

**ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN
FISH, MEAT AND THE EFFECT OF COOKING ON THEIR
CONCENTRATIONS IN MEAT AS SOURCES OF HUMAN EXPOSURE IN
WINAM GULF REGION OF LAKE VICTORIA**

BY

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ABSTRACT

Polycyclic Aromatic Hydrocarbons are of concern in environmental chemistry because they are known or suspected carcinogens and/or toxicants. Fish is a major indicator of the existence of PAHs in aquatic environment and source of exposure to humans. Charcoal-roasted meat can also be a major human exposure to the PAHs. However, the contribution of fish and meat to human exposure to PAHs in the region is not known. The study set out to determine the concentration of the PAHs in the fish and meat; and to establish the effect of roasting on PAH concentration in the meat and then compare the levels with the standards allowed internationally in these foods.

Samples of fish (*Rastrineobola argentea*, *Lates niloticus* and *Oreochromis niloticus*) were obtained from three beaches: Dunga Beach (South West of Kisumu City and South of Kisumu Bay; 4 km from the City Center), Usoma and Usare Beach (West and on the opposite side of Kisumu City across Kisumu Bay about 3 km from each other) in Kisumu Bay (0°6'S, 34°45'E). Samples of meat were from Highway Inn (on Kisumu-Kakamega Highway) for goat meat and beef, Apok Inn for beef (on Kisumu-Nairobi Highway) and pork from Kisumu Hotel (City Center) in Kisumu City. Extraction of PAHs was done using liquid-liquid partition after saponification with alcoholic potassium hydroxide; clean-up using open column chromatography with organic solvents on silica gel stationary phases and final analysis done using GC-MS.

Twelve PAHs were detected in *O. niloticus* (TPAH 3.934 µg/kg); *L. niloticus* had eleven PAHs (TPAH 3.166 µg/kg). In both cases, the highest and lowest PAHs concentrations were Pyrene and Indeno(1,2,3-cd)pyrene respectively, *R. argentea* had two PAHs (TPAH 0.035µg/kg). Overall mean total PAHs was 3.047 µg/kg. Raw beef

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information on PAHs

Polycyclic Aromatic Hydrocarbons (PAH) are of concern in environmental chemistry because they are known or suspected carcinogens and/or toxicants of which the United States Environmental Protection Agency has listed sixteen of them as priority pollutants that need to be periodically monitored in the environment (NRC, 1983). Data from the Nairobi Cancer Registry (NCR) and verbal discussions with practicing physicians indicate that cancer incidence is increasing in Kenya; it now numbers among the top 10 causes of mortality in Kenya (ASCO website, accessed 21-11-2010). According to the Kenya National Cancer Control Strategy Paper (2011-2016), in many developing countries the rapid rise in cancers and other non-communicable diseases has resulted from increased exposure to risk factors which include tobacco use, harmful use of alcohol and exposure to environmental carcinogens (KNCCS, 2011-2016). However, the levels of exposure to these environmental carcinogens of which PAHs are some, is not known.

The PAHs are a group of over 100 hydrocarbon chemicals with a structure of fused rings containing at least two fused benzene rings and may contain other rings of carbon that are not six carbon ring structures. They are colorless, white or pale yellow-green solids with melting points generally above room temperature and boiling points generally above 100°C. They do not dissolve easily in water although some are readily evaporated into air and most do not burn easily (DEH, 2004).

Most of the PAHs have no known commercial use but few are used to make dyes, plastics and pesticides. For example, naphthalene is used to make dyes, explosives, plastics, lubricants and moth repellants; anthracene is used to make dyes, insecticides and wood preservatives (FEHD, 2004). However, PAHs are mainly known because of their existence as environmental contaminants (IARC, 1987).

PAHs are toxicants and/or carcinogens and the population may be exposing themselves by eating fish and roasted meat (Békaert *et al.*, 1999; Bispo *et al.*, 1999; Madill *et al.*, 1999; Fent and Bättscher, 2000). The levels of human exposure from fish and meat in the Winam Gulf region is neither known nor periodically monitored.

1.2 Sources and Sinks of PAHs

Sources of PAHs include; petroleum refineries, fossil fuel power plants, coal-tar production plants, coking plants, bitumen and asphalt production plants, paper mills, wood products manufacture (preserved wood creosote), aluminium production plants, industrial machinery manufacture, asphalt roads and road tar (IPCS, 1998). Another major source is combustion of all types (natural biomass fires, crude oil, shale oil, active volcanoes and motor vehicle exhaust) as well as petroleum fuel spillages (IPCS, 1998). Burning of sugarcane in the sugar belt region around Winam Gulf is common. The sugarcane plantations are generally burned before harvesting, although it is becoming increasingly common to harvest the canes while they are still green (Proserpine, 2010; Lindell and Kroon, 2010). The contribution of these sources to PAH in fish and other animals is not known.

One of the sinks for many pollutants is a water body. Lake Victoria is one such water body. It is the largest African lake and the second largest fresh water lake in the

world. It is a shared natural resource for the three East African countries namely: Kenya, Uganda and Tanzania. The knowledge on PAH pollution on Lake Victoria is limited with only a few studies having been done in Kisumu City Bay of Winam Gulf on sediment and water (Bowa *et. al.*, 2009; Bowa and Lalah, 2009). Decision makers and environmental managers have little information and depend on conjecture when it comes to PAH pollution in the lake. This conjecture is based on the existence of point, diffuse and mobile sources of PAH (Bowa *et. al.*, 2009; Bowa and Lalah, 2009) as well as dumping of consumer products containing PAHs into the lake from the surrounding areas. The PAH concentration in fish and meat (biospheric sinks) as a result of the contribution of these sources is not known.

Kisumu City is located on the Northern tip of Winam Gulf, which is a part of Kavirondo Gulf of Lake Victoria. The point sources of PAHs in Kisumu City are suspected to be Kenya Pipeline depot runoff, car wash activities in the lake; oil spills from vessels at the Pier and Yatch Club, Jua kali sheds and petrol stations runoffs (Bowa *et. al.*, 2009). The diffuse sources are asphalt roads and road tar runoffs, agricultural runoff e.g. Webuye Pan Paper Mills via Nzoia river; natural alteration of organic matter following widespread infestation of the gulf by hyacinth and fires of all types (municipal garbage incineration and burning of sugarcane plantations from the surrounding sugar belt) (Proserpine, 2010; Lindell and Kroon, 2010). A mobile source is motor vehicle exhaust (Lalah and Kaigwara, 2005) while consumer products waste like medicines, plastics and pesticides (FEHD, 2004) dumped into the lake are other sources of PAHs in the Gulf. However, it is not known what the levels of PAHs are in fish and meat in this region.

1.3 Exposure to PAHS

Human beings are exposed to PAHs through smoke (smoking tobacco, burning of wood and petroleum oil combustion), charcoal-grilled food, emissions from creosote treated wood products, foods from soils contaminated with PAHs and industrial workplaces exposure (DEH, 2004). However, the actual amounts of PAHs Kisumu residents are exposed to as a result of roasted meat consumption are yet to be determined. This current study proposed to provide this useful information for future discussion.

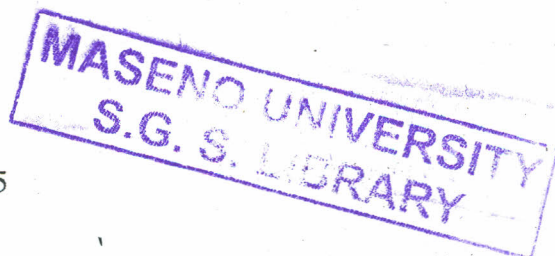
This study investigated the presence of PAHs in fish from Winam Gulf of Lake Victoria and in roast meats in Kisumu in order to determine their importance as sources of human exposure and their baseline quantities. Baseline information is useful in providing guidelines to the levels of PAHs in fish and meat for use by Ministry of Health and nutritionists. This study is the first attempt to provide this crucial information. Contaminated fish pose hazards to people who use it as a source of protein and also to the overall fish industry as a consequence of residue limits of pollutants (Ogutu-Ohwayo *et al.*, 2002). One of the major overseas markets for the fish from Lake Victoria is the European Union (EU), which is known to have very stringent quality standards. Poor quality standards have resulted in fish bans in the past. For example, between 1994 and 1999, the EU imposed several bans on export of chilled fish products from Lake Victoria into the EU due to fish quality concerns and required the East African countries to put in place measures to prevent contamination of fish to safeguard EU consumers (Ogutu-Ohwayo *et al.*, 2002). So far, PAHs on their own have not been a cause for fish banning but there is growing concern on effects of hydrocarbons in the environment according to the Second Schedule of

Environmental Management Coordination Act of Kenya (EMCA), 1999 (Wamukoya and Ludeki, 2002). This study set out to be proactive in avoiding such a ban due to PAHs by determining the current levels of these PAHs and hence give direction on whether there is need for intervention or not.

Many studies concerning PAHs that have been done elsewhere include: crabs samples in a water body having aluminum smelter as a point source (Eickhoff *et al.*, 2003) and water samples in a river and gulf with known sources of PAHs being oil spills from ship traffic, agricultural runoff, fossil fuel combustion and natural alteration of organic matter (Mitra and Bianchi, 2003). Barbecued meat has also been studied in order to determine PAHs in traditional Hong Kong marinated beef (FEHD, 2004) among others. The conditions yielding PAHs in these studies are present in the Winam Gulf and Kisumu City. However, the levels of PAHs in the fish in the Gulf and human exposure to PAHs through roasted meat are not known.

1.4 Statement of the Problem

Fish and meat products of high quality are any consumer's top priority. However, fish is major indicator of PAHs accumulation in aquatic environment and hence a good source of exposure of PAHs to consumers. Roasted meat similarly is a delicious meal which is roasted, smoked or fried through incomplete combustion processes. These two common products are liable to exposure to PAHs although at the moment the level of exposure to humans from these products is not known in the Winam Gulf region of Lake Victoria.



1.5 Objectives of the Study

1.5.1 Broad Objective

The broad objective of the study was to establish if fish and meat in Winam Gulf are potential sources of human exposure to priority PAH pollutants.

1.5.2 Specific Objectives

The specific objectives of this study were:

1. To determine the concentrations of the PAHs in the fish; 'Omena' (*Rastrineobola argentea*), Nile Tilapia (*Oreochromis niloticus*) and Nile Perch (*Lates niloticus*) from Winam Gulf.
2. To determine the concentrations of the PAHs in the meat; beef, goat meat and pork.
3. To establish the effect of roasting on PAH concentrations in the meat
4. To compare the levels of PAHs with the standard limits allowed by the European Commission in these foods.

1.6 Hypotheses (Null)

1. Fish from Winam Gulf are not contaminated with priority pollutant PAHs
2. Meat from Winam Gulf are not contaminated with priority pollutant
3. Roasting as a method of cooking meat does not increases the levels of PAHs significantly
4. The concentration of priority pollutant PAHs in the fish and meat in the region is below the European Commission limits.

1.7 Justification of the Study

Increased industrialization of Kisumu city and its surrounding areas can potentially result in increased levels of PAHs. Dumping of industrial effluents, agricultural runoff and medical waste into the Lake Victoria may have led to increased levels of PAHs in the Winam Gulf. The waters of the gulf contain high organic matter load following the increased water hyacinth infestation and eutrophication of the lake. Natural diagenetic alteration of organic matter is known to lead to formation of PAHs (Mitra and Bianchi, 2003). Sediments and surface water of Winam Gulf have been shown to have high PAH concentration (Bowa *et. al.*, 2009; Bowa and Lalah, 2009). The PAHs could be ending up in the fish that are consumed by human beings; consequently fish eating could be a potential source of exposure. There has been no study to determine the levels of PAHs in fish in Lake Victoria and particularly in the Winam Gulf where contamination is high. Therefore the levels of PAHs in fish from the Gulf are not known and neither is their contribution to human exposure.

The Joint FAO/WHO Expert Committee on Food Additives indicates that there is need to minimize human exposure to benzo(a)pyrene, which is one of the most potent PAHs, as much as it is practicable (WHO, 1991). This compound and other 16 USEPA priority PAH pollutants were analyzed in this study with the aim of determining their levels and comparing them to the internationally acceptable standards and hence assess fish being a potential source of PAHs to humans.

The general populace may be courting cancer due to increased exposure to PAHs as a result of weekend outings into the 'nyama choma' (roasted meat) dens. Roasting of

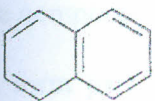
meat potentially yields PAHs since charred food of almost any composition contains PAHs (Phillips, 1999). A fairly consistent association has been observed between grilled or broiled, but not fried, meat consumption and stomach cancer implying that dietary exposure to PAHs may play a role in the development of stomach cancer in humans (WCRF, 1997). According to Dr. Alice Musibi (Medical Oncology Research Officer, Kenya Medical Research Institute (KEMRI); Medical Oncologist, Kenyatta National Hospital (KNH) and Acting Head, Biotechnology and Non-communicable Disease Programme, (KEMRI), there are more cancer cases being reported in Kenya now than 10 years ago, but studies to determine the reasons for the increased prevalence and incidence are not being conducted (ASCO website, accessed 21-11-2010). According to studies in England and Germany, vegetarians were about 40 percent less likely to develop cancer compared to meat eaters (Thorogood et al., 1994; Chang-Claude et al., 1992; Chang-Claude and Frentzel-Beyme, 1993). No study has been conducted locally to determine the extent of increase of PAHs in roast meat prepared using local roasting methods. The commonly roasted meats are from chicken, goat meat, beef (which are charcoal-roasted), pork and sheep mutton (which are oven roasted). It is necessary to determine the extent to which these roasting processes produce PAHs as this will indicate the extent of exposure through these popular foods.

