KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI, GHANA

ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN HONEY

by

SUSSANA ANTWI-BOASIAKO

(PG-2582714)

A Thesis submitted to the Department of Food Science and Technology in the Faculty of Biosciences,

College of Science

Partial fulfilment of the requirements for the degree

MASTER OF SCIENCE FOOD QUALITY MANAGEMENT

27TH MARCH, 2017.

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Supervisor: Gloria Ankar-Brewoo (Mrs)

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CERTIFICATION/DECLARATION

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

Sussana Antwi-Boasiako	**----------**	**----------**
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(Supervisor)	**Date**	**Signed**
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ABSTRACT

The aim of the study was to determine the actual content of polycyclic aromatic hydrocarbons in honey sold in Kumasi. Polycyclic aromatic hydrocarbons are group of different compounds released from burning oils, trash, wood and other organic substances. Samples were collected from hawkers and retailers along the railways and market center of Kumasi Central Market. The content of polycyclic aromatic hydrocarbons in honey samples were determined using high-performance liquid chromatography with fluorescence detector. Concentration of individual polycyclic aromatic hydrocarbons ranged from 9×10^{-4} µg/kg (Pyrene) to 1956960×10^{-4} µg/kg (naphthalene). Naphthalene recorded the highest mean value (150535×10^{-4} µg/kg) which exceeded the limit set for both children and adults. The limit of Benzo (a) pyrene for infant formula (10000×10^{-4} µg/kg) was not exceeded. Compositional pattern of polycyclic aromatic hydrocarbons showed greater percentage of low molecular weight PAH. Pollution emission in honey reported polycyclic aromatic hydrocarbons contamination from burning of wood, grass and coal.

DEDICATION

This work is dedicated to the Almighty God the creator of heaven and earth. To my wonderful husband, Anthony Oppong Kyekyeku, great friends and family people for their love, support, encouragement and prayers during the conduct of this research.

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I am happy and sincerely thankful to the Almighty God, creator of Heaven and Earth for the strength, wisdom and fortitude he bestowed in me during the conduct of this research.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Honey is a natural and biological substance created by honey bees, used as food, diet supplement, as energizer for workers and athletes to overcome fatigue, as medicine (Kumar *et al.,* 2010), antibiotic resistant for killing bacterial (Kwakman *et al.,*2010) and also serve as environmental marker (Ciemniak *et al.,* 2013).

Recently, honey contamination is a public issue and concern since most consumers find it difficult differentiate good honey from bad honey. This concern is health-related and it is mostly derived from chemical pollutant such trace elements (Ribeiro *et al.,* 2014) antibiotics residue (Ullah *et al.,* 2013) pesticides contamination (Blasco *et al.,* 2003) and among them is largely spread polycyclic aromatic hydrocarbons pollutant (Ciemniak *et al.,* 2013, Iwegbue *et al.,* 2016, Lambert *et al,.* 2012, Perugini 2009, Dobrinas *et al.,* 2008). Polycyclic aromatic hydrocarbons, which is a xenobiotic have many adverse effects in humans as well as the environment. Their presence in food, water and air have widely been investigated (Kayarb *et al.,* 2013).

Polycyclic aromatic hydrocarbons are group of compounds made up of carbon and hydrogen compounds. Their presence in food is derived from either pyrogenic or petro genic sources (Lee and Vu, 2010, Abdel-Shafy and Mansour, 2015, Alam *et al.,* 2013, Johnson and Karlson 2007, Nyarko, Botwe and Klubi 2012, Nasher *et al.* 2003, Nkpaa, Wegwu and Essien 2013). They are mostly group based on their molecular weight (high, medium and low) and are characterized as chemicals with higher melting and boiling point, low vapor pressure and very low water solubility (Abdel-Shafy and Mansour, 2015). In the environment, polycyclic aromatic hydrocarbons

contamination is derived from anthropogenic activities but can also be released into the environment through natural sources such as incomplete combustion of organic substance (Lee and Vu, 2010). They are ubiquitous and frequently found in food (Nyarko, Botwe and Klubi (2012), Rose *et al.* (2015), Martorell *et al.* (2010), Guillen and Sopelana (2003), Dost and Deli, (2012), Rey-Salguiro *et al.* (2009)), water (Amoako *et al.* (2011), Essumang (2010), Oros *et al.* (2007)), air (Bortey-Sam (2015), Ravindra *et al.* (2008), Devi *et al.* (2014)), soil and sediments (Bortey- Sam (2014), Samsøe-Petersen *et al.* (2002)). Exposure is mostly through ingestion, inhalation and dermal contact.

Polycyclic aromatic hydrocarbons are strongly carcinogenic and mutagenic to living organisms such as human and laboratory animals (Nkpaa, Wegwu and Essien 2013), marine animals and plants (Boahem *et al.* (2007), Le Bihanic *et al.* (2014)). Its presence in the human body according to Co *et al*. (2014), compete with and displace essential mineral elements, interfering with organsystem function. Exposure to higher levels can produce immunosuppressive effects and cause oxidative stress during metabolism (Kayarb *et al.,* 2013).

Reports exist that honey and beehives products are considerably contaminated with polycyclic aromatic hydrocarbons. Honey contamination with polycyclic aromatic hydrocarbons occur as a result of pollution accumulation in the vegetation (Juhasz and Naidu, 2000) used of synthetic wax made from petroleum for beekeeping which is considered as breach to beekeeping practices (Krell 1996) and worst of all is the introduction of smoke to honey during (Corredera *et al.* (2014), Ciemniak *et al.* (2013)).

Although screening method for polycyclic aromatic hydrocarbons is limited (Liu *et al.,* 2007) high performance liquid chromatography (HPLC) with fluorescence detector method was adopted for

polycyclic aromatic hydrocarbons identification. This analytical method has been used for identification of polycyclic aromatic hydrocarbons in several foods such as smoked fish (Basak *et al.,* 2010), mashed potatoes and toasted bread (Nieva-Cario *et al.,* 2001), decapod crustaceans (Watson *et al.,* 2004).

Identification of possible sources of polycyclic aromatic hydrocarbons contamination in honey was achieve using diagnostic ratio tool. This method has been used by different researchers for different studies such as polycyclic aromatic hydrocarbons contamination in urban street dust (Bhupander et al., (2012), Wang *et al.*, (2009) and fish species (Nyarko, Botwe and Klubi, 2012)

Toxicity sum was achieved by calculating benzo (a) pyrene equivalent quotient (BaP_{eq}). This approach was adopted for the measure of polycyclic aromatic hydrocarbons toxicity sum in oils (Alomira *et al.,* 2010), residential indoors and outdoors air (Jung *et al.,* 2010)

1.2 Problem Statement

Honey is widely consumed as food and medicine and its contamination carries serious health effects. Research conducted by different authors have reported polycyclic aromatic hydrocarbons contamination in honey. Polycyclic aromatic hydrocarbons are known to be carcinogenic, mutagenic and poses health effects to both humans and animals. Reports on polycyclic aromatic hydrocarbons contamination in honey by researchers from different countries were as a result of environmental contamination and harmful beekeeping practices. In Ghana, information concerning the contamination and quality of honey samples in the market is unavailable. This makes

contamination of honey possible since sellers can present contaminated honey for sale as genuine honey. Ingestion of honey without knowing its safety is problematic for consumers.

This study seeks to find out the contamination of polycyclic aromatic hydrocarbons in honey sold at Kumasi central markets by honey retailers.

1.3 Justification

The benefits and uses of honey have made the presence of honey in every country a blessing. The fear of honey adulteration affects the consumption and threatens the food security in the country. Bush burning, fumes from exhaust, dust in atmosphere etc. are mostly the main sources of polycyclic aromatic hydrocarbons in honey. The fear wax adulteration, feeding and treating beeswax with agro chemicals and introduction of smoke from different fuels during honey harvesting have generated concern. All these activities introduce honey to chemical pollutants for which polycyclic aromatic hydrocarbon is one.

The endpoint of polycyclic aromatic hydrocarbon intoxication is cancer. Assessment of polycyclic aromatic in honey sold in Kumasi central market helped researcher to achieve a measurable objective that address quality and safety issues. It also provided information which will be used as a basis for determining the risk of consumers that purchase and consume honey sold in the selected site chosen for the study.

1.4 Goal

The aim of this study is to assess honey contamination by polycyclic aromatic hydrocarbons.

1.5 Objective

The objective of the investigation was to assess the concentration of polycyclic aromatic hydrocarbons in honey, identify their possible source and determine its toxicity.

1.6 Scope / Limitation

The study focused mainly on honey sold by "honey hawkers" at Kumasi Central market. These hawkers sell honeys in small quantities and at affordable prices.

CHAPTER II

2.0 LITERATURE REVIEW

2.1 Introduction

Honey is a supernatural substances created by honey bees and are used by human beings as sweeteners. Since ancient time honey is considered as important beekeeping and biological product used as food and as diet supplementation (Ciemniak *et al.,* 2013). It contains a mixture of different nutrients and often consumed without any additional processing (Corredera *et al.*, 2014). Bogdanov (2015)reported honey as the main nutritional and health relevant substances made up of carbohydrate (fructose, glucose and oligosaccharide), small amount of protein, enzymes, amino acids, mineral, trace elements, vitamins, aromatic compounds and polyphenol.

According to Kuma *et al.* (2010) honey is a highly nutritious food with traces of minerals, vitamins, antioxidant which destroy free radicals and delay ageing. Honey can also be considered as energizer which help workers and athletes to overcome fatigue and gain extra energy without any fear adverse health effects. In the field of medicine, honey is used for the treatment of nausea, cough, cold, osteoporosis, stomach bone cancer and asthma.

Honey is used as an ingredient in hundreds of manufactured foods. It is widely used as a source of sugars for making honey wines and beers, and in the manufacture of many secondary products: breakfast cereals, bakery goods, and a multitude of other value-added products (Bradbear, 2009) and also used to preserve food.

A study conducted by Kwakman (2010) agave a report on honey as antibiotic resistant for the killing of bacterial such *bacillus subtils, staphylococcus aureus, enchericia coli, pseudomonas*

aeruginosa and *enterococcus faecium*. Honey also contain high sugar concentration, hydrogen peroxide, methylglyoxal, peptide bee defensing – 1 and low pH which classify it as antibacterial agent.

In many countries, honey is regarded more as a medicine or special tonic, rather than as an everyday food. The special status of honey among consumers is due to its natural image and to its purported health benefits. It is applied on wounds, burns and taken as syrup for coughs (Rubbin, 2015). In livestock, it is used to treat wounds (lesions) resulting from foot and mouth disease, foot rot (MacOsore, 2005).

