

**STUDIES ON THE ASSESSMENT AND SIMULATION OF DIETARY AND ENVIRONMENTAL EXPOSURES TO POLYCYCLIC AROMATIC HYDROCARBONS IN COMPLEX MATRICES**

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL OF THE UNIVERSITY OF LAGOS IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN ANALYTICAL/ENVIRONMENTAL CHEMISTRY

By

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A Thesis submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy (Ph.D) in the Department of Chemistry, School of Postgraduate Studies, University of Lagos, Lagos, Nigeria.

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**SCHOOL OF POSTGRADUATE STUDIES  
UNIVERSITY OF LAGOS  
CERTIFICATION**

This is to certify that the thesis

STUDIES ON THE ASSESSMENT AND SIMULATION OF DIETARY AND ENVIRONMENTAL  
EXPOSURES TO POLYCYCLIC AROMATIC HYDROCARBONS IN COMPLEX MATRICES

Submitted to  
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is a record of original research carried out by

**ADETUNDE, OLUWATOYIN TIRENIOLUWA**

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## **DEDICATION**

I dedicate this project to The LORD GOD ALMIGHTY who has made it possible to finish this project and to my parents Dr and Mrs Adetunde Anifaleye

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## ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are known for their carcinogenic and mutagenic properties. They are formed from incomplete combustion of organics such as fossil fuels among others. PAHs are found in foods and in the environment. In this study, the concentration of PAHs and their potential risks were determined in smoked fishes, roasted foods, soils and plant samples in the Lagos metropolis, Nigeria. Smoked and raw fishes (*Arius heude loti* (catfish), *Cynoglossus senegalensis* (sole), *Hake Sp* (fresh stock fish)), raw and roasted corn (*Zea mays*), ripe and unripe plantain (*Musa paradisiaca*), yam (*Dioscorea sagittifolia*), plants grown on contaminated soils and soils from activity impacted sites were investigated for their levels and risk of PAHs. The fishes and food were extracted for PAHs by ultrasonication, and analysed using a HPLC (Agilent 1100 model HPLC system coupled to ultraviolet detector (HPLC-UV)). The soil and plant extracts were analysed by a gas chromatograph (GC) (Agilent 6890N GC) equipped with a mass selective detector (MS) (Agilent 5975 MS). The results showed that smoked fish samples (*Hake Sp*, *Cynoglossus senegalensis* and *Arius heudeloti*) processed by charcoal fire gave the lowest sum of PAHs (121, 176 and 1136 µg/kg respectively). Followed by the firewood (781, 1258 and 1321 µg/kg respectively), while the sawdust method had the highest sum of PAHs (856, 1395 and 2058 µg/kg respectively). Based on the mean daily intake (MDI) risk assessment approach consumption of smoked fishes possessed some risks. However, based on the 5.0 µg/kg benzo(a)Pyrene (BaP) maximum level for smoked meat and fish established by the European Commission, no risk was associated with the consumption of the smoked fishes. Higher levels of PAHs were found in the roasted food samples compared with the raw samples. The sum of PAHs for raw and roasted food samples ranged between 0.19 - 0.53 mg/kg and 3.62 – 40.33 mg/kg respectively with roasted ripe plantain having the highest concentration. Based on a comparison with limits for roasted foods, the results from this study showed no potential risk associated with the ingestion of the foods. However, using the benzo (a) pyrene equivalence dose (BaP<sub>eq</sub>) approach to risk assessment, more risk were associated with the consumption of roasted foods compared to the non roasted foods. The sum of PAHs from the soils from activity impacted sites (12 sites) studied ranged between 0.2 to 254µg/g at these sites. The sum BaP<sub>eq</sub> at the sites ranged between 0.0 (a forest soil (k)) and 16.7 µg/g (a lubricating oil depot soil(c)). The MDI of each composite soil sample compared with that of food revealed that some of the individual PAH in samples from sites A (a dump site), C (a depot and loading point for used for black oil), F (a dump site), G (a petroleum depot), H (a roadside) and L (a car park) exceeded the recommended MDI threshold for food, indicating some risk associated with activities on these sites based on the ingestion estimate value. The Fed Organic Estimation Human Simulation Test (FOREhST) used to quantify bioaccessible PAHs in soils showed that not all the PAHs present in soils were bioaccessible. Re-evaluation of the risk based on bioaccessible PAHs showed that although PAHs were present and the amount of bioaccessible PAH based on oral risk assessment will not trigger cancers as depicted in the estimated theoretical cancer risk (ER)  $9.47 \times 10^{-10}$  –  $4.08 \times 10^{-07}$  for an adult. PAHs are lipophilic as a result; bio accumulates in plants and other living organisms reaching levels that cause toxicological effects. It is important to reduce exposures to sites with high levels of PAHs. The study of PAH uptake by plant as a risk assessment approach, showed that the uptake of PAHs by plants was directly proportional to the concentration of PAHs in soils. The amount of PAHs in plants grown on contaminated sites compared to the control site revealed increased concentrations with amounts in the roots being generally higher than in stems followed by leaves for all the plants. *Telfairia occidentale* (*Ugwu*), consistently grew on all the soils which include the contaminated soils while shoko only grew on least contaminated soils

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## LIST OF ABBREVIATIONS

ACP	Acenaphthene
ACN	Acetonitrile
ACY	Acenaphthylene
ANT	Anthracene
BaA	Benzo(a)anthracene
BaP	Benzo(a)pyrene
BaP <sub>eq</sub>	Benzo(a)pyrene Equivalence Dose
BgP	Benzo(g,h,i)perylene
BkF	Benzo(k)fluoranthene
CRM	Certified Reference Material
dPAHs	Deuteriated Polycyclic Aromatic Hydrocarbons (Also called internal Standard)
DhA	Dibenzo(a,h)anthracene
CHR	Chrysene
FLR	Fluorene
FORESHt	Fed Organic Estimation Human Simulation Test
GC-MS	Gas Chromatography –Mass Spectrometer
GI	Gastro intestinal
HOCs	Hydrophobic Organic Contaminants
HPLC	High Performance Liquid Chromatography
IVG	<i>In Vitro</i> Gastrointestinal Method
IcP	Indeno(1,2,3-cd)pyrene
NAP	Naphthalene

PAH	Polycyclic Aromatic Hydrocarbons
PHE	Phenanthrene
PYR	Pyrene
POPs	Persistent Organic Pollutant
PBET	Physiologically Based Extraction Test
RPAH	Alkylated Polycyclic Aromatic Hydrocarbons
Sbet	Simple Bioaccessibility Extraction Test
SHIME	Simulator of Human Intestinal Microbial Ecosystem
SVOC	Semi-Volatile Organic Compounds
TOC	Total Organic Content
TOM	Total Organic matter
SPE	Solid Phase Extraction
USEPA	The United States Environmental Protection Agency
uPAHs	Unsubstituted Polycyclic Aromatic Hydrocarbons
UV/ Vis	Ultraviolet/Visible

# CHAPTER ONE

## 1.0 BACKGROUND

Persistent organic pollutants (POPs) are defined as chemical substances that stay in the environment, bio-accumulate through the food web and possess risk of causing adverse effects to human health and the environment. Adverse human health effects caused by POPs include immunotoxicity, neurotoxicity, developmental toxicity, carcinogenicity, mutagenicity and endocrine disruption potentials (Teran *et al.*, 2012). They resist degradation and are transported through air, water and migratory species, across international boundaries to be deposited terrestrial and aquatic ecosystems there by accumulating far from their places of release (Deniervandergon *et al.*, 2007, Fuoco *et al.*, 2009). Typically they are ‘water-hating’ and ‘fat-loving’ chemicals, i.e. hydrophobic and lipophilic. In aquatic systems and soils, they adhere strongly to solids, notably organic matter, avoiding the aqueous phase. They also partition into lipids in organisms rather than entering the aqueous milieu of cells and become stored in the fatty tissue (Jones and de Voogt, 1999).

Towards the end of the twentieth century, several international initiatives aimed at reducing and/or eliminating emission or production and discharges of POPs, with the view of recognizing the need for global actions to better protect, safeguard human health and the environment have been initiated. POPs have been considered in several internationally accepted protocols and conventions. Some of these include:

i) The United Nations Economic Commission for Europe (UNECE) protocol on POPs was signed in Aarhus (Denmark) on 24th June 1998 and entered into force on 23rd October 2003. The protocol includes the following 16 POPs: aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins, dibenzofurans, chlordecone, hexachlorocyclohexane (HCH) (including lindane), hexabromobiphenyl and polycyclic aromatic hydrocarbons (PAHs) (Lerche *et al.*, 2002, Deniervandergon *et al.*, 2007, Fuoco *et al.*, 2009).

ii) The Stockholm Convention signed on 21st May 2001 and entered into force on 17th May 2004 with a follow up meeting on the 4th to 8th of May 2009. The Convention considered the following 12 POPs: aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (Porta and Zumeta, 2002, Fuoco *et al.*, 2009).

These conventions have led to more studies on the need to quantify the levels of POPs in the environment (Bohlin *et al.*, 2007, Colles *et al.*, 2008) to which humans are exposed to. There is a growing realization that the sum amount of POPs present in the matrix may not relate directly to the environmental or health risk and potential impact on living organisms including humans. Hence the need for bioavailability and bioaccessibility studies of POPs (Dean and Scott, 2004).

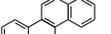
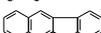
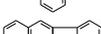
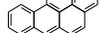
## **1.1 POLYCYCLIC AROMATIC HYDROCARBONS (PAHs)**

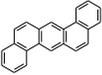
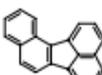
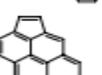
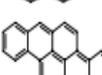
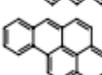
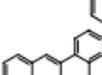
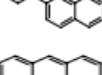
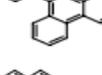
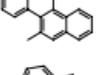
Polycyclic aromatic hydrocarbons (one of the POPs) are commonly occurring or ubiquitous in the environment (Shu *et al.*, 2003) and xenobiotic to most environments (Essumang *et al.*, 2011). PAHs sometimes called polynuclear aromatic hydrocarbons and also known as polyarenes (Amodu *et al.*, 2013), are a group of about 10,000 compounds (Wenzl *et al.*, 2006) which are considered to be one of the most difficult organic contaminants to remove. These compounds, were ranked as the 9th most threatening compounds to human health in 2001 (Iwegbue, 2011).

