quality control of honey products have become an international issue (Wang et al., 2011). Unfortunately, in Ghana, there is a limited literature available on pesticide residues and heavy metals level in honey.

1.3 Objectives

The aim of this work is to determine the concentration of:

1. pesticide residues in honey in the major honey producing forest belts in Ashanti, Brong Ahafo and Western regions of Ghana.
2. heavy metals like Cd, Pb, Zn, Fe and Cu, excess of which could cause danger in individual consumers of honey samples collected from major honey producing forest belts in Ashanti, Brong Ahafo and Western regions of Ghana.

1.4 Justification

A food becomes wholesome when it meets appropriate nutritional and safety standards as well as specific quality attributes. While the nutritional and quality aspects of honey are very important, safety of honey is also critical as it is often used for its medicinal and therapeutic effects. Honey is eaten extensively as both food and medicine in the whole wide world. The occurrence of pesticide residues from nectar may accumulate and get concentrated in honey and that the consumption of small doses of pesticides or heavy metals in food such as honey presents chronic health risk. Unfortunately, there is no data

on heavy metal and pesticides residues levels in honey from Ghana and hence the data that would be obtained will inform policy makers in making decision in pesticides use in the country.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nature of Nectar

Nectar production plays a vital role in the pollination of a flowering plant. Nectars are aqueous solutions that are secreted by plants to attract and reward animal mutualists (González-Teuber & Heil, 2009). Nectar properties (volume, concentration, viscosity) change dynamically in time. Nectar is not a static product remaining outside the plant once produced but is in close contact with the plant system (Nepi & Stpiczynska, 2008). Flowers do bear voluminous, disc-shaped, multi-lobed nectar and maintain a considerable population of nectar thieves indicating that nectar should be present (Astellanos et al., 2002).

The secretion of nectar occurs concomitantly with resorption and that the latter process sometimes continues after secretion has ended (Nepi & Stpiczynska, 2008). The two major functional groups of mutualists comprise pollinators such as insects, birds and bats, which are attracted to floral nectar (FN), and defending arthropods such as ants and parasitoids, which are attracted to extrafloral nectar (EFN). Floral nectar is considered the most common means by which animal-pollinated plants reward their pollen vectors (González-Teuber & Heil, 2009).

Many species can experience impressively high levels of pollinator traffic. For instance, *Penstemonstrictus* flowers can routinely receive 100 bumble bee visits per day (Djurdjevi, 2005). The pollination system is geared to such high visitation rates because pollen is released from anthers very gradually, and a large number of visits are beneficial to successfully moving pollen grains from anthers to stigmas. Another feature of the genus

is that some species are adapted for pollination by bees, whereas other species are adapted for pollination by hummingbirds. Angiosperm flowers produce nectar in order to attract pollinating animals such as insects, birds, small mammals collecting this aqueous solution of sugars for its nutritional properties (Djurdjevi, 2005). Sugar sensing may have a fundamental role in nectar resorption and homeostasis. Due to its direct contact with sugar solutions, nectaries may offer wide scope for insights into this phenomenon which has attracted interest as part of plant signalling systems (Nepi & Stpiczynska, 2008).

The fraction of soluble solids that can be found in nectars mainly comprises mono- and disaccharides and amino acids. However, other compound classes such as proteins, lipids, phenols, alkaloids and volatile organic compounds (VOCs) have also been reported from various nectars (González-Teuber & Heil, 2009). Sugars dominate the total solutes in floral nectar. These are mainly sucrose, fructose and glucose in varying proportions according to the species. Other compounds such as amino acids, phenols, lipids and antioxidants are present in trace quantities (Galetto & Bernadello, 2004).

The main function of nectar compounds is related to the attraction of mutualistic animals. Flowers differ in their pollination syndrome that is they often differ in the amount, concentration and composition of the nectar that they produce. In particular, species adapted for hummingbird pollination produce more nectar that is more dilute and with a higher sucrose: hexose ratio than do congeners adapted for hymenopteran pollination (Astellanos et al., 2002). Field observations showed that a variety of small flies or creeping insects (either landing on the petals or creeping up the pedicel) enter the area of the nectary by penetrating between the filaments. They remain there for a considerable

time period (more than 60 s) without approaching the stigma and showing no interest in pollen, apparently stealing nectar (Manetas & Petropoulou, 2000).

Nectar resorption plays an important ecological function because it can be involved in nectar homeostatic mechanisms that enable regulation of nectar volume, concentration and thus viscosity by reducing the effect of water loss due to evaporation. Since nectar composition and concentration are adapted to the preferences of visitors, reduction of nectar viscosity by sugars resorption may facilitate nectar probing and this mechanism may be important to promote visits by efficient pollinators (Nepi & Stpiczynska, 2008).

Nectar production is a complex physiological process variable due to species-specific characteristics and depends to a great extent upon the environmental conditions. Due to the role of nectar in pollination, it is secreted during the anthesisphenophase and relies among other factors, upon the stage of flower development (Djurdjevi, 2005). The chemical composition of floral nectar and the dynamic control of its secretion affect the reproductive success of plants visited by nectar-seeking pollinators. Its availability and quality can affect a pollinator's decision to visit or not to visit a flower and its behavior while visiting (Astellanos et al., 2002).

The presence of sucrose, fructose and glucose in nectar often impart a particular taste and odour that may be essential for maintaining certain pollinator groups. The nectar sugar ratios together with flower and inflorescence morphology may be good predictors of the pollinators (Galetto & Bernadello, 2004). It has been reported that sugars, amino acids and lipids in nectar have been described as nutritionally valuable components that contribute to its attractiveness for various mutualistic animals, even though VOCs is

identified as further attractive constituents (González-Teuber & Heil, 2009). Nectar is secreted with particular rhythms, throughout the lifespan of a flower, which allow the nectar production dynamics of a species to be determined (Galetto & Bernadello, 2004).

Honey bees (*Apismellifera*) are commonly considered to be the main pollinator. They appear to be effective because they collect both types of pollen (pin and thrum) on a single trip and their foraging and prospecting behaviour, collecting both nectar and pollen, promotes frequent contacts with stigmas. Bee-pollinated flowers secrete comparatively less nectar with higher concentrations; whereas hummingbird pollinated flowers show intermediate values (Galetto & Bernadello, 2004). Insect-pollinated plants that are visited by a large number of pollinator individuals gain reproductive benefits, due to increased import and export of pollen. Repeated visits of the same pollinator to a plant, on the other hand, increase within-plant pollen transfer (geitonogamy), which is genetically equivalent to self-pollination (Keasar et al., 2008).

Nectar production may show diverse patterns according to the different guilds of pollinators that visit the flowers, leading to the assumption that there are coevolutionary relationships between nectar traits and pollinator type (Galetto & Bernadello , 2004). Nectar distribution affects pollinator movements and frequency of foraging bouts, whereas the number of visited flowers affects pollen dispersal and reproductive success of the plant. Therefore measurements of nectar volume and quality are important in understanding pollinator energetics, nutrient requirements, and behavior in floral patches.

2.2 Nature of Honey bees

A foraging honey bee collects large quantities of nectar and pollen and in the process repeatedly exposes itself to the potential hazards of lipophilic food contaminants. In an evolutionary sense feeding on nectar, a purified, carbohydrate-rich food source may have left the honeybee particularly vulnerable to orally ingested insecticides. In the honeybee the highest microsomal oxidase activity is found in the tissues of the midgut, the major site of pollen digestion, while the foregut tissues are largely devoid of oxidase activity. Thus, the honey bee foregut is repeatedly exposed to, and yet cannot efficiently detoxify, foreign substances including insecticides that contaminate nectar. Bees get in touch with pesticides during the foraging activities in an average radius of 3–6 km around the hive. Pesticide residues may provoke toxic effects on honey bees even when they are present at low doses and they have even been suspected to cause a significant decrease of honey bee. In this way, bee's mortality is commonly used as a tool to evaluate the level of agrochemicals in the environment (Flores et al., 2010).

2.3 Importance of Honey

Honey is a natural product produced by *Apis mellifera* bees from the nectar of plants. Its composition is mainly depended by the floral origin of the nectar from which the honey is made (Zacharis et al., 2012). It is regarded as a valuable natural food product: it is normally use as a natural sweetener and additive to bakery products. The healing properties of honey have been known and benefited from in traditional medicine since ancient times (Kujawski & Pinteaux, 2012). Honey as a natural product that must be free of any chemical contaminants and safe for human consumption, because in some

countries it is traditionally used in child, old and ill people and its quality must be proved (Blasco et al., 2004). Honey is a food product with world-wide consumption especially among children and in terms of food safety concern it must be free of chemical contaminants particularly from organochlorine pesticides (OCPs) (Zacharis et al., 2012).

The European Union (EU) legislation has come out with a regulation to regulate the MRLs for three acaricides: amitraz, coumaphos and cyamizole, which are 0.2, 0.1, and 1 mg/kg, respectively and the US Environmental Protection Agency, has also establish MRLs for amitraz (1 mg/kg), coumaphos (0.1 mg/kg), and fluvalinate (0.05 mg/kg) origin reported the European Parliament and of the Councils Regulation as (EC) No. 396/2005 which seek to establish values for the maximum residue levels (MRLs) of pesticides in products of plant and animal origin (Blasco et al., 2004). Since September 1st 2008, the European Commission set new MRLs in food and feed of plant and animal. These pesticides include MRLs in honey and pollen between 10 and 50 ngg^{-1}. The residues of pesticide compounds and their metabolites are found in different environmental and food samples (Blasco et al., 2011; Flores et al., 2010).

Though pesticides are lipophilic in nature, and that they enter into the food chain by accumulating in fats, yet they can also be present in non-fatty products. They can be present in honey because of the plant treatment or by migration from wax to honey. Since honeybees travel long distances and come close to many plants, honey may be an easily accessible environmental pollution indicator (Erdo, 2007). Honey may be contaminated by pesticides specifically applied in agriculture and forestry which maybe in the environment during the production and harvesting of honey. These contaminants may be

carried on bee bodies or with the forages to the hive, from where they eventually find their way into honey (Kujawski & Pinteaux, 2012).

The determination of contaminants and residues in honey and other bee products has become necessary, especially as these contaminants may diminish the beneficial properties of honey and if present in significant amounts, may pose a serious threat to human health (Kujawski & Pinteaux, 2012).The determination of pesticides in honey at trace levels is a challenging task owing to the complex matrix of honey and its high sugar content. Clean-up is often necessary prior to analysis in order to eliminate interferents (Kujawski & Pinteaux, 2012).

2.4 Medicinal Uses of Honey

As a natural product, honey is famous for its richness in nutrients and being a valuable remedy as it is used to treat diseases such as gastrointestinal disorders or wound healing (Akbari et al, 2012). Honey possesses numerous nutritional, healing, and prophylactic properties. These are a direct consequence of its chemical composition (Iuliana & Cecilia, 2005). The moisturising action of honey around a wound facilitates the healing process and the high viscosity of honey inhibits infections to penetrate into the body (Akbari et al., 2012).

Nowadays, honey has attracted attention for its potency in prevention and treatment of illnesses and also its ability to cause well-being. Also honey, because of its flavour, colour and sweetness, could be used as an ingredient or preservative in foodstuffs; for example, it has been shown that honey is able to prevent lipid peroxidation in meat (Akbari et al., 2012). In order to have a beneficial effect, honey must be free of any

contaminating agents. Any heavy metals present in honey above the admitted levels by pollution standards, are threats to human body through the possible negative effect of the contaminants. Even though a lot of research focuses on pollution in general, not a lot of work has been related to pollution with heavy metals as well as pesticide residue in honey has been done in Ghana.

2.5 Characteristics of Pesticides

Agrochemicals are used in modern agriculture to control insects, weeds, plant diseases, worms and rodents. In biological systems, several of these chemicals are potentially carcinogenic and may cause alternations in endocrine, reproductive and nervous systems (Erdo, 2007). Pesticides are used in agriculture to increase production, treat infections or for prophylactic reasons (Chauzat & Faucon, 2007). The use of pesticides to protect crops against plagues and insects is one of the most important ways to ascertain agricultural quality and to increase productivity (Chauzat & Faucon, 2007). A great productivity gains can be achieved in agriculture by the use of appropriate and adequate pesticides. Indeed, they are needed to meet the world's demand on foodstuffs and no other alternative can compete to be used in such a large scale. However, the slow degradation of pesticides in the environment and its extensive or inappropriate use by farmers can lead to environmental contamination of the water, soil, air, several other types of non-targeted crops and indirectly humans (Rissato et al., 2007).