Research on honey conducted by Ciemniak *et al.* (2013) reported honey as an environmental marker due to its ability to contain harmful pollutants that come from environment. Examples are trace elements, pesticides, antibiotics and largely spread is polycyclic aromatic hydrocarbons and beekeeping practices such as smoking, which according to Deshpande (2002) is a major polycyclic aromatic hydrocarbons contributing factor in foods.

Ribeiro *et al.* (2014) researched into the determination of trace elements in honey from different regions in Brazil. From 160 honey samples, the study reported higher rates of essential and non - essential elements in honey from some regions. The was abundant of K and Ca elements in all samples.

Ullah *et al.* (2013) conducted a study on the detection and quantification of antibiotics residues in branded and unbranded honey samples. This research reported 12.5% abundance of polycyclic aromatic hydrocarbons concentration in branded honey and 19.96% abundance in unbranded honey samples.

For pesticides contamination, a research conducted by Blasco *et al.* (2003) studied on pesticides residues in honey samples from Portugal and Spain. Results from the study reported

organochlorides in honey. Among them were gamma- HCH, HCB and other isomers of HCH. Residues of DDT and their metabolites were detected in some samples.

Polycyclic aromatic hydrocarbons are widely distributed pollutant in the environment. They belong to the family of organic pollutants with alarming properties and negative impacts on human, living organisms and the environment (Ramesh *et al.*, 2004). More than hundred (100) polycyclic aromatic hydrocarbons have been characterized in nature, sixteen of which were classified as priority pollutants according to the U.S Environmental Protection Agency. Its pathway entry into food chain include both natural and anthropogenic mechanisms. Several studies in different countries have reported polycyclic aromatic hydrocarbons concentration in honey. Among such study includes Ciemniak *et al.* (2013).

2.2 Sources of Polycyclic aromatic hydrocarbons in an Environment

Pyrogenic polycyclic aromatic hydrocarbons are typically formed during incomplete combustion of organic materials at temperature > 500ºC. Polycyclic aromatic hydrocarbons formed from this source have four to six (4-6) benzene rings which include flouranthene, pyrene, benzo (b) flouranthene, benzo (a) pyrene, benzo (k) flouranthene and dibenz (ah) anthracene. Lee and vu (2010) classified these polycyclic aromatic hydrocarbons compounds as high molecular weight which are emitted in particulate phase. Sources of pyrogenic polycyclic aromatic hydrocarbons in environment originate from forest fires, incomplete combustion of fossil fuels, tobacco smoke (Hussein and Mansour, 2015).

Petro genic originate and are formed over a long period of time in low temperatures between 100-300◦C and at high pressure environments where organic matter is converted into petroleum and coal. Petro genic polycyclic aromatic hydrocarbons have two or three (2-3) benzene rings, among

them are naphthalene and it derivatives, acenaphthene, flourene and anthracene. These polycyclic aromatic hydrocarbons according to Lee and vu (2010) have low or light molecular weight and are emitted in gaseous phase. Petro genic polycyclic aromatic hydrocarbons are common in the environment due to the widespread of transportation, storage and the use of crude oil products (Hussein and Mansour, 2015).

Research conducted by Alam *et al*. (2013) on atmospheric measurement of polycyclic aromatic hydrocarbons and Quinone compounds at roadside and urban background concluded that polycyclic aromatic hydrocarbons with low molecular weight compound are susceptible to atmospheric processing.

Study conducted by Johnson and Karlson (2007) on diffuse polycyclic aromatic hydrocarbons contamination of surface soil reported on continuous input of pyrogenic polycyclic aromatic hydrocarbons in urban and pristine topsoil.

Study conducted by Nyarko, Botwe and Klubi (2012) on polycyclic aromatic hydrocarbons levels in two commercially important fish species from coastal waters of Ghana and their carcinogenic health risks. Results from the study showed predominance of high molecular weight polycyclic aromatic hydrocarbons as compared to low molecular polycyclic aromatic hydrocarbons. This study concluded the bioavailability of pyrogenic polycyclic aromatic hydrocarbons in two important commercially fish species.

Nasher *et al.* (2003) studied on concentration and sources of polycyclic aromatic hydrocarbons in seawater around Longkawi Island, Malaysia. The study collected four jetties and three marine fish around Langkawi and analyzed for the presence of 18 polycyclic aromatic hydrocarbons. Total polycyclic aromatic hydrocarbons concentrations ranged from 6.1± 0.43 to 4 ±0.42 μgL^{-1} which is above the maximum acceptable concentration in water (0.2 μgL^{-1}). The calculated diagnostic of

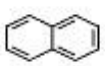
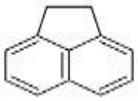
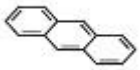
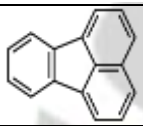
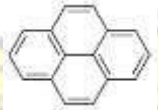
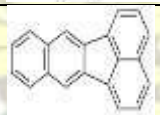
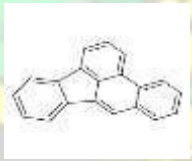
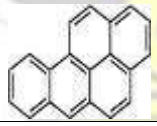
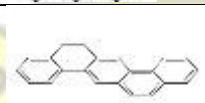
benzo (a) anthracene + chrysene of polycyclic aromatic hydrocarbons in major stations studied suggests that polycyclic aromatic hydrocarbons are derived from pyrogenic.

Nkpaa, Wegwu and Essien, (2013) studied on the assessment of polycyclic aromatic hydrocarbons levels in two commercially important fish species (*tilapia queneesis* and *Liza falcipies)*. The results showed predominance of higher molecular weight polycyclic aromatic hydrocarbons than low molecular weight polycyclic aromatic hydrocarbons.

2.3 Characteristics of Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are classified by their melting, boiling point, vapor and water solubility depending on their structures. An increase in molecular weight leads to increase it solubility in non-polar organic solvent and soluble in polar water. In aqueous system, they are essentially insoluble. Solubility of polycyclic aromatic hydrocarbons in water is crucial characteristics for distribution of polycyclic aromatic hydrocarbons in foods (Lerario *et al*, 2003). The water solubility of polycyclic aromatic hydrocarbons increases with temperature and decreases with increasing molecular weight (Yu *et al*., 2015). Relatively high solubility is therefore likely to have a higher environmental mobility and is mostly found in higher concentration in aquatic samples (Gilbert and Şenyuva, 2009). They are highly lipophilic and readily adhere to particles of food (Lawley, Curtis and Davis, 2012). As lipophilic compounds they can easily cross lipid membranes and have the potential to accumulate in aquatic organism (Yu *et al*., 2015) and also accumulates in the soils by binding to the organic water. The transportation of polycyclic aromatic hydrocarbons in the atmosphere is influenced by their volatility.

Table 2.1: Physiochemical properties of selected polycyclic aromatic hydrocarbons (US EPA cited in Lee and Vu, 2010)

POLYCYCLIC AROMATIC HYDROCARBONS	STRUCTURE	CHEMICAL FORMULAR	MOLECULAR FORMULAR	MELTING POINT(◦C)	BOILING POINT (◦C)
Naphthalene		$C_{10}H_8$	128.17	80.26	218
Acenapthene		$C_{12}H_{10}$	154.21	95	96
Flourene		$C_{13}H_{10}$	166.2	116-117	295
Anthracene		$C_{14}H_{10}$	178.2	100	340
Flouranthene		$C_{16}H_{10}$	202.26	110.8	375
Pyrene		$C_{16}H_{10}$	202.3	156	393-404
Benzo(k) Flouranthene		$C_{20}H_{12}$	252.3	215.7	480
Benzo(b) Flouranthene		$C_{20}H_{12}$	252.3	168.3	No data
Benzo(a) Pyrene		$C_{20}H_{12}$	252.3	179-179.3	495
Dibenz(ah) Anthracene		$C_{22}H_{14}$	278.35	262	No data

Polycyclic aromatic hydrocarbons of lower molecular mass (generally those with three or fewer aromatic rings) are numerous in the atmosphere and those with higher molecular mass enter the

environment and adsorbed into particulate matter (Gilbert and Şenyuva, 2009). Polycyclic aromatic hydrocarbons also have low vapor pressures and vapor pressure tends to decrease with increasing molecular mass (Johnson *et al.,* 2005).

Polycyclic aromatic hydrocarbons according Hautumm (2012) can be characterized as been persistent (substances remain in the environment for a long time and are hardly decomposed there), bio-accumulative (accumulate in organisms including the human body) and toxic (it can no longer remove from the environment).

Above is Table 2.1 showing the physiochemical properties of selected Polycyclic Aromatic Hydrocarbons (USEPA cited in Lee and Vu, 2010).

2.4 Exposure of Polycyclic aromatic hydrocarbons in the Environment

Exposure of polycyclic aromatic hydrocarbons that occurs regularly in the environment are through air, water, soil and food. Routes of exposure include ingestion, inhalation and dermal contact either by occupation or non – occupational settings. Non – workers are expose through diet, smoking and burning of fuels.

2.4.1 Polycyclic aromatic hydrocarbons in Air

Polycyclic aromatic hydrocarbons in the atmosphere are mainly from residential heating, vehicle traffic, industrial or other point sources of pollution. The levels of individual substances vary over several orders of magnitude and are mainly adsorbed to airborne particulate matter (WHO, 2013).

In the air polycyclic aromatic hydrocarbons exist in gaseous phase. A research conducted in Kumasi by Bortey- Sam *et al.* (2015) into the levels, potential sources and human health risk of

polycyclic aromatic hydrocarbons in particulates matter. The study reported a total ranged of concentration from 0.51 – 16 ng/m^3 (KNUST) and 19 - 38 ng/m^3 (CC). Estimated B(a)P equivalent concentration was 70% and it was 18 times higher than recorded percentage at KNUST. Study conducted by Ravindra *et al.* (2008) on atmospheric polycyclic aromatic hydrocarbons served as a database to identify and characterize the emission sources of polycyclic aromatic hydrocarbons. The results of the study high levels of low molecular weight polycyclic aromatic hydrocarbons in vapor phase which is more probable human carcinogenic polycyclic aromatic hydrocarbons were found to be associated with particulate matter, especially in fine mode particles in ambient air.