PAHs consist of fused aromatic ring compounds (Ashraf and Salam, 2012). They are built from two or more benzene rings (Ajiboye *et al.*, 2011, Ashraf and Salam, 2012) and may also be composed of unsaturated four-, five-, and six-member rings (non-alternant PAHs) (Boström *et al.*, 2002). Although the term PAH has traditionally been applied to compounds with carbon and hydrogen skeletons that do not contain hetero-atoms (Rhodes *et al.*, 2005, Wenzl *et al.*, 2006, Alexander *et al.*, 2008), it now encompasses chemicals that contain hetero-atoms such as nitrogen, sulphur, and oxygen. PAHs may exist in substituted forms i.e., alkyl-, nitro-, amino- or halogen-substituted (Boström *et al.*, 2002). PAHs are classified as hydrophobic organic contaminants (HOC) (Pazos *et al.*, 2010), as POPs by the UNECE protocol (Deniervandergon *et al.*, 2007) and Semi-Volatile Organic Compounds (SVOC) (Anyakora *et al.*, 2011).

The United States Environmental Protection Agency (USEPA), European Union Scientific Committees of Food (SCF) and the World Health Organization/Food and Agriculture Organization's Joint Expert Committee on Food Additives and Contaminants (JECFA) have listed sixteen, fifteen and fourteen un-substituted PAHs respectively as priority pollutants (Wenzl *et al.*, 2006, Quilliam *et al.*, 2012) on the basis of their occurrence and carcinogenicity (Barranco *et al.*, 2003, Bishnoi *et al.*, 2005, Quilliam *et al.*, 2012). The 16 USEPA priority PAHs and Benzo (a) Pyrene have been the main focus of research community (Purcaro *et al.*, 2012) with the best known being benzo(a)pyrene (BaP) (WHO, 2000). A list of USEPA, SCF and JECFA priority PAHs are as shown in Table 1.1.

**Table 1.1: Priority PAHs, their Structure and Abbreviation (Wenzl *et al.*, 2006).**

PAHs	Abreviation	Structure	USEPA	SCF	JECFA
Naphthalene	NAP		x		
Acenaphthylene	ACY		x		
Acenaphthene	ACP		x		
Fluorene	FLR		x		
Phenanthrene	PHE		x		
Anthracene	ANT		x		
Fluoranthene	FLT		x		
Pyrene	PYR		x		
Benzo(a)anthracene	BaA		x	x	x
Chrysene	CHR		x	x	x
Benzo(b)fluoranthene	BbF		x	x	x
Benzo(k)fluoranthene	BkF		x	x	x
Benzo(a)pyrene	BaP		x	x	x
Indeno(1,2,3-cd)pyrene	IcP		x	x	x

Dibenzo(a,h)anthracene	DhA		x	x	x
Benzo(g,h,i)perylene	BgP		x	x	
Benzo(j)fluoranthene	BjF			x	x
Cyclopenta(c,d)pyrene	CPP			x	
Dibenzo(a,h)pyrene	DhP			x	x
Dibenzo(a,i)pyrene	DiP			x	x
Dibenzo(a,e)pyrene	DeP			x	x
Dibenzo(a,l)pyrene	DlP			x	x
5-methylchrysene	5MC			x	x
Benzo (c)flourene	BcL				x

## 1.2 PROPERTIES AND CLASSES OF PAHs

The physical and chemical properties of PAHs are determined by their conjugated pie ( $\pi$ ) electron systems, which are dependent on the number of aromatic rings and the molecular mass (Skupinska *et al.*, 2004). The structures of some selected PAHs are as seen in Table 1.1. Their biochemical persistence in the environment arises from dense clouds of  $\pi$  electrons on both sides of the ring structures making them resistant to nucleophilic attack (Fagbote and Olanipekun, 2010) and recalcitrant (Zhan *et al.*, 2010). The compounds are generally lipophilic, a property that increases with increase in their molecular mass. PAHs occur as colourless, white/pale yellow solids with low solubility in water and low vapour

pressure (Pazos *et al.*, 2010). The compounds differ in the number and position of aromatic rings. Un-substituted lower molecular weight PAH compounds (LPAHs), contain two or three rings (e.g., naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene) while the high molecular weight PAHs (HPAHs), contain four to seven rings (fluoranthene, pyrene, BaP, and benzo(a)fluoranthene) (Stanković *et al.*, 2008, Zhang and Wang, 2011). Thus LPAHs are more volatile, water soluble and less lipophilic than the HPAHs.

Accordingly, PAHs of different molecular weight vary substantially in their behaviour, distribution in the environment and in their biological effects. With increasing molecular weight, aqueous solubility decreases while melting point, boiling point and the log  $K_{ow}$  (octanol/water partition coefficient) increases (Table 1.2), suggesting increased solubility in fats, a decrease in resistance to oxidation and reduction, and a decrease in vapour pressure. PAHs are very difficult to degrade; the difficulty is due to their complex and stable molecular structures. As a result of these properties they can be adsorbed rapidly onto soil particles, particularly on soil organic matter (Dai *et al.*, 2008). They are non-ionic (Conte *et al.*, 2001) neutral, nonpolar, organic molecules (Stanković *et al.*, 2008). Environmental concern has focused on the 16 USEPA priority PAHs with a molecular weight (g) range from 128.16 (naphthalene, 2-ring structure) to 276 (Indeno(1,2,3-cd)pyrene and Benzo(g,h,i)perylene) (Eisler, 1987). The 16 priority PAHs and their properties are as seen in Table 1.2.

**Table 1.2: Chemical Formula and Properties of 16 Priority PAHs (CCME, 2008).**

PAH	Formular	No of rings	Molecular weight (g)	Aqueous solubility (mg/l)	Vapour pressure (Pa)	Log $K_{ow}$
Naphthalene	C <sub>18</sub> H <sub>8</sub>	2	128	31	1.0x10 <sup>-2</sup>	3.37
Acenaphthylene	C <sub>12</sub> H <sub>8</sub>	3	152	16	9.0x10 <sup>-1</sup>	4.00
Acenaphthene	C <sub>12</sub> H <sub>10</sub>	3	154	3.8	3.0x10 <sup>-1</sup>	3.92
Fluorene	C <sub>13</sub> H <sub>10</sub>	3	166	1.9	9.0x10 <sup>-2</sup>	4.18
Phenanthrene	C <sub>14</sub> H <sub>10</sub>	3	178	1.1	2.0x10 <sup>-2</sup>	4.57
Anthracene	C <sub>14</sub> H <sub>10</sub>	3	178	0.045	1.0x10 <sup>-3</sup>	4.54
Fluoranthene	C <sub>16</sub> H <sub>10</sub>	4	202	0.26	1.2x10 <sup>-3</sup>	5.22
Pyrene	C <sub>16</sub> H <sub>10</sub>	4	202	0.13	6.0x10 <sup>-4</sup>	5.18
Benz(a)anthracene	C <sub>18</sub> H <sub>12</sub>	4	228	0.011	2.8x10 <sup>-5</sup>	5.91
Chrysene	C <sub>18</sub> H <sub>12</sub>	4	228	0.006	5.7x10 <sup>-7</sup>	5.91
Benzo(b)fluoranthene	C <sub>20</sub> H <sub>12</sub>	5	252	0.0015	-	5.80
Benzo(k)fluoranthene	C <sub>20</sub> H <sub>12</sub>	5	252	0.0008	5.2x10 <sup>-8</sup>	6.00
Benzo(a)pyrene	C <sub>20</sub> H <sub>12</sub>	5	252	0.0038	7.0x10 <sup>-7</sup>	5.91
Dibenz(a,h)anthracene	C <sub>22</sub> H <sub>12</sub>	5	278	0.0006	3.7x 10 <sup>-10</sup>	6.75
Indeno(1,2,3-cd)pyrene	C <sub>22</sub> H <sub>14</sub>	6	276	0.00019	-	6.50
Benzo(g,h,i)perylene	C <sub>22</sub> H <sub>12</sub>	6	276	0.00026	1.4x10 <sup>-8</sup>	6.50

The fused benzene rings of PAHs are arranged in linear, angular or cluster arrangements as seen in Table 1.1. PAHs may be further categorized as alternant and non-alternant PAHs. Alternant PAHs are those with a core ring structure composed entirely of benzenoid rings (e.g naphthalene, phenanthrene, anthracene, pyrene, benz(a)anthracene, chrysene, benzo(a)pyrene, benzo(g,h,i)perylene, dibenz(a,h)anthracene). Non-alternant forms also include four-, five-, and six-member, non-aromatic ring structures in addition to benzoid rings (for example, acenaphthene and benzo(k)fluoranthene) (CCME, 2008). The

compounds have large number of isomers thus exist with great number of structures (isomers) which depend on the complexity of the PAHs (Zhang and Wang, 2011).

### **1.3 TOXICITY OF PAHs**

The great interest in PAH compounds stems from the observations that some of these compounds may cause tumours in humans. In fact, the turning point with respect to PAH toxicity was the observation in 1775 by the British surgeon, Sir Percival Pott that scrotal cancer in chimney sweepers originated from occupational exposure to soot (Jacob, 1996 , Boström *et al.*, 2002, Šimko, 2002). He noted the high incidence of cancer of the scrotum among chimney sweepers that often climbed up to sweep down the soot. Although he deduced correctly that the soot was responsible for cancer, at that time it was not possible to determine the compounds responsible for such tissue damage. A century later (1875), another milestone was the observation of the occurrence of elevated skin cancers in workers in the coal tar industry (Boström *et al.*, 2002).

In the early 1900s, it was widely recognized that soot, coal tar, and pitch were carcinogenic to man when Japanese workers discovered that painting extract of soot onto skin of mice caused tumours of skin. In 1929, the first pure chemical carcinogen dibenzo(a,h)anthracene was isolated from soot at the Chester Beauty Research Institute, Kennaway furtherore, Doll in 1953, on the basis of wide epidemiological and statistical analysis proved that cigarette smoking was a prime cause of lung cancer. Careful analysis of smoke and tar from cigarettes showed that it contained many PAHs (Šimko, 2002). Taiwanese and Chinese women in

1984 were observed to exhibit high incidences of lung cancer. These women smoke little but were contracting lung cancer rather frequently. Early pathological investigation showed that Chinese women tend to contract adenocarcinoma, rather than squamous cell carcinoma, implying that the cause of lung cancer may not be from smoking. Epidemiological surveys indicated indoor air contamination derived from cooking practices might be an important risk factor for lung cancer in Chinese women. The survey and found an 8.3 fold excess risk to contract lung cancer among non-smoking women exposed to fumes from various cooking oils used for preparing Chinese food without a fume extractor (Chiang *et al.*, 1999, Ko *et al.*, 2000).

Various industrial workplaces are now associated with a significant increase to certain cancer diseases which has been attributed to an unusually high exposure to PAH. For instance, PAH exposure is high in coke plants, coal tar and pitch producing and manufacturing industries, aluminium plants, iron and steel foundries, creosote, rubber, mineral oil, soot and carbon black-producing or manufacturing companies. Other highly exposed occupational groups include chimney sweepers, roadmen (pavement-tarring) and roofers (roof-tarring) are also under increased risk (Srogi, 2007).