CHAPTER TWO

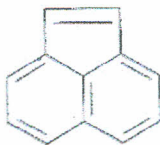
2.0 LITERATURE REVIEW

2.1 What are PAHs?

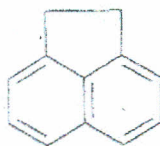
Polycyclic Aromatic Hydrocarbons (also referred to variously as polyaromatic hydrocarbons, polyaromates, polynuclear aromatic hydrocarbons or PAHs) is a group of over 100 different compounds formed during incomplete combustion of coal, oil and gas, garbage or other organic substances e.g. tobacco or charbroiled meat (ATSDR, 1995; Codina *et al.*, 1994). They consist of carbon and hydrogen arranged in two or more fused five or six membered aromatic rings. The sixteen USEPA priority PAHs (DEH, 2004) are shown below:



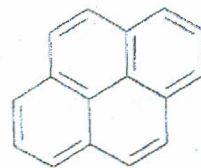
Naphthalene



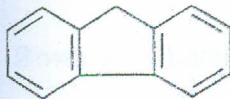
Acenaphthylene



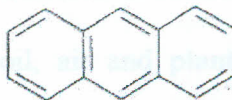
Acenaphthene



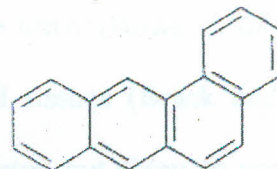
Pyrene



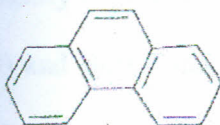
Fluorene



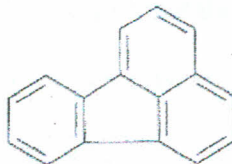
Anthracene



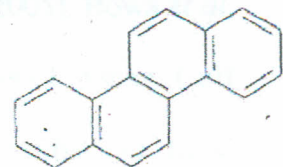
Benzo[a]anthracene



Phenanthrene



Fluoranthene



Chrysene

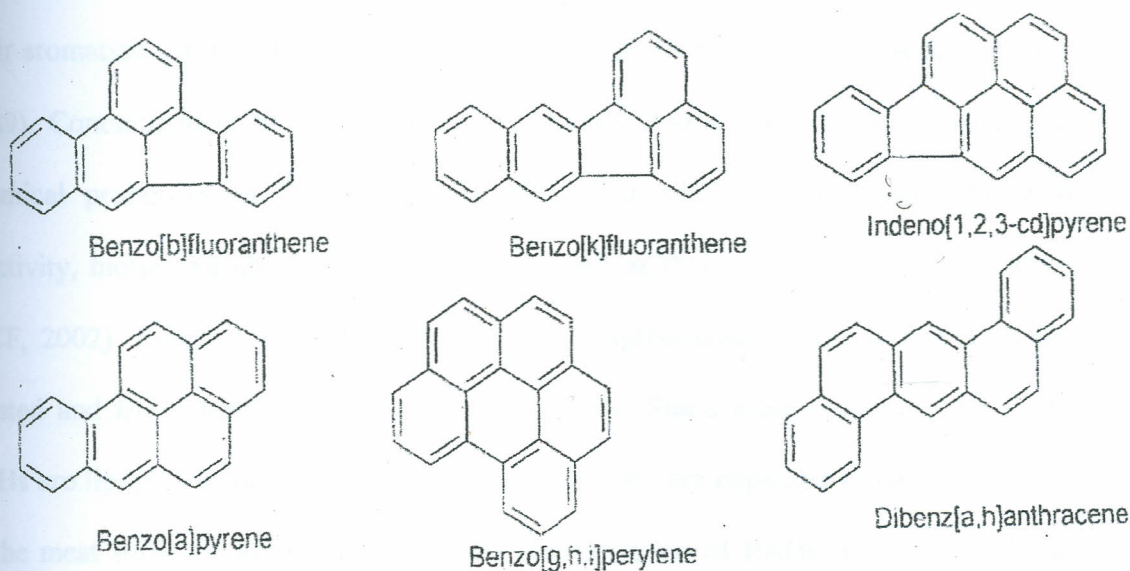


Figure 2.0.1: The structures and names of sixteen USEPA listed priority PAHs

These compounds appear to be ubiquitous in the biosphere (Gu *et al.*, 2003). Fish and meat form part of the biosphere and as such they are expected to contain PAHs. However, the PAH concentration in the fish and meat in Winam Gulf region has not been established. They have been detected in sediments, surface water (Bowa *et al.*, 2009; Bowa and Lalah, 2009), soil, air and plant and animal tissues (Brack and Schirmer, 2003). PAHs bioaccumulate in organisms living in water, but show do not biomagnify in food chains due to their rapid metabolism (WVITC, 2005). Bowa *et al.* (2009) and Bowa and Lalah (2009) studied the waters and sediments of Winam Gulf and found them to be PAH contaminated. It follows that the fish in these contaminated waters could be having PAHs in them. However, this has not been established in the Winam Gulf.

Most of the PAHs are formed as byproducts from any fuel burned under oxygen deficient conditions (McVeety and Hites, 1988). The PAHs are crystalline solids with

high melting point; low vapor pressure and low water solubility, which coupled with their aromaticity, make them exhibit strong adsorption affinity for surfaces (Onuska, 1989). Contamination of food with environmental PAHs depends on physical and chemical properties of PAHs, for example, their solubility, volatility, chemical reactivity, biotic and abiotic degradability as well as their being lipophilic in nature (SCF, 2002). Roasting and frying involves incomplete combustion and as such the roasted and fried meat could be containing PAHs. Since meat comprise lipids and PAHs are lipophilic and therefore when formed, they are expected to be adsorbed on to the meat surfaces. However, so far the concentration of PAHs in the roasted and fried meats has not been established in the Winam Gulf. This study is an attempt to establish this.

Some PAHs and metabolites of many high molecular weight PAHs have been shown to be carcinogenic or to increase their carcinogenicity and/or mutagenicity (WVITC, 2005). There is therefore need to monitor the levels of these compounds in the foods to assess the safety or quality of the food to consumers since this is of particular health concern.

2.2 Sources of PAHs

Sources of PAHs are coal coking, forest fires (Onuska 1989), aluminium production (Eickhoff *et al.*, 2003), foundries, domestic and residential heating, cooking (food itself, fuel and cooking oil) (DEH, 2004), motor vehicle exhaust; petrol and diesel fuel and exhaust emissions (Lalah and Kaigwara, 2005), coal fired power plants and incineration of refuse (IPCS, 1998; Gu *et al.*, 2003; Codina *et al.*, 1994; Takatsuki *et al.*, 1985). There is evidence that PAHs accumulate in tissues of aquatic organisms

above those levels found in water and in the biota and that their uptake processes are related to their hydrophobic character (Onuska, 1989). An unusually high prevalence of oral, dermal and hepatic neoplasms have been observed in bottom dwelling fish caught in PAH-contaminated waters and sediments (Couch and Harshbarger, 1985). Concentration of PAHs in the tissues of aquatic organisms is highest and most verifiable in organisms collected close to point sources (Eickhoff *et al.*, 2003). Fires occur in the region as a result of burning of Sugarcane plantations (Proserpine, 2010; Lindell and Kroon, 2010) and municipal waste incineration. Roasting and frying of meat are some of the cooking methods used in the region. Kisumu City has a considerable size of motor vehicles used by dwellers and also given that it is a transit town to heavy commercial vehicles bound for Uganda as well as being a principal port with motorized vessels. All these are potential sources of PAHs in the study region and yet so far no study had been undertaken to determine the extent to which the PAHs get to the fish and meat and as such being potential routes of human exposure.

PAHs find their way into a water body through both natural and anthropogenic (which is the major contributor) deposition; by surface run-off from contaminated soils and other surfaces, as well as through dumping of industrial waste (Onuska, 1989). In the aquatic environment, they distribute themselves in water but since they are hydrophobic, most are found deposited in the sediments. They are also lipophilic and as such find their way into the aquatic animals in which they bioaccumulate (Onuska, 1989). Most PAHs in water are believed to result from urban runoff, atmospheric fall out, asphalt abrasion and naturally occurring biological processes (Holoubek *et al.*, 1990; Codina *et al.*, 1994). These conditions exist in the Winam Gulf namely; urban

area (Kisumu), two annual rainy seasons to aid atmospheric fall out, asphalt covered roads and infestation of Lake Victoria by water hyacinth to aid naturally occurring biological processes. Despite all these conditions being present, the levels of concentration of the PAHs in fish and meat have not been determined.

They also result from point sources e.g. petroleum drilling activities, oil spills (Car wash activities in Winam Gulf) or chronic leakage (accumulating PAHs at the $\mu\text{g/g}$ level), erosion and effluent discharges (Volkman *et al.*, 1992; Onuska, 1989). Most PAHs in the marine environment are associated with particulate materials and seem to be resistant to degradation and desorption (McGroddy and Farrington, 1995). The individual PAHs in these sources can be as high as up to 50 ng/l while the highly polluted rivers contain up to 600 ng/l (IPCS, 1998). PAH contents of plants and animals may be higher than contents in soil or water in which they live (ATSDR, 1995). This is partly because PAHs have a high affinity for organic fractions in sediments, soils and biota. They accumulate in organisms in water, sediments and in their foods (IPCS, 1998). High concentrations of PAHs in aquatic organisms can therefore indicate recent contamination. According to Bowa and Lalah (2009), there is high concentration of PAHs in water and sediments of the Winam Gulf and this begs the question of how much of these are in the fish found in the same area and this is the question that is addressed in this study.

Food is one source of PAHs (FEHD, 2004). When food, particularly meat, meat products, and fish, is smoked, roasted, barbecued, and grilled, PAHs are formed as a result of incomplete combustion or thermal decomposition (pyrolysis) of the organic material. If the meat is in direct contact with the flame, pyrolysis of the fats in the

meat generates PAH that become deposited on the meat (SCF, 2002). PAH production by cooking over charcoal (barbecued, grilled) is a function of both the fat content of the meat and the proximity of the food to the heat source [Puzanowska-Tarasiewicz and Tarasiewicz, 1999]. For example, it has been reported that cereals were found to contain PAHs at levels of 6-14 $\mu\text{g}/\text{kg}$, fats and oils at 8-11 $\mu\text{g}/\text{kg}$ and seafood at 7-8 $\mu\text{g}/\text{kg}$, respectively (FSA, 2002; Falco *et al.*, 2003). PAHs are mainly formed on the outer surface of barbecued food (IPCS, 1998), brought onto the surface of the food and are adsorbed (Wu *et al.*, 1997). They are generated through incomplete combustion of charcoal (Dyremark *et al.*, 1995) and food processing or cooking steps e.g. roasting, barbecuing, grilling and smoking. However, the extent of human exposure to PAHs as a result of consuming fish, roast and fried meats have not been determined in the Winam Gulf region and this study ventured into availing this data for future direction. This is of concern in the region. Data from the Kakamega Hospice Registry (2010) indicate that cancer cases are rampant in the region. These include cancer of the oesophagus, cervical cancer, prostate cancer and breast cancer (Kakamega Hospice Registry, 2010 as quoted by Lisouza *et al.*, 2011).

2.3 Genotoxicity and Carcinogenicity of PAHs

PAHs are potent toxins and genotoxins (Békaert *et al.*, 1999; Bispo *et al.*, 1999; Madill *et al.*, 1999; Fent and Bätischer, 2000). These compounds can induce endocrine disrupting activities mediated by mammalian estrogen receptor and/or aryl-hydrocarbon receptor (Tran *et al.*, 1996; Clemons *et al.*, 1998; Fielden *et al.*, 2000). In mammalian cells PAH undergo metabolic activation to diol epoxides that bind covalently to cellular macro-molecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process [Rodriguez *et*

al., 1997]. An increased risk of respiratory tract cancer in cooks and bakers has been reported (Coggon *et. al.*, 1986).

The International Agency for Research on Cancer (IARC) of the World Health Organization has evaluated the carcinogenicity of some PAHs based on evidence in human and experimental animals. Below is a summary of the classification:

Table 2.3.1: IARC classification of some PAHs on genotoxicity and carcinogenicity

Common Name	Genotoxicity	IARC Classification
Acenaphthene	Questionable	Not yet evaluated
Acenaphthylene	Questionable	Not yet evaluated
Anthracene	Negative	3
Benz[a]anthracene	Positive	2A
Benzo[b]fluoranthene	Positive	2B
Benzo[k]fluoranthene	Positive	2B
Benzo[ghi]perylene	Positive	3
Benzo[a]pyrene	Positive	2A
Chrysene	Positive	3
Dibenz[a,h]anthracene	Positive	2A
Fluoranthene	Positive	3
Fluorene	Negative	3
Indeno[1,2,3-cd]pyrene	Positive	2B
Phenanthrene	Questionable	3
Pyrene	Questionable	3

IARC Classification

Group 1: The agent is carcinogenic to humans

Group 2A: The agent is probably carcinogenic to humans

Group 2B: The agent is possibly carcinogenic to humans

Group 3: The agent is not classifiable as to its carcinogenicity to humans

Source: FEHD, 2004.

Concerning the carcinogenicity of the three PAHs which are listed above as probably carcinogenic:

- benz[a]pyrene, when administered by the oral route, produced tumours of the gastrointestinal tract (forestomach), liver, lung and mammary glands of mice and rats (SCF, 2002; WHO, 1991; USDHHS, 2002).
- Dibenz[a,h]anthracene and benz[a]anthracene produced tumours of the gastrointestinal tract (forestomach), lungs and liver in mice (SCF, 2002; USDHHS, 2002).
- Benz[a]anthracene also induced papillomas of the forestomach in mice when administered by gavage, and induced lung adenomas and hepatomas in mice in another gavage study (USDHHS, 2002).

According to the Agency for Toxic Substances and Disease Registry (ATSDR, 1995), mice fed on high levels of one PAH during pregnancy had difficulty reproducing. Similar observations were made on the offspring with the offspring having higher rates of birth defects and lower body weights. Animal studies have shown harmful effects on skin and body fluids and low ability to fight disease after short and long-term exposures. US Department of Health and Human Services (DHHS) has determined that some PAHs are reasonably carcinogenic when touched or breathed over long periods. Laboratory animals that breathed some PAHs were found to have lung cancer (USDHHS, 2002). Ingestion of food resulted in stomach cancer, and application on skin resulted in skin cancer (IPCS, 1998).