The potential presence of pesticide residues and other contaminants in food substance is an important issue in the field of food safety and quality. The large number of permitted and commercially available pesticides and veterinary drugs have caused a steady increase

of the number of analytes to be monitored (Chauzat & Faucon, 2007). The use of pesticides in beehive treatment during honey harvesting is another possible route of honey contamination (Kujawski & Namieśnik, 2011).

2.6 Categories of Pesticides

Pesticides can be categories into four groups based on their functional group, namely; Organonitrogen, Organochlorine, Organophosphorus and synthetic pyrethroid; which is regarded as the fourth major generation of synthetic organic insecticides developed (Li et al., 2013).

2.6.1 Organochlorine Pesticide

Organochlorine pesticides (OCPs) are very toxic, carcinogenic and highly resistant to physical, chemical and biological degradation. Due to their lipophilic character these compounds can be bio-accumulated in various tissues of living organisms and they can move between the various compartments of an ecosystem contaminating the food chain (Zacharis et al., 2012). Contamination of organochlorine pesticides (OCPs) in foods has been considered a serious threat to human health because of their environmental persistence, high accumulation, and high mammalian toxicity. They are ubiquitous in the environment because of their moderate vapor pressure, low solubility, and low reactivity. OCPs production, usage and disposal into the environment have been regulated or prohibited in many countries since 1980s (Wang et al., 2010).

Although the introduction of DDT and other organochlorine pesticides was beneficial originally for both farming and public health, the massive use of these products has

proved undesirable (Mui et al., 1994). The use of OCPs has been restricted by many countries (Stockholm Convention of Certain Persistent Organic Pollutants), however these compounds are still detected in the environmental water samples and in foodstuffs as well (Zacharis et al., 2012). The occurrence of organochlorine compounds in the food chain constitutes one of the most important groups of dangerous organic contaminants. The environmental contamination by persistent organochlorine pesticide (OCPs) residues has been widely documented in several countries, such as Portugal and Spain in medicinal plants, water, milk, and biological fluids (Zacharis et al., 2012).

Organochlorines are lipophilic substances and consequently are soluble and stable in bees wax. Therefore, an amount of these substances gradually migrates from wax into the stored honey (Erdo, 2007). Due to its lipophilic nature, OCPs enter into the food chain by accumulating in fats (Blasco et al., 2004) and finally into various tissues of living organisms. Thus can move between the various compartments of an ecosystem contaminating the food chain (Zacharis et al., 2012). These organochlorine pesticides and their metabolites have been extensively used and are still present in the environment, owing to their high persistence (Erdo, 2007).

Even though organochlorine pesticides have been restricted by European countries and USA in 1987 yet they are still found in the environment due to their persistence and bioaccumulation (Ion, et al., 2011). Extreme use of organochlorine pesticides possess still dangerous effects such as cancer, immune systems, reduced bone mineral density and the disruption of hormonal functions in human (Koc & Karakus, 2011).

The chronic toxicity associated with continual ingestion of small doses over long periods of time includes teratogenic, carcinogenic, oestrogen-inducing, hepatotoxic, immuno-

suppressive and neurotoxic effects whist acute toxicity effects caused by the ingestion of relatively large doses may include neuronal alterations, irritation of skin and mucosa, tremor, convulsions, and even shock and death (Mui et al., 1994).

Honey bees can bring many pollutants deposited on plants into the hive. Therefore, plant protection products used in agriculture cannot only cause mass poisoning of bees but may also enter bee products, especially honey affecting its quality, properties and posing a particular threat to human health (Slebioda & Namie, 2013).

Pesticides especially the persisted organochlorine pesticides have been a major concern since honey bees are greatly affected by pesticides and transport them to the colony as contaminated nectar which ends as a contaminated honey (Tahboub et al., 2006). Some organochlorine pesticides structures are given in Figure 1 below.

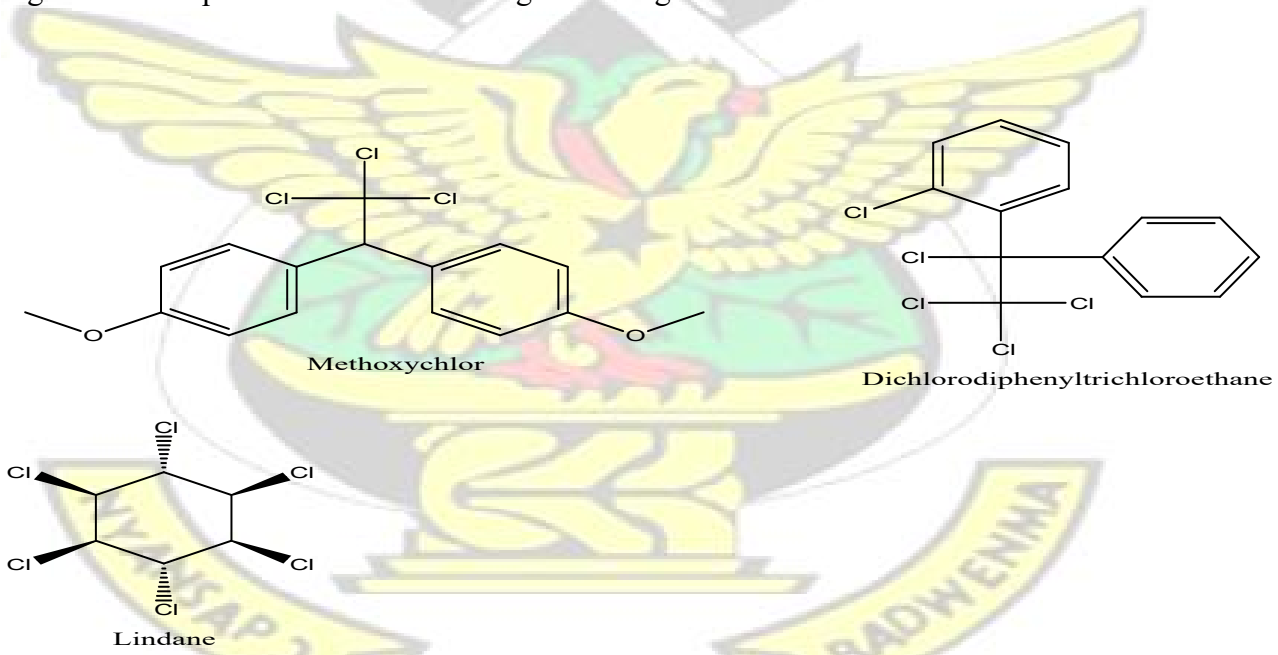


Figure 1: Structures of Some Organochlorine Pesticides

2.6.2 Organophosphorus Pesticide

Organophosphorus (OPs) compounds were first developed by Schrader shortly before and during the Second World War. They were first used as an agricultural pesticide. In these compounds, the OPs are a group of both synthetic and biogenic OP compounds, characterized by the presence of the binding covalent and carbon to phosphorus (C-P) bond (Kazemi et al., 2012).

About 79% of the insecticides in current use in Europe are Organophosphorus (OPs) and (closely related) carbamates (Blasco et al., 2011). Organophosphates pesticides (OPPs) are widely used in agricultural practices for pests and diseases control. Slow degradation of pesticides in the environment and extensive or inappropriate use by farmers can lead to environmental contamination (Fontana et al., 2010).

Organophosphorus and carbamates chemicals primarily affect the nervous system by inhibiting acetylcholinesterase (AchE) enzyme activity (Blasco et al., 2011). The widespread distribution of pesticides caused several problems to apiculture industry including residues in hives products (honey and wax). Honey bees are greatly affected by pesticides and transport them to the colony as contaminated nectar which ends as a contaminated honey. These residues finally get to the consumers. This has a greater potential risk for human health, because of their sub-acute and chronic toxicity (Fontana et al., 2010). Some structures of organophosphorus pesticides are given in Figure 2 below.

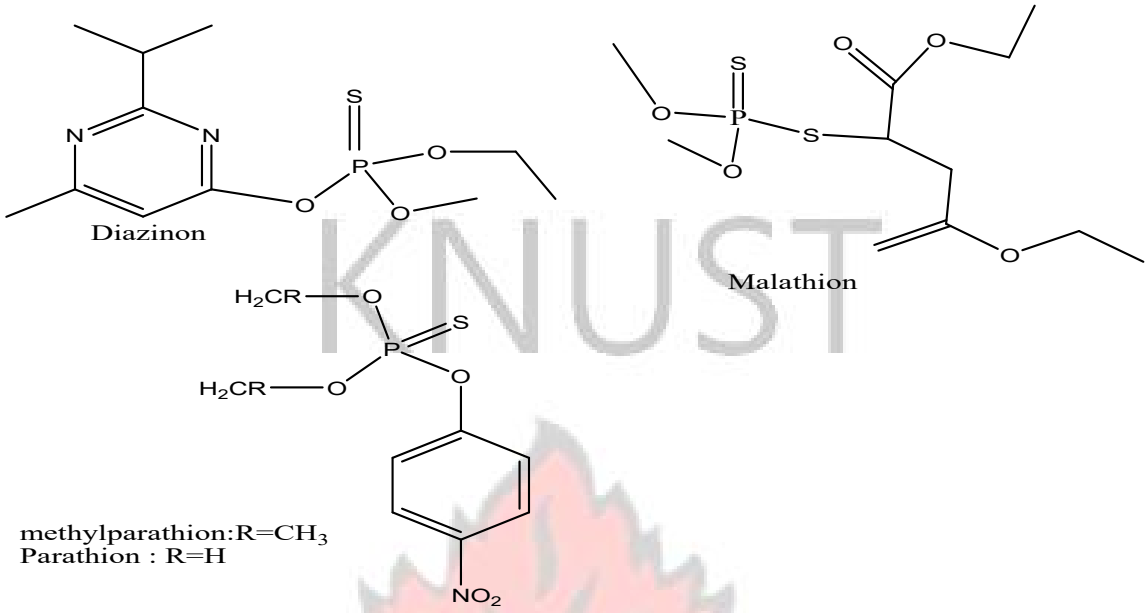


Figure 2: Structures of Some Organophosphorus Pesticides

2.6.3 Synthetic Pyrethroid Pesticide

Pyrethroids with structures typically containing 2–3asymmetric carbon atoms (chiral centers), are synthetic pyrethrins that present a high stability and insecticidal activity for a large spectrum of pests. In recent decades, pyrethroids have attracted much more attention and have been widely used due to their selectivity in action, their relatively lower mammalian toxicity and lower environmental persistence compared to their predecessors. However, the wide spread residue from pyrethroids in the environment could lead to chronic exposure and long term toxicity effects. Additionally, the toxicity effects of pyrethroids on aquatic organisms and insects, including fish and some arthropods, is of great concern because of their very low LC_{50} values (less than 0.5 mg L^{-1}) (Li et al., 2013). Some structures of synthetic pyrethroids pesticides are given in Figure 3 below.