As a result, follow up experiments were set up to study the cancer causing ability of PAHs in animals. Relevant data for PAHs from laboratory studies on rodents are available, for example through EPA's Integrated Risk Information System (IRIS). Considerable laboratory data currently exist for rat, mouse or other mammalian surrogates of human health for many

contaminants, which are then used by the USEPA, US National Institute of Occupational Safety and Health, World Health Organization, Health Canada, and other risk assessment/management agencies for deriving human health protective benchmarks; i.e., reference doses or concentrations (RfDs and RfCs respectively), allowable or tolerable daily intakes (ADIs and TDIs, respectively), and so on (USEPA, 2007a).

A typical study on carcinogenicity of PAHs was by Grimmera *et al.*, (1983) on automobile exhaust condensate. Using topical application onto the skin of mice, gasoline-driven automobile condensate provoked local tumours after long-term application to the dorsal skin of mice. The tumour incidence occurred and clear cut dose-response relationships in the mice were observed. The fraction of PAH which contained more than 3 rings accounted for about 84–91% of the sum carcinogenicity of automobile exhaust condensate. This fraction represented only about 3.5% by weight of the condensate. The content of BaP (0.414 mg/g) made up 6–7.6% of the sum carcinogenicity of automobile exhaust condensate. Regarding the minor effect of the PAH-free fraction (about 83% by weight), no hints for a carcinogenic activity was observed.

Pinkney *et al.*, (2009 and 2011) investigated the levels of PAHs in four rivers around the Chesapeake Bay tributary from 1992 to 2006 and showed that the PAHs levels increased. There was an increase in skin tumours for bull head fishes from all four Rivers (Chesapeake Bay tributary). Liver tumour was also prevalent (0 % upto 6 %) in one of the river collection. Others had a prevalence of upto 20 %. They were able to identify PAH-like biliary metabolites and (32) P-DNA adducts in these fishes which they used as biomarkers

of exposure and response to PAHs. A picture of bull head fish with tumour is as seen in Plate 1.1



**a**

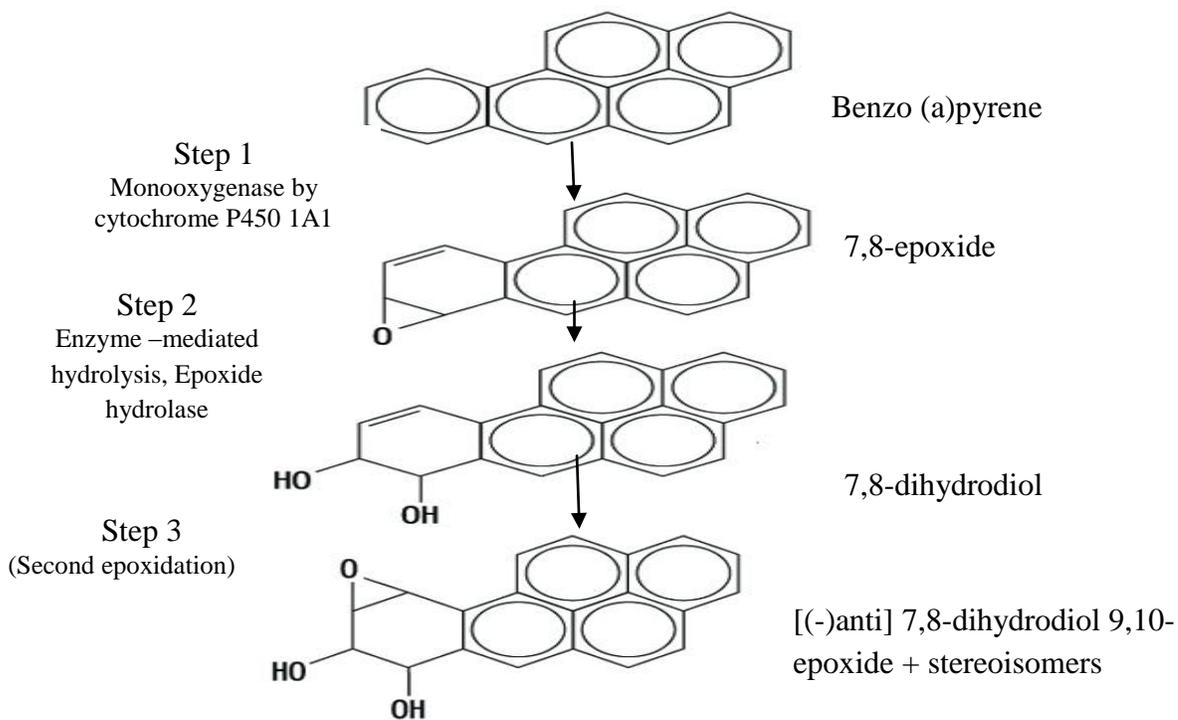


**b**

**Plate 1.1 a and b: Brown Bull Head Fish (*Ameiurus aebulosus*) with Severe Mouth Lesions (Tumour) from Chesapeake Bay Tributary, US Later Diagnosed to Contain as Squamous Cell Carcinomas (Pinkney *et al.*, 2009, Pinkney *et al.*, 2011 )**

PAHs are now well known for their mutagenic and carcinogenic effects. Some PAHs while not carcinogenic (the LPAHs), may act as synergists (Ajiboye *et al.*, 2011). Some of its health effects include growth retardation, low birth weight, small head circumference, low IQ, damaged DNA in unborn children and the disruption of endocrine systems, such as estrogens, thyroid and steroids. Skin changes (thickening, darkening, and pimples) and reproductive related effects such as early menopause due to destruction of ovum have also been identified with PAHs. They are dangerous and increase cancer risk by creating advance glycogen end product (Essumang *et al.*, 2006, Essumang *et al.*, 2011). PAH compounds and metabolites can have estrogenic, antiestrogenic or antiandrogenic activity and teratogenic abilities (Armstrong *et al.*, 2007). PAHs are harmful mainly because they may cause genetic

alterations (Alcántara *et al.*, 2009). Some tests have shown BaP to be genotoxic. PAHs have cytostatic and immunostatic effects. The carcinogenic products of transformation in the cell have negative effect on reproduction (Badyga *et al.*, 2005). They have produced stomach tumours, mammary gland tumours, lung and respiratory tumours, and hepatic tumours in laboratory animals. The carcinogenesis of PAHs is basically as a result of their ability to bind to the DNA thereby causing a series of disruptive effects that end up in tumour initiation. The aromatic hydrocarbon receptor plays a very important role in the binding process. Inactivated aromatic hydrocarbon receptors are located in the cytoplasm of specific cell types. PAH induced carcinogenesis involves three stages as shown in Figure 1.1.



**Figure 1. 1: A Schematic Representation of Reaction in the Stages of PAH Induced Carcinogenesis using BaP as an Example, (Boström *et al.*, 2002, CCME, 2008,)**

The first stage is the enzymatic activation of the compound into metabolites. Some PAH metabolites are as seen shown in Figure 1.2.

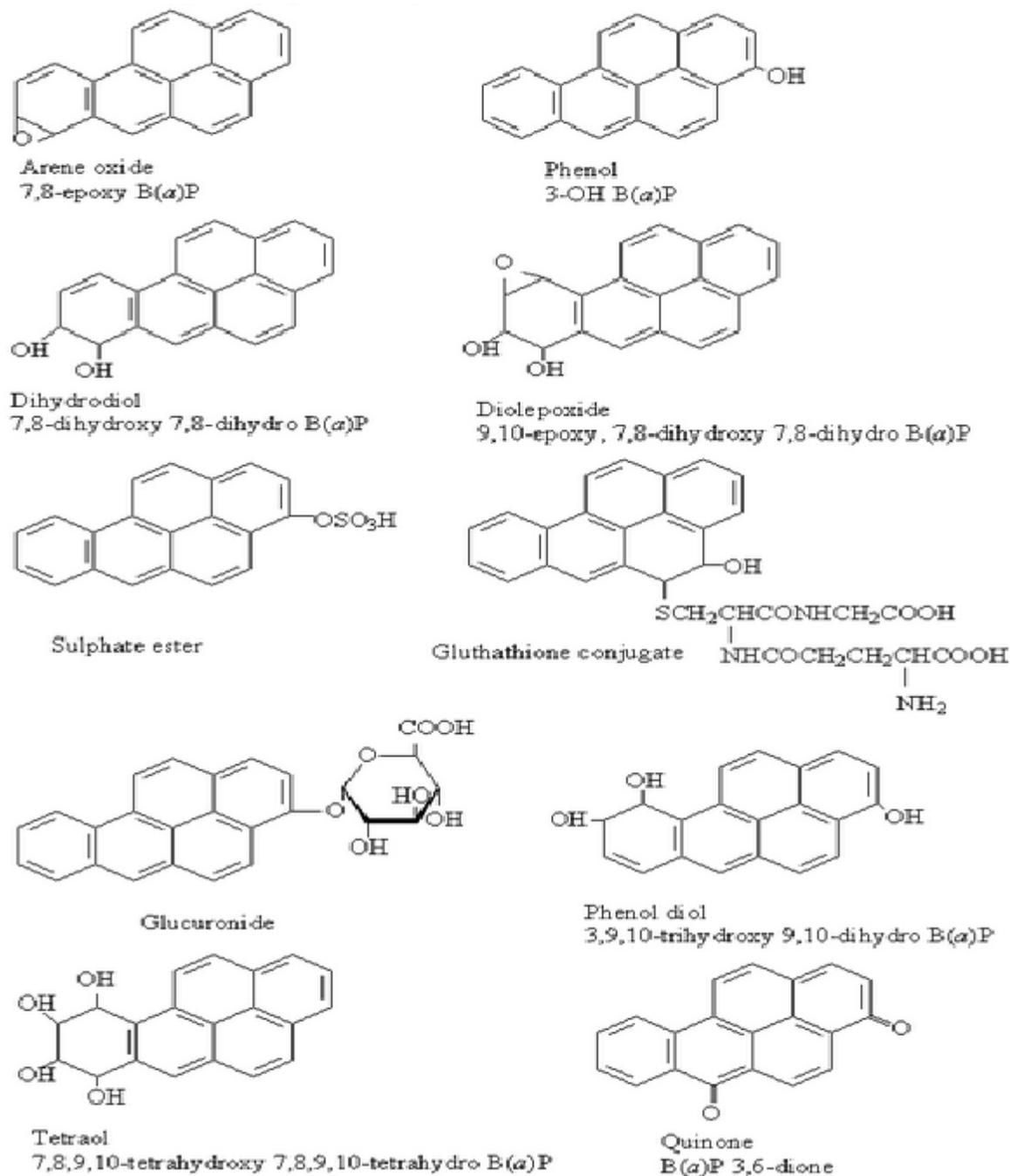


Figure 1.2: Structure of Some PAH Metabolites (Juhasz and Naidu, 2000)

The second stage in the carcinogenesis of PAH is the covalent bonding (ligand binding) of PAH metabolite to DNA to form adducts. The last stage is the induction of mutations that serve to initiate the transformation process. This is done by the translocation of adducts into the nucleus to dimerize with another protein to increase transcription of the mixed function oxidase enzymes cytochrome P450 (CYP) 1A1, CYP1A2, and CYP1B1 (Boström *et al.*, 2002, Lodovici *et al.*, 2004 , Armstrong *et al.*, 2007, Anyakora *et al.*, 2008).