According to Australian Department of Environment and Heritage (DEH, 2004), LD₅₀ values of individual PAHs indicate low to moderate acute toxicity while some PAHs

showed genotoxic effects in both *in vitro* mammalian cell lines and *in vivo* in rodents cell lines and prokaryotes. Many of the PAH compounds are known or are suspected carcinogens (NRC, 1983; USDHHS, 2002; WHOROE, 2000; SCF, 2002; WHO, 1991; FEHD, 2004; DEH, 2004; Delistraty, 1997; Kubiak *et al.*, 1999, Gu *et al.*, 2003). High molecular weight PAHs, for example, benzo(a)pyrene, and benzo(a)anthracene are more potent carcinogens (Eickhoff *et al.*, 2003). While studies on petroleum indicate that multiple ring PAHs are not toxic in oils but naphthalenes and phenolic compounds are mainly responsible for the acute toxicity of petroleum oils (James and Bend, 1980). Onuska (1989) notes that PAH toxicity increases with increase in molecular size till the 4- and 5-ring molecules are reached while the general relationship of structure to carcinogenic activity seems to suggest that 4-, 5- and 6- membered ring PAHs rather than smaller (3-ring structures) or larger (7-ring structures) are more toxic.

PAHs are toxicants and carcinogens, the population may be exposing themselves by eating fish and roast meat. Cancer research in Kenya has been minimal, as most of the funding is directed toward malaria, HIV/AIDS, and tuberculosis research. The government of Kenya and politicians had not recognized cancer to be among priority areas. However, the Country Programme Framework (CPF, 2011-2016) signed between the Kenyan Government and International Atomic Energy Agency identified cancer as one of the priority areas to be addressed in nuclear technology. Cancer is of priority because it is the third cause of death in the country after infectious diseases and cardiovascular diseases. In 2005, deaths attributed to cancer were approximately 18,000 of which 60% were of people under the age of 70 (KNCCS 2011-2016). However, there is need for diagnostics, surveillance and treatment. There are also no

well-equipped laboratories to undertake the relevant research, especially in cancer, and the few that have the capacity are donor-driven to focus on infectious diseases. Cancer control, prevention, and screening programs have not yet taken root in Kenya, considering the heavier burden imposed on the Ministry of Health by infectious diseases (ASCO website, accessed 21-11-2010). Therefore there is need to determine PAH in the food to find out whether they may be a source of exposure to carcinogenic PAHs and so cause preventive measures to be taken to reduce the burden of treatment.

2.4 PAHs in Fish

Studies have been done in fish from various locations in the world. In several fish species collected from the North and Main Arms of the Fraser River, British Columbia by Swain and Walton (1989a, b) PAHs were found in both muscle and liver tissues. For example, muscles of six species of fish in Lake Ontario were found to contain 3-8 $\mu\text{g}/\text{kg}$ ww of total PAH (Eisler, 1987). Goyette and Boyd (1989) examined PAH levels in the liver and muscle tissues of English Sole from Vancouver Harbour. Concentrations ranging from 0.001-0.037 $\mu\text{g}/\text{g}$ dw of low molecular weight PAH (LPAH) and trace-0.074 $\mu\text{g}/\text{g}$ of high molecular weight PAH (HPAH) were detected in the fish livers from the outer Harbour. The inner Harbour fish liver samples contained 0.013 $\mu\text{g}/\text{g}$ fluoranthene, 0.001 $\mu\text{g}/\text{g}$ anthracene and 0.014 $\mu\text{g}/\text{g}$ phenanthrene. In Port Moody Arm, only phenanthrene was detected at 0.019 $\mu\text{g}/\text{g}$ dw. By comparison, the muscle samples contained non-detectable levels of both LPAH and HPAH except for phenanthrene and fluoranthene which were present in trace amounts and 0.013 $\mu\text{g}/\text{g}$ dw, respectively. There is need to determine whether fish in the Winam Gulf which has been reported to have PAH contaminated water and sediments (Bowa and Lalah, 2009) is PAH contaminated.

2.5 PAHs in Meat

PAHs have been found to exist in roasted and smoked foods. Djinovic *et al.* (2009), found that the sum of 16 EU priority PAHs in smoked beef and pork ham is ($\mu\text{g}/\text{kg}$) was: beef ham - 3.9 in Traditional Smokehouse, 1.9 in Industrial Smoke house; pork ham - 4.9 in Traditional Smokehouse, 4.2 in Industrial Smokehouse in Serbia. This is consistent with the findings of Janoszka *et al.* (2004), who studied roast meats in Poland prepared according to recipes used for cooking in Upper Silesia (roasted, fried) and in grilled dishes. They found that the total PAH content was within the range 2.43–16.10 ng g^{-1} meat. Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food expressed on 4th December 2002 reports that, levels as high as 200 $\mu\text{g}/\text{kg}$ in food have been found for individual PAH in smoked fish and meat (SCF, 2002). In barbecued meat, 130 $\mu\text{g}/\text{kg}$ has been reported whereas the average background values are usually in the range of 0.01–1 $\mu\text{g}/\text{kg}$ in uncooked foods (SCF, 2002). The association of the heavier PAH with particulate matter makes atmospheric fall out a principal route of contamination. Consequently, vegetables with large leaves and browsing cattle and pecking poultry, which may ingest particulate matter from contaminated grass and soil, are susceptible to contamination by PAH adsorbed to particles (Edwards, 1983; Nielsen *et al.*, 1996).

PAH formation during charcoal grilling was shown to be dependent upon the fat content of the meat, the duration of cooking and the temperature used. For example a heavily barbecued lamb sausage contained 14 $\mu\text{g}/\text{kg}$ of the sum of six PAH, considered by IARC (IARC, 1987) to be carcinogenic (Mottier *et al.*, 2000). A comparison of PAH levels in duck breast steaks undergoing various processing and

cooking treatments for 0.5 hour to 1.5 hours, showed that charcoal grilled samples without skin contained the highest amount of total PAH (320 µg/kg), followed by charcoal grilling with skin (300 µg/kg), smoking (210 µg/kg), roasting (130 µg/kg), steaming (8.6 µg/kg) and liquid smoke flavouring (0.3 µg/kg) (Chen and Lin, 1997). Meat has background PAHs (FSA, 2002) since PAHs are ubiquitous (Gu *et al.*, 2003). These studies show that method of cooking influences the concentration of PAHs. However, this had not yet been established in the Winam Gulf to determine the extent of human exposure.

2.6 PAH Exposure and Exposure Limits

Common sources of exposure are contact with water, drinking contaminated water, breathing air containing PAHs in workplace such as coking, coal-tar, asphalt production; municipal trash incineration, breathing PAH containing cigarette smoke, wood smoke, vehicle exhausts, asphalt roads or agricultural refuse smoke (ATSDR, 1995), eating grilled or charred meats, contaminated food, processed or pickled foods (DEH, 2004; ATSDR, 1995, IPCS, 1998). The main source of exposure to PAHs for an adult is food, which contributes more than 90% of the total exposure (IPCS, 1998; SCF, 2002). However for smokers, significant contribution is through cigarette smoking (FSA, 2002). The PAH pollution at home also depends on the heating system, food preparation and ventilation habits (IARC, 1993).

The American government has set regulations to protect people from the possible health effects of eating, drinking, or breathing PAHs. The Environmental Protection Agency (EPA) has suggested that taking into your body each day the following amounts of individual PAHs is not likely to cause any harmful health effects: 0.3

milligrams (mg) of anthracene, 0.06 mg of acenaphthene, 0.04 mg of fluoranthene, 0.04 mg of fluorene, and 0.03 mg of pyrene per kilogram (kg) of your body weight. Estimates for total exposure in the United States population have been listed as 3 mg/day (ATSDR, 1995).

EPA has regulated that, if the following amounts of individual PAHs are released to the environment within a 24-hour period, then it must be notified: 1 pound (0.454 kg) of benzo[b]fluoranthene, benz[a]pyrene, or dibenz[a,h]anthracene; 10 pounds (4.54 kg) of benz[a]anthracene; 100 pounds (45.4 kg) of acenaphthene, chrysene, fluoranthene, or indeno[1,2,3-c,d]pyrene; or 5,000 pounds (2270 kg) of acenaphthylene, anthracene, benzo[k]fluoranthene, benzo[g,h,i]perylene, fluorene, phenanthrene, or pyrene (ATSDR, 1995).

The American Conference of Governmental Industrial Hygienists (ACGIH) recommends an occupational exposure limit for coal tar products of 0.2 mg/m³ for an 8-hour workday, within a 40-hour workweek. The Occupational Safety and Health Administration (OSHA) has established a legally enforceable limit of 0.2 mg/m³ averaged over an 8-hour exposure period and Permissible Exposure Limit (PEL) for mineral oil mist is 5 mg/m³ averaged over an 8-hour exposure period. National Institute of Occupational Safety and Health (NIOSH) established a recommended occupational exposure limit, time-weighted average (REL-TWA) for coal tar products of 0.1 milligram of PAHs per cubic meter of air (0.1 mg/m³) for a 10-hour workday, within a 40-hour workweek and has established a recommended occupational exposure limit (REL-TWA) for mineral oil mists of 5 mg/m³ for a 10-hour work day, 40-hour work week, with a 10 mg/m³ Short Term Exposure Limit (STEL). Mineral oil

mists have been given an IARC classification of 1 (sufficient evidence of carcinogenicity) (ATSDR, 1995).

The European Union in Commission Regulation (EC) No. 1881/2006 for polycyclic aromatic hydrocarbons has given the Maximum levels of PAH. In smoked meats, smoked meat products, muscle meat of smoked fish and smoked fishery products the level is set at 5.0 µg/kg wet weight while muscle meat of fish other than smoked fish is set at 2.0 µg/kg (Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, as amended by Regulations 1126/2007 and 629/2008).

The local population around Winam Gulf consumes fish from a PAH-contaminated lake (Bowa and Lalah, 2009) which could be exposing them to PAHs. They also use frying and cooking as methods of cooking meat and this too may expose them to PAHs. However, this exposure has not been determined. It was necessary to compare the extent of exposure to PAHs in comparison to international standards (since no local standards) exist to ascertain whether the human exposure is acceptable or not.

2.7 Importance of Area of Study

According to Lake Victoria Environmental Management Program (LVEMP) Fisheries Management Component of Lake Victoria (K) fish landings bulletin 2005 report, *Rastrineobola argentea* ('omena') gave the highest yield of 54,016.7 metric tonnes (LVEMP, 2005). Its market is exclusively local for human consumption and animal feed formulation. The second highest yield was from Nile perch (*Lates niloticus*), 51,400.2 metric tonnes. Its market is almost exclusively foreign, the EU and Israel

being the largest importers, unless the fish is damaged by the time it lands. Tilapiine species catches reached 21,608.6 metric tonnes. The most dominant of this species is the Nile tilapia, *Oreochromis niloticus*, although the traditional *Oreochromis esculentus* is making a comeback after a long depletion due to introduction of *Lates niloticus* (Ogutu-Ohwayo *et al.*, 2002). Given that Nile perch, Tilapia and 'Omena' are the most important commercial species they were studied to determine the amounts of PAHs in them, which would show the extent of human exposure. Highest densities of Nile tilapia (i.e. 65% of all the fish biomass) occur in shallow macrophyte-dominated habitats in less than 4 meters in deep areas within 500 meters from the shoreline (Ogutu-Ohwayo *et al.*, 2002). The sampling of this species therefore was carried out on catches from vessels that cover this range of the lake. Since PAHs are hydrophobic (Onuska, 1989) the concentration in the water at these places is relatively higher due to proximity to deposition sites or runoff from land into water than in the open lake before they settle in the sediments hence the fish here are more exposed to the PAHs. Due to the expanding Nile perch export market Nile tilapia is currently the main fish for the local consumers (Ogutu-Ohwayo *et al.*, 2002).

The three species are the most important commercially. They are the most consumed and therefore are better indication of the extent of human exposure to PAHs which is not known.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Sampling

3.1.1 Area of Study and Sample Locations

Lake Victoria is located at $0^{\circ}21'N-3^{\circ}0'S$, $31^{\circ}39'-34^{\circ}53'E$ astride the equator at an altitude of 1134 m above sea level with a surface area of 68,800 sq.km. (Ogutu-Ohwayo *et al.*, 2002). The study area is Winam Gulf, which is one-sixth of the lake on the Kenyan side. This area of the Lake is surrounded by intense agricultural and industrial activities as well as a number of towns, the largest being Kisumu City. Kisumu City is located at $0^{\circ}6'S$, $34^{\circ}45'E$ on the North tip of Winam Gulf, which is part of the Kavirondo Gulf of Lake Victoria. It was the headquarters of the former Nyanza Province and the third largest city in Kenya as well as the principal port of the lake on the Kenyan side. Kisumu City has a population of 322,724 (1999 census) although the projected population as at 2011 is 451,614 (derived using Kenya Central Bureau of Statistics growth rate of 2.8%).

Samples of fish were collected from the three main beaches that land fish in Kisumu Bay ($0^{\circ}6'S$, $34^{\circ}45'E$) i.e. Dunga Beach (Southwest of Kisumu City and south of Kisumu Bay; 4 km from the City Center), Usoma Beach and Usare Beach (West and on the opposite side of Kisumu City across Kisumu Bay about 3 km from each other). Beaches were chosen around Kisumu City because the concentration of PAHs in tissues of aquatic organisms is highest and most verifiable in organisms collected close to point sources (Eickhoff *et al.*, 2003). Samples of meat were collected from randomly chosen meat roasting places in Kisumu town, that is, Highway Inn (on

Kisumu-Kakamega Highway) for goat and beef, Apok Inn (on Kisumu-Nairobi Highway) for beef, while pork was collected from Kisumu Hotel (City Center).