Devi *et al.* (2014) assessed the atmospheric polycyclic aromatic hydrocarbons in Manipur of the Northeast, India in the Journal of Polycyclic Aromatic Compounds. Active sampling was conducted in ambient air, rural and mountain of Manipur. The polycyclic aromatic mean concentration of urban air (235.6 ng/m^3) were high compared to rural air (206.3 ng/m^3) and mountain (202.1 ng/m^3). The result reported predominant concentration of 2-3 aromatic rings. Monthly variation of polycyclic aromatic hydrocarbons showed high concentration during autumn and winter as compared to summer and spring. The study then concluded that polycyclic hydrocarbons were from diesel powered and gasoline powered vehicle while coal burning source polycyclic aromatic hydrocarbons in rural Manipur.

2.4.2 Polycyclic aromatic hydrocarbons in Soil

Soil contain measurable amount of polycyclic aromatic hydrocarbons, primary from airborne and fallout. Tay and Biney (2013) studied on polycyclic aromatic hydrocarbons in selected irrigated

urban agricultural soils in Accra, Ghana. The study assessed and compared levels of contamination as well polycyclic aromatic hydrocarbons sources from four urban agricultural soils. The study showed that polycyclic aromatic hydrocarbons levels in the soil ranged from 12 ng/kg in soil collected from Dzorwulu, 2.95 ng/kg to Ghana broadcasting cooperation vegetable irrigation site. The results showed the presence of more water soluble polycyclic aromatic hydrocarbons (2-4) rings and there were significant levels of carcinogenic polycyclic aromatic hydrocarbons (5-6) rings present at percentage abundance of 47.1, 37.5, 46.8 and 50.8%.

Again a research conducted by Bortey-sam *et al.* (2014) on the occurrence, distribution, sources and toxic potential of polycyclic aromatic hydrocarbons in surface soils from Kumasi. From the study, there was a mean concentration of all polycyclic aromatic hydrocarbons ranged from 14.78 (Ahissan) to 2084 ng/kg (Adum) with an average concentration of 442.5 ± 527.2 ng/kg. The study concluded that polycyclic aromatic hydrocarbons in surface soils samples were mainly from fuel combustion.

Samsøe-Petersen *et al.* (2002) also investigated into the uptake of trace elements and five polycyclic aromatic hydrocarbons in crop plants grown on different sites. The results of the study showed elevated levels of polycyclic aromatic hydrocarbons and trace elements in vegetables grown in contaminated soil than fruits grown in uncontaminated soil.

2.4.3 Polycyclic aromatic hydrocarbons in Food

Dietary intake of polycyclic aromatic hydrocarbons is the major sources of human exposure. Food is thought to be the main source of polycyclic aromatic hydrocarbons exposure for humans

especially non-smokers (Lawley, Curtis and Davis (2012), Tuteja *et al.* (2011)). Although, polycyclic aromatic hydrocarbons are not naturally present in food and do not have nutritional value (Jakszyn *et al.,* 2011)but they extremely occur through the consumption of contaminated food. Doses from one meal are typically so small to notice its toxic effect after eating (Stelljes, 2008). Human exposure to polycyclic aromatic hydrocarbons is not restricted to individual compounds but a mixture of these compounds and depends on occupational or environmental situations (WHO, 2003).

Most food contains measurable levels of polycyclic aromatic hydrocarbons, because of the widespread distribution of polycyclic aromatic hydrocarbons in the environment (Yu *et al*., 2015). It has been reported that cereals and cereals products, milk, vegetables and fruits are the highest contributors to total polycyclic aromatic hydrocarbons intake (Tuteja *et al.*, 2011).

Nyarko, Botwe and Klubi (2012) studied on polycyclic aromatic hydrocarbons levels in two commercially important fish species from the coastal waters of Ghana and their carcinogenic health risks. The results from the study recorded average polycyclic aromatic hydrocarbons concentration from0.01 g/kg wet wt. to 34.04 ± 0.56 g/kg wet wt. and 0.01 g/kg wet wt. to 54.13 ± 5.22 g/kg wet wt.

Rose *et al.* (2015) also studied on the formation of polycyclic aromatic hydrocarbons in food preparation in home. This study focused on cooking methods such as toasting, barbecuing, frying, grilling and roasting. The effects of frying, grilling, toasting and roasting on the formation of 27 different polycyclic aromatic hydrocarbons showed little evidence of polycyclic aromatic hydrocarbons formation regardless of the distance of the heat. Barbecuing with charcoal plus wood chips resulted in the formation of benzo (a) pyrene in most tested foods.

In the Environmental International Journal Volume 36 Issue 5 Martorell *et al.* (2010) published their work on Polycyclic aromatic hydrocarbons in foods and estimated polycyclic aromatic hydrocarbons intake by the population of Catalonia, Spain: Temporal trend. They determined polycyclic aromatic hydrocarbons in various foodstuffs randomly purchased in Catalonia (Spain) during November and December 2008. They compared their results after dietary intake containing polycyclic aromatic hydrocarbons was estimated according to age and sex for the overall population of Catalonia to those of previous studies performed in 2000 and 2006. The current polycyclic aromatic hydrocarbons concentration estimated was comparably lower than for previous studies.

Phillips (1999) in his work on "Polycyclic aromatic hydrocarbons in the diet" published in the Journal of Mutation Research discussed the source of polycyclic aromatic hydrocarbons in uncooked foods and source of polycyclic aromatic hydrocarbons in cooked foods. He explained the major dietary sources of polycyclic aromatic hydrocarbons and biomonitoring procedures that has been developed to assess human exposure to polycyclic aromatic hydrocarbons. Phillips agreed to proponent who supported the wide distribution of polycyclic aromatic hydrocarbons in the environment making human exposure inevitable. Cooked foods with polycyclic aromatic hydrocarbons were attributed to the cooking processes by Phillips. He concluded diet to be a major source human exposure to polycyclic aromatic hydrocarbons.

Guillen and Sopelana (2003) reviewed Polycyclic aromatic hydrocarbons in diverse foods in the Journal of Food Safety where they discussed the chemical nature of polycyclic aromatic hydrocarbons, their distribution in foods, metabolism, toxicity, carcinogenicity, and risk assessment.

Dost and Deli (2012) determined polycyclic aromatic hydrocarbons in edible oils and barbecued foods. The edible oils investigated for polycyclic aromatic hydrocarbons hand different values ranging from 0.44 - 98.92 gL^{-1}. They concluded that the processing method had increased the concentration of the polycyclic aromatic hydrocarbons in the range of 2- to 8-fold in the food samples. The work was published in the Journal of Food Chemistry volume 133.

In the Journal of Food Chemistry, Rey-Salgueiro *et al.* (2009) published their work on "Occurrence of polycyclic aromatic hydrocarbons and their hydroxylase metabolites in infant foods". Their results showed that, the limit of benzo (a) pyrene an indicator for the presence of polycyclic aromatic hydrocarbons was not exceeded and hydroxyl polycyclic aromatic hydrocarbons metabolites was not detected. They analyzed commercial milk and infant cereals for two (2) hydroxylase polycyclic aromatic hydrocarbons metabolites (1-OH-Pyr and 3-OH-B[a]P) and their conjugates together with other eleven (11) polycyclic aromatic hydrocarbons. The purpose of their survey was to determine and assess the exposure of babies and infants to polycyclic aromatic hydrocarbons and for comparison with proposed limits that were being considered at the time of their survey.

2.4.4 Polycyclic aromatic hydrocarbons in Water

Polycyclic aromatic hydrocarbons in water are leached from soil, industrial affluent and accident spills during shipment. Polycyclic aromatic hydrocarbons intake from drinking-water could be equal to or even exceed other dietary intakes (WHO, 2013). Polycyclic aromatic hydrocarbons can reach water bodies mainly through dry and wet deposition, road runoff, industrial wastewater, leaching from creosote-impregnated wood, petroleum spills, and fossil fuel combustion (Karyab

et al., 2013). The main source of polycyclic aromatic hydrocarbons contamination in drinkingwater is usually not the raw water sources but the coating of the drinking-water distribution pipes for effective protection against corrosion (WHO, 2013).

A study conducted in Ghana by Amoako *et al.* (2011) on levels of polycyclic aromatic hydrocarbons in Densu river basin showed higher concentrations levels. Samples were collected from nine stations. The study reported total polycyclic aromatic hydrocarbons concentrations of 13.0 – 80.0 µg/mL with mean value of 37.1 µg/mL. The 2-3 ring polycyclic aromatic hydrocarbons were dominant in the Densu river basin. Polycyclic aromatic hydrocarbons levels from all sites exceeded the limit set by Agency for Toxic Substances and Disease Registry (ATSDR). The study concluded that the presence of polycyclic aromatic hydrocarbons was an indicator of contamination of water sources.

Essumang (2010) also studied on the distribution levels and risk of polycyclic aromatic hydrocarbons in some water bodies along the coastal belt of Ghana. From the results, the average total polycyclic aromatic hydrocarbons ranged from 6.3 – 16.1 µg/L. The presence of the polycyclic aromatic hydrocarbons was a concern since water from these bodies are used for domestic and fishing activities. Polycyclic aromatic hydrocarbons were found in all the studied sampling sites. Lower- molecular weight and methylated polycyclic aromatic hydrocarbons which are less carcinogenic were dominant at all sites. The presence of compound such as benzo (a) anthracene, benzo (b) flouranthene, benzo (j, k) flouranthene was of concern since they pose a greater health risk. From the analysis conducted there was indication of possible risk cancer among inhabitants depending those water bodies. Sources of polycyclic aromatic hydrocarbons contamination into these water bodies were associated with deposition of airborne particulate,

surface run-off from roads and land surfaces, direct inputs from industrial and sewage effluents and fossil fuel products and burning of solid waste.

Oros *et al.* (2007) studied on polycyclic aromatic hydrocarbons in San Francisco Bay. The study analyzed water from the bay, sediments and mussel. Total polycyclic aromatic hydrocarbons concentration from the data showed that there were very few significant (< 0.05) increasing or decreasing temporal trends in total polycyclic aromatic hydrocarbons concentration in the Bay during the period of 1993-2001. Input of wet and dry season showed no influence on total polycyclic aromatic hydrocarbons in water. Polycyclic aromatic hydrocarbons pathways were ranked as storm water runoff > tributary inflow > waste water treatment plant effluent > atmospheric deposition > dredged material disposal.