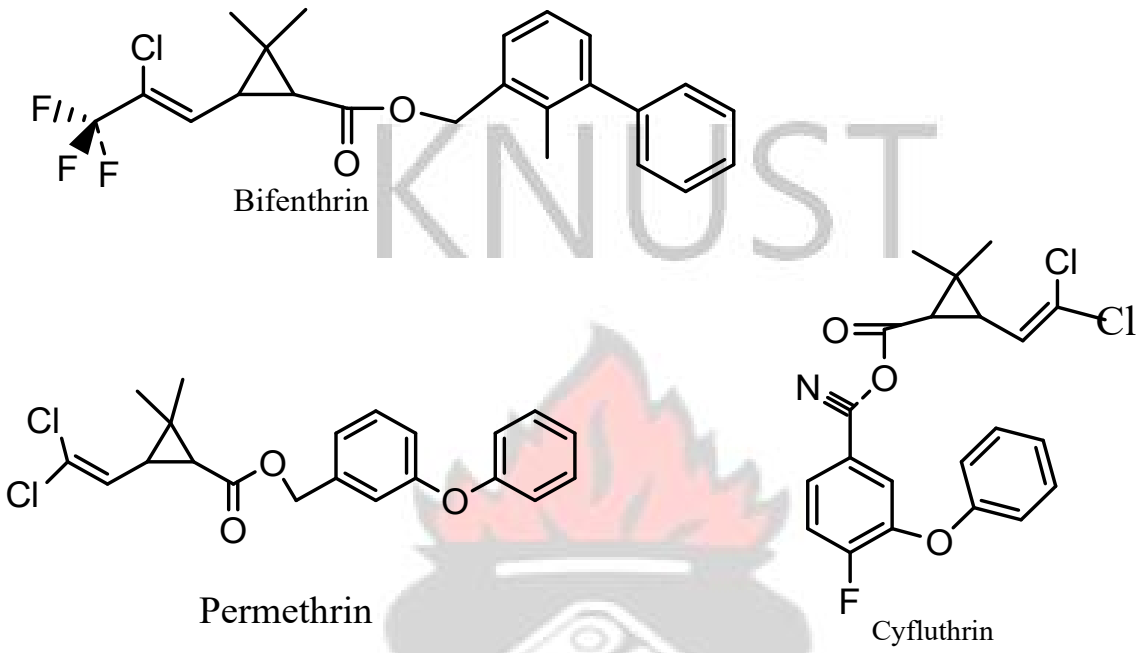


Figure 3: Structures of some Synthetic Pyrethroid Pesticides

2.7 Nature of Heavy Metals

The high concentration of heavy metals in honey can be a source of illness to human beings. Heavy metals are chemical elements with a specific gravity that is at least five times the specific gravity of water (Ajibola et al., 2012). Some Heavy metals are important in daily diets because of their essential nutritious value. Metals like iron, copper, zinc and manganese are essential metals since they play an important role in biological systems; whereas lead and cadmium, etc. are non-essential metals which can be toxic even in trace amounts (Tuzen & Soylak, 2005). In small quantities, some heavy metals are nutritionally essential for a healthy life. These are referred to as the trace elements (Ajibola et al., 2012). The essential metals can also have harmful effects when

their intakes exceed the recommended quantities significantly. Honey is an important food for the human nutrition (Tuzen & Soylak, 2005). The occurrence of heavy metals above the admitted levels in honey is a threat to human health (Singh et al., 2014).

Heavy metals present in the atmosphere may be deposited on the hairy bodies of bees and may be brought back to the hive with pollen or absorbed together with the nectar of the flowers, or through water and honeydew. On other hand, the nectar, from which the honey is made, may contain heavy metals absorbed by the roots from the polluted soil (Tuzen & Soylak, 2005). As food stuff used for healing purposes, honey must be free of objectionable contents. It should contain only small amounts of pollutants, such as trace metals (Singh et al., 2014).

At high levels, heavy metals are undesirable because of their known or supposed toxicity and can be a source of health hazards to human beings. Heavy metals in honey are of interest mainly for quality control and nutritional aspects along with the determination of the level of toxic substances in the environment (Singh et al., 2014). Therefore, high concern exists about the contribution of these ingredients to the total dietary intake of trace metals that may be present as contaminants. Heavy metals are emitted in a continuous manner by various natural and anthropological sources. Since they are not degraded, they are continuously kept entering the physical and biological cycles (Singh et al., 2014).

Environmental pollution such as those from non-ferrous metallurgy, industrial smelter and factories and agrochemicals such as cadmium-containing fertilizers, and organic mercury and arsenic-based pesticides factors may contribute to the presence of heavy

metals in honey (Bilandžić et al., 2012). In addition to the environmental importance, the determination of heavy metals is important regarding to quality control of honey and because the fact that today's total production of honey in the world is increasing. Different heavy metals content have been determined in different honey samples in European and monitoring their concentrations is very important (Bilandžić et al., 2012).

Heavy metals in the atmosphere are deposited on flowers, water, air, soil and hairs of bee and are transported the hives along with pollen grains. It is known that trace metals such as sodium, potassium, calcium, iron, zinc and copper can be considered essential for human health (Silveira et al., 2013). Heavy metals are released in a continuous manner into the environment by various natural and anthropic sources and as they do not decay and are characterized by latent toxicity, they are continuously present in the environment and enter into the biological cycles (Ruschioni et al., 2013). Honey has attracted attention for its potency in prevention and treatment of illnesses and also its ability to cause well-being (Akbari et al., 2012).

The concentration of minerals in honey ranges between 0.04% and 0.2% in pale and dark honeys, respectively. This concentration could be greatly affected by the soil type of the source plants. Most abundant elements found in honey are potassium, calcium, magnesium and sodium. Providing the best possible quality of food will protect public health and preserve consumer confidence (Akbari et al., 2012). For this purpose, recently results for antibiotic residues in honey were published. In this study, 10 of the most famous honey brands in Iran market were collected, and their heavy metal/trace element contents, specifically cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), aluminium (Al), iron (Fe), manganese (Mn), zinc (Zn), selenium (Se) and copper (Cu), were

determined and compared with results of honey sample analyses published in the literature from different countries (Akbari et al., 2012).

The essential metals can also have harmful effects when their intakes significantly exceed the recommended quantities. Since food is one of the main source of heavy metal ions for human, the analysis of food samples for trace/heavy metal contents have been continuously performed. Other metals such as lead, cadmium, mercury, aluminum are classified as micro-contaminants of the environment, toxic or non-essential to living organisms and at high concentrations they are lethal (Silveira et al., 2013).

Heavy metals are important in daily diets because of their essential nutritional value and possible harmful effects. Metals like iron, copper, zinc and manganese are essential metals since they play an important role in biological systems; whereas lead and cadmium, etc. are non-essential metals which can be toxic even in trace amounts (Tuzen & Soylak, 2005). The occurrence of heavy metals above the admitted levels in honey is a threat to human health (Singh et al., 2014)

Heavy metals, pesticides, radioactivity, and antibiotics all endanger bee families. The division of the metals into required elements, neutral and toxic may be inaccurate and misleading, because all the required elements in small doses become toxic and very toxic in large doses. The difference between the concentrations in which they are useful and in which they are harmful can sometimes be very small (Porrini et al., 2003).

The low metal levels could be explained by mineral and trace element content in plant materials from which nectar honey has been produced. It is important to know that the plants contribute to accumulation of trace elements or heavy metals in their tissues,

according to their different availability in the soil. Pb is known to be rather immobile in the soil and it is poorly translocated to the vegetative parts, while Cd is easily adsorbed; their presence reflects the environmental pollution trend (Naccari et al., 2014).

The fundamental aspect that differentiates heavy metals from other pollutants, like pesticides, is their introduction into the territory and their environmental fate. Pesticides are scattered both in time and space and, depending on the type of chemical compound, they are degraded by various environmental factors over a longer or shorter period of time. Heavy metals, on the other hand, are emitted in a continuous manner by various natural and anthropical sources and are not degradable by any other means. Heavy metals in the atmosphere are deposited on the hairy bodies of bees and are sent into their hive together with the pollen, or they may be absorbed together with the nectar of the flowers, or through the water or the honeydew (Porrini et al., 2003). Iron is essential to life and plays irreplaceable roles in the functioning of critical enzyme systems (Matei et al., 2004). The other heavy metals of public health importance found in honey apart from As, Cd and Pb are chromium (Cr) and zinc (Zn). It is important to note that when honey comes in contact with stainless steel surfaces during storage can generate a high Cr content, due to the corrosive effect of honey acidity. It has also been documented that honey storage in galvanized containers can be a source of Zn contamination. Therefore it is important to take into consideration the type and quality of ware used to store honey after harvesting as the possible sources of honey contamination with heavy metals. In recent times, the increasing overwhelming demand of natural honey necessitates the promotion of all feasible activities towards ensuring quality and safety of the product (Ajibola et al., 2012).

2.8 Effects of Heavy Metals in Honey

Among many pollutants accumulating in the environment, there are elements of toxic properties like Cadmium, Chromium, Lead, Mercury and Arsenic may cause vascular diseases, kidney or bone damage, irregular functioning of human and animal reproductive system. They can easily penetrate to the cell membranes and internal organs as well as cause denaturation of proteins in the blood or mucous membranes and penetrate to the tissues (Singh et al., 2014). Heavy metals are dangerous because in the processing of food the metals do not decompose and that on the contrary their concentration referred to the mass unit increases. Secondly, the metals posse the feature to accumulate in the human organism slowing or even blocking the intracellular biochemical processes. Thirdly, the majority of the metals possess carcinogenic and mutagen proprieties. Once they are assimilated is very difficult to remove them from the human organism (Mujić et al., 2011).

Areas with intensive industry are associated with heavy metal pollution of the environmental, which is a first step of contamination of food sources. Iron, manganese and zinc are essential elements, very important for biological systems in small quantities, which can have a harmful effect when their concentration exceeds well known quantities. The excess of iron in food is correlated, in principal, with degenerative brain diseases. A higher concentration of zinc in human diet can interfere with white blood cells and other defense systems against infections and cancers. An increase of the doses of manganese in food can produce the nervous system disturbances (Dima & Popescu, 2012).

Heavy metals are toxic because they cause DNA damage and their carcinogenic effects in humans are caused by their mutagenic ability. A very important biological property of metals is their tendency to bioaccumulate. A potential threat is that heavy metals are not readily degradable and without intervention may progressively bioaccumulate in the body (Achudume & Nwafor, 2010). Symptoms of heavy metals in honey after intake may include dizziness, nausea, vomiting, convulsions, headache, palpitations and death in some cases. Furthermore, long term ingestion of honey containing heavy metals such as Cu and Fe may lead to significant reactions including gastrointestinal disorders (Achudume & Nwafor, 2010).

Lead and cadmium are not essential and undesirable elements in human and animal organisms as they do not perform any physiological functions but show strong toxic properties even when present at very low levels, causing several health effects. Moreover, the continuous exposure to low levels of these metals can result in bioaccumulation and negative health effects. However, FAO/WHO recommend that a tolerable intake for lead of (25 µg/Kg) and for cadmium of (7 µg/Kg) body weight/week (Khammas, Ghali, & Kadhim, 2012). The intracellular release of cadmium is responsible for the generation of reactive oxygen species, glutathione depletion, lip- id peroxidation, protein cross-liking, DNA damage, culminating ultimately in oxidant-induced cell death (Abdel-moneim & Ghafeer, 2007).

It has been reported that Pb can cause damage of brain, kidney, nervous system and red blood cells. The other health problems caused by heavy metals toxicity include headache, metabolic abnormalities, respiratory disorders, nausea and vomiting. These three heavy metals (As, Cd, Pb) have been identified in the priority list of top 20 hazardous

substances compiled by an agency of the United States of America Department of Health and Human Services, known as The Agency for Toxic Substances and Disease Registry (ATSDR) in 2001 (Ajibola et al., 2012).

2.9 Analytical Approach

2.9.1 Analytical Approach to Pesticide Residue in Honey

The most universally accepted extraction method to analyze a wide range of pesticides is the "QuEChERS Method". This method consists in two steps, liquid-liquid extraction and purification by dispersive Solid Phase Extraction. QuEChERS is an acronym which refers to Quick, Easy, Cheap, Efficient, Rugged and Safe (Wiest et al., 2011). The QuEChERS Method is a streamlined approach that makes it easier and less expensive for analytical chemists to examine pesticides residues in food sample preparation technique followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and initially validated according to the SANCO guidance 10684/2009 "Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed". The simplicity, robustness, rapidity and low solvent consumption are attractive parameters for the analytical chemist (Pirard et al., 2007).