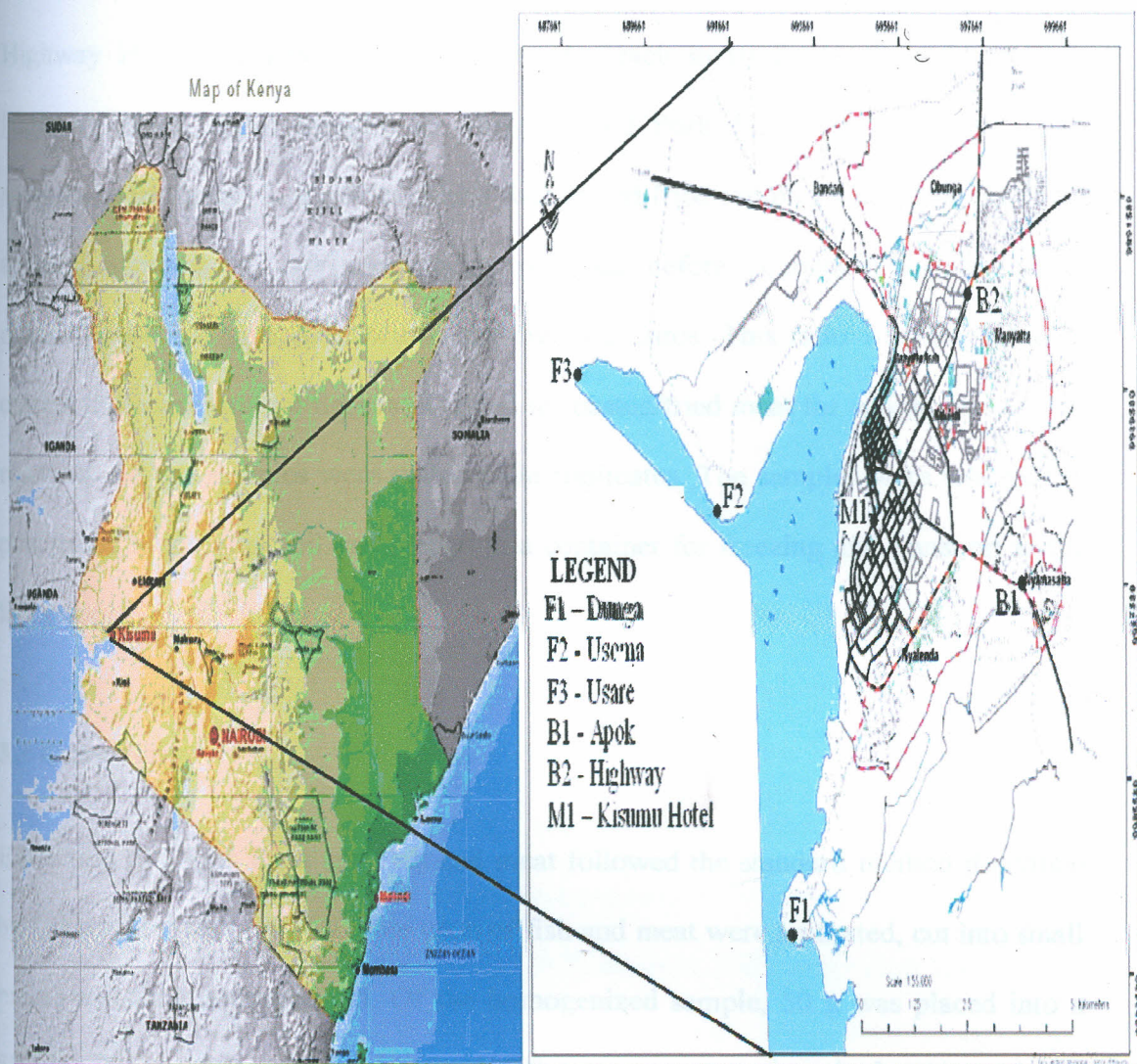


Figure 3.0.1: Map of Kenya showing Kisumu City and Bay ($0^{\circ}6'S$, $34^{\circ}45'E$) with sampling locations

3.1.2 Samples and Sample Treatment

Samples were raw fish: *Rastrineobola argentea* ('Omena') from Dunga Beach (three samples in triplicate); *Lates niloticus* (Nile perch) from the three beaches (three samples each in triplicate) and *Oreochromis niloticus* (Nile tilapia respectively) from

the three beaches (three samples each in triplicate) from Winam Gulf of Lake Victoria. These species were chosen because they were the most important commercially. Samples of meat (raw and roasted) were also collected; Beef from Highway Hotel and Apok Inn, three samples each in triplicate; Goat Meat from Highway Hotel, three samples in triplicate and Pork from Kisumu Hotel, three samples in triplicate. The raw meat and the roasted/fried samples were from the same animal. Samples of raw meat were weighed before roasting/frying and after roasting/frying to give the raw:roasted/raw:fried ratios. This ratio was later used to correct the concentration figures obtained in roasted/fried meat for weight loss during roasting. All the samples were collected in triplicates. The samples were wrapped in precleaned aluminium foil and placed in a container for freezing and transport to the laboratory.

3.2 Extraction

Extraction of PAHs from raw fish and meat followed the standard method described by Takatsuki *et al.* (1985). Edible parts of fish and meat were separated, cut into small pieces and then homogenized. Of the homogenized sample, 50 g was placed into a 500 ml flask. Ethanol 200 ml, 35 ml 50% aqueous potassium hydroxide and 2 g sodium sulphide ($\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$) were then added followed by reflux on a water bath for 2 hours. The mixture was then cooled to 40°C and portions of 150 ml of n-hexane added with occasional swirling. The alkaline digestion mixture was poured into 500 ml glass separation funnel containing 150 ml water, the flask rinsed twice with 10 ml ethanol and the rinses added to the funnel. The funnel was then vigorously shaken and set on a stand to separate into aqueous and organic layers. The aqueous layer was extracted twice with 150 and 100 ml portions of n-hexane respectively. All the hexane

extracts were combined and washed with 100 ml deionized water before being dried over anhydrous sodium sulphate. The dried n-hexane extract was transferred to a flask covered with aluminium foil then taken to a rotary evaporator and concentrated to about 3-5 ml.

The method of extraction was modified for extraction in the samples of roasted meat. They were saponified with methanol (instead of ethanol) following the same procedure as described above. The samples were then extracted with cyclohexane in place of n-hexane as per the method of Lawrence and Webber (1984).

3.3 Clean-Up

The method used was modified from the Takatsuki *et al.* (1985) method. The chromatographic column for clean-up and separation was packed by tapping 8 g silica (in slurry) into the column and 3 g anhydrous sodium sulphate added on top. The column was washed with 30 ml n-hexane and the wash discarded. The solvent was drained to just top of column but always above the column to avoid contact of air with column packing. The column was protected from light with aluminium foil. Using 300 ml amber rotary evaporator flask as receiver, the concentrated extract was transferred to the column, the receiver rinsed twice with 2 ml hexane and rinses added to column. Stopcock of column was opened and when n-hexane just reached the column top, 150 ml of 10% ethyl ether in n-hexane was added and the PAHs eluted under gravity. After elution, 2-3 boiling chips were added to the receiver, the solvent extract evaporated to 1-2 ml, residual solvent evaporated under mild stream of Nitrogen with gentle warming and the residue was taken up in 1 ml with pentane, (Sigma, St. Louis, MO, USA) and stored in sealed vials for analysis.

3.4 Analysis

The samples were analyzed by GC-MS on a 7890A stand-alone gas chromatograph (Agilent Technologies, Inc., Beijing, China) and a 5975 C mass selective detector (Agilent Technologies, Inc., Santa Clara, CA, USA) by using the following conditions: Inlet temp 270° C, transfer line temp of 280° C, and column oven temperature programmed from 35 to 285° C with the initial temperature maintained for 5 min. then 10° C/min. to 280° C for 10.5 min. and then raised to the final temperature at 5° C/min. to 285° C for 29.9min. The GC was fitted with a HP-5 MS low bleed capillary column (30 m × 0.25 mm i.d., 0.25- μ m) (Restek, Bellefonte, PA, USA). Helium, at a flow rate of 1.25 ml/min, served as carrier gas.

The Agilent 5975 C mass selective detector maintained an ion source temperature of 250°C and a quadrupole temperature of 180°C. Electron impact (EI) mass spectra were obtained at acceleration energy of 70eV. A 1.0 μ l aliquot of extract was automatically injected in the split/ splitless mode using an auto sampler 7683 (Agilent Technologies, Inc., Beijing, China). Fragment ions were analyzed over 40-550 m/z mass range in the full scan. The filament delay time was set as 3.3 min. Library -MS searches using NIST/EPA/NIH Mass Spectral Library (NIST 05) and NIST Mass Spectral Search Program Version 2.0d, Chemecol and Adams data base were used for characterization purposes in the GC-MS data system.

3.5 Recovery Experiment

To evaluate the recovery of PAH separated and analysed by use of this procedure, and to account for matrix effects on peak positions in GC-MS chromatograms, spiked and

unspiked samples were analysed under the same conditions. The results obtained were then corrected based on percentage recovery for each PAH.

3.6 Effect of Cooking Ratios

To investigate this, the raw meat weight: roasted meat weight ratio for beef and goat meat were used while for pork the raw pork weight: fried pork weight was used. These ratios were obtained by weighing the raw meat which was ready for roasting/frying and recording its weight. Roasting/frying was done followed by reweighing.

3.7 Statistical Analysis

The results analyzed using INSTAT for Descriptive Statistics (Appendix 1) on Fish and Raw Meats. The effect of cooking was analyzed using a two factorial design ANOVA (SAS, MSTAT-C) with PAH type as the main treatment and meat type as the subtreatment (Appendix 1), while the charts were performed using MS-Excel Statistical packages.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Qualitative determination of the PAHs was restricted to those PAHs whose spectra matched those in the NIST, Adams and Chemecol mass spectra. However, the quantification was only done for the sixteen USEPA listed priority PAHs because their external standard mix was available. Descriptive statistics was calculated by assuming the values below the detection limits were zero.

The GC-MS results and the TIC Chromatograms can be found in the appendix section on pages 69 – 82. Statistical analysis results are on pages 83 – 115.

4.1 Determination of types and the concentrations of the PAHs in raw fish

A number of PAHs were detected in the fish with the concentration range from 0.004 to 0.886 $\mu\text{g}/\text{kg}$ while TPAHs ranged from 0.035 to 3.934 $\mu\text{g}/\text{kg}$ (Table 4.1.1, Table 4.1.2, Figure 4.0.1 and 4.0.2).

Table 4.1.1: Mean PAH Concentration in Fish from different locations ($\mu\text{g}/\text{kg ww}$)

SITE	FISH TYPE	PAH TYPE													
		1	2	3	4	5	6	7	8	9	10	11	12	13	TPAH
Dunga	<i>L. niloticus</i>	0.19	0.60	0.22	0.34	0.16	0.51	0.31	0.59	nd	0.15	0.12	0.14	nd	3.32
	<i>O. niloticus</i>	0.21	nd	0.11	0.45	0.23	0.81	0.40	0.90	0.34	0.21	0.08	0.09	0.14	3.96
	<i>R. argentea</i>	0.01	0.03	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.04
Koma	<i>L. niloticus</i>	0.18	0.52	0.20	0.34	0.16	0.50	0.31	0.58	nd	0.16	0.14	0.14	nd	3.22
	<i>O. niloticus</i>	0.23	nd	0.11	0.47	0.23	0.80	0.41	0.90	0.31	0.22	0.07	0.09	0.16	3.99
Bare	<i>L. niloticus</i>	0.20	0.18	0.21	0.33	0.15	0.51	0.32	0.58	nd	0.16	0.14	0.13	nd	2.92
	<i>O. niloticus</i>	0.20	nd	0.13	0.42	0.22	0.78	0.41	0.87	0.32	0.21	0.07	0.09	0.14	3.84
	LSD (p=0.05)	0.06	1.06	0.04	0.05	0.02	0.05	0.01	0.04	0.03	0.02	0.04	0.01	0.02	
	CV(%)	7.75	140.15	7.70	3.56	2.41	2.06	0.97	1.69	4.62	3.40	11.40	2.68	9.20	
	R ²	0.40	0.99	0.98	0.99	0.99	0.99	0.99	0.98	0.99	0.96	0.97	0.95	0.99	

Note: nd = not detected/below detection limit of the machine (detection limit = 10^{-12} g)

Key: 1 – Naphthalene, 2 – Acenaphthylene, 3 – Acenaphthene, 4 – Fluorene, 5 – Phenanthrene, 6 – Anthracene, 7 – Fluoranthene, 8 – Pyrene, 9 – Chrysene, 10 – Benz(k)fluoranthene, 11 – Indeno(1,2,3-cd)pyrene, 12 – Dibenzo(a,h)anthracene, 13 – Benz(g,h,i)perylene.

The PAH concentrations was not significant ($P \leq 0.05$) with reference to location. This could be due to the fact that the sites were simply landing beaches while the fishing sites could as well have been the same since the fishermen do not confine themselves to one place when fishing inshore. *R. argentea* was only found at Dunga beach.