Karyab *et al.* (2013) investigated into the distribution and seasonal variation of sixteen priority polycyclic aromatic hydrocarbons of Tehran drinking water, the capital of Iran. Single and total polycyclic aromatic hydrocarbons detected ranged from 3.01 - 38.96 and 32.45 – 733.10 ng/L. High molecular weight polycyclic aromatic hydrocarbons average occurrence was 79.55% with maximum concentration of 438.96 ng/L. Ratio for carcinogenic and non – carcinogenic polycyclic aromatic hydrocarbons was 63.84. Benzo (a) pyrene concentration which is an indicator of water pollution to polycyclic aromatic hydrocarbons was lower the guideline value proposed by World Health Organization (WHO). According to the study, total concentration of polycyclic aromatic hydrocarbons in drinking water of Tehran varies according to geographical location. And the results also indicated the presence of carcinogenic polycyclic aromatic hydrocarbons in drinking water of Tehran.

2.5 Polycyclic aromatic hydrocarbons Toxicity

The earliest human polycyclic aromatic hydrocarbons related epidemiological study was reported in 1936 by investigators in Spain and Japan. According to Nkpaa, Wegwu and Essien (2013) polycyclic aromatic hydrocarbons was the first and largest set of compounds that was strongly mutagenic to laboratory animals and human. Studies have established the link between polycyclic aromatic hydrocarbons exposure and incidence of immune toxicities and cancer. The end point of polycyclic aromatic hydrocarbons toxicity is cancer.

Polycyclic aromatic hydrocarbons have low degree acute toxicity in human and it include effects such as headache, nausea, respiratory and dermal irritation. Chronic exposure to non-carcinogenic effects of polycyclic aromatic hydrocarbons include pulmonary, gastrointestinal, renal and dermatologic systems (EHME, 2009).

In laboratory animals, the carcinogenicity of certain polycyclic is well established. Research by different authors have reported the incidence of skin, lung, bladder, liver and stomach cancers as well as injection sites sarcomas in animals. Animals exposed to levels of some polycyclic aromatic hydrocarbons over long periods in laboratory studies developed lung cancer from inhalation, stomach cancer from ingesting polycyclic aromatic hydrocarbons in food and skin cancer from skin contact (Boström *et al.,* 2002).

In the Journal of Environmental Science and Pollution Research, Le Bihanic *et al.* (2014) research the developmental toxicity of polycyclic aromatic hydrocarbons mixtures in fish early life stages compared the developmental toxicity of polycyclic aromatic hydrocarbons complex mixtures on Japanese medaka (Oryzias latipes) embryos. Endpoints recorded for the different developmental stages were acute and included induced developmental abnormalities, disrupted larvae summing activity, and damaged DNA at environmental concentrations. Fractions of the polycyclic aromatic

hydrocarbons mixtures from petro genic source containing a high proportion of alkylated polycyclic aromatic and low molecular weight polycyclic aromatic hydrocarbons were more toxic to the Japanese medaka (Oryzias latipes) early life stages than the pyrolytic fraction.

Ibiam *et al.* (2005) in their work on "RNA/DNA ratios as a sub lethal endpoint for large-scale toxicity tests with the nematode Caenorhabditis elegans" proposed RNA/DNA ratio, after 48-h exposure to metals at a median effective concentration (EC50) values of 0.05, 0.6, 6.1 and 35 mg/L for Cu, Pb, Cd, and Zn respectively to be slightly more sensitivity toxicity endpoint than inhibitions of growth, reproduction, movement, and feeding rate which are the already proposed sub lethal toxicity endpoints. They explained the constant cell number of C. elegans to mean that different stages in the life history have very different RNA/DNA ratios even in the absence of toxins.

Assessing the sub lethal toxicity of metals to C. elegans shows that it is sensitive particularly to Cu. This work was published in the Journal of Environmental Toxicology and Chemistry.

The work on "In vitro mammalian mutagenicity of complex polycyclic aromatic hydrocarbon mixtures in contaminated soils" by Lemieux *et al.* (2015) published in the Journal of Environmental Science and Technology concluded mutagenic activity of synthetic polycyclic aromatic hydrocarbons mixtures that mimic the polycyclic aromatic hydrocarbons content of the soils. The study used in vitro assay to assess mutagenicity from 10 polycyclic aromatic hydrocarbons- contaminated sites. Their work was inspired by the absence of risk assessment protocols for unidentified polar compounds that also contribute to the overall hazard associated with polycyclic aromatic hydrocarbons-contaminated soils.

In the Journal of Regulatory Toxicology and Pharmacology volume 73, Sewell *et al.,* (2015) in their work on "A global initiative to refine acute inhalation studies through the use of 'evident toxicity' as an endpoint: Towards adoption of the fixed concentration procedure" used data to

propose changes to TG433 that incorporates a clear indication of the clinical signs that define evident toxicity. The background for their work was that current accepted methods for chemical hazard identification and characterization use death as an endpoint (OECD TG403 and TG436), whereas the fixed concentration procedure (FCP) (draft OECD TG433) uses fewer animals and replaces lethality as an endpoint with 'evident toxicity.' With evidence toxicity according to the findings of the working group, signs such as body weight loss (>10% pre-dosing weight), irregular respiration, tumors and hypo-activity, seen at least once in at least one animal after the day of dosing are highly predictive (positive predictive value > 90%) of severe toxicity or death at the next highest concentration. There was therefore not the need to use death as final endpoint in chemical hazard identification and characterization in an acute inhalation studies.

Chunrong Jia and Batterman (2010) also reported on naphthalene (Nap) toxicity. This research explained naphthalene exposure as a linked to a number of health effects. From non-carcinogenic endpoint are hyperplasia and metaplasia in the respiratory and olfactory epithelium. And from the endpoint of cancer are nasal tumors.

2.6 Polycyclic aromatic hydrocarbons in Honey

According to some authors, honey is considered an environmental marker due to its ability to contain harmful substances coming from polluted environment (Batelková, Borkovcová, Čelechovská, Vorlová, and Bartáková, 2012). The relationship between honey contamination and environmental pollution has also been confirmed. Ciemniak *et al.* (2013), stated that high levels of polycyclic aromatic hydrocarbons occurred in honey and beehives products are usually related to the degree of environmental pollution. And honey usually produced from polluted industrialized area contain up to 14 $\mu g/kg^{-1}$ benzo (a) pyrene.

In the wilderness, honey bees live on plants and polycyclic aromatic hydrocarbons according to Juhasz and Naidu (2000) accumulate in vegetation. Honey can be contaminated with polycyclic aromatic hydrocarbons are as a result of forest fires, stubble burning, location of beehives near industrial sites, vehicle pass etc. Honey contamination with polycyclic aromatic hydrocarbons can also be inherited from its constituents such as honey bees, pollen and nectar. Flying bees are exposed to atmospheric dust and contact contamination when gathering pollen from flowers. Also bees feed on nectar and pollen, so when plants and soils are contaminated they could be exposed to contamination (Lambert *et al.,* 2012).

In commercial farms, beeswax harvested from wilderness or fields are sold to commercial beeswax processors for the production of commercial beeswax. Processors then produced commercial beeswax by blending natural harvested beehives with synthetic cheap petroleum based wax (plastic wax) and then distribute them throughout the beekeeping industry for the production of commercial honey. This according to Krell (1996) is a breach to the beekeeping practices, since chemical contamination (such as polycyclic aromatic hydrocarbons) can occur from the use of synthetic wax. Bees reared by commercial beekeepers are fed and treated with chemical which may contain chemical pollutants for which polycyclic aromatic hydrocarbons is one.

The common method mostly used by farmers during honey harvesting is smoking honey bees. Beekeepers and honey harvesters blow smoke into the beehives during handling to calm the bees and keep them from attacking the beekeeper (Corredera *et al.*, 2014). Ciemniak *et al.* (2013) also stated that polycyclic aromatic hydrocarbons often get into honey from smoke used by beekeepers during the collection of honey. Smoke is a major contributory factor to the levels of polycyclic aromatic hydrocarbons found in foods (Desphande, 2012). Smoke is made up of collection of gaseous products from burning materials especially organic materials such as wood, papers,

plastics, nylons, rubber products etc. visible by the presence of small particles of incomplete combustion (carbon). Excessive use of smoke during honey harvesting flavor honey quickly no matter the fuel used (Krell, 1996) and smoke flavoring may contaminate food with polycyclic aromatic hydrocarbons (Lawley, Curtis and Davis, 2012).

Research conducted by Iwegbue *et al.* (2016) on concentrations, health risks and sources of polycyclic aromatic hydrocarbons in Nigerian honey reported concentration of Σ16 polycyclic aromatic hydrocarbons in honey samples. Polycyclic aromatic hydrocarbons compositional pattern of the analyzed sample ranged followed the order of 5 rings > 4 rings > 3 rings > 6 rings > 2 rings. From the results, there was no indication of risk associated with the consumption these honey. Analysis indicated that combustion of fossil fuels, natural gas, biomass and automobile emissions were the main sources of polycyclic aromatic hydrocarbons contamination in honey consumed in Nigeria.

Ciemniak *et al.* (2013) on the assessment of honey contamination with polycyclic aromatic hydrocarbons in Journal of Environmental Science and Health. Six species of honey as well as rape blossom and soil from villages were sampled. Twenty-three (23) polycyclic aromatic hydrocarbon were studied and the results of honey showed high concentration of non-carcinogenic polycyclic aromatic hydrocarbons with low molecular weights.

Lambert *et al.* (2012) analyzed four (4) polycyclic aromatic hydrocarbons in bees, honey and pollen in the journal of Chemosphere. Samples for the study were collected during four different periods. Results from the analysis reported polycyclic aromatic hydrocarbons contamination levels in honey as the lowest (min = 0.03 $\mu g/kg^{-1}$, max = 50.80 $\mu g/kg^{-1}$, mean = 0.82 μ/kg^{-1}, SD = 1.17). This research concluded that the presence of polycyclic aromatic hydrocarbons concentration in bee products were as a results of the used of smoke by beekeepers during manipulation in hives.

Perugini (2009) studied on polycyclic aromatic hydrocarbons in bees and honey. This study was published in Journal of Agricultural and food chemistry. The work used honey bees and honey as a detector of polycyclic aromatic hydrocarbons for several areas with different environmental pollution degree. All sampling results according to the results showed the presence of all the studied polycyclic aromatic hydrocarbons. Honey showed the presence of only phenanthrene, anthracene and chrysene. The results again reported highest polycyclic aromatic hydrocarbons concentration in honey bees due to the use of smoke by beekeepers for maintenance of apiary. Dobrinas *et al.* (2008) also studied on the assessment of polycyclic hydrocarbons in honey and propolis produced from different flowering trees and plants in Romania. Samples were collected at 15 different regions from beekeepers and retailers at local markets. Fifteen (15) polycyclic aromatic hydrocarbons were studied and the results showed the presence of some polycyclic aromatic hydrocarbons in honey and propolis within the range of 0.6 – 665.0 ng/kg.