There are several analytical procedure reported. Among the different reported analytical procedures are: liquid–liquid extraction (LLE) with immiscible solution (Blasco et al., 2011; Fontana et al., 2010; Pirard et al., 2007; Tahboub et al., 2006) solid-phase extraction (SPE) or matrix solid phase dispersion (MSPD) (Blasco et al., 2011) with various absorbers (Blasco et al., 2011; Fontana et al., 2010; Pirard et al., 2007; Tahboub et al., 2006) Solid-phase micro-extraction (SPME) (Blasco et al., 2011; Fontana et al.,

2010; Pirard et al., 2007; Tahboub et al., 2006) and supercritical fluid extraction (SFE) (Fontana et al., 2010; Tahboub et al., 2006) on column liquid–liquid extraction (OCLLE) (Blasco et al., 2011; Pirard et al., 2007) have been employed. Liquid–liquid extraction (LLE is the most popular technique for difficult matrix as honey). However, LLE requires large amounts of solvent, is time consuming, laborious and not well suited for automation (Fontana et al., 2010; Pirard et al., 2007). Moreover, due to the low concentration of analytes in the samples, large sample volumes are typically required to ensure detectability (Fontana et al., 2010).

Whereas SPE is based on the retention of selected analytes on cartridge sorbents and their elution with appropriate solvent, MSPD consists in the dispersion of the matrix on a free-adsorbent and its homogeneous packing on a column prior to elution of compounds with organic solvent allowing the extraction of semi-solid and solid samples. The other side of the coin is its poor capability for high sample input. Solid-phase microextraction (SPME) (Blasco et al., 2011; Fontana et al., 2010; Pirard et al., 2007) is a fast, simple and solvent-free extraction technique. The main drawbacks of this technique are the fragility and cost of the fibers, in addition to possible sample carry-over effects between runs (Fontana et al., 2010).

The supercritical fluid extraction (SFE) (Pirard et al., 2007) and stir-bar sorptive extraction (SBSE) still remain quite marginal in this area until now (Blasco et al., 2011). A number of reviews have indicated that compared to conventional GC methods, LC–MS are very straightforward, sensitive, fast and more reliable. However, for LC–MS and/or LC with tandem MS (LC–MS^2), it is still important to apply a good extraction and preparation method, as matrix effects can impact on detection systems, generating

significant noise, or altering ionization efficiency and what is more, can impact on limits of detection and quantification. To establish what method is better than other, comparison of the results is the best way.

Mass spectrometery represents the most selective detector for pesticides as it provides structural information allowing unequivocal confirmation and its use in a multi-residue screening context. Although GC is often reported as the most powerful separation tool, it involves a derivatization step for thermally unstable compounds. This introduces additional handling and reaction, thus potentially reducing reproducibility and recovery rates (Pirard et al., 2007). Most pesticides are separated by LC prior to MS detection except dimethoate and fipronil, which are reported to be analyzed by GC–MS or SPME-GC–MS.

To avoid derivatization step and allow a less rugged clean-up were the reasons which led us to use liquid instead of gas chromatography. The configuration of the Z-spray source designed at first to prevent fragmentation during ionization enhances LC robustness in terms of matrix related interferences, as only charged species enter in the detector. The use of tandem mass spectrometry confers high specificity and reduces the risk of potential interferences related to the complexity of the matrix. Each precursor ion was fragmented by collision-induced dissociation and the two most abundant produced ions were monitored. In addition to this gain of selectivity, the use of the MS/MS mode substantially increases sensitivity by limiting the high background noise related to the honey matrix (Pirard et al., 2007).LC–MS/MS is well-known for its great sensitivity and as a reliable tool for quantification (Wiest et al., 2011).

In the determination of pesticide residue in honey samples collected from the major honey producing forest belts in Ghana, GC coupled with ECD and PFPD was adapted successfully for the pesticide analysis because (Wiest et al., 2011) it has been proposed that it is among the most efficient technique available to analysts (Astellanos et al., 2002).

2.9.2 Analytical Approach to Heavy Metals Analysis in Honey

The heavy metals content (cadmium, copper zinc, iron and lead) in honey samples are often determined using Atomic Absorption Spectrophotometer (AAS) (Ahmida et al., 2013). There are many other approaches to determine the concentration of heavy metals in honey. For instance in the Federal Institute of Consumer Health Protection and Veterinary Medicine in Berlin, (Germany) Concentrations of metals were determined using double focusing and high resolution ICP–MS model "Element" (Finnigan MAT, Bremen). This method enables determination of the amounts of metals that are lower than 10^{-12} g/kg (Matusevicius et al., 2010).

A Shimadzu 1650 PC model double-beam UV-Vis spectrophotometer (Japan) equipped with 10-mm optical path cell were used for the scanning study of absorption spectra of the complexes formed, while absorbance measurements were carried out with spectrophotometer Sunny UV-7804C (China). The effect of temperature was investigated by using a water bath WB 710 model (OPTIMA, Japan). A microprocessor pH meter 211 model (Triup International Corp., Italy) with a combined electrode was used for pH measurements (Khammas et al., 2012) are also use in the determination of heavy metal concentration in honey samples. Ultraviolet-visible (UV-Vis) spectroscopy has got a lot of advantages over others because it is simple instrument, cheap, easy operated, rapid

response time, available in many laboratories and offers acceptable analytical figures of merit when dealing with trace levels of metals in different matrices. But due to its low detection power, extraction and preconcentration procedures are a must which can dramatically improve the detection limit as well as the selectivity of the technique (Khammas et al., 2012).

Inductively coupled plasma atomic emission spectrometer (ICP-AES).—Optima 3100 (Perkin-Elmer Co., Norwalk, CT), axial view, equipped with cross-flow type nebulizer with Ryton Scott chamber and polychromator with echelle grating (ruling density 79 lines/mm), combined, in turn, with a Schmidt cross disperser. Signal detection was achieved by a simultaneous solid-state segmented-array charged-coupled device detector (SCD). The wavelength range was 165–403nmwith maximum resolution of 0.006nm at 200 nm (Forte et al.,, 2001). Recently, the metals determination in various grades of honey by atomic spectrometric techniques (FAAS, ETAAS and ICP-OES) is well reviewed. Inductively coupled plasma-mass spectrometry total reflection X-ray fluorescence spectrometry and ion chromatography and voltammetry have also been applied for the determination metals in honey (Khammas et al., 2012).

CHAPTER THREE

METHODOLOGY

3.1 Reagents and Solutions

Table 1: Information Pertaining to the Name, Grade and Source of Reagents and Solutions used in this Research Work are given in Table1 below.

Reagent	Grade	Source
Acetonitrile	Pesticide	BDH Laboratory Supplies, England
Sodium chloride	AnalaR	BDH Laboratory Supplies, England
Disodium hydrogencitratesesquihydrate	AnalaR	BDH Laboratory Supplies, England
Trisodium citrate dihydrate	AnalaR	BDH Laboratory Supplies, England
Sodium hydroxide	AnalaR	BDH Laboratory Supplies, England
Bondesil-PSA 40 µm	-	Varian
Ethyl acetate	Pesticide	BDH Laboratory Supplies, England
Magnesium sulphate anhydrous	AnalaR	BDH Laboratory Supplies, England
Formic acid conc. (>95%ig)	AnalaR	BDH Laboratory Supplies, England
Pesticide Standards	Certified	Dr. Ehrenstorfer GmbH

3.2 Description of the Study Sites

Honey samples were collected from the major honey producing forest belts in Ashanti, Brong Ahafo and Western Regions of Ghana. All sites are located in agricultural landscapes where various pesticides are continuously applied to either control insects or weeds. The product formed by the honey bees after visiting various flowers for their nectars are used in this research to determine whether the bees carry the applied pesticides into their hive. Two sets of honey are used. These are those honeys from the wild forest and the other set were those from the bee keepers. Two sets of honey samples were collected from each site and analysed separately. The first set was the honey

collected from wild forest whiles the second set was honeys collected directly from the bee keepers. All the honey samples were collected from February to June 2014 within the harvesting season.

A purposive sampling method approach was used in sample collection because the samples at the sample sites were not many. Most of the honey samples were collected by the researcher with the aid of fire to drive away the honey bees and protective gown against the bees bite. After harvesting, the honey was squeezed from the wax followed by filtration to remove any foreign materials that may be present in the honey. In all, a total of 45 honey samples consisting of 30 from the wild forest and 15 from the beehive were obtained for the researcher. About 500 mL of each of the samples was placed in separate plastic containers and sent to the laboratory for the analysis.

3.3 Gas Chromatography

The samples obtained were extracted and analyzed for pesticides residues using standard method. Varian CP-3800 Gas Chromatograph with a CombiPAL Autosampler, Electron Capture Detector for Organochlorine and synthetic pyrethroids pesticides whilst Pulse Flame Photometric Detector was used for Organophosphorus pesticides because it has been proposed that it is among the most efficient technique available to analysts (Wiest et al., 2011).

3.4 Honey Sample Preparation

Forty five honey samples from three botanical origins namely; Ashanti, Brong Ahafo and Western regions of Ghana were obtained from beekeepers as well as wild forest. The

samples were put in glass bottles and wrapped with aluminium foil before they were taken to the laboratory. All samples were kept at ambient temperature until the analysis.

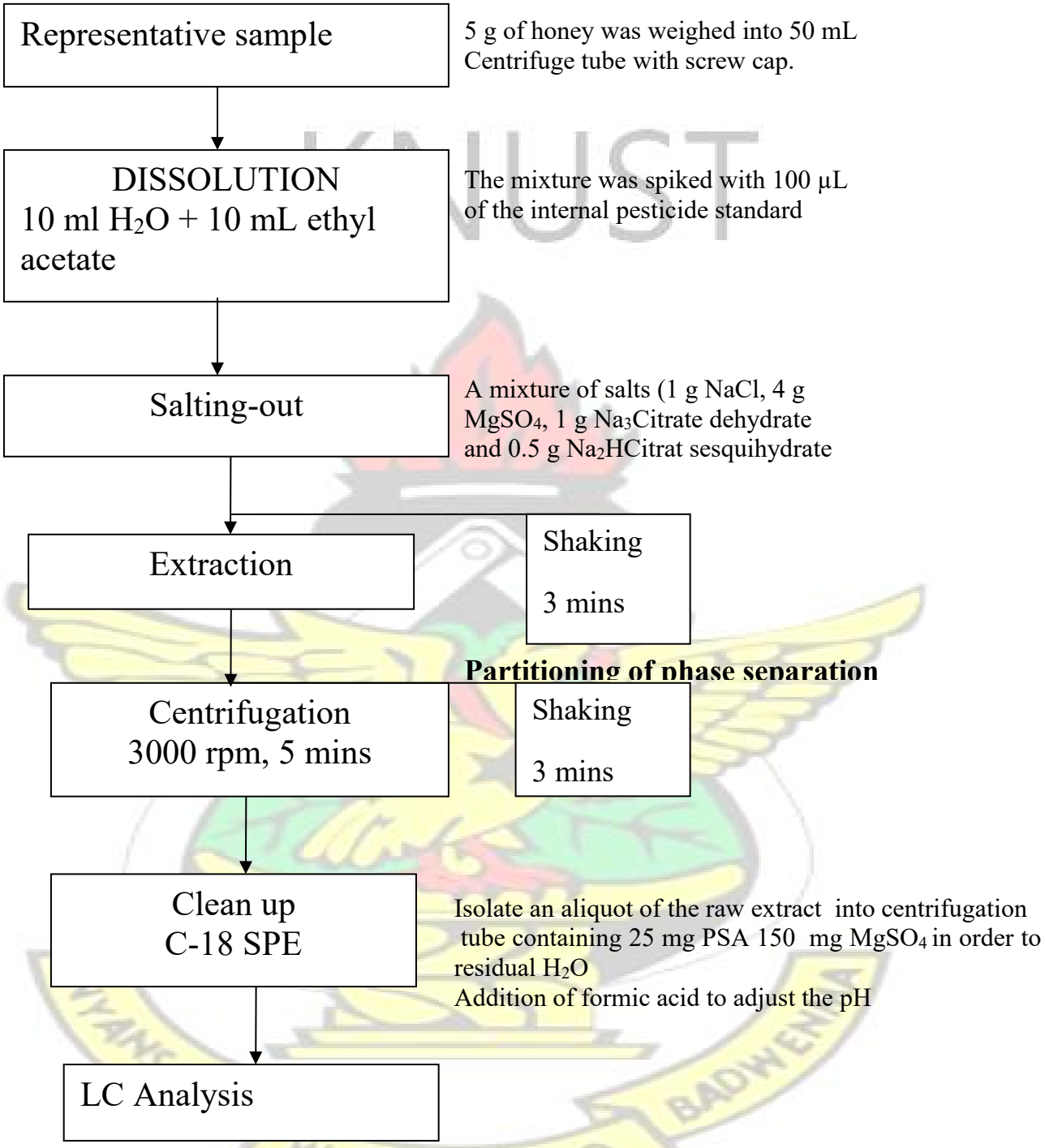
3.5 Extraction Procedure

In order to analyze a number of pesticide residues in honey, QuECHER method, a multiresidue method for the analysis of pesticide residue in low fat matrix was used. A 5 g of the homogenized honey sample was weighed in an Erlenmeyer flask and spiked with 100 µL of the internal pesticide standard solution and mixed with 10 ml of water and homogenized by shaking to reduce its viscosity and facilitate its handling. The sample was mixed with 10 ml of acetonitrile solvents tested and subjected to extraction by agitating for 3 mins. A mixture of salts (1 g sodium chloride, 1 g disodium hydrogencitrate sesquihydrate, 0.5 g trisodium citrate dehydrate and 4 g anhydrous magnesium sulphate) were added to the sample and vortex for 3 mins for extraction with separation.