PAHs can have detrimental effects on both the flora and fauna of affected habitats through uptake and accumulation in food chains. They can disturb physiological processes in plant vegetation (Barakat *et al.*, 2011a). PAHs from soil and water are very dangerous to human and aquatic health because plants takes them up through their roots bioconcentrate and translocate them (Essumang *et al.*, 2011).

#### **1.4 SOURCES OF PAHs**

The sources of PAHs are both anthropogenic and natural in origin, and include several source classes, mainly; pyrogenic, petrogenic, and diagenic. They can also be classified as point source and non point sources, mobile and stationary source. Anthropogenic sources are mainly from combustions. The pyrogenic source of PAHs includes from the incomplete combustion of fossil fuels and other organic materials; such as vehicular and other exhaust emissions, crankcase oil, asphalt (road-building material), coal tar (roofing material), creosote (wood preservative) and wood burning (forest fires, open fire place, heating and cooking stoves. The combustions are usually for energy supply (e.g. coal, gas, wood) or for

waste minimalization (Oleszczuk and Pranagal, 2007)). The mixture of PAH compounds produced during these processes depends mainly on the starting material, temperature, and fuel-to-air ratio in combustion and generally results in high percentages of parent PAHs with four or more rings (Sanders *et al.*, 2002). At rapid, high temperature combustion (>700°C) of motor (automobile), bunker (shipping) and power plant (coals and petroleum) fuels pyrogenic PAHs are formed. They are also formed at intermediate temperatures (400–600°C) that are reached in the processing of coals into coal tars and coal tar products (e.g., creosote or coal tar pitch used in aluminium smelters). Formation temperatures are directly related to the types and complexities of PAHs formed (Liu *et al.*, 2009). Complex mixtures of PAHs are usually formed through combustion (pyrogenesis) processes and produces mainly un-substituted PAHs (Rhodes *et al.*, 2005).

Other important anthropogenic sources of pyrogenic PAHs include cracking of crude petroleum (Kim *et al.*, 2009), leaching of asphalt, tyre wear, drips of crankcase oil (Karlsson and Viklander, 2008), incomplete combustion or pyrolysis of organic matter during various industrial processes, waste incineration (Akhlaq, 1997) and those formed during food preparation processes where roasting and drying are involved (Ziegenhals *et al.*, 2008). Combustion of wood and oil, industrial emission and manmade forest fires and so on are also pyrogenic sources for PAHs.

The release of hydrocarbons through geologic seepage, petroleum spills, and petroleum refined products account for petrogenic input, which contain assemblages of PAHs. These

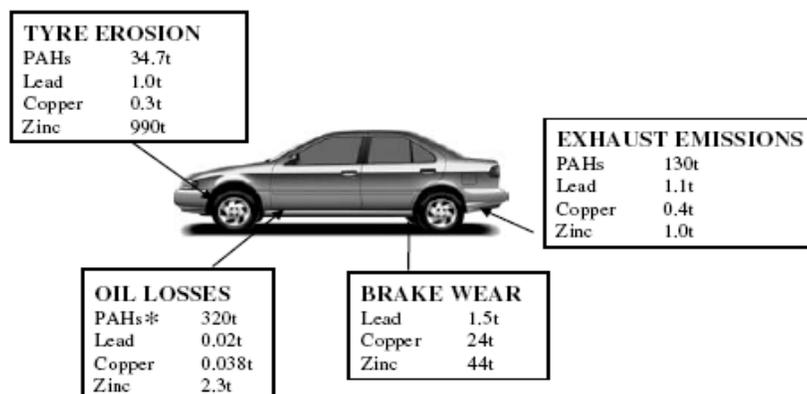
assemblages have high percentages of three or fewer rings and contain a wide range of alkyl-derivatives ((Sanders *et al.*, 2002, Barranco *et al.*, 2003). Petrogenic PAHs are formed through slow, long-term, moderate temperatures (100–300°C) in the earth or seas and are associated with fossil fuels (petroleum and coal) formation (Pazos *et al.*, 2010, Anyakora *et al.*, 2011).

Natural sources of PAHs include PAHs of diagenetic and biogenic origins. The natural sources of PAHs are derived from terrestrial precursors, including plant debris under reduced oxygen or anoxic conditions through the process of diagenesis (biodegradation) to produce alkylated PAHs e.g petroleum (Rhodes *et al.*, 2005). One of such naturally derived PAH is perylene, which arises in anaerobic environments from soils and sediments which can be used as a marker for diagenetic sources (Budzinski *et al.*, 1997, Malawska and Ekonomiuk, 2008). PAHs which occur as a transformation product of natural sources such as volcanic eruption and microbial degradation of organic matter are also said to be diagenetic. Each of these sources produces a characteristic PAH pattern and its distribution is governed by chemical and physical properties (Sanders *et al.*, 2002). Another class of natural PAHs include those derived from biogenic precursors like terpenes, pigments and steroids. Examples include naphthalene, phenanthrene, and perylene produced biologically (Budzinski *et al.*, 1997). Some plant species such as *Magnolia* flowers, *Annonaceae* species from the Amazon rain forest, *Muscodor vitigenus*, an endophytic fungus of a lianer growing in the Peruvian Amazon region all produce naphthalene. Naphthalene synthesis by termites or associated microorganisms has also been reported and there are indications that

phenanthrene can be produced biologically from alkylphenanthrene precursors in plant debris (Wilcke, 2007). Compared with other unsubstituted PAHs, only trace or small amounts of perylene are produced during combustion or by abnormal thermal exposure of organic materials, probably due to its thermodynamic instability (Malawska and Ekonomiuk, 2008). Other natural PAHs are introduced into the terrestrial environment directly from forest fires and volcanoes. In view of the overall contamination, the amount of PAHs in the environment originating from natural sources is minimal in comparison to the amount released from human activities (anthropogenic) (Malawska and Wiołkomirski, 2001). Pollutants in environments are derived from both point and non-point sources. Point source of pollution is the emissions of pollutants that are located at an identifiable point in space. They are associated with a point location such as a toxic-waste spill site. For PAHs, it includes industrial wastewater, sewage treatment plants, creosoted timber and diesel or petroleum contaminated sites (Boonyatumanond *et al.*, 2007). Non-point sources include inputs of PAHs from various sources into soils and rivers (Pies *et al.*, 2008). Point sources when compared to nonpoint sources of pollutants characteristically are (i) easier to control, (ii) more readily identified and measured and (iii) generally more toxic. Point sources include hazardous spills, from underground storage tanks, storage piles of chemicals, mine-waste ponds, deep-well waste disposal, industrial or municipal waste outfalls, leachate from municipal and hazardous waste dumpsites, and septic tanks. Characteristically, non-point source pollutants (i) are difficult or impossible to trace to a source, (ii) enter the environment over an extensive area and sporadic timeframe, (iii) are related (at least in part) to certain uncontrollable meteorological events and existing geographic/geomorphologic conditions,

(iv) have the potential for maintaining a relatively long active presence in the global ecosystem, and (v) may result in long-term, chronic (and endocrine) effects on human health and soil-aquatic degradation (Loague and Crowin, 2005). Examples of nonpoint source for PAHs include air or soot by the road side, PAHs from urban and industrial runoff, erosion associated mining and forest harvesting activities.

Sources of PAHs can also be grouped as mobile and stationary, industrial and domestic, fires and smokes. Mobile sources such as cars, buses, trucks, ships, and aircrafts are known sources of PAHs as illustrated by the findings of Napier *et al.*, (2008) in Figure 1.3. PAHs from mobile sources stems from the combustion of the fuels, wear and tear of the parts. Industrial sources such as power generation, steelworks, coke, ovens, aluminium production, and cement kilns and oil refining are stationary sources. Domestic sources include combustion for heating and cooking especially solid fuel using coal and wood. Domestic sources are also stationary sources. Fires and smokes resulting from burning of vegetation in agricultural process, bushfires, grilling of food, or tobacco smoke result in the release of large amount of PAHs into the environment (Essumang *et al.*, 2006, Djinovic *et al.*, 2008, Ferguson, 2010).



**Figure 1.3: Derived Values for Estimated Annual Pollutant Emissions from Car Components in the UK (2003) (Napier *et al.*, 2008).**

## 1.5 SOURCE CHEMICAL FINGERPRINTING

Chemical fingerprinting is an aspect of environmental forensic investigation which involves analysis of contaminants and associated chemicals to provide source specific information. PAHs fingerprinting involves the determination of a number of quantitative diagnostic ratios of source and specific marker PAH compounds. Different finger printing methods have been used to determine the probable sources of PAHs and the common methods include:

### 1.5.1 Amount of a Particular PAH Compound

Huang *et al.*, 2005 used the amount of particular PAHs to apportion sources of PAHs. In general, the pyrogenic PAHs are composed of larger ring systems than the petrogenic PAHs. PAH compounds in a petrogenic exposure often contain one or more methyl- ethyl- or butyl- and sometimes higher alkyl groups on one or more of the aromatic carbons (Barranco *et al.*,

2003, Pampanin and Sydnes, 2013). Petrogenic PAHs include the unsubstituted parent and alkyl homologues of naphthalenes, fluorenes, phenanthrenes, dibenzothiophenes, and chrysenes where the alkyl homologues are more abundant than the parent PAH. Pyrogenic PAHs are generally represented by greater abundances of parent compounds and a predominance of the three to five ring PAHs such as anthracene and benzo[*a*]pyrene (Thorsen *et al.*, 2004). The concentration pattern of the C0–C4 alkylated PAH can be used as a diagnostic tool to determine the weathering status of the oil, as more highly substituted alkyl-PAHs tend to dominate after the oil is weathered (C0 < C1 < C2 < C3 < C4) (Saravanabhavan *et al.*, 2007).