2.7 Analytical Methods for Polycyclic aromatic hydrocarbons in Honey

2.7.1 High performance liquid chromatography (HPLC)

Literature on polycyclic aromatic hydrocarbons screening method is limited (Liu *et al*., 2007). HPLC with fluorescence detection has been widely used for detection of polycyclic aromatic hydrocarbons in several food matrices. HPLC method for determination of polycyclic aromatic hydrocarbons in smoke meat, meat products and smoke flavoring additives (Simko, 2002) and edible oils and fat (Moret and Conte, 2000) have been reviewed

Nieva-Cano *et al.* (2001) studied on sixteen (16) polycyclic aromatic hydrocarbons in mashed potato and toasted bread samples. An HPLC with fluorescence detection was used. Mean recoveries of polycyclic aromatic hydrocarbons were in the range of 70-86% for mashed potato,

potato and toasted bread samples. Ortiz toasted bread showed the presence of chrysene which is a known carcinogen.

Ames (1993) in his book on Food Chemistry volume 46 discusses food analysis by HPLC. He presents an exhaustive compilation of analytical methods every practicing food chemist needs. Conte *et al*. (2011) also reviewed the use of HPLC in food analysis in the Journal of chromatographic Science Service, handbook of HPLC.

Isaac *et al.* (2013) in their work on "Indigenous polycyclic aromatic hydrocarbons-degrading bacteria from oil-polluted sediments in Caleta Cordova, Patagonia Argentina" used HPLC to detect the residual substrate of bacteria from the genus Pseudomonas, Marinobacter, Salinibacterium and Brevibacterium ability to grow and degrade naphthalene, phenanthrene and pyrene. He presence of this ability is an indication of the possibility to study further growth stimulation in situ of these bacteria, in order to harness their intrinsic potential for bioremediation opportunities in other polluted harbors.

In the work on "HPLC/fluorescence determination of anti-BPDE-DNA adducts in m ononuclear white blood cells from polycyclic aromatic hydrocarbons-exposed humans" Pavanello *et al.* (1999) compared (+/-) – r - 7, t - 8- dihydroxy – t - 9, 10 – oxy - 7, 8, 9, 10 – tetrahydrobenzo(a)pyrene (anti-BPDE) - DNA adduct levels in groups of humans subjected to various levels of polycyclic aromatic hydrocarbon (benzo (a) pyrene) exposure. After one hundred and thirty (130) human subjects belonging to different categories were exposed to polycyclic aromatic hydrocarbons, HPLC/ Fluorescence method was used to detect the levels of the polycyclic aromatic hydrocarbons by specifically checking anti-BPDE-DNA adducts in mononuclear white blood cells from the human subjects. The samples for the assessment was from urinary excretion of the human subjects. The results of the research were published in the Journal of Carcinogenesis volume 20, issue 3.

In the Journal of Environmental Toxicology and Pharmacology, Volume 30 issue 3, Beyer *et al.* (2010) reviewed the Analytical methods for determining metabolites of polycyclic aromatic hydrocarbons pollutants in fish bile. High performance liquid chromatography with fluorescence detection (HPLC-F) was one of the analytical methods mentioned for biliary polycyclic aromatic hydrocarbons metabolite levels measurement. The review however discussed the limitations and advantages in using different methods based on the analytical performance towards different polycyclic aromatic hydrocarbons metabolites structures and suitability for different monitoring strategies.

Watson *et al.* (2004) published "Rapid assessment of polycyclic aromatic hydrocarbon exposure in decapod crustaceans by fluorimetric analysis of urine and haemolymph" in the Journal of Aquatic Toxicology. They found out that, while fluorimetric techniques could recognize a distinction between 1-OH pyrene equivalents and parent pyrene, identification of specific metabolites was only possible with HPLC/F analysis. It was the first study to use direct flourimetry to detect polycyclic aromatic hydrocarbons equivalents in exposed crustacean urine and a great alternative but HPLC/F was still the preferred choice and hence fluorimetric results will still have to correlate well with those obtained for HPLC/F technique.

Basak *et al.* (2010) in the Turkish Journal of Fisheries and Aquatic Science published "The Detection of Potential Carcinogenic polycyclic aromatic hydrocarbons using HPLC procedure in two different smoked fish, Case Study: Istanbul/Turkey". Levels of polycyclic aromatic hydrocarbons causing Cancer from twenty-four (24) samples were analyzed for smoked fish product. HPLC was used to test the polycyclic aromatic hydrocarbons in Salmon and Rainbow trout samples. Polycyclic aromatic hydrocarbons compounds with the potential to cause cancer on food codex Alimentarius list was detected. In addition, benzo (a) anthracene, benzo (b)

fluoranthene, benzo (k) fluoranthene, benzo (g, h, i) perylene, compounds with potential to cause cancer for humans were also found. With a positive correlation between fish fat and polycyclic aromatic hydrocarbons concentration, smoked salmon observed a higher polycyclic aromatic hydrocarbons levels compared to Rainbow Trout. The researchers also observed variations of the total polycyclic aromatic hydrocarbons of the smoked samples and attributed the variation to the non-homogenous smoking method implored by conventional ovens for smoking fish.

2.7.2 Identification of polycyclic aromatic hydrocarbons sources in honey

Polycyclic aromatic hydrocarbons diagnostic ratio is used for identification of pollution emission sources. According to Tobiszewski and Namieśnik (2012) diagnostic ratio is a tool for identifying and assessing sources of polycyclic aromatic hydrocarbons pollution. The method is applicable to polycyclic aromatic hydrocarbons determination in different media by identifying pollution originality from petroleum products, petroleum combustion and biomass or coal burning. The diagnostic ratio is normalized as : $a/a+b$. Polycyclic aromatic hydrocarbons used have the similar physiochemical properties such as volatility, solubility and chemical reactivity.

According to Tobizewski (2014), the characteristics ratio values for particular pollution sources are listed in table 2. The concentration of $FLT/(FLT+PYR)$ is an indicator of different process and for different media.

Table 2.2 Characteristics values for particular pollution sources.

Diagnostic ratio	Petro genic	Fuel combustion	Coal, grass and wood burning
$ANT/(ANT+PHE)$	< 0.1	0.1	-
$FLT/(FLT+PRY)$	< 0.4	0.4 – 0.5	> 0.5
$BaA/(BaA+CHR)$	< 0.2	0.35	0.2 – 0.35
$IcdP/(IcdP+BghiP)$	< 0.2	0.2 – 0.5	0.5

**Ant* (Anthracene), *PHE* (Phenethrane), *FLT* (Flouranthene), *PYR* (Pyrene), *BaA* (Benzo (a) Anthracene), *CHR* (Chrysene), *IcdP* (Indeno (cd) Perylene), *BghiP* (Benzo (ghi) Pyrene).*

Bhupander *et al.* (2012) researched into the distribution, composition profiles and source identification of polycyclic aromatic hydrocarbons in road side soils in Delhi, India. The diagnostic ratios of individual polycyclic aromatic hydrocarbons (LMW and HMW) were calculated and applied to assessed the possible sources of polycyclic aromatic hydrocarbons.

Research conducted by Wang *et al*. (2009) on Polycyclic aromatic hydrocarbons in urban street dust and surface soils reported the source of contamination as a principal component of the analysis. Percentage abundance for various samples were calculate to determine the source of contamination for street dust and surface dust.

Nyarko, Botwe and Klubi (2012) studied on polycyclic aromatic hydrocarbons levels in two commercially important fish species from coastal waters of Ghana and their carcinogenic health risks. Results of individual polycyclic aromatic hydrocarbons (LMW and HMW) were calculated to determine the source of predominate source of polycyclic aromatic hydrocarbons.

2.7.3Toxicity assessment of Polycyclic aromatic hydrocarbons in Honey

Toxic equivalence quotient depict an assemblage measure of toxicity based on a number of contributing compounds. Basically, polycyclic aromatic hydrocarbons are determined as individual or single compounds. Total polycyclic aromatic hydrocarbons are usually calculated as the sum of individual polycyclic aromatic hydrocarbons present irrespective of toxicity. Benzo (a) pyrene toxicity equivalence quotient used by researchers provide a toxicity sum of polycyclic aromatic hydrocarbons concentration for a specific list of polycyclic aromatic hydrocarbons. This toxicity sum is presented as single and objective concentrations for health investigation levels.

Benzo (a) pyrene concentration is often used as polycyclic aromatic hydrocarbons indicator and regarded by World Health Organization (WHO) as index for polycyclic aromatic hydrocarbons associated carcinogenicity (Yassaa and Cecinato, 2005). This index defined the proposed parametrized carcinogenicity better than having recourse of only benzo (a) pyrene. This approach is suitable as far as sources do not emit benzo (a) pyrene at the extents comparable to other carcinogenic polycyclic aromatic hydrocarbons.

Alomirah *et al.* (2010) conducted a research on benzo (a) pyrene and total polycyclic aromatic levels in oils. This studies determined polycyclic aromatic hydrocarbons concentration in 115 samples of olive oil, cooking oil, and fats collected from retail stores in Kuwait. Concentrations of carcinogenic polycyclic aromatic hydrocarbons in oils and other fats were determined using Benzo (a) pyrene equivalent quotient and eight (8) polycyclic aromatic hydrocarbons.

Jung *et al.* (2010) also studied on the assessment of benzo (a) pyrene equivalent carcinogenicity and mutagenicity of residential indoors and outdoors. The use of this method by researchers provided an accurate risk assessment for calculation environmental exposure to polycyclic

aromatic hydrocarbons. Data were gathered as carcinogenic equivalents and mutagenic equivalent were used for the sum of polycyclic aromatic hydrocarbons.

CHAPTER III

3.0 METHODOLOGY

3.1 Sampling site

The area for study is the Central market located in Kumasi, the second the largest city in Ghana. It is one of the biggest open market in the city of Kumasi. Range of food items to non-food items are sold at the Kumasi central market. This market is known to house diverse informal sector activities to open space trading (Baah-Ennumh and Adom-Asamoah, 2012). Geographically, the central market is located at a latitude of 6 ^{0}N and longitude of 1^{0}W from Kwame Nkrumah University of Science and Technology (KNUST).