The organic phase was separated from the inorganic phase by centrifugation at 3000 rpm for 5 minutes. The supernatant was collected and the residue was re-extracted with 10 ml of acetonitrile. The extract was transferred into PP single use centrifugation tube, which contained 25 mg PSA and 150 mg $MgSO_4$ per mL. The addition of $MgSO_4$ was to absorb the residual water. The tube was vortex for 1 min followed by centrifugation at 3000 rpm for 5 minutes. After the centrifugation, the cleaned extract was transferred into a screw cap vial and the pH was quickly adjusted to ca.5 by adding a 5 % formic acid solution in acetonitrile (vol/vol) (pro mL extract ca. 10 µL). The pH adjusted extract in filled into

vials for gas chromatography and is used for further analysis (Basavarajappa & Raghunandan, 2013).

For honey fortification 5 g of the control sample was heated in a water bath at 40^{o}C for 20 min and spiked by adding an appropriate volume of standard working solution to reach the concentrations 0.02 and 0.20 mg/kg. The mixture was mechanically stirred in a blender to ensure homogenization and then subjected to the extraction step (Basavarajappa & Raghunandan, 2013). The flow chart for the extraction of pesticide residues in honey are given in Figure 4.



(Bargańska, Ślebioda, & Namieśnik, 2014; Rissato et al., 2007)

Figure 4: The flow chart for the extraction of pesticide residues in honey

3.6 Chromatographic conditions for Organochlorine and Synthetic Pyrethroids Pesticides Residue Content in Honey Using GC-ECD

All compounds were determined and quantified with the aid of a gas chromatograph equipped with electron capture detector (GC-ECD), an autosampler and a split-splitless injector, 30 m + 10 m EZ Guard x 0.25 mm internal diameter fused silica capillary coated with VF-5ms (0.25 µm film) from Varian Inc or equivalent. The carrier gas was nitrogen at a flow rate of 1 ml/min at constant flow rate. Oven temperature was maintained initially at 70 ^{O}C for 2 min, increased at 25 ^{O}C/min to 180 ^{O}C, then at 5 ^{O}C/min to 300 ^{O}C. The injection volume was 1µL, injected in splitless mode at injection temperature of 270 ^{O}C whilst the detector temperature was 300 ^{O}C.

3.7 Chromatographic conditions of Organophosphorus Pesticides Residue Content in Honey Using GC-PFPD

All compounds were determined and quantified with the aid of a gas chromatograph equipped with pulse flame phosphorus detector (GC-PFPD), an autosampler and a split-splitless injector, 30 m x 0.25 mm internal diameter fused silica capillary coated with VF-1701ms (0.25 µm film) from Varian Inc. The carrier gas was nitrogen at a flow rate of 2 ml/min at constant flow rate. Oven temperature was maintained initially at 70 ^{O}C for 2 min, increased at 25 ^{O}C/min to 200 ^{O}C/1 min, then at 20 ^{O}C/min to 250 ^{O}C. The injection volume was 1µL, injected in splitless mode at injection temperature of 270 ^{O}C whilst the detector temperature was 280 ^{O}C.

The average retention time for organochlorine and synthetic pyrethroids of both the internal pesticide standards and the honey samples collected from the forest belts namely:

Ashanti, Brong Ahafo and Western regions of Ghana using GC-ECD are given in the table below.

3.8 Limits of Detection of the Pesticides

The detection limits of the GC coupled with either (ECD/PFPD) were determined for each pesticide category by successive dilution of the standard mixed pesticide solution followed by injection into the GC-volume several times. Serial dilution experiments provided the necessary information to calculate the detection limits. The detection limits for all the pesticide categories were found to be 0.01 mg/kg.

3.9 Quality Control

The quality control for the analysis of pesticides in honey consisted of two samples, one honey spiked and one blank spike with five calibration standards (ranging from 0.010 to 2.00 mg/kg of mixed pesticide solution standards), a calibration check standard and ethyl acetate rinses. The honey spike was selected from a set of several free pesticide samples and consisted in fortifying the honey with a mixed pesticide spike standard. The honey and blank samples were fortified at 0.020 mg/kg and analyzed from 60% to 130%. The positive results in the honey samples were confirmed by comparing the retention time and identifying the main ions in relation to those of a pesticide standard. Retention times were within ±0.20 min of the expected retention times (Basavarajappa & Raghunandan, 2013).

3.10 Digestion Procedure for the Heavy Metals

A 5 mL of honey samples was weighed into 100 mL volumetric flask, 10 mL of Conc. HNO_3 was added and heated on a hot plate at 200 ^{O}C until a clear solution was obtained. The digest was allowed to cool in a fume hood and filtered into 50 mL volumetric flask. The filtrate was topped up with distilled water to the 50 mL mark.

In this study, concentrations of heavy metals for Cd, Pb, Fe, Zn and Cu were determined in forty five honey samples collected from the major honey producing forest belts in Ghana. The quality of data was checked by analysis of the recovery rate with spiked honey samples for Cd, Pb, Fe, Zn and Cu and showed good accuracy, with recovery rate for metals of 95.9 % to 99.2 %. The limit of detection (LODs, mg/kg) was 0.001 for all the heavy metal analysed.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

The use of pesticides to protect crops against plagues and insects is one of the most important ways to assure quality and productivity of agricultural products. However, wrong application practices may result in the contamination of different environmental compartments and animal species (Flores, et al., 2010). However, this study is focusing on determination of all the pesticides groups or families that are used in agriculture as well as heavy metals in the honey sample collected from the major honey producing forest belts in Ashanti, Brong Ahafo and Western regions of Ghana.

The method was evaluated according to the "Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed" (SANCO/10684/2009) (SANCO/2007/ 3131, 2007) for its repeatability, linearity, recovery, limit of detection and quantification (Bargańska et al., 2013).

Retention time of the internal pesticide standard and the average retention time for the organochlorine and synthetic pyrethroids in the honey from the three regions are given in Table 2 below.

Table 2: Retention time of the internal pesticide standard and the average retention time for the organochlorine and synthetic pyrethroids in the honey from the three regions

Types of pesticide	Standard (min)	Ashanti (min)	Brong Ahafo (min)	Western (min)
β-HCH	11.288	10.735	10.734	10.907
γ-HCH	11.357	11.083	11.863	ND
δ-HCH	12.221	11.859	ND	11.866
Heptachlor	13.036	12.266	12.368	12.244
Aldrin	14.060	13.220	13.229	13.202
Allethrin	14.970	14.606	14.660	14.764
γ-chlordane	15.954	15.208	15.263	15.275
α-endosulfan	16.336	ND	ND	15.840
p,p'-DDE	16.622	16.358	16.346	16.230
Dieldrin	16.719	16.361	16.360	ND
Endrin	17.226	17.106	16.993	17.126
β-endosulfan	17.916	17.552	17.519	17.614
p,p'-DDT	18.152	17.959	17.953	17.948
P,p'-DDD	18.393	ND	ND	ND
Endosulfan sulfate	19.636	19.168	19.060	19.063
Bifenthrin	20.449	20.478	20.638	20.759
Fenpropathrin	20.920	20.662	ND	ND
Methoxychlor	21.201	21.061	21.040	ND
λ-cyhalothrin	22.252	ND	22.628	ND
Permethrin	23.840	24.054	24.054	24.054
Cyfluthrin	24.909	25.387	25.387	ND
Cypermethrin	25.480	25.999	25.999	25.999
Fenvalerate	27.040	ND	ND	ND
Deltamethrin	28.257	28.933	28.928	28.792

ND = Not Detected

The average retention time for organophosphorus residues of both the internal pesticide standards and the honey samples collected from the forest belts namely: Ashanti, Brong Ahafo and Western regions of Ghana using GC-PFPD are given in Table 3 below.

Table 3: Retention time of the internal pesticide standard and the average retention time for the organophosphorus residues in the honey samples for the three regions

Types of pesticides	Standard (min)	Ashanti (min)	Brong Ahafo (min)	Western (min)
Methamidophos	7.021	6.760	6.777	6.748
Ethoprophos	8.335	ND	ND	ND
Phorate	8.696	ND	ND	ND
Diazinon	9.092	8.994	8.957	8.960
Fonofos	9.340	ND	ND	ND
Dimethoate	9.572	ND	ND	9.837
Pirimiphos-methyl	10.134	ND	ND	10.074
Chlorpyrifos	10.337	10.211	10.753	10.222
Malathion	10.561	ND	ND	ND
Fenitrothion	10.682	ND	ND	ND
Parathion	10.904	ND	10.755	10.766
Chlorfenvinphos	11.080	ND	ND	ND
Profenofos	11.750	ND	ND	11.630

ND = Not Detected

The average concentrations in mg/kg of Organophosphorus in honey from the forest belts namely: Ashanti, Brong Ahafo and Western regions of Ghana for both beehive and wild forest honey are given in the Table 4 below.

Table 4: The average concentrations in mg/kg of Organophosphorus in honey from the forest belts

Types of pesticide	Average Concentrations obtained (mg/kg)						EU MRLs
	Ashanti		**Brong Ahafo**		**Western**		
	Beehive	**Wild**	**Beehive**	**Wild**	**Beehive**	**Wild**	
Diazinon	ND	ND	0.01	ND	0.01	ND	0.01
Chlorpyrifos	ND	0.01	ND	0.01	ND	ND	0.05
Pirimiphos methyl	ND	ND	ND	ND	ND	ND	0.05
Parathion	ND	ND	ND	ND	ND	ND	0.05
Methamidophos	ND	ND	ND	ND	ND	ND	0.01
Phorate	ND	ND	ND	ND	ND	ND	0.05
Dimethoate	0.01	ND	ND	ND	ND	ND	0.02
Methoxychlor	ND	0.01	ND	0.01	ND	ND	0.01
Phosmet	ND	ND	ND	ND	ND	ND	0.05
Fenitrothion	ND	ND	ND	ND	ND	ND	0.01
Malathion	ND	ND	0.01	ND	0.01	ND	0.02
Profenofos	ND	ND	ND	ND	ND	ND	0.05

ND = Not Detected EU MRs = European Union Maximum Residue Limits

The average concentrations in mg/kg of Organochlorine and Synthetic Pyrethroids in honey from the forest belts namely: Ashanti, Brong Ahafo and Western regions of Ghana for both beehive and wild forest honey are given in the Table 5 below.