Table 4.1.2: The Mean PAH Concentration in Fish by Type (\pm standard deviation $\mu\text{g}/\text{kg}$ ww)

PAH	Fish Type		
	<i>Oreochromis niloticus</i>	<i>Lates niloticus</i>	<i>Rastrineobola argentea</i>
Naphthalene	0.214 \pm 0.016	0.192 \pm 0.025	0.004 \pm 0.000
Acenaphthylene	nd	0.432 \pm 0.193	0.031 \pm 0.000
Acenaphthene	0.108 \pm 0.011	0.219 \pm 0.021	nd
Fluorene	0.447 \pm 0.022	0.338 \pm 0.010	nd
Phenanthrene	0.228 \pm 0.006	0.162 \pm 0.008	nd
Anthracene	0.796 \pm 0.017	0.506 \pm 0.015	nd
Fluoranthene	0.408 \pm 0.006	0.309 \pm 0.008	nd
Pyrene	0.886 \pm 0.035	0.581 \pm 0.007	nd
Chrysene	0.324 \pm 0.010	nd	nd
Benz(k)fluoranthene	0.212 \pm 0.009	0.158 \pm 0.007	nd
Indeno(1,2,3-cd)pyrene	0.073 \pm 0.003	0.133 \pm 0.015	nd
Dibenzo(a,h)anthracene	0.093 \pm 0.002	0.136 \pm 0.008	nd
Benz(g,h,i)perylene	0.145 \pm 0.012	nd	nd
Total PAH (TPAH)	3.934	3.166	0.035

Note: nd = not detected/below detection limit of the machine (detection limit = 10^{-12} g)

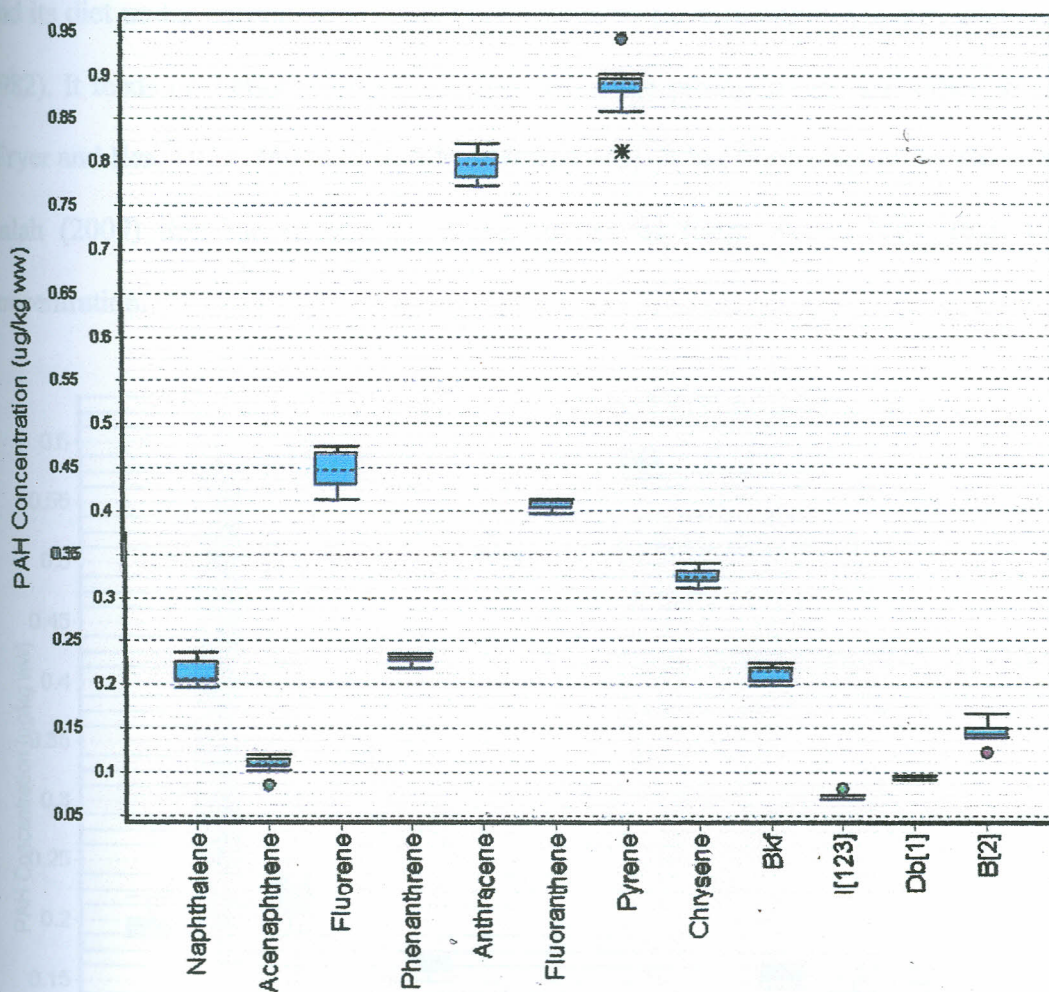


Figure 4.0.1: Means, Ranges, Minima and Maxima of PAH Concentration in *O. niloticus* ($\mu\text{g}/\text{kg ww}$). Bkf = Benzo(k)fluoranthene; I[123] = Indeno(1,2,3-cd)pyrene; Db[1] = Dibenz(a,h)anthracene; B[2] = Benzo(g,h,i)perylene

Oreochromis niloticus was found to have twelve of the priority PAHs ranging from 0.073 $\mu\text{g}/\text{kg}$ to 0.886 $\mu\text{g}/\text{kg}$ (Table 4.1.2). The highest individual mean PAH concentration recorded was Pyrene in *O. niloticus* at Dunga Beach (0.886 $\mu\text{g}/\text{kg}$) while the lowest was Indeno(1,2,3-cd)pyrene at Usoma Beach (0.073 $\mu\text{g}/\text{kg}$) (Table 4.1.1). The mean total PAH was found to be 3.934 $\mu\text{g}/\text{kg}$ (Table 4.1.1 and Table 4.1.2). *O. niloticus* recorded significantly higher levels than *L. niloticus* ($P \leq 0.05$).

This could be due to the fact that *O. niloticus* like most other tilapias is a herbivore, and its diet under natural conditions is restricted to phytoplankton (Jauncy and Ross, 1982). It feeds on bottom deposits derived from the plankton rain and other sources (Fryer and Iles, 1972; Moriarty and Moriarty, 1973). It has been showed by Bowa and Lalah (2009) that the sediments at the bottom of these waters have high PAH concentration.

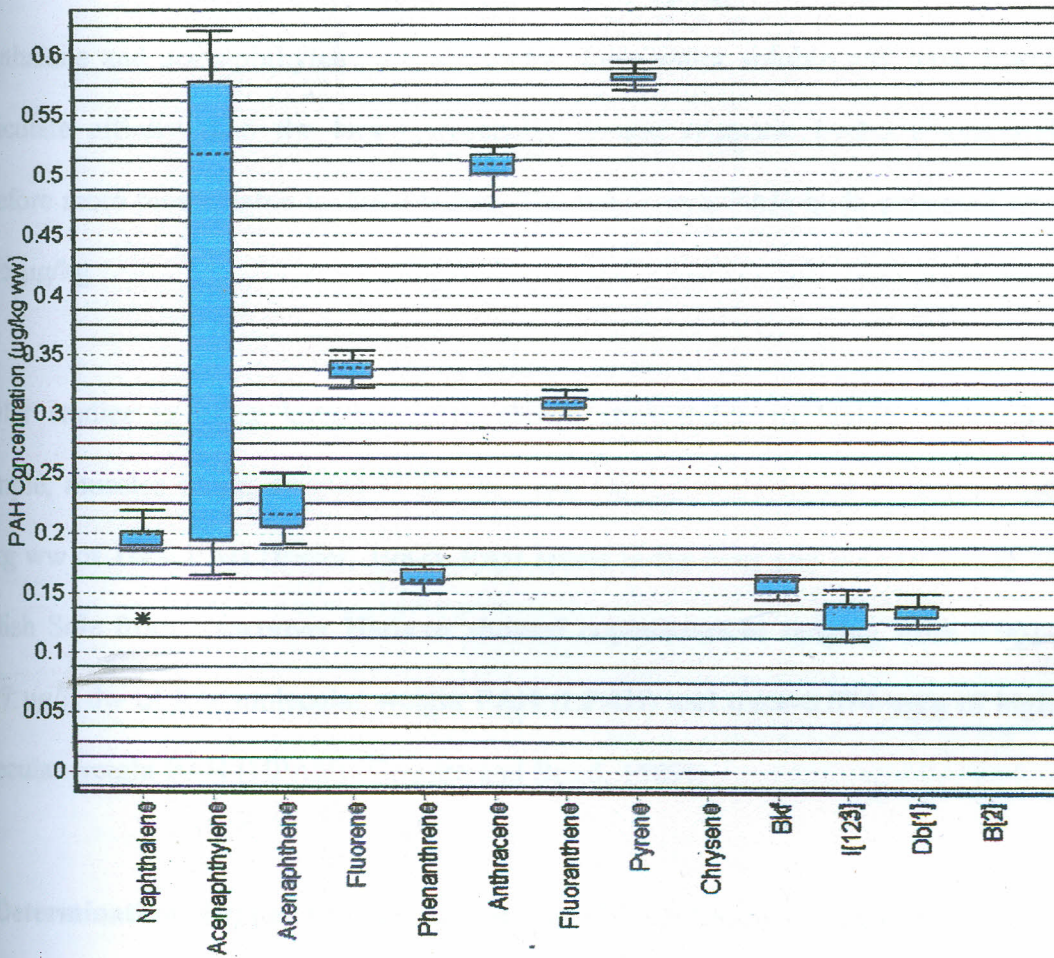


Figure 4.0.2: Means, Ranges, Minima and Maxima of PAH Concentration in *Lates niloticus* (µg/kg ww). Note: Bkf = Benzo(k)fluoranthene; I[123] = Indeno(1,2,3-cd)pyrene; Db[1] = Dibenz(a,h)anthracene; B[2] = Benzo(g,h,i)perylene.

Lates niloticus was found to have eleven of the priority PAHs with concentrations in the range of 0.133 to 0.581 µg/kg while the mean total PAH was found to be 3.166 µg/kg. The highest individual mean PAH concentration recorded was Pyrene in *L. niloticus* (0.581 µg/kg) while the lowest was Indeno(1,2,3-cd)Pyrene (0.133 µg/kg).

Rastrineobola argentea was found to contain only two of the priority PAHs, Naphthalene 0.004 µg/kg and Acenaphthylene 0.031 µg/kg. This could be because naphthalene and acenaphthylene are somewhat more water soluble and have lower particulate affinity than the larger molecular weight aromatic hydrocarbons and therefore more bioavailable to these fishes. The mean total PAH in *R. argentea* was 0.035 µg/kg.

Similar studies in other locations have shown comparable to higher values for example, muscles of six species of fish in Lake Ontario were found to contain 3-8 µg/kg ww of Total PAH (Eisler, 1987). PAH levels in the liver and muscle tissues of English Sole from Vancouver Harbour showed concentrations ranging from 0.001-0.037 µg/g dw of low molecular weight PAH (LPAH) and trace-0.074 µg/g of high molecular weight PAH (HPAH) (Goyette and Boyd, 1989).

4.2 Determination of types and concentrations of the PAHs in the raw meat

Three types of meat were tested namely; beef, goat meat and pork (Table 4.2.1, Figures 4.0.2, 4.0.3 and 4.0.4).

Table 4.2.1: The mean PAH concentration in raw meat (\pm standard deviation $\mu\text{g}/\text{kg}$ ww)

PAH	Meat Type				
	BEEF			GOAT Highway	PORK Kisumu Hotel
	Apok	Highway	Mean		
Naphthalene	0.087 \pm 0.003	0.118 \pm 0.015	0.102 \pm 0.019	0.105 \pm 0.007	nd
Acenaphthylene	0.070 \pm 0.001	0.124 \pm 0.004	0.097 \pm 0.030	0.031 \pm 0.004	0.097 \pm 0.005
Acenaphthene	0.053 \pm 0.001	0.086 \pm 0.002	0.069 \pm 0.018	0.074 \pm 0.004	0.072 \pm 0.003
Fluorene	nd	0.310 \pm 0.009	0.155 \pm 0.170	0.229 \pm 0.020	nd
Anthracene	nd	0.870 \pm 0.001	0.435 \pm 0.477	1.033 \pm 0.045	nd
Pyrene	nd	0.934 \pm 0.010	0.467 \pm 0.512	nd	nd
Benz(k) fluoranthene	nd	Nd	nd	0.332 \pm 0.009	nd
Indeno(1,2,3- cd)pyrene	nd	0.097 \pm 0.002	0.067 \pm 0.073	0.097 \pm 0.003	nd
Dibenzo(a,h)A	nd	Nd	nd	0.102 \pm 0.022	nd
Benz(g,h,i)P	nd	Nd	nd	0.127 \pm 0.011	nd
Total PAH (TPAH)	0.210	2.105	1.392	2.201	0.169

Note: nd = not detected/below detection limit of the machine (detection limit = 10^{-12} g)

Raw beef was found to contain seven of the sixteen priority PAHs with a mean concentration range of 0.067 - 0.467 $\mu\text{g}/\text{kg}$ (Table 4.2.1).

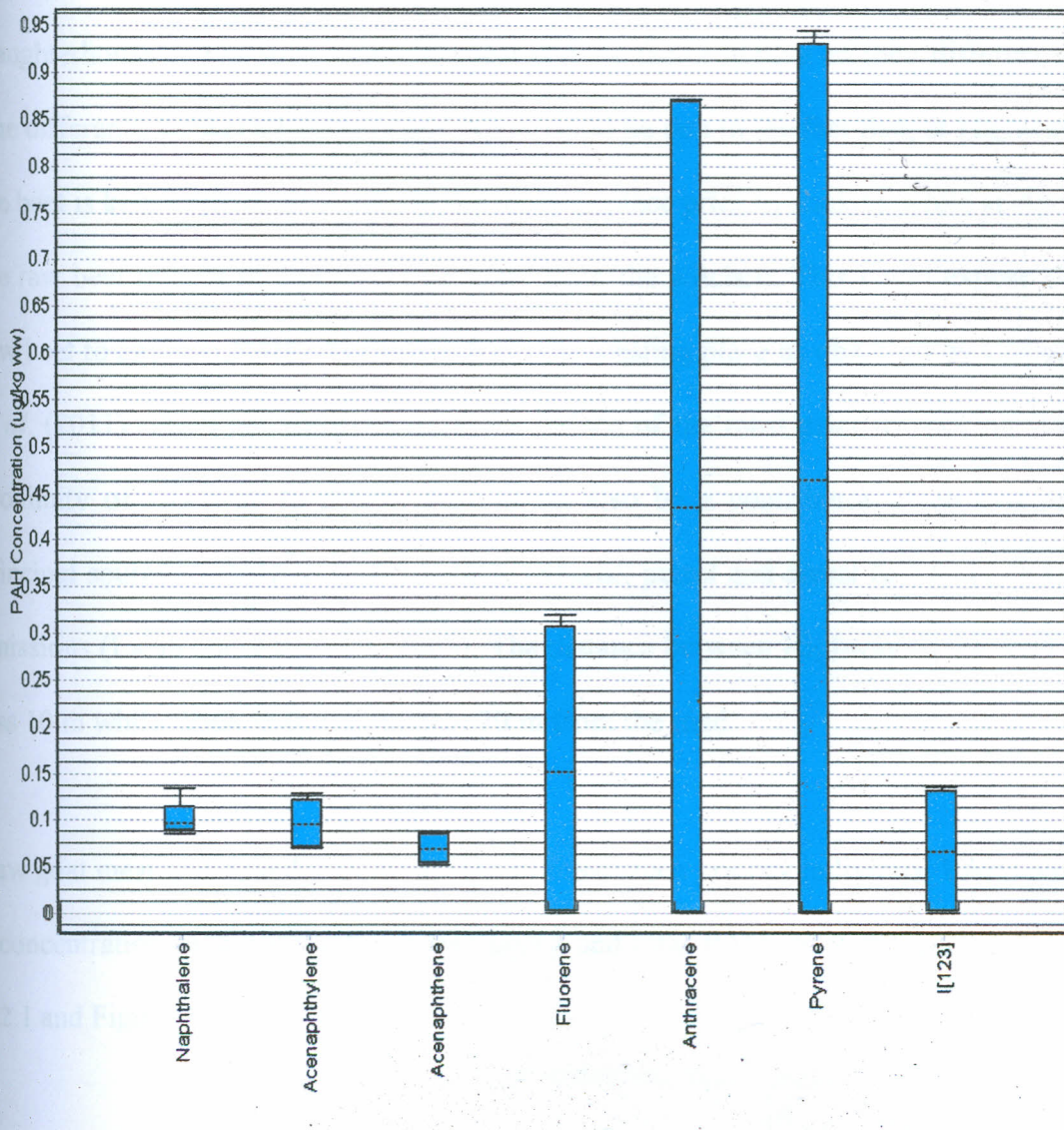


Figure 4.0.3: Means, Ranges, Minima and Maxima of PAH Concentration in Raw beef ($\mu\text{g}/\text{kg ww}$). Note: I[123] = Indeno(1,2,3-cd)pyrene.