### 1.5.2 Fossil Fuel Pollution Index (FFPI)

FFPI is a diagnostic ratio used to approximate the percentage of PAHs from fossil fuel, relative to the sum of PAHs in a sample (ASTM, 1990, Huang *et al.*, 2005, Dahle *et al.*, 2008). It has been used in source apportioning of PAHs. 
$$FFPI = \frac{[\text{naphthalene} + \text{phenanthrene} + 0.5 \times (\text{phenanthrene} + 2\text{-methylphenanthrene} + 1\text{-methylphenanthrene}) + \text{dibenzothiophene}]}{[\text{sum PAH} + 2\text{-methylphenanthrene} + 1\text{-methylphenanthrene}] \times 100}$$
 (Boehm and Farrington, 1984, Gao *et al.*, 2007).

### 1.5.3 PAH Abundance Ratios

PAHs show different abundance ratio depending on their origin though their distribution (Huang *et al.*, 2005). For example, Phenanthrene / Anthracene + phenanthrene, fluoranthene/Pyrene + pyrene and phenanthrene/anthracene ratios have been used to determine the source of PAHs. A phenanthrene/anthracene ratio lower than 10 and fluoranthene/fluoranthene + pyrene ratio higher than 0.4 are an indication of pyrolytic origin whereas values higher than 15 for the former and less than 0.4 for the latter ratio are attributed to petrogenic origin (Okedeyi *et al.*, 2012). These quantitative diagnostic ratios may be used to distinguish petrogenic PAHs including phenanthrene/anthracene, benzo(a)anthracene/chrysene, fluoranthene/pyrene, phenanthrene/(phenanthrene + anthracene) and indeno(1,2,3-cd)pyrene /indeno(1,2,3-cd)pyrene + benzo(ghi)perylene from other sources (Saravanabhavan *et al.*, 2007, Lorenzi *et al.*, 2011). The relative concentrations of these PAHs can give several important clues as shown in Table 1.3 but cannot be said to be 100% accurate (Saravanabhavan *et al.*, 2007, Anyakora *et al.*, 2011). Yunker *et al.*, (2002), in their review of indicative ratios stated that petrogenic sourced PAHs contain more alkylated PAHs than the parent homologue.

**Table 1.3 Ratio of PAHs and Suggested Source/Origin (Fagbote and Olanipekun, 2013)**

PAHs	Ratio of PAH	Inference
Phenanthrene / Anthracene	3	Motor vehicle exhaust
Phenanthrene / Anthracene	50	From mineral oil
Fluoranthene / Pyrene	1	Paralytic process
Fluoranthene / Pyrene	1	Pyrogenic process
Pyrene / Benzo(a)pyrene	1 to 50	Diesel fuel powered truck exhaust
Naphthalene / Phenanthrene	1	Pathogenic sources
Benzo(b)fluoranthene / Benzo(k)fluoranthene	0.8-1.1, 1.1-1.5, 2.5-2.9, 3.5-3.9	Wood combustion

## 1.6 RISK ASSESSMENT

Risk assessment is a science-based process that consists of effects and exposure analyses. It is the application of science for informed risk management program. Decisions in risk assessment include; no risk, moderate risk and significant risk. These indicate the magnitude of the risk and ways to mitigate the potential exposure (Pittinger *et al.*, 1997, Djinovic *et al.*, 2008, Santos *et al.*, 2011). Approaches to risk assessment include;

1. Comparison of contaminant concentration with limits and guideline values established by legislations.
2. The annual mean daily intake estimate ( $D_a$ ) based on estimated exposure
3. Calculation of BaP equivalence ( $BaP_{eq}$ ) also called the potency factor approach
4. Calculation of cancer risk estimate
5. Bioavailability and bioaccessibility assessment studies

### **1.6.1 Comparison of Contaminant Concentration with Limits and Guideline Values Established by Legislations.**

Countries have limits or guidelines for selected contaminants such as dioxins, PCBs, PAHs and heavy metals in water, soil, sediments and food. Even in regions with comparable economic development and a desire to enforce control measures, there can be different technical approaches to regulations. For example, USA uses a slightly different subset of

PAHs compared with the EU as seen in Table 1.1 possibly reflective of uncertainties in the evaluation of the underpinning scientific data.

In Europe, the maximum permitted levels of these contaminants for defined foodstuffs are based on toxicological evidence and as stated in Regulation EC (No.) 1881/2006. This regulation includes maxima for the sum of dioxins and dioxin like PCBs. With regard to PAHs, the maximum levels are defined for BaP only (used as an indicator substance) but there are instructions to monitor other PAHs with a view to including those in future legislation (EFSA, 2008). European Union Legislation (EUL) sets a maximum allowed concentration for BaP in different food products and BaP in liquid smoke flavouring of 20  $\mu\text{g}/\text{kg}$ . BaP recommended allowable daily intakes range from 0.04 to 0.42  $\mu\text{g}/\text{day}$  in Italy, whereas in Spain, the daily recommended allowable intakes for BaP and sum PAHs were 0.14 and 8.6  $\mu\text{g}/\text{day}$ , respectively (Al-Rashdan *et al.*, 2010).

The EU has recommended the analysis of three additional PAH next to BaP, namely, chrysene, benzo(a)anthracene, benzo(b)fluoranthene as biomarkers. Maximum limits exists for some PAHs which, if exceeded, should result in food being withdrawn from sale. Further more, there are action limits, which, if exceeded, should trigger an investigation by the industry or the authorities to identify sources and take necessary action in order to remove or control the product distribution (Rose *et al.*, 2009). A vivid example is as seen in Table 1.4 and 1.5 of regulatory limits of BaP in foods across Europe while there is a general limit for BaP, specific countries in Europe have limits for other PAHs as seen in Tables 1.4 and 1.5.

**Table 1. 4: Maximum Levels of BaP in food, Specified in European Commission Regulation (EC) 208/2005 (Wenzl *et al.*, 2006, FSAI, 2009, Kumari *et al.*, 2012).**

<b>Product</b>	<b>Maximum Level (ppb) wet weight</b>
Oil and fats intended for direct consumption or use as an ingredient in foods	2.0
Foods for infants and young children	1.0
Smoked meats and smoked meat products	5.0
Muscle meat of smoked fish and smoked fishery products excluding bivalve mollusks	5.0
Muscle meat of fish, other smoked fish	2.0
Crustaceans, cephalopods, other than smoked bivalve Molluscs	5
Bivalve mollusks	10.0
Infant formulae and follow on formulae including infant milk and follow on milk	1.0
Dietary foods for special medical purposes intended specially for infants	1.0
Processed cereal based foods and baby food for infants	1.0
Cocoa butter	5

**Table 1.5: Overview of Maximum Limits for PAHs in Certain Types of Food, Set in Certain EU Member States Before Commission Regulation (EC)208/2005 Came into Force**

Country	Food type	PAH monitored	Limits (µg/kg)
Czech Republic	Meat products	BaA, BbF, BkF, DhA,DhP,DiP,IcP,CHR	3.0
	Roasted coffee, cereals, fruits, vegetables, Fats and oils.	BaA, BbF, BkF, DhA,DhP,DiP,IcP,CHR	2.0
	Fish, Spirits, flavourings	BaA, BbF, BkF, DhA,DhP,DiP,IcP,CHR	1.0
	Beverages, beer, wine, tea	BaA, BbF, BkF, DhA,DhP,DiP,IcP,CHR	0.5
Slovak Republic	Smoked meat products and non smoked fish products.	BaP	1.0
	Smoked fish products		2.0
	Oils and fats of plant origin		5.0
	Other foodstuffs of plant origin		10.0
Italy	Olive pomace oil and refined olive pomace oil	BaP, BeP, BaA, BbF, BkF, DgP, IcP	2.0 each
		Sum of above	5.0
Germany	Cheese and cheese products, smoked or containing smoke flavourings, cheese products manufactured by using smoked or flavoured food ingredients.	BaP	1.0
	Smoked meat and meat products. Meat products, partly containing smoked food ingredients.		1.0
	Food ready for consumption containing		(Maximum)

	food flavourings, with the exception of food treated with freshly produced smoke.		0.03 may be added by adding flavourings
Poland	Smoke flavourings	BaP	(Maximum) 0.03 may be added by adding flavourings
Belgium	Smoked meat and meat preparations	BaP	2.0
Spain	Olive pomace oil	BaA, BbF, BkF, DhA, IcP, BaP, BeP, BgP Sum of above	2.0 each 5.0
Greece	Olive pomace oil	BaA, BbF, BkF, DhA, IcP, BaP, BeP, BgP Sum of above	2.0 each 5.0
Sweden	Olive pomace oil	BaA, BbF, BkF, DhA, IcP, BaP, BeP, BgP Sum of above	2.0 each 5.0

BaP: benzo[a]pyrene; BbF: Benzo(b)fluoranthene; BkF: Benzo(k)fluoranthene; BeP: benzo[e]pyrene; BgP : benzo[g,h,i]perylene; DiP: Dibenzo(a,i)pyrene; DhA : Dibenzo(a,h)anthracene; DgP: dibenzo[g,h,i]pyrene; IcP: Indeno(1,2,3-cd)pyrene (Wenzl *et al.*, 2006)

Some guideline values also exist for PAHs in air as seen in Table 1.6. According to EU directives, the concentrations of PAHs in the air should be constantly monitored. Monitoring of ambient air is required in member states by the European Commission's Fourth Air Quality Daughter Directive, with a target value of 1 ng/m<sup>3</sup> being placed on the allowable annual average concentration of BaP the chosen 'marker' PAH at any given monitoring station (Brown and Brown, 2012). However, UK Expert Panel on Air Quality Standards in 1999 recommended a standard of 0.25 ng/m<sup>3</sup> expressed in terms of the concentration of BaP as annual average (EU, 2001).

**Table 1. 6: Work Place Standards and Regulations for PAHs (ATSDR, 1995, USEPA, 2007a, ATSDR, 2009)**

Agency	Focus	Level	Comments
American Conference of Governmental Industrial Hygienists	Air: Work Place	0.2mg/m <sup>3</sup> for benzene -soluble coal tar pitch fraction	Advisory :*TLV (8-hour *TWA)
National Institute for Occupational Safety and Health Administration (NIOHA)	Air: Work Place	0.2mg/m <sup>3</sup> for coal tar pitch fraction volatile agents	Advisory: *REL (8 hour *TWA)
Occupational Safety and Health Administration (OSHA)	Air: Work Place	0.2mg/m <sup>3</sup> for benzene -soluble coal tar pitch fraction	Regulation: (benzene soluble fraction of coal tar volatiles) *PEL (8-hour workday)
U.S Environmental Protection Agency (USEPA)	Water	0.0001mg/L	*MCL for benzo(a)anthracene
		0.0002 mg/L	*MCL for benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene
		0.0003 mg/L	*MCL for dibenzo(a,h)anthracene
		0.0004 mg/L	*MCL for indeno(1,2,3-c,d)pyrene

\*TLV: threshold limit value, \*REL (recommended exposure limit): The recommended airborne exposure limit for coal tar pitch volatiles (cyclohexane- extractable fraction) averaged over 10 hour work shift, \*PEL (permissible exposure limit): The legal airborne permissible exposure limit (PEL) for coal tar pitch volatiles (benzene soluble fraction) averaged over 8 hour work shift, \*MCL: maximum contaminant level,\*TWA :Time weighted average.