Table 5: The average concentrations in mg/kg of Organochlorine and Synthetic Pyrethroids in honey from the forest belts

Types of pesticides	Average Concentrations obtained (mg/kg)						EU MRLs
	Ashanti		**Brong Ahafo**		**Western**		
	Beehive	**wild**	**Beehive**	**wild**	**beehive**	**wild**	
Aldrin	ND	ND	ND	0.01	ND	0.01	0.01
Dieldrin	ND	ND	ND	ND	ND	ND	0.01
Bifenthrin	ND	ND	ND	ND	ND	ND	0.05
Cyfluthrin	ND	0.02	ND	0.02	ND	0.02	0.02
Cypermethrin	ND	ND	ND	ND	ND	ND	0.05
Heptachlor	ND	ND	ND	ND	ND	ND	0.01
ΣEndosulfan	0.01	ND	ND	0.01	ND	0.01	0.05
Lindane	ND	ND	ND	ND	ND	ND	0.01
Methoxychlor	ND	ND	ND	ND	ND	ND	0.01
Lambda cyahalothrin	ND	ND	ND	ND	ND	ND	0.02
Permethrin (cis/trans)	ND	0.04	0.01	0.04	ND	0.04	0.05
Gamma-chlordane	ND	ND	ND	ND	ND	ND	0.01
Deltamethrin	ND	ND	ND	ND	ND	ND	0.05
Fenvalerate	0.01	0.01	ND	ND	ND	0.01	0.02
β-HCH	ND	ND	ND	ND	ND	ND	0.01
α-HCH	ND	ND	ND	ND	ND	ND	0.01
ΣDDT	0.01	ND	ND	ND	0.01	ND	0.01

ΣEndosulfan (sum of isomers) Σ DDT (sum of p,p´ DDT, o,p´-DDT, p-p´-DDE)

ND= Not Detected EU MRs = European Union Maximum Residue Limits

4.1 Discussions

Honey samples collected from the major honey-producing forest belts in Ashanti, Brong Ahafo and Western Regions of Ghana revealed the presence of different categories of

pesticide based on their functional group (Organophosphorus, Organochlorine and Pyrethroids) and are within the limit of detection that is 0.01 mg/kg. However only a few of Organophosphorus, Organochlorine and Pyrethroids were detected and most of them were below detection limit.

For Organophosphorus pesticide, only chlorpyrifos, dimethoate, methoxychlor and malathion were detected and the concentrations at which these were detected were all within the EU MRLs. The concentrations at which these pesticides were detected were 0.01 mg/kg for each pesticide. None of the Organophosphorus pesticides were having concentration greater than the detection limit or the European Union Maximum residue limit of honey and that consumption of any honey from the forest belts of the country would not pose any health risk to the consumer.

For the organochlorine and synthetic pyrethroids aldrin, cyfluthrin, cypermethrin permethrin deltamethrin, fevalerate, endosulfan and DDT were detected. The concentrations at which these pesticides were detected were 0.01 mg/kg for each with the exception of cyfluthrin and permethrin which were detected at 0.02 and 0.04 mg/kg respectively. The efforts made to restrict pesticide application provided some relief but there were no mechanism to control the residual activity of some pesticides effectively.

According to European Union (EU) Regulations, honey as a natural product, must be free of any chemical contaminants and safe for human consumption (Blasco et al., 2004). On this basis, all the samples analysed agreed with this regulation.

The average concentrations in (mg/kg) of some heavy metals in honey samples namely: Fe, Zn, Cd, Cu, and Pb collected from the major honey producing forest belts in Ashanti, Brong Ahafo and Western Regions of Ghana are presented in Table 6 below.

Table 6: The average concentrations in (mg/kg) of some heavy metals in honey samples collected from the forest belts namely: Ashanti, Brong Ahafo and Western regions of Ghana.

Test Conducted (mg/kg)	Ashanti		Brong Ahafo		Western		Average Conc.	Standard deviation
	Wild	beehive	Wild	beehive	Wild	beehive		
Zn	0.22	0.21	0.20	0.18	0.24	0.29	0.22	0.47
Cu	3.22	3.12	3.15	3.43	3.20	3.24	3.23	9.92
Pb	0.88	0.81	0.84	0.86	0.85	0.79	0.84	0.59
Fe	2.21	2.24	2.30	2.33	2.31	2.36	2.30	5.04
Cd	0.03	0.03	0.03	0.02	0.02	0.02	0.03	0.16

Conc. = Concentrated

The purpose was to determine heavy metal concentrations in honey samples collected from the major honey-producing forest belts in Ashanti, Brong Ahafo and Western regions of Ghana. The investigation revealed that, there are different heavy metal contaminants in the honey samples with average concentration ranging from Cd 0.02 < Zn 0.22 < Pb 0.84 < Fe 2.30 < Cu 3.23 mg/kg. Cadmium recorded the lowest concentration of 0.02 mg/kg whilst copper recorded the highest concentration of 3.23 mg/kg.

Pb, Cd, Cu and Zn belong to the group of the biogenic elements whose concentrations of which in honey is regulated by maximum tolerance limit (MTL) or standards of microelements in nutrition products (Matusevicius, et al., 2010).

According to published data, only in Macedonia is the maximum permitted value for Cd and Cu in honey is set to be 0.03 mg/kg for Cd and 1 mg/kg for Cu (Bilandžić et al., 2012). This study revealed that Cd is in line with the published data but Cu recorded a concentration which was about three times higher than the literature value. Cadmium in multifloral honey levels observed were lower than the contents obtained in different geographical regions of Turkey and Macedonia (3.63 mg/kg) and to previously reported levels in multifloral honey samples from different regions in Croatia (1.51 mg/kg). But the level of cadmium obtained was 0.02 mg/kg which were far smaller than the heavy metal analysis done in Turkey and Croatia. Cd reaches humans through air, water and food. This metal had no beneficial role in human metabolism and produces a progressive toxicity and can cause health disorders such as fatigue, sleeplessness, hearing and weight loss and that Cd would not pose any health risk to individual who consume honey for any purpose be it food or medicine (Bilandžić et al., 2012).

Copper has a nutritional value which is very important in human diet but at higher concentrations may generate toxic effects such as dermatitis, liver cirrhosis and neurological disorders, while acute Cu poisoning causes symptoms of nausea, vomiting and abdominal and muscle pain. The provisional permitted daily intake for Cu determined in an average adult with 60 kg body weight is 3 mg (Bilandžić et al., 2012). The level of concentration of Cu obtained from the analysis was 3.23 mg/kg which is higher than the daily required intake by adult human.

Zinc is an essential element that occurs in the human body in larger quantities. Its concentration in humans is about 1.8 g (Mujić et al., 2011). In this study, the average concentration of Zn content in all honey samples was 0.22 mg/kg which was much lower than the concentration of zinc in humans. According to the RDA, allowed daily allowance for men is 15 mg, and for women 12 mg. Consumption of 100 g honey from honey samples collected from the major honey-producing forest belts in Ashanti, Brong Ahafo and Western regions of Ghana with its portion of zinc, one consumes 0.22 mg of zinc, which meets 1.47 % of the allowed limit reached by the daily input of zinc, which cannot be called a critical value. These levels of metals may be appropriate for honey (Âelechovská & Vorlová, 2001).

Fe is essential to life and plays irreplaceable roles in, for example, the functioning of critical enzyme systems (Matei et al., 2004). The average concentration of this metal was found to be 2.30 mg/kg. Pb is relatively unavailable to plants when the soil pH level is above 6.5. In case of pH values less than 6.5, there is actual increase of Pb uptake by the plant itself from soil (Bilandžić et al., 2012) and that might be the root cause of Pb in honey.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In all forty five honey samples were purposively collected from both wild forest and beehives from three regions in Ghana, namely; Ashanti, Brong Ahafo and Western Regions and analysed for both heavy metals and pesticides residues. The pesticide residue analysis which was done with GC-coupled with ECD/PFPD revealed only 15 pesticides residues in all the analysis and none of the pesticides residues detected had a concentration which was higher than the accepted value set by the EU. The pesticide residues detected were aldrin, γ-HCH, β-HCH, Σendosulfan, cyfluthrin, cypermethrin, deltamethrin, permethrin methoxychlor, ΣDDT, chloripyrifos, fenvelerat, malathion, dimethoate and diaxinon. For the heavy metal analysis using AAS, the concentrations were in the order as: Cd 0.02 < Zn 0.22 < Pb 0.84 < Fe 2.30 < Cu 3.23 mg/kg. Cd recorded the lowest concentration 0.03 mg/kg whilst Cu recorded the highest concentration of 3.23 mg/kg.

The determination of Organophosphorus, Organochlorine and Synthetic Pyrethriod pesticides and heavy metals in 45 different honey samples collected from the three forest belts in Ghana revealed the presence of the following pesticides. The study signifies the importance of heavy metals and pesticide analysis in order to know the level of contamination, safeguard the consumer's health and to maintain honey as a natural product devoid of any contaminants. Honey is a highly valuable food, which has also other potential uses, like sweeteners and treatment of different illnesses or food preservative. The results from the investigation clearly indicate that, there is no

significant contamination of heavy metals and pesticides residues in honey from the major honey-producing forest belts in Ashanti, Brong Ahafo and Western Regions of Ghana.

5.2 Recommendations

Due to the potential health risk associated with heavy metals and pesticides residues in honey, nationwide investigation is necessary to make general conclusion since this work was restricted to only the forest belts in Ghana to ascertain the concentrations of pesticide residues. Policy makers should regulate the production and the use of the synthetic pyrethroids since permethrin recorded significant concentration and was detected in all the regions.

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Appendices

Appendix 1: Sample chromatograms from the GC-PFPD analysis of Organophosphorus pesticide residues

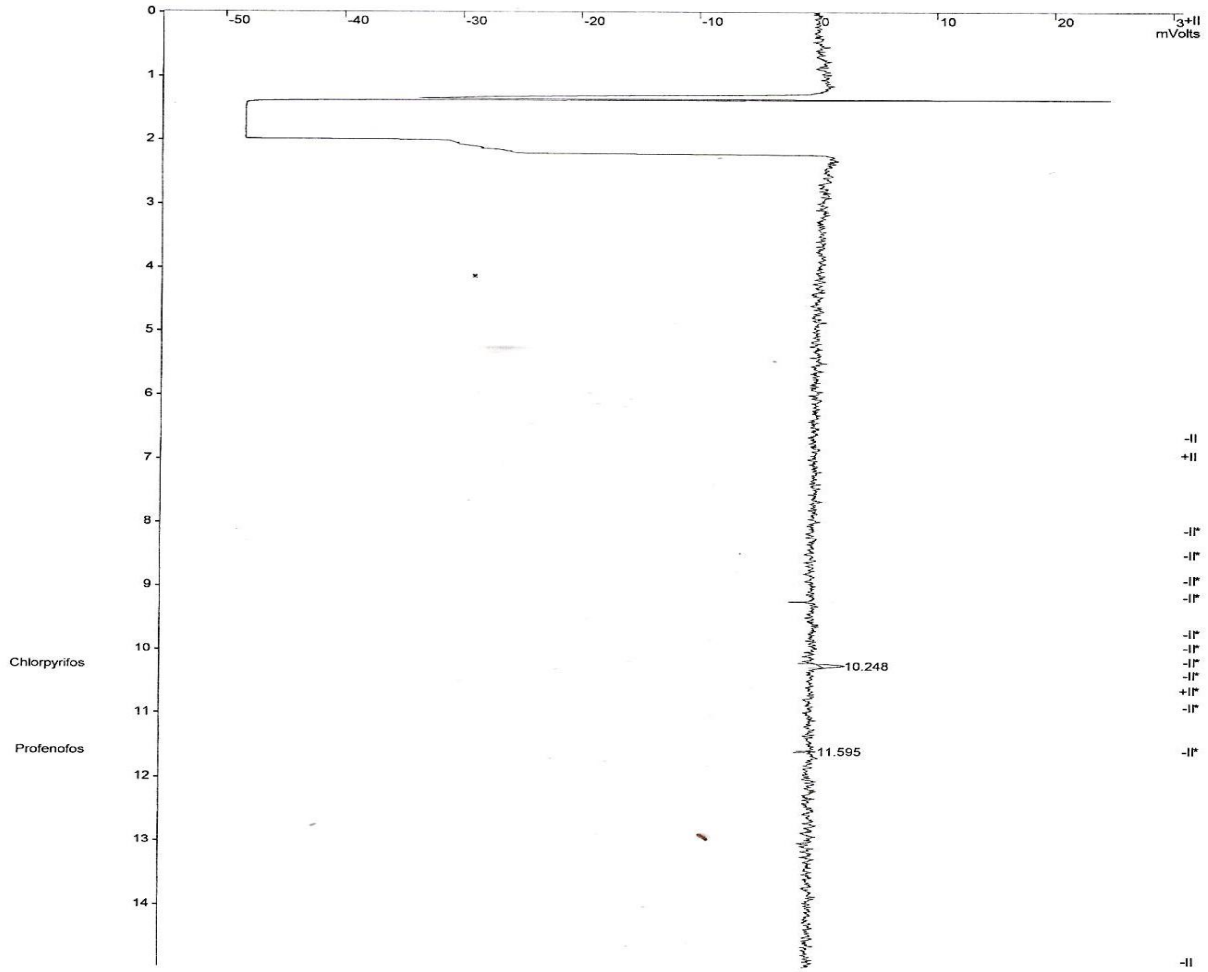

```
tle       :
n File    : C:\star\data\2014\Normal Analysis\Mar\No. 7 (2014-03-18)\2014-03-18 op\reg blk 14-03-18.run
thod File : c:\star\data\2014\normal analysis\mar\no. 9 (2014-03-26)\2014-03-26 oc\spk 173_pes2-14-rear.mth
mple ID   : Reg Blk 14-03-18

jection Date: 3/20/2014 2:14 AM    Calculation Date: 4/4/2014 11:11 AM

erator   : SKF                    Detector Type: 3800 (10 Volts)
rkstation: OS                     Bus Address  : 44
strument : Varian CP-3800 GC      Sample Rate  : 10.00 Hz
annel    : Rear = PFPD            Run Time     : 14.987 min

 GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

art Speed =    1.34 cm/min   Attenuation = 36          Zero Offset = 63%
art Time  =   0.000   min     End Time    = 14.987  min   Min / Tick  = 1.00
```