The raw beef sampled from Highway contained seven of the sixteen priority PAHs in the concentration range of 0.086 - 0.934 $\mu\text{g}/\text{kg}$ (Table 4.2.1) while beef from Apok exhibited three of the priority PAHs in the concentration range of 0.053 - 0.087 $\mu\text{g}/\text{kg}$ (Table 4.2.1). Total PAH was found to range between 0.210 - 2.105 $\mu\text{g}/\text{kg}$ (Table 4.2.1). Average background values are usually in the range of 0.01-1 $\mu\text{g}/\text{kg}$ in uncooked foods (SCF, 2002) and the values obtained at Highway are above that

range. Apok and Highway source their beef from the same place, that is, Mamboleo slaughterhouse and as such source of meat is not a factor in play on their differences. The difference between Highway and Apok could be due to the fact that, at Highway, the beef is kept in close proximity to the roasting place prior to cooking while at Apok the raw beef is kept in a butchery separate from the Kitchen. This could expose the raw beef to ambient PAHs originating from the roasting place as observed by Lisouza *et al.* (2011) which get adsorbed onto the surface of the meat prior to cooking. The proximity of Highway to a busy road could also be a contributor given that one principal source of PAHs is motor vehicle exhaust; petrol and diesel fuel and exhaust emissions (Lalah and Kaigwara, 2005). The distance between Highway to the road is less 10 m while Apok is approximately 70 m from the road.

Raw goat meat was found to contain nine of the sixteen priority individual PAHs with a concentration range of 0.031 - 1.033 $\mu\text{g}/\text{kg}$ and total PAHs of 2.201 $\mu\text{g}/\text{kg}$ (Table 4.2.1 and Figure 4.0.4).

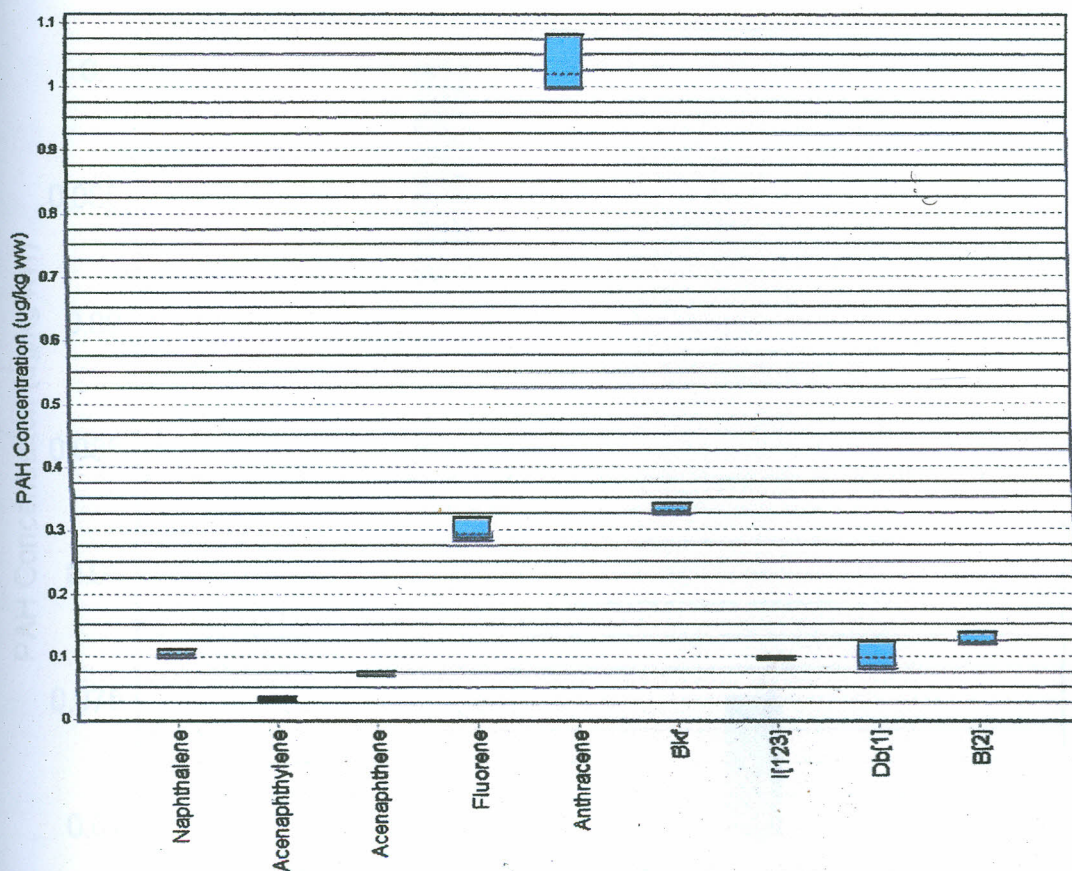


Figure 4.0.4: Means, Ranges, Minima and Maxima of PAH Concentration in Raw Goat Meat ($\mu\text{g}/\text{kg}$ ww). Bkf = Benzo(k)fluoranthene; I[123] = Indeno(1,2,3-cd)pyrene; Db[1] = Dibenz(a,h)anthracene; B[2] = Benzo(g,h,i)perylene.

The individual and total concentration of PAHs in the current study ($2.201 \mu\text{g}/\text{kg}$) are within the average background values of $0.01\text{-}1 \mu\text{g}/\text{kg}$ for uncooked foods (SCF, 2002) with the exception of Anthracene ($1.033 \mu\text{g}/\text{kg}$) which was also the highest mean PAH concentration recorded.

Raw pork contained two of the sixteen priority PAHs in the concentration range of $0.072\text{ - }0.097 \mu\text{g}/\text{kg}$ (Table 4.2.1). These values are within the average background values for uncooked foods (SCF, 2002).

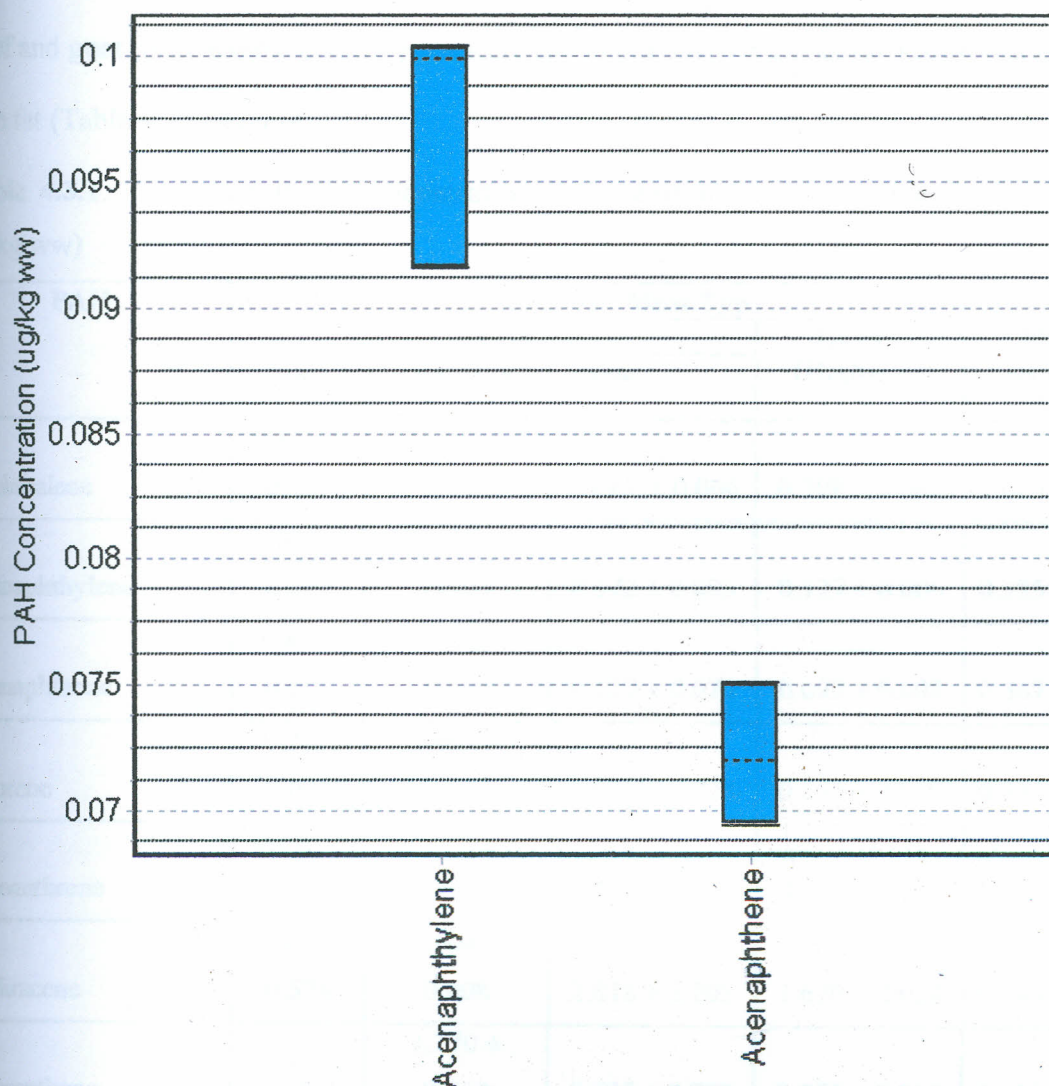


Figure 4.0.5: Means, Ranges, Minima and Maxima of PAH Concentration in Raw Pork ($\mu\text{g}/\text{kg ww}$)

The low number and concentration found in pork could be because the PAHs which are known to be lipophilic (Onuska, 1989), were mainly in the fatty portion of meat which is usually trimmed off during preparation. The pork used in this study are also zero grazed and as such are only exposed to the PAHs in the commercial feeds which may be lower than what the rest of the animals were exposed to in the environment. The difference in the number, types and concentration of PAHs found in the meats could be attributed to the fact that different animals metabolise PAHs differently.

4.3: Determination of types and concentrations of the PAHs in the cooked meat

Beef and goat meat were cooked by roasting while pork was cooked by frying it in its own fat (Table 4.3.1, Figure...

Table 4.3.1: The mean PAH concentration in cooked meat (\pm standard deviation $\mu\text{g}/\text{kg}$ ww)

PAH	Meat Type				
	BEEF			GOAT (Highway)	PORK (Kisumu Hotel)
	Apok	Highway	Mean		
Naphthalene	0.113 \pm 0.004	0.233 \pm 0.008	0.173 \pm 0.066	0.308 \pm 0.005	0.074 \pm 0.004
Acenaphthylene	0.087 \pm 0.002	0.253 \pm 0.005	0.172 \pm 0.093	0.127 \pm 0.006	0.325 \pm 0.005
Acenaphthene	0.062 \pm 0.005	0.192 \pm 0.002	0.129 \pm 0.070	0.092 \pm 0.008	0.367 \pm 0.022
Fluorene	0.255 \pm 0.006	1.604 \pm 0.013	0.930 \pm 0.738	0.892 \pm 0.010	0.341 \pm 0.016
Phenanthrene	0.223 \pm 0.010	nd	0.112 \pm 0.122	0.207 \pm 0.005	nd
Anthracene	0.832 \pm 0.574	4.804 \pm 0.008	2.818 \pm 2.205	1.670 \pm 0.028	0.543 \pm 0.019
Fluoranthene	nd	1.270 \pm 0.032	0.635 \pm 0.700	0.251 \pm 0.014	0.491 \pm 0.008
Pyrene	0.395 \pm 0.004	3.648 \pm 0.064	2.021 \pm 1.782	nd	0.701 \pm 0.522
Benzo(k) fluoranthene	0.143 \pm 0.005	0.202 \pm 0.003	0.172 \pm 0.033	0.414 \pm 0.005	0.237 \pm 0.025
Benz(a)pyrene	nd	Nd	nd	0.422 \pm 0.004	Nd
Indeno(1,2,3- cd)pyrene	0.097 \pm 0.016	0.253 \pm 0.009	0.175 \pm 0.086	0.121 \pm 0.007	0.386 \pm 0.028
Dibenz(a,h)anthracene	nd	5.032 \pm 0.062	2.516 \pm 2.756	0.113 \pm 0.004	Nd
Benz(g,h,i)pyrene	nd	0.385 \pm 0.008	0.193 \pm 0.211	0.164 \pm 0.008	Nd
Total PAH (TPAH)	2.207	17.875	10.041	4.781	3.464

Note: nd = not detected/below detection limit of the machine (detection limit = 10^{-12} g)

Twelve of the sixteen priority PAHs were found in roast beef, twelve were found in roasted goat meat while fried pork exhibited nine priority PAHs (Table 4.3.1). Individual PAH range in roast beef was found to be within the range of 0.053 - 5.032 $\mu\text{g}/\text{kg}$ while total PAH range was 2.207-17.875 $\mu\text{g}/\text{kg}$. This is comparable to what is found in Literature. Janoszka *et al.* (2004) who studied roast meats in Poland found that the total PAH content was within the range 2.43–16.10 ng g^{-1} meat while Reinik *et al.* (2007) found 16 $\mu\text{g}/\text{kg}$ in smoked meat and ham as part of Estonian Food Safety Monitoring. Martonell *et al.* (2010) however, reported higher levels at 33.99 $\mu\text{g}/\text{kg}$ in meat and meat products in population exposure study of Catalonia, Spain.