There are no general soil guideline values (SGVs) for PAHs in soil (Environment Agency, 2009), however some other documents establish the generic assessment criteria between 0.83-2.1 mg/kg for BaP in residential soils and allotments soils with organic matter ranging from 1 to 6 %. In countries such as Denmark and Belgium, the threshold level for a unique PAH is generally fixed at 1 mg/kg. This is confirmed by the Dutch environmental regulation which estimates 1 mg/kg as a level where there is a potential risk and a value of 40 mg/kg for the sum of 10 PAHs. In the UK, the threshold value for sum PAH content was fixed at 50 mg/kg for residential and domestic areas, but those values have not been considered to be up-to-date (Lorenzi, 2011). Maximum acceptable limits for soils are SGVs used in the

legislation of contaminants in soils for specific purposes such as residential, allotment and commercial/industrial premises. Examples of some SGV are as seen in Table 1.7.

**Table 1. 7: Maximum Concentration (ng/g) (also called SGV) of PAHs Allowed in Soils for Different uses : data from the Catalonia and Canadian legislations (Nadal *et al.*, 2004).**

	Catalonia		Canada		
	Non-industrial	Industrial	Agriculture	Residential	Commercial/ Industrial
Naphthalene	5000	15,000	100	600	22,000
Acenaphthylene	–	–	–	–	–
Acenaphthene	–	–	–	–	–
Fluorene	–	–	–	–	–
Phenanthrene	5000	10,000	100	5000	50,000
Anthracene	100,000	1,300,000	–	–	–
Fluoranthene	15,000	1,000,000	–	–	–
Pyrene	–	–	100	10,000	100,000
Chrysene	–	–	–	–	–
Benzo[a]anthracene	10,000	535,000	100	1000	10,000
Benzo[k]fluoranthene	50,000	535,000	100	1000	10,000
Benzo[b]fluoranthene	–	–	100	1000	10,000
Benzo[a]pyrene	80	7,500	100	700	700
Indeno[123-cd]pyrene	50,000	535,000	100	1000	10,000
Dibenzo[ah]anthracene	–	–	100	1000	10,000
Benzo[ghi]perylene	–	–	–	–	–

Some other guideline values include the effects range low (ERL) and the effects range median (ERM) values. The ERL (the concentrations of PAHs above which environmental threat can be caused) and ERM (the concentration of PAHs above which biological impairment to organisms in the sediment can be caused) have been used to assess the aquatic sediment with a ranking of low to high impact values. Long *et al.*, (1995) and Xu *et al.*, (2007) noted that correlation between impacts and chemical concentrations were fairly good for individual PAHs and sum PAH, so that ERL and ERM values are useful in addressing quality issues and provide qualitative guidelines on what needs to be done to effectively protect the environment. The ERL and ERM values are intended to define chemical

concentration ranges that are rarely, occasionally or frequently associated with adverse biological effects (Xu *et al.*, 2007). Some ERM and ERL values are as shown in Table 1.8.

**Table 1. 8: Standard Pollution Criteria of PAH Components for Soil Matrices (Xu *et al.*, 2007).**

<b>16 priority USEPA PAHs</b>	<b>ERL (µg/g)</b>	<b>ERM (µg/g)</b>
Naphthalene	0.160	2.100
Acenaphthylene	0.044	0.640
Acenaphthene	0.016	0.500
Fluorene	0.019	0.540
Phenanthrene	0.240	1.500
Anthracene	0.853	1.100
Fluoranthene	0.600	5.100
Pyrene	0.665	2.600
Benz(a)anthracene	0.261	1.600
Chrysene	0.384	2.800
Benzo(b)fluoranthene	NA	NA
Benzo(k)fluoranthene	NA	NA
Benzo(a)pyrene	0.430	1.600
Indeno(1,2,3-cd)pyrene	NA	NA
Dibenz(a,h)anthracene	0.0634	0.260
Benzo(g,h,i)perylene	NA	NA
<b>Sum PAHs</b>	<b>4.000</b>	<b>44.792</b>

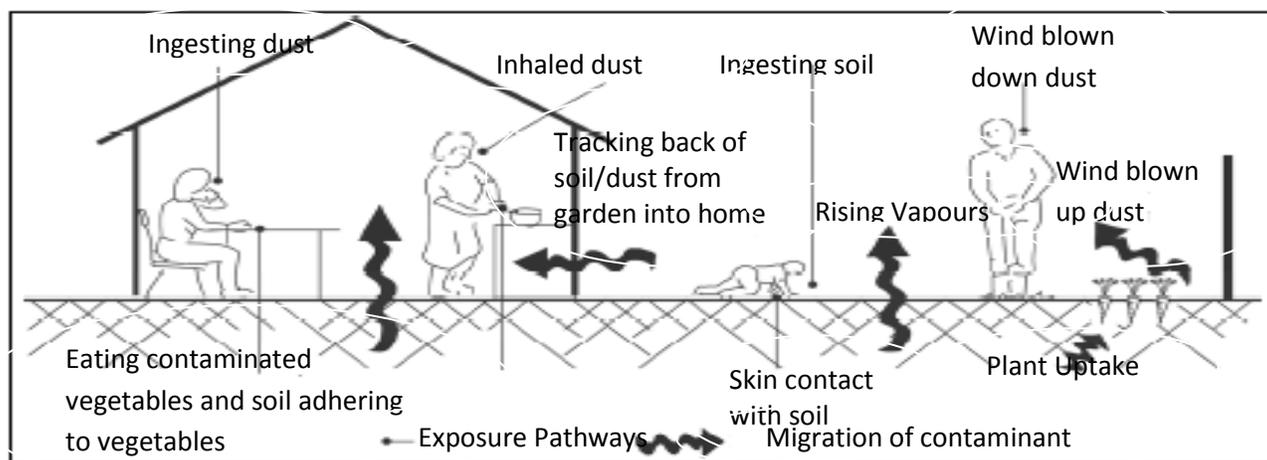
### **1.6.2 Annual Mean Daily Intake Estimate ( $D_a$ ) Based on Estimated Exposure**

In the estimation of annual mean daily intake, different ways of exposure from chemicals present in environmental matrices to human living, working and/or playing on contaminated land, over significant period of time is considered (Environment Agency, 2009). In this approach, information on the toxicity of soil contaminants with estimates of potential exposure by adults and children, over a long period of time and to predict the amount of

contaminant to which they might be exposed based on a given soil contaminant concentration is utilized for estimation. By comparing predicted exposure with health criteria values (HCV) on tolerable or acceptable contaminant intakes, soil guideline values (SGV) are generated that establish a contaminant concentration in soil that is protective of human health (DEFRA, 2002). Considering the ingestion of soil, the risk is currently based on the possible ingestion of 100 mg/day of soil or dust by a children aged between 1 and 6 years. This value has been established and used by the USEPA and the Netherlands National Institute for Public Health and the Environment (Dutch: Rijksinstituut voor Volksgezondheid en Milieu or simply RIVM) (Oomen *et al.*, 2003, USEPA, 2008a, Kim *et al.*, 2009) and these values are generally used nowadays when considering the study of the possible ingestion of contaminants via soil or dust. 150 or 200 mg/day of soil or dust ingested per day represent a realistic worst case scenario. The Contaminated Land Exposure Assessment supplies a software program where risk assessors can enter contaminant levels, estimates and assumptions about the factors that influence chemical exposure on a site (USEPA, 2008b). Estimates of exposure based on average annual intake dose (i.e. the amount of contaminant that can be in contact with the human, defined by mg/kg, Body weight (bw)/day), rather than on the uptake (i.e. the dose of contaminant that can potentially reach the systematic circulation mg/kg) (Figure 1.4).

The level where there is no appreciable risk to human health is the threshold and is called the Tolerable Daily Intake (TDI) and is expressed on a body weight basis (mg/kg bw/day). When the intake is from soil, it is called Tolerable Daily Soil Intake (TDSI). The non-

threshold substances will involve risks at any level of exposure and to regulate non-threshold substance in soil, a minimal risk to human health level is used. In this case an Index Dose (ID) will be required to define the risk and it is soil specific. The Mean Daily Intake (MDI) can also be used when other matrices than soils are considered for the exposition of humans, such as food, water, and air. The MDI is defined in units of mass per day ( $\mu\text{g}/\text{day}$ ) (Lorenzi, 2011, DEFRA, 2002)



**Figure 1.4: Illustration of the Potential Exposure Pathways in the CLEA Model (DEFRA, 2002, Environment Agency, 2009)**

### 1.6.3 Potency Factors Approach in Risks Assessment

Assessment of human exposure to environmental chemicals such as PAHs can be also accomplished through potency factor approach. The potency factors approach for calculating the potential health risks from PAHs with the characteristic “Bay-K region,” a structural distinction that defers carcinogenic properties to BaP and the other carcinogenic PAHs, has

been developed by USEPA (NDEP, 2009). Out of all the known potentially carcinogenic PAHs, BaP has been characterized as the most potent of the carcinogenic PAH compounds and it is the only PAH for which its toxicological potency is labelled (Huang *et al.*, 2005, NDEP, 2009). Hence, the toxicities of other PAHs are determined in relation to BaP and expressed as toxicity equivalence factor (TEF). TEF is used to determine BaP equivalent dose ( $BaP_{eq}$ ).

#### **1.6.4 Cancer Risk Estimation**

Theoretically, cancer risk is defined as the number of additional cases of cancer in a population due to exposure to a toxic substance during a lifetime of exposure. A cancer risk of  $1 \times 10^{-5}$ , for example, refers to one additional case of cancer per one hundred thousand individuals (ODH, 2011). With regard to the carcinogenic risk associated with the dietary intake of PAHs, on the basis of animal carcinogenicity data, an acceptable daily intake of BaP was computed as the quantity that would be associated with a  $1 \times 10^{-6}$  which is one additional case or risk of cancer in one million individuals for an adult of 70 kg (Falco *et al.*, 2003). The estimated theoretical cancer risk from exposure to contaminants is calculated by multiplying the estimated exposure dose by the Cancer Slope Factor (CSF) for a suspected or known carcinogenic substance. Due to the conservative safety factors used to calculate the CSFs, using these values provides only a theoretical estimate of risk with the true or actual risk being unknown and could be as low as zero (ODH, 2011).