3.2 Sampling and sampling size

Samples of honey were collected for a period of November and December the central market in Kumasi- Ghana, using simple random sampling technique. Ten (10) honey samples were collected every two weeks from honey sellers for a period of two months in Kumasi central market.

The collected samples were stored in tight glass bottles and stored in refrigerator before analysis. This was done to avoid contamination into the sampled honey before the analysis. At the end of sampling, a total of fifty honey samples were collected. All samples were immediately taken for analysis at the Kwame Nkrumah University of Science and Technology Mycotoxin Laboratory.

3.3 Calibration curve preparation

A standard mix of polycyclic aromatic hydrocarbons was purchased from Sigma Aldrich, Japan. Standard mix contained 100 ug/mL each of Anthracene, Benzo[a]anthracene, Benzo(b)fluoranthene, Benz(k)fluoranthene, Fluoranthene, Pyrene; 200 ug/mL each of Benzo(ghi)perylene, Fluorene and 1000 ug/mL each of 1-Methylnaphthalene, 2Methylnaphthalene, Naphthalene, Acenaphthene. Calibration standards of 5 – 125 ng/g per 100µ L were prepared from the standard mix at the mycotoxin laboratory (KNUST).

3.4 Sample preparation for polycyclic aromatic hydrocarbons extraction

Fifty grams (50 g) of homogenate honey samples were placed into a 50 mL centrifuge tube. 10 mL of HPLC grade acetonitrile were added and vortex for 1 minute. Agilent Bond Elut QuEChERS AOAC extraction salt packet containing 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaOAc was added to the tube. The sample tubes were vortex again for 1 minute and the centrifuge at 4000 rpm for 5 minutes.

3.5 Dispersive SPE Cleanup

About 6.0 mL of aliquot of the upper acetonitrile layer was transferred into a 15 mL Bond Elut QuEChERS AOAC Dispersive SPE tube which contains 400 mg of PSA, 40 mg of C18 EC and 1200 mg of anhydrous $MgSO_4$. The tube was vortex for 1minute and then centrifuged at 4000 rpm for 5 minutes. A 4ml aliquot of the extract was filtered through a 0.45 um PVDF syringe filter, then 100 µL of the extract was inject into the HPLC system.

3.6 Set-up for High Performance Liquid Chromatography

HPLC Set-up was based on protocol by Shimadzu Application Note (LC-022) Demuro (2010). A Cecil-Adept Binary Pump HPLC coupled with Shimadzu 10AxL fluorescence detector (Ex: 254 nm, Em: 390) with Phenomenex HyperClone BDS C18 Column (150 x 4.60 mm, 5 um). Mobile phase composition was Pump A (Acetonitrile) and Pump B (Deionized Water) at 0.8 mL/mim. Gradient elution was used with the following combination, 0 minute – 5 minutes = 60% A, 40% B; 5 minutes; 15 minutes = 90% A, 10% B; 20 minutes 100% A, 0% B; 28 – 30 minutes = 60%A, 40% B. Polycyclic aromatic hydrocarbons in samples were identified using the retentions times against the standards and quantified using the calibration curve obtained.

3.7 Polycyclic aromatic hydrocarbons Analysis and Quality Control

Analysis of samples was carried out in duplicate and average of two analyses was used in calculation of individual polycyclic aromatic hydrocarbons concentration. Limit of detection for all polycyclic aromatic hydrocarbons ranged from 0.10 – 6.46 ppb.

Minitab 17 version software developed by Minitab Incorporation from Pennsylvania State University was used for the statistical analysis of data. One-way anova was used to determine whether polycyclic aromatic hydrocarbons were present at different concentrations. Significance was accepted at 95 % confidence interval ($p < 0.05$).

3.8 Identification of polycyclic aromatic hydrocarbons source using diagnostic ratio

Sources of polycyclic aromatic hydrocarbons contamination in honey was identified by calculating diagnostic ratios. The ratio allows to distinguish between polycyclic aromatic hydrocarbons pollution originating from petro genic and pyrogenic source.

Below is the applicable equation (Tobizewski, 2014): $FLT/(FLT + PYR)$

Where FLT is flouranthene and PYR is pyrene.

Result from the calculation was compared to diagnostic ratios values (Tobizewski, 2014)

X < 0.4, represents polycyclic aromatic hydrocarbons from petro genic source

0.4 – 0.5, represent polycyclic aromatic hydrocarbons from fuel combustion

X > 0.5, represent polycyclic aromatic hydrocarbons from burning of coal, grass and wood.

3.9 Toxicity Assessment

Quantitative assay was used to determine polycyclic aromatic hydrocarbons contamination in honey. Individual chemicals were evaluated as separate chemical and discussed as group because polycyclic aromatic hydrocarbons are commonly found as mixture of two or more compounds in the environment and often treated similarly because of their similar structures and toxicities. The main pathways of long life exposure of polycyclic aromatic hydrocarbons contamination for human are ingestion, dermal contact and inhalation. In this study honey ingestion was considered as exposure route of polycyclic aromatic hydrocarbons for human health risk assessment. Benzo (a) pyrene toxicity equivalence concentration using stipulated toxicity equivalent factors (appendix

B) of individual polycyclic aromatic hydrocarbons. Benzo (a) pyrene equivalent concentration was calculated by multiplying the concentration of individual polycyclic aromatic hydrocarbons identified in the honey by its toxicity factor

CHAPTER FOUR

4.0 RESULTS AND DISCUSION

4.1 Concentration of polycyclic aromatic hydrocarbons in sampled honey from Kumasi Central Market

The mean concentrations of all (12) twelve polycyclic aromatic hydrocarbons in 50 honey samples from Kumasi Central Market ranged from 9×10^{-4} µg/kg (pyrene) to 1956960×10^{-4} µg/kg (naphthalene) (appendix C) with mean value of $150535 \times 10^{-4} \pm 510840 \times 10^{-4}$ µg/kg (Table 4.1). One-way anova test ($p > 0.05$) showed no significant difference between mean concentrations of individual polycyclic aromatic hydrocarbons.

The results showed predominance of naphthalene (Nap) and its derivatives (1 methylnaphthalene (1CH-Nap) and 2 methylnaphthalenes (2CH-Nap). According to ATSDR (2005), 1 methylnaphthalene and 2 methylnaphthalene compounds are similar to naphthalene and they act as naphthalene in ubiquitous environment. Naphthalene recorded the highest concentrations between the range of $9 \times 10^{-4} - 1848000 \times 10^{-4}$ (Table 4.1). Recorded total concentration of naphthalene (Nap) was 1956960×10^{-4} µg/kg (appendix C) with mean value of $150535 \times 10^{-4} \pm 510840 \times 10^{-4}$ µg/kg. (Table 4.1). The recommended intake of naphthalene according to US EPA, (2002) ranged from 410×10^{-4} - 2370×10^{-4} µg/kg per day for children and $2040 \times 10^{-4} - 9040 \times 10^{-4}$ µg/kg per day for adult.

Studies by Wakefield (2007), Jia and Batterman (2010) and EPA (2000) reviewed the health effects of naphthalene as hemolytic anemia, damage to the liver and neurological damage, long

term exposure by ingestion has been reported to cause cataracts. It is a hazardous pollutant according to U.S. EPA (Han *et al.,* 2014). The Department of Health and Humans Services (DHHS) also concluded that naphthalene is reasonably anticipated to be a human carcinogen. And the International Agency for Research on Cancer (IARC) (2016) also concluded that naphthalene is possibly carcinogenic to humans. From the results mean concentration for naphthalene (150535 × 10^{-4} µg/kg) (Table 4.1) exceeded the recommended intake for both children and adult.

Flourene (Fl), Acenapthene (Ace), Flouranthene (Flt), Anthracene (Ant), and Pyrene (Pyr) recorded mean concentrations of 117 ×10^{-4}, 100 × 10^{-4}, 8 × 10^{-4}, 23 × 10^{-4} and 3 × 10^{-4} µg/kg (Table 4.1)). Although the presence was lower as compared to naphthalene, chronic exposure to these non-carcinogenic polycyclic aromatic hydrocarbons according to Environmental Health and Medicine education (2009) affects the pulmonary, gastrointestinal, renal and dermatological systems.

Total polycyclic aromatic hydrocarbons concentrations of Dibenz (a h) Anthracene (DahA), Benzo (b) Flouranthene (BbF), Benzo (a) Pyrene (BaP), Benzo (k) Flouranthene (BkF) were 266 × 10^{-4}, 25 × 10^{-4}, 11 × 10^{-4} and 10 × 10^{-4} µg/kg, respectively (Appendix C). Recorded mean concentrations ranged from 67 × 10^{-4} ± 61 × 10^{-4}, 13 × 10^{-4} ± 4 × 10^{-4}, 6 × 10^{-4} ± 1 × 10^{-4} and 5 × 10^{-4} ± 4 ×10^{-4} µg/kg (Table 4.1). Chronic exposure to higher levels of Dibenz (a h) Anthracene (DahA) according to WHO (2003) results in health effects such as pulmonary adenomotosis, alveologenic carcinoma, mammary carcinoma and heaman goedotheliomas. Benzo (b) Flouranthene (BbF) and Benzo (k) Flouranthene are genotoxic and carcinogenic. Chronic exposure results in tumor formation (WHO, 2003). The carcinogenicity of polycyclic aromatic hydrocarbons has reported the evidence of skin, lung, bladder, livers and stomach cancer (Bostrom *et al.,* 2002)

Table 4.1: Polycyclic Aromatic Hydrocarbons mean concentration, standard deviation and percentage of abundance in honey (µg/kg).