Individual PAH range in roast goat meat was found to be within the range of 0.092 - 1.670 $\mu\text{g}/\text{kg}$. However, total PAH range was found to be between 2.201-4.781 $\mu\text{g}/\text{kg}$. This is relatively low as compared to the literature quoted above and the concentration in roasted beef. This could be due to the fact that the goat meat tested was relatively leaner than the beef and therefore there was less dripping of the fat into the heating source leading to less formation of PAHs (FEHD, 2004).

The concentration of individual PAH in fried pork was found to be in the range of 0.075 - 0.560 $\mu\text{g}/\text{kg}$ while total PAH was found in the range of 0.170-3.464 $\mu\text{g}/\text{kg}$. This is much lower than the values seen in roasting beef and goat meat showing that shallow pan frying of meat generates less PAHs than roasting or smoking which is the method Janoszka *et al.* (2004) investigated.

4.4: Effect of cooking on PAH concentrations in cooked meat

The concentrations of PAHs in roasted/fried meat were corrected to cater for weight loss during roasting/frying using the effect of cooking ratios (Section 3.6 of chapter 3). The ratios used were as shown:

Raw beef: roasted beef ratio	=	100:57
Raw goat meat: roasted goat meat ratio	=	100:73
Raw pork: fried pork ratio	=	100:65

4.4.1: Effect of roasting on the PAH concentration in roast beef

Roasting significantly ($P \leq 0.05$) increased the concentration of all the PAHs concentration with the exception of the low molecular weight PAHs (LPAH), whose concentrations were significantly ($P \leq 0.05$) reduced at Apok (Table 4.4.1).

Table 4.4.1: Effect of roasting on the PAH concentration in beef (in µg/kg ww)

SITE	BEEF TYPE	PAH TYPE												TPAH
		1	2	3	4	5	6	7	8	9	10	11	12	
Apok	Raw	0.09	0.07	0.05	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.21
	Roast	0.06	0.05	0.03	0.15	0.13	0.47	nd	0.23	0.08	0.06	nd	nd	1.26
Eway	Raw	0.12	0.12	0.09	0.31	nd	0.87	nd	0.93	nd	0.13	nd	nd	2.11
	Roast	0.16	0.18	0.13	1.17	nd	3.51	0.93	2.67	0.15	0.18	3.68	0.29	13.07
	Raw Mean	0.10	0.10	0.07	0.16	nd	0.44	nd	0.47	nd	0.07	nd	nd	1.40
	Roast Mean	0.12	0.12	0.10	0.68	0.08	2.06	0.47	1.48	0.12	0.13	1.84	0.15	7.35
	LSD (P=0.05)	0.02	0.02	0.02	0.17	0.04	0.54	0.21	0.39	0.01	0.06	0.84	0.06	

Note: nd = not detected/below detection limit of the machine (detection limit = 10^{-12} g)

Key: 1 - Naphthalene, 2 - Acenaphthylene, 3 - Acenaphthene, 4 - Fluorene, 5 - Phenanthrene, 6 - Anthracene, 7 - Fluoranthene, 8 - Pyrene, 9 - Benzo(k)Fluoranthene, 10 - Indeno(1,2,3-cd)Pyrene, 11 - Dibenz(a,h)Anthracene, 12 - Benzo(ghi)Perylene

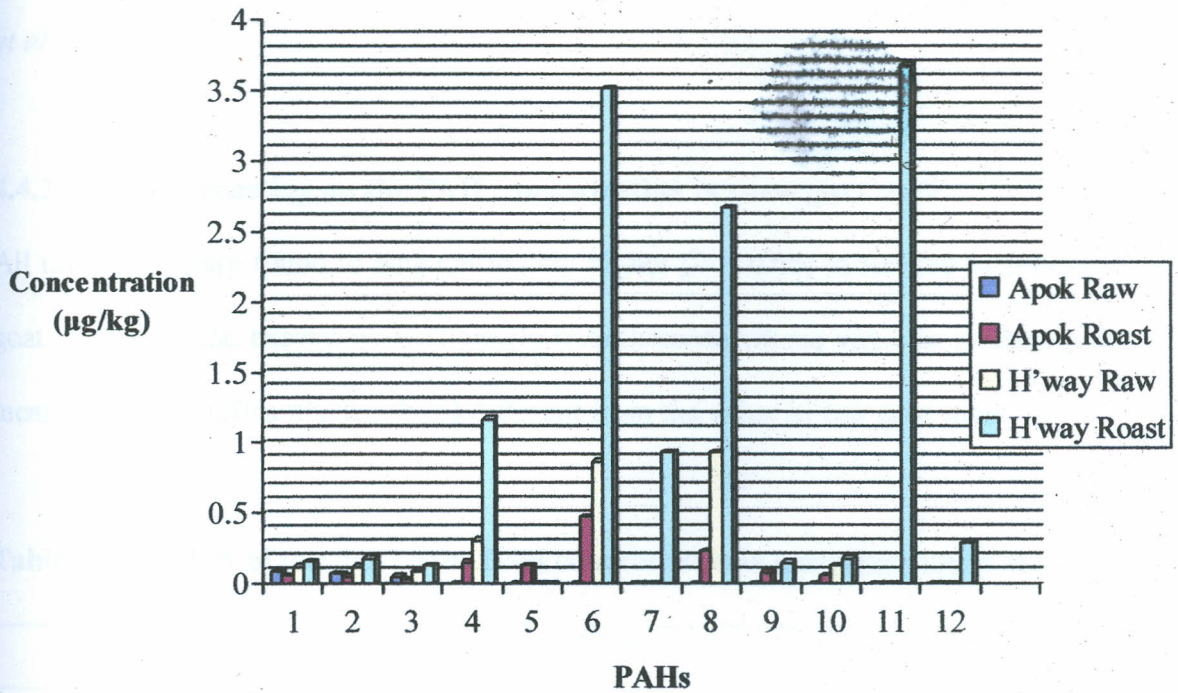


Figure 4.0.6: Effect of Roasting on PAH concentration in Beef ($\mu\text{g}/\text{kg}$ ww).

1 - Naphthalene, 2 - Acenaphthylene, 3 - Acenaphthene, 4 - Fluorene, 5 - Phenanthrene, 6 - Anthracene, 7 - Fluoranthene, 8 - Pyrene, 9 - Benzo(k)Fluoranthene, 10 - Indeno(1,2,3-cd)Pyrene, 11 - Dibenz(a,h)Anthracene, 12 - Benzo(ghi)Perylene

These LPAHs were Naphthalene, Acenaphthylene and Acenaphthene at Apok. It also introduced new PAHs. At Apok, the new PAHs introduced were Fluorene, Phenanthrene, Anthracene, Pyrene, Benzo(k)Fluoranthene and Indeno(1,2,3-cd)Pyrene while the new ones at Highway were Dibenz(a,h)anthracene, Benzo(k)fluoranthene and Benzo(ghi)perylene. Therefore roasting is a dynamic process where some PAHs are burnt out while new ones are generated. The concentrations of PAHs were also significantly different with respect to location with Highway showing higher levels than Apok ($P \leq 0.05$). This could be due to the fact that Apok used an indoor grill to prepare their meat while Highway uses open air

charcoal stove which perpetuates production of PAHs as noted in a study by Lisouza *et al.* (2011).

4.4.2: Effect of roasting on the PAH concentration in roast goat meat

All the PAHs were found to be significantly higher ($P \leq 0.05$) in roasted than in raw goat meat with the exception of Dibenz(a,h)Anthracene whose value in roasted goat meat was not significantly ($P \leq 0.05$) different from the value in raw goat meat.

Table 4.4.2: Effect of roasting on the PAH concentration in goat meat ($\mu\text{g}/\text{kg ww}$)

GOAT TYPE	PAHs													
	1	2	3	4	5	6	7	8	9	10	11	12	TPAH	
Raw	0.105	0.031	0.075	0.299	nd	1.033	nd	0.332	nd	0.097		0.102	0.127	2.201
Roast	0.225	0.093	0.067	0.651	0.151	1.219	0.181	0.302	0.308	0.088		0.085	0.120	3.490
LSD ($P=0.05$)	0.026	0.007	0.022	0.073	0.020	1.305	0.035	0.024	0.011	0.10		0.069	0.020	

Note: nd = not detected/below detection limit of the machine (detection limit = 10^{-12} g)

Key: 1 – Naphthalene, 2 – Acenaphthylene, 3 – Acenaphthene, 4 – Fluorene, 5 – Phenanthrene, 6 – Anthracene, 7 – Fluoranthene, 8 - Benz(k)fluoranthene, 9 - Benz(a)pyrene, 10 - Indeno(1,2,3-cd)pyrene, 11 – Db(a,h)Anthracene, 12 – B(ghi)Perylene

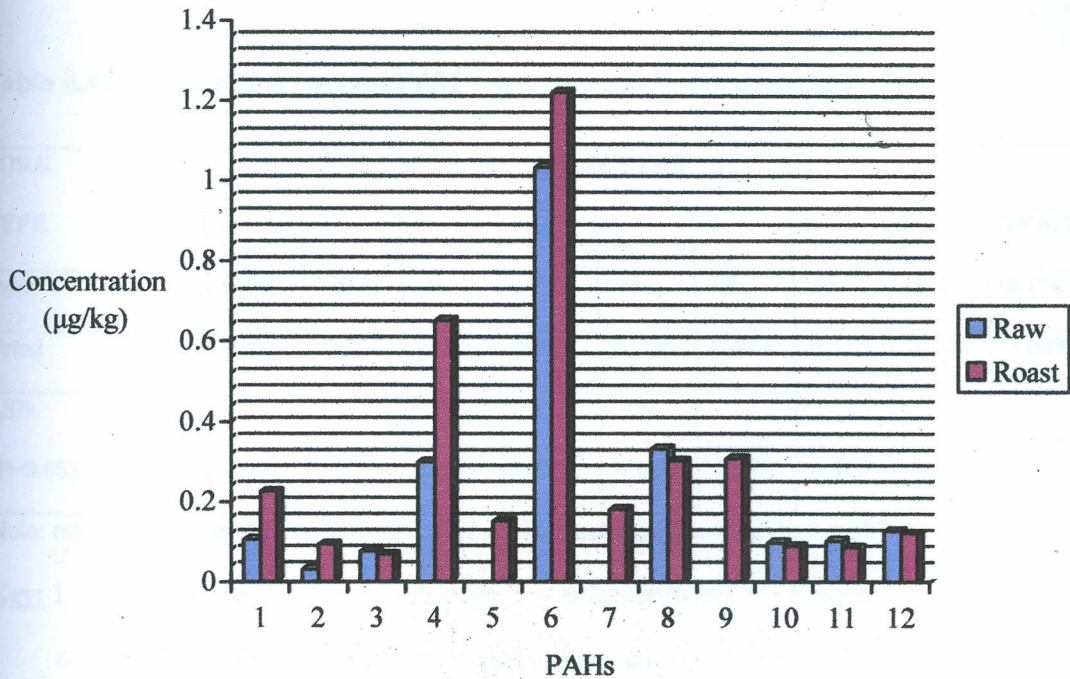


Figure 4.0.7: Effect of roasting on PAH Concentration in Goat Meat ($\mu\text{g}/\text{kg}$ ww).

1 – Naphthalene, 2 – Acenaphthylene, 3 – Acenaphthene, 4 – Fluorene, 5 – Phenanthrene, 6 – Anthracene, 7 – Fluoranthene, 8 - Benz(k)fluoranthene, 9 - Benz(a)pyrene, 10 - Indeno(1,2,3-cd)pyrene, 11 – Db(a,h)Anthracene, 12 – B(ghi)Perylene

The number of PAHs in roasted goat increased to twelve as compared to the nine found in raw goat meat (Table 4.4.2). The new PAHs generated were Phenanthrene, Fluoranthene and the potent Benz(a)Pyrene whose percentage in comparison to total PAH concentration was 8.83%.

4.4.3: Effect of frying on PAH concentration in pork

Values of PAH concentration in fried pork were significantly higher ($P \leq 0.05$) than the values in raw pork with the exception of pyrene which was not significantly different ($P \leq 0.05$) (Table 4.4.3).