### **1.6.5 Bioavailability and Bio-Accessibility Approach**

Bioavailability and bio-accessibility are often used as the key indicator of potential risk that chemicals pose to the environment and human health (Stokes *et al.*, 2006). Thus measuring the sum concentration of organic contaminants present at contaminated sites may lead to over conservative risk assessments as only the bio available fractions can cause toxic effects.

### **1.7 STATEMENT OF PROBLEM**

Nigeria is faced with enormous environmental and agricultural challenges which threaten the livelihood of the tens of millions of its teeming population. One of the environmental challenges include contaminated lands as a result of oil (petroleum) spills especially in the Niger Delta area of the country. Examples are spills of petroleum refined products during loading into tankers for distribution, petroleum products leakage from distribution tanks during accident or due to rust of tank and disposed lubricating oils in soils around automobile workshops among other (Anoliefo *et al.*, 2006). PAHs found in petroleum products have been found in many contaminated sites. As a result of PAHs persistence, they can be transferred to water bodies, soil and plant surfaces. From the, soil PAHs can be taken up by plants and food crops through their roots (Li *et al.*, 2009). They bio-accumulate in plants and other living organisms reaching levels that cause toxicological effects (Manoli *et al.*, 2004).

One of the other major routes of human exposure to PAHs in non-smoking people is food (Wang *et al.*, 2011a). PAHs have been found in different food products, such as dairy products, vegetables, fruits, oils, coffee, tea, cereals, smoked fishes, smoked meat and roasted food stuffs. Certain food processing, have been found to contribute/increase the levels of

PAHs in food. Therefore the analysis of PAHs in food is a matter of concern (Plaza-Bolaños *et al.*, 2010). These have far reaching agricultural, health and environmental effects such as are cancers and so on. Cancer has become a major source of morbidity and mortality globally. A sum of 1,660,290 new cancer cases and 580,350 cancer deaths were projected to occur in the United States in 2013 by Siegel *et al.*, (2013). In Nigeria, 182.5 per 100,000 male admissions were reported by Badmus *et al.*, (2010) as the hospital prevalence rate of prostate cancer from their study in South Western Nigeria. Breast cancer age standardized incidence rate at the Ibadan based cancer registry, Nigeria was found to be 52.0 per 100,000 and 64.6 per 100,000 in Abuja cancer based registry, Nigeria. For cervical cancer, it was 36.0 per 100,000 and 30.3 per 100,000 respectively for Ibadan and Abuja (Jedy-Agba *et al.*, 2012).

Human health, agricultural development and the ecosystems are all at risk unless water and land systems are effectively managed. Fruits and vegetables accumulation of PAHs may cause an indirect exposure pathway to human through consumption. The identification, quantification and risk assessment of organic contaminants in different matrices have been traditionally achieved using exhaustive chemical extraction techniques, which determine the sum amount of PAHs. Examples of exhaustive chemical extraction techniques include; Soxhlet, microwave and ultrasonic extractions among others. However, experimental evidence has clearly demonstrated that such measurements are not representative of contaminants availability to human guts. Risk can be estimated by determining their estimated cancer risk (ER), estimated annual daily dose ( $D_a$ ), BaP equivalent dose ( $BaP_{eq}$ ), mean daily intake (MDI)

and bioaccessibility assessment and comparison with standards/limits (Davoli *et al.*, 2010, Yang *et al.*, 2012).

Bioavailability is the fraction of a chemical that can be absorbed, by the gastro-intestinal tract system, pulmonary system and the skin. It is also defined as the fraction of a compound that is released from the gastro-intestinal tract and thus becomes available for internal absorption i.e enters the blood stream (Oomen *et al.*, 2003) while bioaccessibility sometimes called “bioaccessible fraction” is the fraction of the sum amount of a chemical present in ingested food, water, or ingested soil and sediment particles, that at maximum can be released during digestion. There are some *in vivo* assessments but these have limitation because of the ethical issues, disparities between human and animal absorption system. The *in vitro* assessment overcomes these limitations (Intawongse and Dean, 2006). Many studies have been undertaken on pharmacokinetic uptake of pollutants but almost no studies have investigated organic contaminant bioavailability/ bioaccessibility in soils (Ng *et al.*, 2010).

In Nigeria, limited data are available in the literature on the concentration and distribution pattern of PAHs in soils and foods. From literature search, the study of PAH level in roasted Nigerian food and smoked fishes have also not been reported to the best knowledge of the researcher. Studies on the uptake of PAHs by edible Nigerian vegetables from contaminated soils are not also available to the best knowledge of the researcher. In-depth risk assessments

of PAHs in Nigerian soils and foods have not been undertaken to the best knowledge of the researcher.

## **1.8 SIGNIFICANCE OF STUDY**

PAHs occur naturally in the environment, due to their formation during forest fires and volcanic eruptions. However, the largest amount of PAHs is released into the environment by human activities such as cracking of crude petroleum, burning of fossil fuels, oil spills during dispensing, disposal of used lubricating oil, incineration of industrial and domestic waste, heating, cigarettes, fireplaces, *etc.* They are also found in roasted and smoked foods. These pollutants are semi-volatile organic compounds and can be deposited on water bodies, soil and plant surfaces (Bakker *et al.*, 2000). From the soil they can be taken up by plants and food crops through their roots. Roasted foods which contain PAHs, fruits and vegetables bioaccumulated PAHs, may cause indirect exposure to humans and many of which are carcinogens or mutagens (Li *et al.*, 2009). The concentration of PAHs in the environment and food are sources concern to regulators. This requires accurate quantification.

In view of the need for sensitive, selective and more specific method, an optimised method for the quantification of trace level PAHs by gas chromatograph will be provided. The study will for the first time in Nigeria, determine the concentration of PAHs in roasted foods (yam, corn and plantain) and smoked fishes. The risk associated with food and fish

samples will be assessed. PAH ingestion can also occur through plant consumption, inadvertent ingestion of soil on hands or food items, mouthing of objects, ingestion of dust (involuntary ingestion), or through intentional ingestion (pica behaviours). This research will also study PAHs in sites of various activities, uptake of PAHs by edible plants and bioassessable PAHs to human guts. Therefore a comprehensive risk assessment of PAHs to humans on sites of different activities for the first time in Nigeria soils will be investigated.

### **1.9 AIMS AND OBJECTIVES**

The research aims at determining and assessing the risk associated with the levels of PAHs in selected smoked fishes, roasted food and the soil samples in Lagos, Nigeria.

The objectives of this study include:

1. Validation/optimisation of analytical method for quantification of PAHs in complex matrices.
2. Quantification and risk assessment of the PAHs level in smoked fishes, roasted foods (yam, corn and plantain).
3. Quantification of the sum and bioassessable PAHs in soils from sites of different anthropogenic activities using exhaustive and *in vitro* techniques respectively.
4. Utilization of other risk assessments approaches to assess human exposure to PAHs from sites of different anthropogenic activities.
5. Determination of the level of uptake of PAHs from soil by some tropical edible plants.