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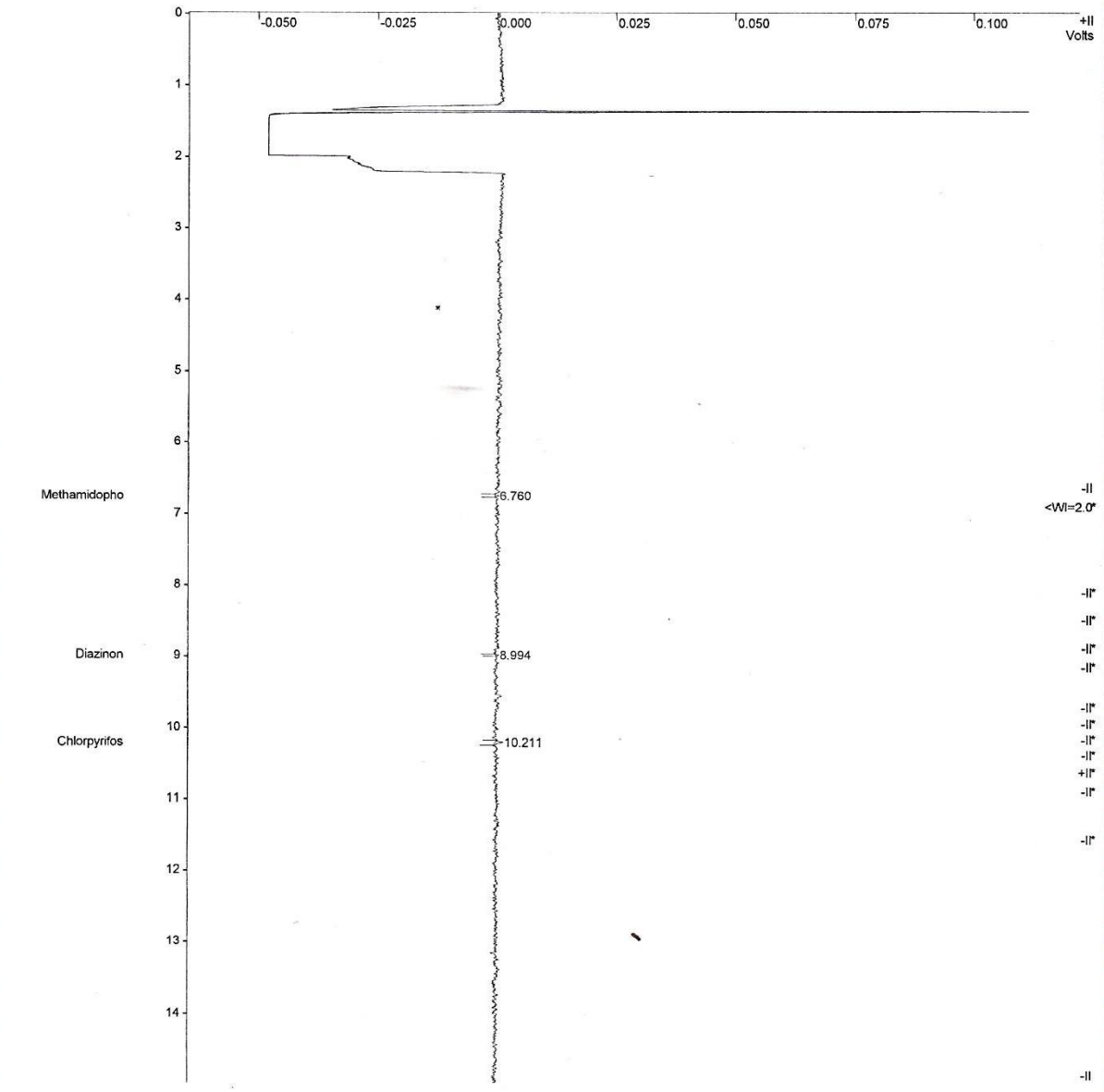
thod File : c:\star\data\2014\normal analysis\mar\no. 9 (2014-03-26)\2014-03-26 oc\spk 173_pes2-14-rear.mth
mple ID   : 169-PES2-14

jection Date: 3/20/2014 2:33 AM    Calculation Date: 4/4/2014 11:11 AM

erator   : SKF                  Detector Type: 3800 (10 Volts)
rkstation: OS                   Bus Address  : 44
strument : Varian CP-3800 GC    Sample Rate  : 10.00 Hz
annel    : Rear = PFPD          Run Time     : 14.987 min

 GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

art Speed =    1.34 cm/min   Attenuation = 78          Zero Offset = 34%
art Time  =   0.000   min     End Time    = 14.987  min   Min / Tick  = 1.00
```



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mple ID   : 171-PES2-14

jection Date: 3/20/2014 3:12 AM    Calculation Date: 4/4/2014 11:11 AM

erator   : SKF                  Detector Type: 3800 (10 Volts)
rkstation: OS                   Bus Address  : 44
strument : Varian CP-3800 GC    Sample Rate  : 10.00 Hz
annel    : Rear = PFPD          Run Time     : 14.987 min

 GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

art Speed =    1.34 cm/min   Attenuation = 75          Zero Offset = 34%
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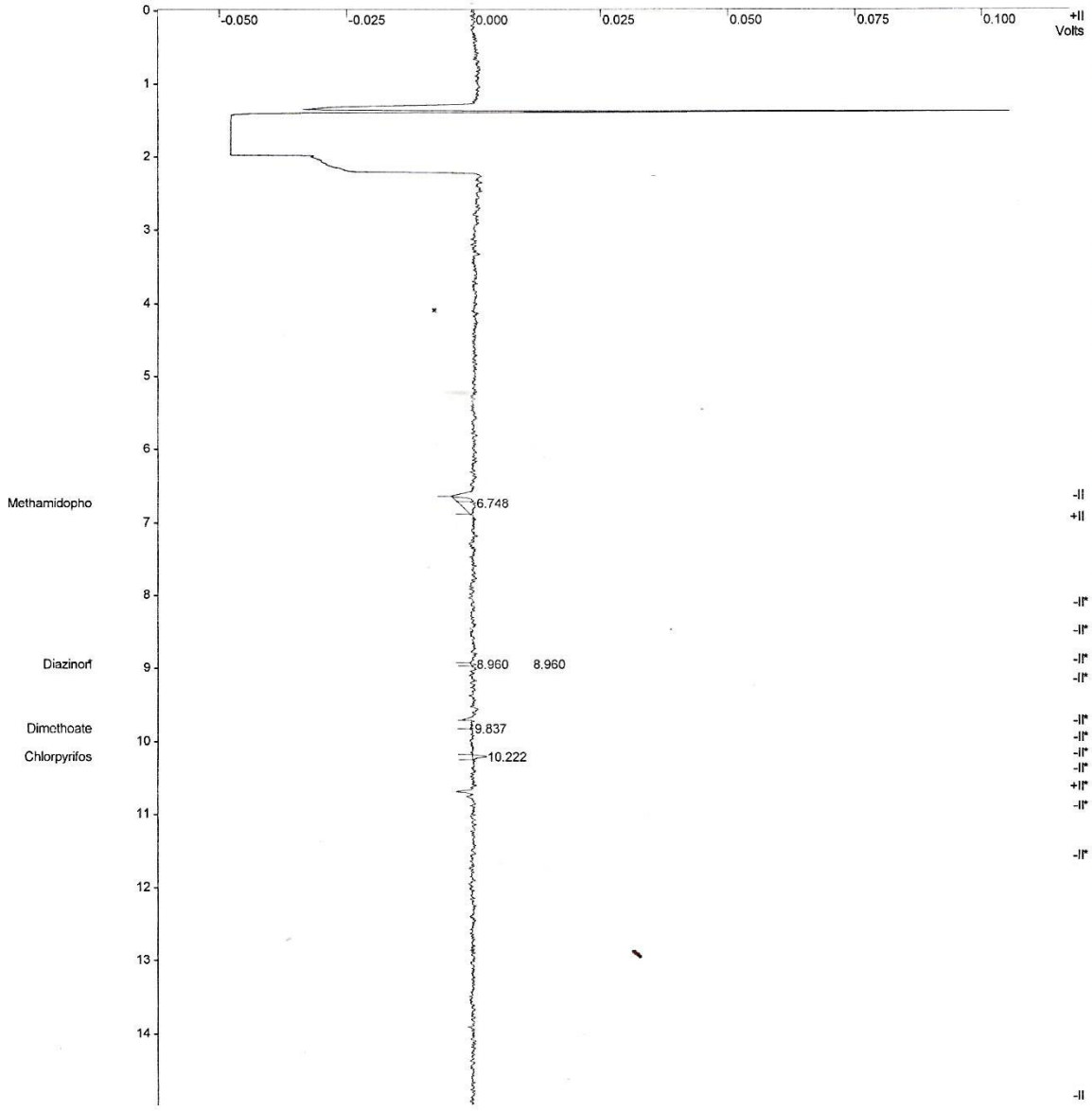
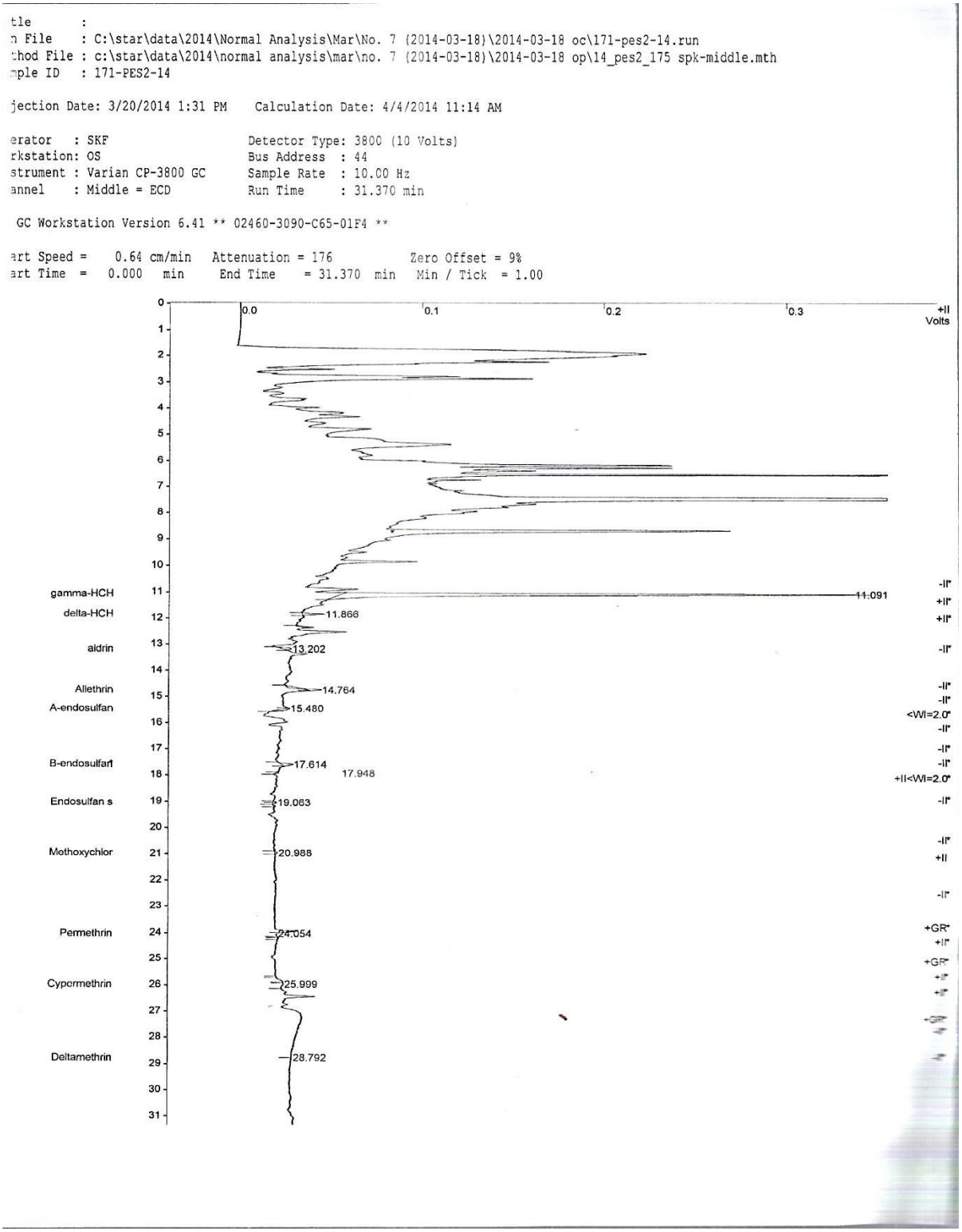


Appendix 2: Sample chromatograms from the GC-ECD analysis of Organochlorine and Synthetic Pyrethroids pesticide residues



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thod File : c:\star\data\2014\normal analysis\mar\no. 7 (2014-03-18)\2014-03-18 op\14_pes2_175 spk-middle.mth
mple ID   : 171-PES2-14

jection Date: 3/20/2014 1:31 PM    Calculation Date: 4/4/2014 11:14 AM

erator   : SKF                  Detector Type: 3800 (10 Volts)
rkstation: OS                   Bus Address  : 44
strument : Varian CP-3800 GC    Sample Rate  : 10.00 Hz
annel    : Middle = ECD         Run Time     : 31.370 min

 GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

art Speed =    0.64 cm/min   Attenuation = 176          Zero Offset = 9%
art Time  =   0.000   min     End Time    = 31.370  min   Min / Tick  = 1.00
```
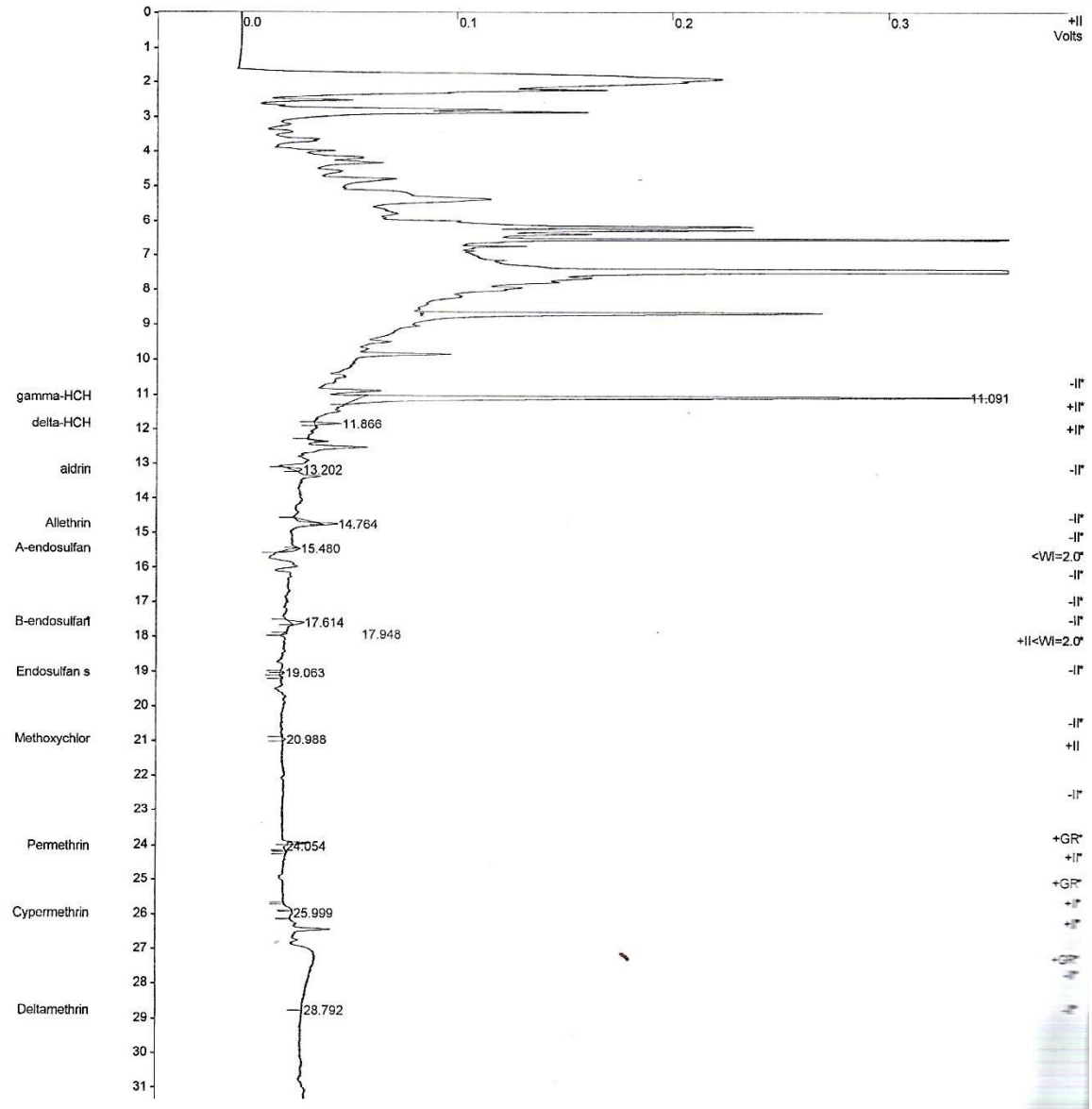



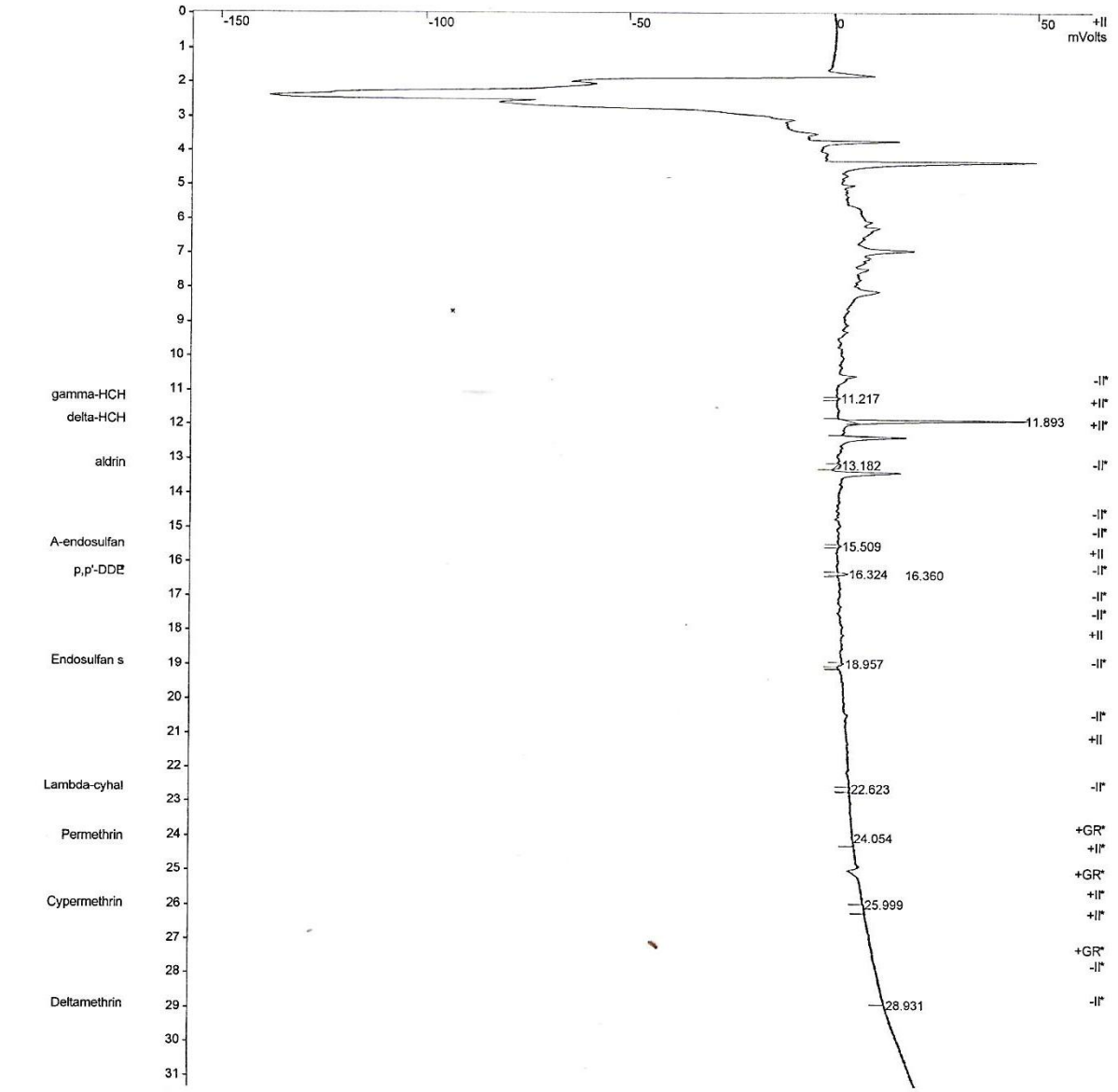
tle :
n File : C:\star\data\2014\Normal Analysis\Mar\No. 7 (2014-03-18)\2014-03-18 oc\reg blk 14-03-18.run
thod File : c:\star\data\2014\normal analysis\mar\no. 7 (2014-03-18)\2014-03-18 op\14_pes2_175 spk-middle.mth
mple ID : Reg Blk 14-03-18

jection Date: 3/20/2014 11:44 AM Calculation Date: 4/4/2014 11:14 AM

erator : SKF Detector Type: 3800 (10 Volts)
rkstation: OS Bus Address : 44
strument : Varian CP-3800 GC Sample Rate : 10.00 Hz
annel : Middle = ECD Run Time : 31.368 min

GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

art Speed = 0.64 cm/min Attenuation = 92 Zero Offset = 70%
art Time = 0.000 min End Time = 31.368 min Min / Tick = 1.00



```
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thod File : c:\star\data\2014\normal analysis\mar\no. 7 (2014-03-18)\2014-03-18 op\14_pes2_175 spk-middle.mth
mple ID   : 169-PES2-14

jection Date: 3/20/2014 12:20 PM    Calculation Date: 4/4/2014 11:14 AM

erator   : SKF                  Detector Type: 3800 (10 Volts)
rkstation: OS                   Bus Address  : 44
strument : Varian CP-3800 GC    Sample Rate  : 10.00 Hz
annel    : Middle = ECD         Run Time     : 31.370 min

 GC Workstation Version 6.41 ** 02460-3090-C65-01F4 **

art Speed =    0.64 cm/min   Attenuation = 1776          Zero Offset = 9%
art Time  =   0.000   min     End Time    = 31.370  min   Min / Tick  = 1.00
```