Polycyclic aromatic hydrocarbons	Min Con.	Max Con.	Mean	STD	% Abundance
Nap	9×10^{-4}	1848000×10^{-4}	150535×10^{-4}	510840×10^{-4}	25.0
1 CH3-nap	7×10^{-4}	332×10^{-4}	76×10^{-4}	1040×10^{-4}	19.2
2 CH3-nap	16×10^{-4}	262×10^{-4}	119×10^{-4}	128×10^{-4}	5.9
Ace	12×10^{-4}	88×10^{-4}	100×10^{-4}	54×10^{-4}	3.8
Fl	2×10^{-4}	576×10^{-4}	117×10^{-4}	228×10^{-4}	11.5
Ant	2×10^{-4}	78×10^{-4}	23×10^{-4}	37×10^{-4}	3.8
Flt	7×10^{-4}	9×10^{-4}	8×10^{-4}	1×10^{-4}	7.8
Pyr	3×10^{-4}	4×10^{-4}	3×10^{-4}	1×10^{-4}	5.8
BbF	7×10^{-4}	18×10^{-4}	13×10^{-4}	4×10^{-4}	3.8
BkF	2×10^{-4}	8×10^{-4}	5×10^{-4}	4×10^{-4}	3.8
BaP	5×10^{-4}	6×10^{-4}	6×10^{-4}	1×10^{-4}	3.8
DahA	3×10^{-4}	125×10^{-4}	67×10^{-4}	61×10^{-4}	5.8
Σ12 PAH	76×10^{-4}	1849506×10^{-4}	151072×10^{-4}	512399×10^{-4}	100.0
PAH (LMW)	49×10^{-4}	1849330×10^{-4}	150970×10^{-4}	512327×10^{-4}	69.2
PAH (MMW)	10×10^{-4}	13×10^{-4}	11×10^{-4}	2×10^{-4}	13.6
PAH (HMW)	17×10^{-4}	157×10^{-4}	91×10^{-4}	70×10^{-4}	17.2
PAH (carcinogenic)	17×10^{-4}	157×10^{-4}	91×10^{-4}	70×10^{-4}	17.2
PAH (non -carcinogenic)	59×10^{-4}	1849394×10^{-4}	150981×10^{-4}	512329×10^{-4}	82.2

**polycyclic aromatic hydrocarbons*(PAH), *min concentrations*(min con), *maximum concentrations* (max con), *standard deviation* (STD), *naphthalene* (nap), *1methylnapthalene* (1-CH nap), *2 methylnaphtahlene* (2-CH nap), *acenapthanene* (Ace), *Flourene* (Fl), *Anthracene (Ant), flouranthene* (Flt), *pyrene* (Pyr), *benzo (b) flouranthene* (BbF), *Benzo (k) flouranthene* (BkF), *Benzo (a) pyrene* (Bap), *Dibenz (ah) anthracene* (DahA).*

Benzo (a) pyrene (BaP) is always of great interest in terms of potential cancer hazard (Bortey-Sam, 2014). It is a good indicator for polycyclic aromatic occurrences and toxicity. BaP according to WHO (2003) is human carcinogenic and administration induces tumor of the forestomach, skin tumors, lungs and respiratory tumors. The maximum recommended maximum set limits for benzo (a) Pyrene in uncontaminated infant formulae is 10000×10^{-4} µg/kg (Batelková *et al.,* 2012). Recorded mean concentration ($6 \times 10^{-4} \pm 1 \times 10^{-4}$ µg/kg) (Table 4.1) for benzo (a) Pyrene in honey was less than the recommended limit set.

4.2 Classification of polycyclic aromatic hydrocarbons based on molecular weights

The studied priority polycyclic aromatic hydrocarbons were classified into three groups, according to their number of aromatic rings as low molecular weight (LMW), medium molecular weights (MMW) and high molecular weight (HMW). Polycyclic aromatic hydrocarbons with low molecular weight (2-3 rings) (naphthalene, 1 methylnaphthalene, 2 methylnaphthalenes, acenapthene, flourene and anthracene) and polycyclic aromatic hydrocarbons with medium molecular weight (4 rings) (flouranthene and pyrene) and polycyclic aromatic hydrocarbons with high molecular weight (5-6 rings), benzo (b) flouranthene, benzo (k) flouranthene, benzo (a) pyrene and dibenz (a h) anthracene).

The concentration of benzo (b) flouranthene (BbF), benzo (k) flouranthene (BkF), benzo (a) pyrene (BaP) and dibenz (ah) anthracene (DahA) (high molecular weight) ranged from 17×10^{-4} – 157×10^{4}µg/kg, (flouranthene (Flt) and pyrene (pyr)) (medium molecular weight) ranged from 10×10^{-4} –

13× 10^{-4} µg/kg and (naphthalene (Nap), 1 methylnaphthalene (1 CH- Nap), 2 methylnaphthalenes (2 CH- Nap), acenapthene (Ace), flourene (Flu), anthracene (Ant), (LMW) ranged from 0.0049 – 1849330× 10^{-4} µg/kg respectively (Table 4.1).

Studies conducted by Lee and Vu (2010) reported bioavailability of polycyclic aromatic hydrocarbons with low molecular weights as gaseous substances and are susceptible to atmospheric processing (Alam *et al.* (2010). The compositional pattern of polycyclic aromatic hydrocarbons in the analyzed honey samples from Kumasi central market is as follows: 2 - 3 rings > 5 - 6 rings > 4 rings (Figure 4.1).

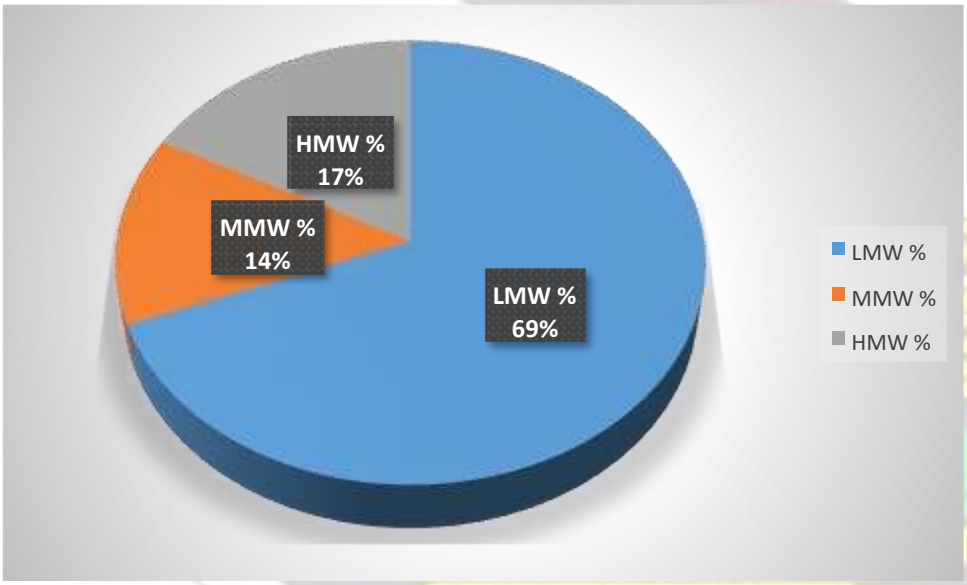


Figure 4. 1. Percentage abundance of polycyclic aromatic hydrocarbons molecular weights ***LMW* *(low molecular weight), *MMW*(medium molecular weight) *HMW* (high molecular weight), *%*(percentage), *polycyclic aromatic hydrocarbons* (polycyclic aromatic hydrocarbons).**

4.3 Identification of Possible Source of Polycyclic aromatic hydrocarbons using Diagnostic Ratio

The use of diagnostic ratio formula ratio was applicable to apportion the pollution emission of polycyclic aromatic hydrocarbons in honey. From Table 4.1, concentration of flouranthene (flt) ranged from 7×10^{-4} - 9×10^{-4} µg/kg with mean value of $8\times 10^{-4} \pm 1\times 10^{-4}$ µg/kg and concentration of pyrene (pyr) ranged from $3\times 10^{-4} - 4\times 10^{-4}$ with mean value of $3\times 10^{-4} \pm 1\times 10^{-4}$ µg/kg. The result of the diagnostic ratio calculation (13750×10^{-4}) (Figure 4.2) which was greater than 5000×10^{-4} ($13750\times 10^{-4} > 5000\times 10^{-4}$) was an indication of polycyclic aromatic hydrocarbons from burning of wood, grass and coal (Tobizewski, 2014). Excessive use of smoke according to Krell (1996) flavors honey and contaminate it with polycyclic aromatic hydrocarbons (Lawley, Curtis and Davis, 2012).

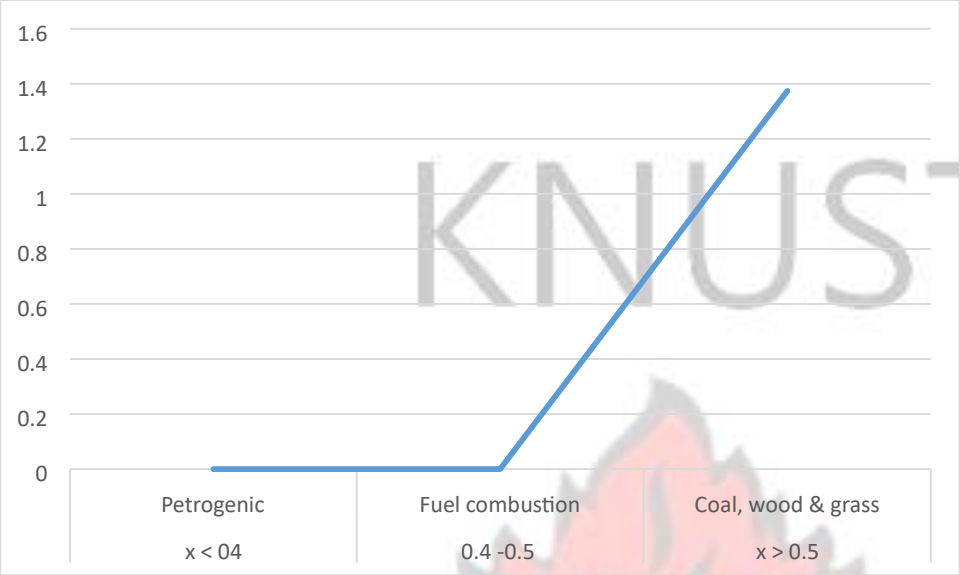


Figure 4.2: Diagnostic ratio chart of the sources of polycyclic aromatic hydrocarbons in honey samples.

4.4 Toxicity Assessment of Honey

Benzo (a) pyrene toxic equivalent factor for the priority polycyclic aromatic hydrocarbons were derived and used for the calculation and quantification of the cancer risks. Estimated benzo (a) pyrene toxicity equivalent for the study was presented in Table 4.2.

Estimated benzo (a) Pyrene equivalent concentration for 12 studied polycyclic aromatic hydrocarbons (Table 4.2) ranged from 0 – 128× 10^{-4} µg/kg. Benzo (a) pyrene total equivalent concentration is the sum of estimated cancer potency for individual polycyclic aromatic hydrocarbons concentrations. The estimated benzo (a) pyrene total toxicity equivalent concentration (Table 4.2, Appendix B) was 146× 10^{-4} µg/kg.