Table 4.4.3: Effect of frying on PAH concentration in pork ($\mu\text{g}/\text{kg}$)

PORK TYPE	PAH TYPE									
	1	2	3	4	5	6	7	8	9	TPAH
Raw	nd	0.097	0.072	nd	nd	nd	nd	nd	nd	0.170
Fried	0.045	0.196	0.221	0.206	0.327	0.296	0.423	0.143	0.233	2.089
LSD (P=0.05)	0.011	0.025	0.055	0.039	0.047	0.020	1.295	0.063	0.069	

Note: nd = not detected/below detection limit of the machine (detection limit = 10^{-12} g)

Key: 1 – Naphthalene, 2 – Acenaphthylene, 3 – Acenaphthene, 4 – Fluorene, 5 – Anthracene, 6 – Fluoranthene, 7 – Pyrene, 8 – Benz(k)fluoranthene, 9 – Indeno(1,2,3-cd)pyrene

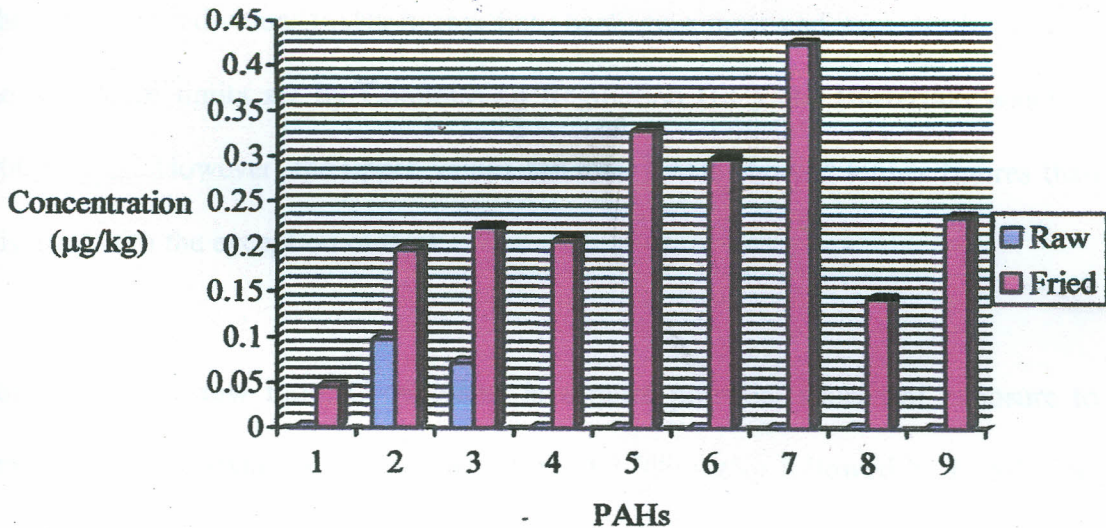


Figure 4.0.8: Effect of frying on PAH concentration in pork ($\mu\text{g}/\text{kg}$).

1 – Naphthalene, 2 – Acenaphthylene, 3 – Acenaphthene, 4 – Fluorene, 5 – Anthracene, 6 – Fluoranthene, 7 – Pyrene, 8 – Benz(k)fluoranthene, 9 – Indeno(1,2,3-cd)pyrene

Frying significantly ($P \leq 0.05$) increased the concentration of PAHs in all cases. It also introduced new PAHs namely; Fluorene, Anthracene, Fluoranthene, Pyrene, Benzo(k)fluoranthene and Indeno(1,2,3-cd)Pyrene.

4.5 Comparison of levels of PAHs with the European Commission standards

Table 4.5.1: Comparison of Maxima of PAH Concentration Range in Tested Food to Maximum Limits Set by European Commission Regulation (2006) (in $\mu\text{g}/\text{kg}$)

Sample Tested	Fish		Beef		Goat Meat		Pork		EU Stds
	Individual PAH Conc.	TPAH Conc.	Individual PAH Conc.	TPAH Conc.	Individual PAH Conc.	TPAH Conc.	Individual PAH Conc.	TPAH Conc.	
Raw	0.886	3.994	0.934	2.105	1.033	2.201	0.097	0.169	2.000
Cooked	-	-	5.032	17.875	1.670	4.781	0.701	3.464	5.000

The values of individual PAHs in raw fish, beef, goat meat and pork are lower than the maximum limits set by Commission Regulation (EC) No 1881/2006 which is $2.000 \mu\text{g}/\text{kg}$. However, the upper values for total PAHs showed higher figures than this limit with the exception of Pork.

Considering the raw food; *O. niloticus* is a more potential source of exposure to humans before cooking with an upper value of $3.990 \mu\text{g}/\text{kg}$ followed by *L. niloticus* ($3.321 \mu\text{g}/\text{kg}$), Goat Meat ($2.199 \mu\text{g}/\text{kg}$), Highway beef ($2.105 \mu\text{g}/\text{kg}$), Apok beef ($0.210 \mu\text{g}/\text{kg}$), Pork ($0.170 \mu\text{g}/\text{kg}$) and the least potential source of exposure to humans before cooking is *R. argentea* ($0.0311 \mu\text{g}/\text{kg}$).

Upper values for individual PAHs in roast/fried meats were found to be much lower than the maximum limits set by Commission Regulation (EC) No 1881/2006 which is

5µg/kg with the exception of roast beef. The concentration of Dibenz(a,h)anthracene in roast beef which was above this limit at 5.032 µg/kg while total PAHs' concentration in roast beef's upper values was 17.875 µg/kg which again was well above the maximum limit.

For roast/fried foods studied; Highway beef is a more potential source of exposure to humans with an upper value of 17.875 µg/kg followed by Goat meat (4.780 µg/kg), Pork (3.464 µg/kg) and the least potential source of exposure to humans after cooking is Apok beef (2.207µg/kg).

The recommended daily dietary allowances for adults (www.nutrition.com.sg/he/herda-adt.asp accessed 27/6/2011) puts daily protein allowance as Men (68 g), Women (58 g), Pregnant Women (67 g) and Lactating Women (83 g first six months and 77 g after six months). These values were used to calculate the weekly (daily exposure x 7) exposure assuming all daily protein allowance was provided by fish or meat.

Table 4.5.2: Weekly Exposure to Total PAHs assuming all daily dietary protein allowance is provided by the given foods ($\mu\text{g}/\text{kg}$)

GROUP	FISH			COOKED MEAT		
	<i>O. niloticus</i> (3.934 $\mu\text{g}/\text{kg}$)	<i>L. niloticus</i> (3.166 $\mu\text{g}/\text{kg}$)	<i>R. argentea</i> (0.035 $\mu\text{g}/\text{kg}$)	BEEF (10.06 $\mu\text{g}/\text{Kg}$)	GOAT (4.781 $\mu\text{g}/\text{Kg}$)	PORK (3.464 $\mu\text{g}/\text{Kg}$)
Men	1.873	1.507	0.017	4.789	2.276	1.649
Women	1.597	1.285	0.014	4.084	1.941	1.406
Pregnant Women	1.845	1.485	0.016	4.718	2.242	1.625
Lactating Women (first six months)	2.286	1.839	0.020	5.845	2.778	2.013
Lactating Women (after six months)	2.120	1.706	0.019	5.422	2.577	1.867

These values show that, assuming that all the PAHs being taken in are not being broken down by natural processes in the body, it only takes one week for lactating women taking the daily protein requirement of 83 or 77 g to reach the limit set by European Commission Regulation 1881/2006 while the rest of the groups would need just over a week. For goat meat, about two weeks would be needed to reach this limit. This shows that the human exposure in the region is very high especially given that there are other sources of PAHs e.g. water (Bowa *et al.*, 2009; Bowa and Lalah, 2009) and smoke during cooking, especially the cooks preparing these foods given that PAHs are entrained in the smoke as noted by Lisouza *et al.* (2011) among others. People may take more than the prescribed daily allowance.

CHAPTER FIVE

5.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY

1. Twelve priority PAHs were detected in *O. niloticus* (TPAH was 3.934 $\mu\text{g}/\text{kg ww}$) while *L. niloticus* had 11 priority PAHs detected (TPAH was 3.166 $\mu\text{g}/\text{kg ww}$). In both cases, the PAHs with the highest and the lowest concentrations were Pyrene and Indeno(1,2,3-cd)pyrene respectively. *R. argentea* had only 2 priority PAHs (TPAH was 0.035 $\mu\text{g}/\text{kg ww}$). The overall mean TPAH was 3.047 $\mu\text{g}/\text{kg ww}$.
2. Beef had 7 priority PAHs (TPAH was 1.392 $\mu\text{g}/\text{kg ww}$) with Pyrene having the highest concentration and Indeno(1,2,3-cd)pyrene having the lowest concentration; Goat meat had 9 priority PAHs (TPAH was 2.201 $\mu\text{g}/\text{kg ww}$) with the concentration of Anthracene being the highest and Acenaphthylene being the lowest while Pork had only 2 priority PAHs (TPAH was 0.169 $\mu\text{g}/\text{kg ww}$).
3. Roasting increased the number and concentration of existing PAHs detected in raw meat. Roast beef had five new PAHs (TPAH was 10.06 $\mu\text{g}/\text{kg ww}$) with potent Dibenz(ah)anthracene being detected. Roast goat meat had three new PAHs (TPAH was 4.781 $\mu\text{g}/\text{kg ww}$) with potent Benz(a)pyrene being detected. Fried pork had seven new PAHs (TPAH was 3.464 $\mu\text{g}/\text{kg ww}$) detected.
4. Raw fish (3.934 $\mu\text{g}/\text{kg ww}$) and beef from Highway Hotel (2.105 $\mu\text{g}/\text{kg ww}$) had TPAHs in quantities that are higher than the 2 $\mu\text{g}/\text{kg}$ limit. Roast beef had individual PAH (5.032 $\mu\text{g}/\text{kg ww}$ Dibenz(ah)anthracene) and TPAHs at Highway Hotel (17.875 $\mu\text{g}/\text{kg ww}$) higher than the 5 $\mu\text{g}/\text{kg}$ limit.

5.2 CONCLUSIONS

1. This study has revealed that the PAH concentration in raw fish, both individual and total, are in the $\mu\text{g}/\text{kg}$ range. The figures for total PAHs concentration indicate levels higher than maximum allowable limits indicative of fish being a significant potential human exposure to PAHs. *O. niloticus* is a more potential source of exposure followed by *L. niloticus* while *R. argentea* is the least potential source of exposure. *O. niloticus* is a herbivore which feeds on bottom phytoplanktons more in contact with the sediments at the bottom of these waters that have been shown to have high PAH concentration.
2. The PAH concentration in raw meats, both individual and total, were in the $\mu\text{g}/\text{kg}$ range. The figures for total PAHs concentration indicate levels higher than maximum allowable limits indicative of significant potential human exposure to PAHs. Goat meat posed the highest potential for exposure followed by beef while pork was the least potential source of exposure. Raw goat meat was the leanest of all the three meats. PAHs are lipophilic and as such the highest concentration would be found in the fats that are usually trimmed off for beef and pork but there is no trimming done for goat.
3. Roasting/frying increased the PAH concentration in the meat significantly ($P \leq 0.05$) in all cases. It also introduced new PAHs namely; Fluorene, Phenanthrene, Fluoranthene, Anthracene, Pyrene, Benzo(k)fluoranthene, Indeno(1,2,3-cd)pyrene, Benzo(ghi)perylene and the more potent Dibenz(ah)anthracene and Benz(a)pyrene.
4. The values of individual PAHs in raw fish, beef, goat meat and pork are lower than the maximum limits set by Commission Regulation (EC) No 1881/2006 but the upper values for total PAHs showed higher figures than this limit with the

exception of Pork. Similarly, the upper values for individual PAHs in roast/fried meats were found to be lower than the maximum limits with the exception of roast beef. The concentration of Dibenz(a,h)anthracene in roast beef which was above this limit while total PAHs' concentration in roast beef's upper values was well above the maximum limit. The potential of human exposure to PAHs' in the Winam Gulf higher is than acceptable.

5.3 RECOMMENDATIONS

1. The population should be encouraged to consume *R. argentea* since it posed the least potential source of exposure to humans before cooking. Consumption of *O. niloticus* and *L. niloticus* should not be frequent due to the high potential they pose for PAH exposure before cooking. Otherwise, food preparation methods that are known to reduce PAH concentration should be employed before consumption. For, example sun-drying since PAHs are photodegradable as noted by Onuska (1989).
2. Fried pork is relatively safe for eating since it posed the least potential source of exposure to humans before cooking. Consumption of goat meat and beef should be minimized due to the high potential they pose for PAH exposure before cooking. There is need to minimize poor handling practices such as keeping raw meat in close proximity to roasting places/kitchens. The meat could be contaminated by the ambient PAHs.
3. Roast beef should be avoided in the diet since it contains in excess of three times the allowed maximum limit. Roast goat meat should also be avoided because although it has less than the maximum limit allowable, it is the only meat out of the ones tested that gave positive results for the most potentially carcinogenic.

PAH, Benz(a)pyrene. Use of frying as a method of cooking is preferred to open fire roasting since it yields less PAHs. Grilling is a better method of cooking than open air charcoal stove.

4. Open air charcoal roasting of meat is a greater source of human exposure yielding in excess of three times the allowable maximum limit of PAHs in the diet. This method of cooking should be avoided by all means. Although open air has more oxygen, this presence of oxygen increases the cooking temperatures such that there is more charring, yielding more PAHs.
5. These values show that the Human exposure to PAHs' in Kisumu around the lake is higher than acceptable hence the need for the relevant authorities like the Ministry of Public Health and nutritionists to address this by sensitizing the population on exposure to PAHs and effect of cooking methods on exposure especially given that they are suspected to be toxic, and/or carcinogenic. This study could form a basis on which health workers could advise those who love roasted meat on best practices.

5.4 SUGGESTIONS FOR FUTURE STUDY

1. Research should be done on burrowing fishes and bottom feeders that are more in contact with sediments which generally show higher concentrations of PAH e.g. mudfish (*Protopterus aethopicus*) and catfish (*Clarias gariepinus*) since their demand for consumption is increasing due to the fact that they are relatively cheaper than *Oreochromis niloticus* and *Lates niloticus*.
2. Research should be done on fish from pristine areas to determine whether the background levels in raw fish are due to pollution or whether there are other factors causing the high background concentrations.

3. There is need to trace the origin of the animals slaughtered for beef, goat meat and pork in order to understand the reason for high background levels in the raw meats.
4. This study investigated beef, goat meat and pork only. Other meats should be studied to determine the potential exposure of the population to PAHs.
5. Cooking conditions influence PAH concentration in foods therefore there is need to compare PAH concentration based on roasting methods e.g. open fire or cooking ovens and conditions that are currently used in the region. This is in order to advice on best practices when it comes to roasting to ensure low yield of PAH.
6. Other methods of cooking meat should also be investigated. This study only investigated PAHs in raw fish. Studies should in future analyze PAHs in fried, stewed and smoked fish.
7. There is need to investigate ways in which the high human exposure to PAHs in the region can be mitigated e.g. by better handling and processing procedures.

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