Table 4.2: Benzo (a) Pyrene Toxicity Equivalence Concentration (µg/kg). (Refer to Appendix B)

Polycyclic aromatic hydrocarbons	TEF Values	Mean C ± STD	TEQs
Benzo (a) Pyrene	10000×10^{-4}	$0.0006\times 10^{-4} \pm 0.0001\times 10^{-4}$	0.0007×10^{-4}
Benzo(k) flouranthene	1000×10^{-4}	$5\times 10^{-4} \pm 4\times 10^{-4}$	9×10^{-4}
Dibenz(ah) Anthracene	10000×10^{-4}	$67\times 10^{-4} \pm 61\times 10^{-4}$	128×10^{-4}
Benzo(b) Flouranthene	1000×10^{-4}	$13\times 10^{-4} \pm 4\times 10^{-4}$	2×10^{-4}
Flouranthene	10×10^{-4}	$8\times 10^{-4} \pm 1\times 10^{-4}$	0
Pyrene	10×10^{-4}	$3\times 10^{-4} \pm 1\times 10^{-4}$	0
Total			146×10^{-4}

**BaP_{eq}* (Benzo (a) Pyrene equivalence concentration),*TEQs*(Toxicity equivalence concentrations, *SD* (Standard deviation).*

The maximum residue levels (MRLs) for polycyclic aromatic hydrocarbons in honey is not set. The Commission regulation only set limits of benzo (a) pyrene for fats, oil, smoked fish, infant formula and dietary foods for special medical purposes intended for specifically for infants. Results of this study was compared with limits of benzo (a) pyrene in uncontaminated food matrix for infant formula (10000×10^{-4} µg/kg) (Batelková, Borkovcová, Čelechovská, Vorlová, and Bartáková, 2012). The result recorded from the study did not exceed the regulated limit

CHAPTER V

5.0 CONCLUSION, SUMMARY OF FINDINGS AND RECOMENDATION

5.1 Conclusion

The concentration of both benzo (a) Pyrene and other polycyclic aromatic hydrocarbons were quantified. Individual mean concentration and range of occurrences were reported. The study showed the presence of polycyclic aromatic hydrocarbons in honey at different concentration levels. The analysis identified naphthalene, 1methylnaphthalene, 2 methylnaphthalenes,

acenaphthene, flourene, pyrene, anthracene, benzo (k) flouranthene, benzo (b) flouranthene, benzo (a) pyrene and dibenz (ah) anthrancene.

The study recorded higher concentration level of naphthalene (150535×10^{-4} µg/kg) which exceeded the recommended intake for both children and intake stipulated by US EPA (2002). Benzo (a) Pyrene which is an indicator for polycyclic aromatic hydrocarbon occurrences and toxicity recorded level ($13\times 10^{-4} \pm 1\times 10^{-4}$ µg/kg) which was far lower than the maximum limit (1.0 µg/kg). Composition pattern of polycyclic aromatic hydrocarbons according to molecular weight showed greater percentage of low molecular weight PAH. This is an indication of polycyclic aromatic hydrocarbons that exists in gaseous phase and are susceptible to atmospheric processing.

Pollution emission in honey was (13750×10^{-4} µg/kg), and this is an indication of polycyclic aromatic hydrocarbons from burning wood, grass and coal. Toxicity assessment of polycyclic aromatic hydrocarbons in honey was low (146×10^{-4} µg/kg) and this confirmed that honey sold in Kumasi central market by honey hawkers are safe and of good quality.

5.2 Summary of Findings

Honey is a supersaturated sweetener rich in variety of nutrients and have medicinal efficacy. Environmental pollution as well harmful beekeeping practices has been known to contaminate honey with polycyclic aromatic hydrocarbons.

Polycyclic aromatic hydrocarbon is a xenobiotic that is basically formed from incomplete combustion of organic substances and also emit from crude products. In foods they are formed from processing methods, domestic cooking and by contamination.

Fifty (50) honey samples were collected from Kumasi central market for period of two months. HPLC coupled with fluorescence detector was used for polycyclic aromatic hydrocarbons

identification in honey. The study recorded the presence of the twelve studied polycyclic aromatic hydrocarbons at varying concentrations.

Results showed different concentrations of polycyclic aromatic hydrocarbons but predominance existence of naphthalene (Nap) compound which exceeded the set limit set for both children and adults. Pyrene (Py) recorded lowest total mean concentration. Diagnostic ratio showed the bioavailability of source of polycyclic aromatic hydrocarbons in honey sold at Kumasi central market.

5.3 Recommendations

The study design was find the levels, sources and toxicity of polycyclic aromatic hydrocarbons in honey. The following suggestions are given to improve the study for further research;

1. Honey from different source (wild honey and honey from apiculture) should tested for polycyclic aromatic hydrocarbons.
2. Honey from different regions in the country should also be compared to which part of the country produced contaminated honey with polycyclic aromatic hydrocarbons.

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APPENDICES

Appendix A. Synthetic Wax for beekeeping

Appendix B. Toxicity Equivalence Factor for Individual polycyclic aromatic hydrocarbons

PAH	TEF
Benzo (a) Pyrene	10000×10^{-4}
Pyrene	10×10^{-4}
Benzo (k) Flouranthene	1000×10^{-4}
Fluoranthene	10×10^{-4}
Benzo (b) Fuoranthene	1000×10^{-4}

Dibenz (a,h) Anthracene 10000×10^{-4}

Total Toxicity Equivalence Concentration (TTEC) = $\sum$ Cn * TEFn Where:

TTEC = Total Toxicity Equivalent Concentration C_n = Concentration of the individual congener or cPAH in the mixture TEF_n = Toxic equivalency factor of the individual congener or CPAH associated with its respective mixture.

$TEQ_{Bap} = \sum(TEF_1 * C_1)$.

Benzo (a) Pyrene = $(6\times 10^{-4} + 1\times 10^{-4}) \times (10000\times 10^{-4}) = 7 \times 10^{-4}$

Benzo (k) Flouranthene = $(5\times 10^{-4} + 4\times 10^{-4}) \times (1000\times 10^{-4}) = 9 \times 10^{-4}$

Dibenz (ah) Anthracene = $(67\times 10^{-4} + 61\times 10^{-4}) \times (10000 \times 10^{-4}) = 128 \times 10^{-4}$

Benzo (b) Flouranthene = $(13\times 10^{-4} + 4\times 10^{-4}) \times (1000\times 10^{-4}) = 17 \times 10^{-5}$

Flournthene = $(8\times 10^{-4} + 1\times 10^{-4}) \times (10\times 10^{-4}) = 0$

Pyrene = $(3\times 10^{-4} + 1\times 10^{-4}) \times (10\times 10^{-4}) = 0$

$$TEQ_{Bap} = 7\times 10^{-4} + 9\times 10^{-4} + 128\times 10^{-4} + 2\times 10^{-4} = 146 \times 10^{-4}$$

Appendix C. Mean concentration (ug/ml) of polycyclic aromatic hydrocarbons in honey sampled from Kumasi central market.

Samples	Nap	1MN	2MN	Ace	Fl	Ant	Flt	Py	BbF	BkF	BaP	DahA
1	38969.55	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
2	32.92	Nd	Nd	Nd	0.4	Nd	Nd	nd	Nd	Nd	Nd	Nd
3	103.2	Nd	Nd	Nd	57.6	Nd	Nd	Nd	Nd	Nd	Nd	Nd
4	184799.6	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	nd
5	Nd	33.2	26.2	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
6	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
7	Nd	8.2	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
8	Nd	15.27	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
9	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	12.47
10	11.5	11.2	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	7.7
11	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
12	Nd	Nd	8	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
13	14.3	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
14	Nd	1.8	Nd	Nd	Nd	Nd	Nd	0.4	Nd	0.8	Nd	Nd
15	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
16	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
17	Nd	Nd	Nd	Nd	0.2	0.23	Nd	Nd	Nd	Nd	Nd	Nd
18	162	Nd	Nd	8.8	10.1	7.8	Nd	0.3	Nd	Nd	Nd	Nd
19	21.9	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd

20	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
21	Nd	1.11	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
22	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
23	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
24	Nd	Nd	nd	Nd	Nd	Nd	Nd	Nd	Nd	0.2	Nd	Nd
25	Nd	0.84	Nd	Nd	Nd	Nd	Nd	0.2	Nd	Nd	Nd	Nd
26	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.7	Nd
27	Nd	15.77	Nd	Nd	Nd	1.8	Nd	Nd	Nd	Nd	Nd	Nd
28	Nd	Nd	Nd	Nd	Nd	1	Nd	Nd	Nd	Nd	Nd	Nd
29	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
30	Nd	Nd	Nd	Nd	Nd	Nd	0.9	Nd	Nd	Nd	Nd	Nd
31	35.44	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
32	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
33	Nd	1.3	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
34	10478.43	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
35	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.6	Nd
36	0.9	Nd	Nd	Nd	Nd	Nd	0.7	Nd	Nd	Nd	Nd	Nd
37	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
38	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
39	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
40	Nd	0.74	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
41	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
42	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
43	Nd	2.2	Nd	1.2	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd

44	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
45	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
46	Nd	Nd	Nd	Nd	Nd	0.3	Nd	Nd	Nd	Nd	Nd	Nd
47	15.76 Nd		Nd	Nd	Nd	0.21	Nd	Nd	Nd	1.8	Nd	Nd
48	Nd	Nd	1.6	Nd	Nd	Nd	Nd	Nd	Nd	Nd	0.5	Nd
49	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
50	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Σ12	**19555.023**		**75.86**	**35.8**	**10**	**70.2**	**9.33**	**1.6**	**0.9**	**2.5**	**1**	**1.1**	**26.6**
PAH (µg/mL)						**6**						
Total	**1629.59**		**0.075**	**0.035**	**0.0**	**0.07**	**0.00**	**0.0**	**0.0**	**0.00**	**0.0**	**0.00**	**0.0266**
Mean (µg/kg)			**9**	**8**	**1**	**03**	**93**	**016**	**009**	**25**	**01**	**11**	

**nd (not detected), *Nph* (naphthalene), *1CH* (1 methyl naphthalene), *2CH* (2 methyl naphthalene), *Ace* (acenaphthene), *fl* (flourene), *Ant* (anthracene), *flt* (flouranthene), *Py* (pyrene), *BbF* (Benzo b Flouranthene), *BkF* (benzo k flouranthene), *BaP* (benzo a pyrene), *DahA* (dibenz a h anthracene)*