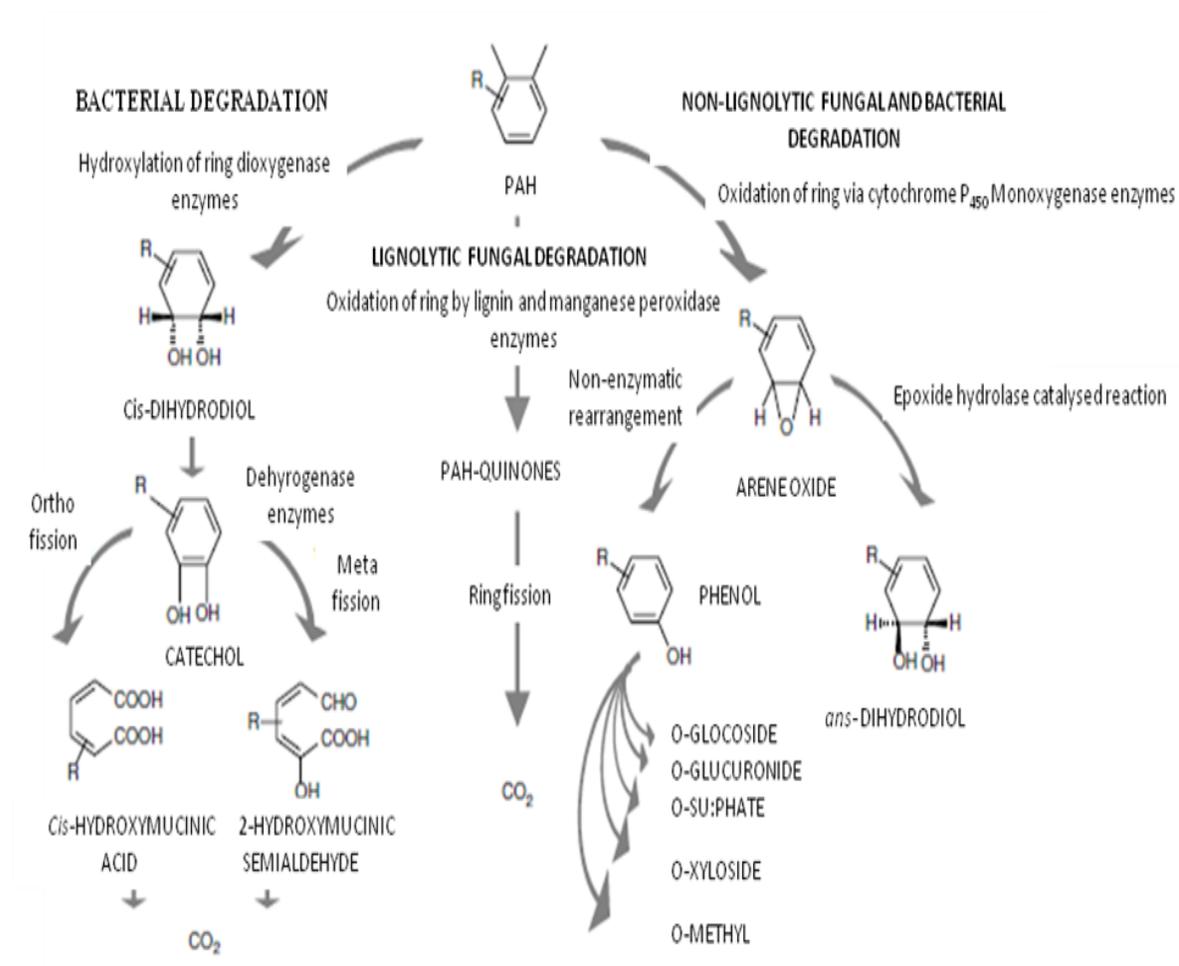
## CHAPTER TWO

### 2.0 LITERATURE REVIEW

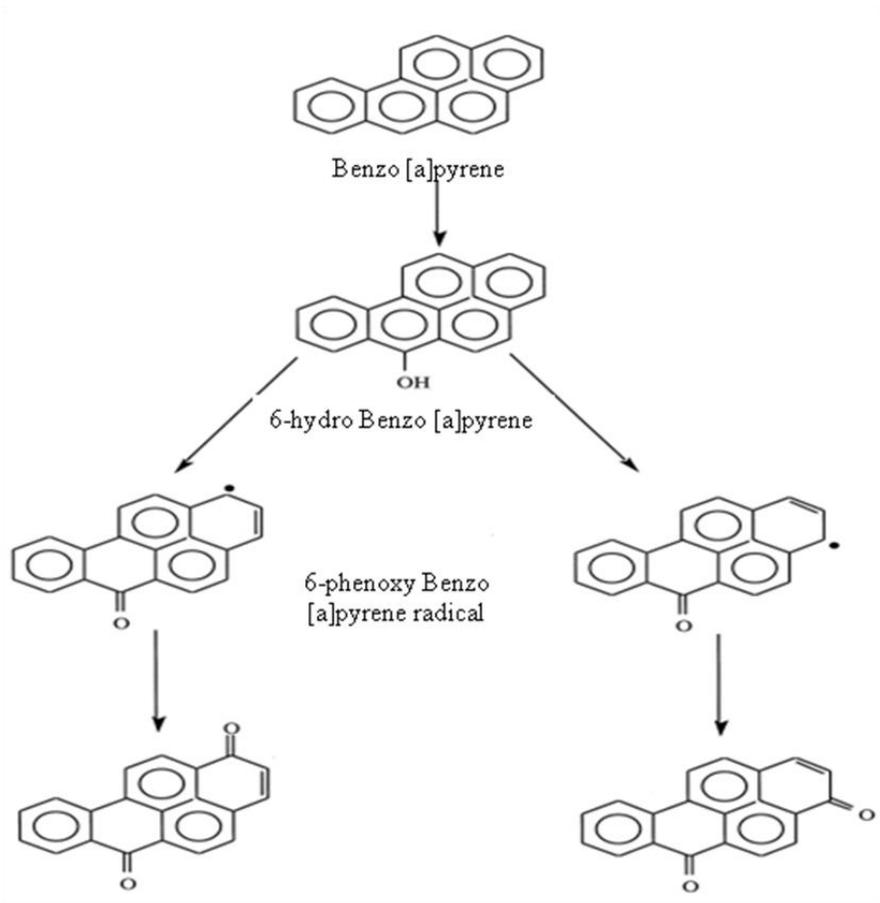
#### 2.1 PAHs IN THE ENVIRONMENT

PAHs, once produced from source have three possible fates in the environment:

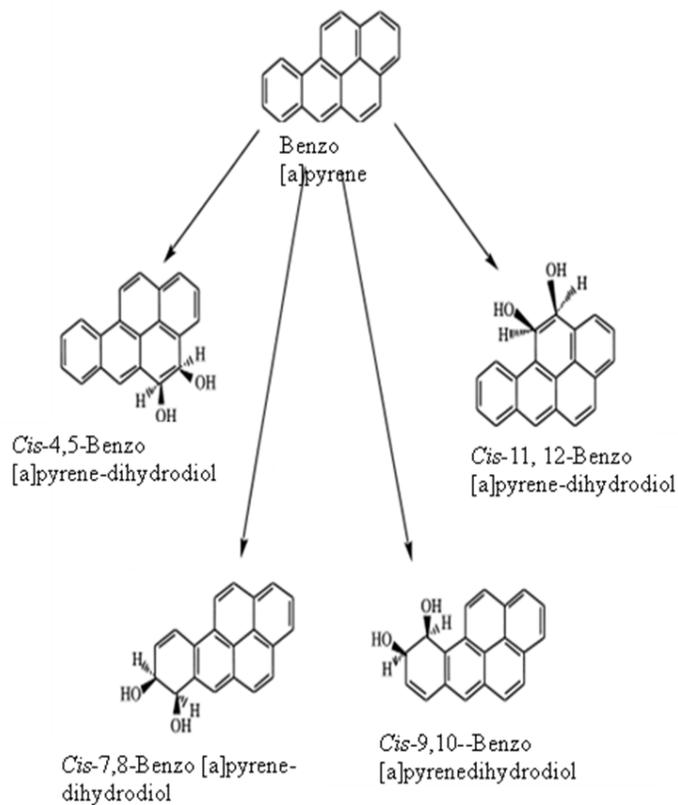
1. All or a portion might remain unchanged in their present location (Persistence) (Sharak-genthner *et al.*, 1997, Conte *et al.*, 2001).
2. All or a portion might be carried elsewhere by transport processes (Björseth and Lunde, 1967, Teran *et al.*, 2012, Long *et al.*, 2012, Schwienteka *et al.*, 2013) due to a combination of their physicochemical properties as in **Section 1.2** (Nagy *et al.*, 2012). This was the basis for the establishment of a task force on POPs under the framework of the convention on long-range trans-boundary air pollution (CLRTAP) of UNECE initiatives on POPs in 1998 when it first began (Lerche *et al.*, 2002). The transportation processes include:
  - a. Movement to other phases (air, water, soil or plant) by volatilization, dissolution, adsorption and precipitation (Schwienteka *et al.*, 2013).
  - b. Movement within a phase under gravity, diffusion and advection.
3. All or a portion might be transformed into other chemical species by natural chemical and biological processes. Possibilities are:
  - a. Biodegradation (aerobic and anaerobic): Pollutants are altered structurally by biological Processes, mainly the metabolism of microorganisms present in aquatic and soil environments (Haritash and Kaushik, 2009, Dandie *et al.*, 2010). Example is shown in Figure 2.1. and Figure 2.2



**Figure 2.1-Degradation of PAH Showing Three Main Pathways by Fungi and Bacteria (Bamforth and Singleton, 2005).**



I



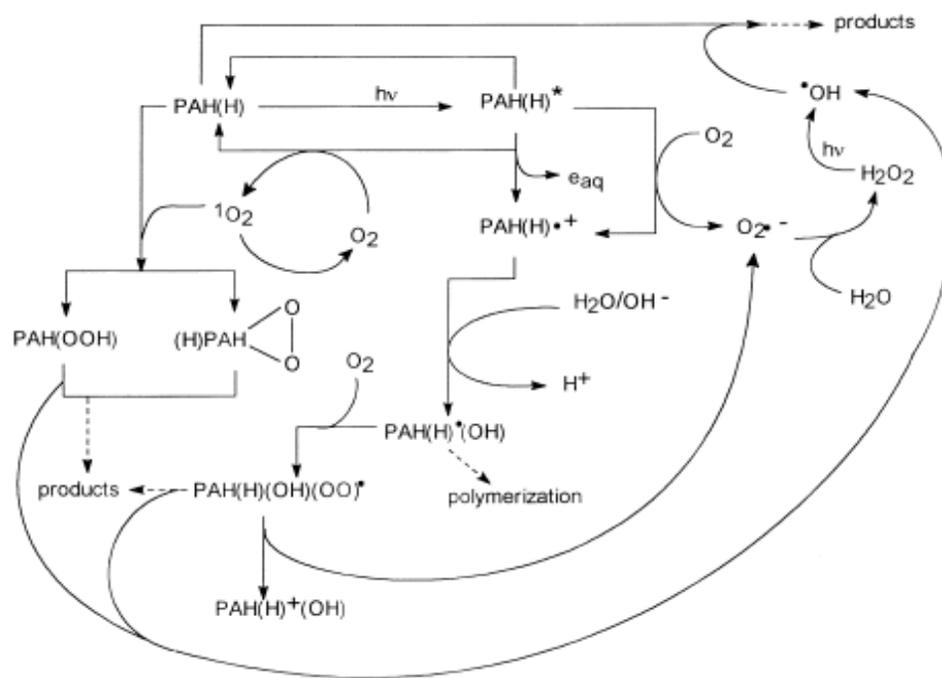
II

**2.2: I-BaP Quinones Produced from the Fungal Oxidation of BaP, II-Algal Transformation Products of Benzo[a]pyrene (Juhasz and Naidu, 2000)**

- b. Bioaccumulation: Pollutants accumulate in plant and animal tissues to higher concentrations than in their original environmental locations (Meudec *et al.*, 2005).
- c. Weathering: Pollutants undergo a series of environmental non-biological chemical changes by processes such as oxidation-reduction, acid-base, hydration, hydrolysis, complexation, and photolysis reactions (Alexander *et al.*, 2008, Nagy *et al.*, 2012). PAHs can undergo photodecomposition when exposed to ultraviolet light from solar radiation (Srogi, 2007).

### **2.1.1 PAHs in Air**

After the formation of PAHs, distribution occurs between the gaseous and particulate phases of the atmosphere according to their vapour pressures. In the atmosphere, they can be dispersed over large areas and transferred by dry and wet deposition to soil or other environmental surfaces, such as water bodies and vegetation. PAHs can return into the atmosphere by volatilization or even by soil or dust particle re suspension (Pereira-Netto *et al.*, 2004). In the atmosphere, PAHs react with pollutants such as ozone, nitrogen oxides and sulfur dioxide, yielding diones, nitro- and dinitro- PAHs, and sulfonic acids respectively (Srogi, 2007). Example is as seen in Figure 2.3.



**Figure 2. 3: Scheme of PAHs Photo-Degradation Pathways in the O<sub>2</sub>/H<sub>2</sub>O System (Miller and Olejnik, 2001).**

PAHs are found in the ambient air which include indoor air, dusts at home and in the workplace (Ishii *et al.*, 2000, Bohlin *et al.*, 2007). Additional contributions to ambient air levels arise from tobacco smoking while the use of heating sources can increase PAH concentrations in indoor air. Natural sources such as forest fires and volcanic eruptions are less important. Naphthalene because of its lowest boiling point and highest volatility of all the PAHs occurs almost completely in the vapour-phase. Therefore, its surface deposition will be relatively small in comparison with other PAHs. The observed high levels of naphthalene in domestic environments might be related to the popular use of camphor balls in the wardrobe (Srogi, 2007). The highest concentrations and risk associated with human exposure to atmospheric PAHs can be found in the urban environment whilst PAHs have

been turned into omnipresent environmental pollutants due to the increasing vehicular traffic and industrialization considering the population density. Although these lighter compounds have lower carcinogenic/mutagenic properties, they are the most abundant in the urban atmosphere and can react with other pollutants to form more toxic derivatives. The health consequence of air pollution demonstrates a strong association between air pollution, asthma and chronic obstructive pulmonary diseases (Fromme *et al.*, 2004).

According to EU directives, the concentrations of PAHs in the air should be constantly monitored. Monitoring of ambient air is required in member states by the European Commission's Fourth Air Quality Directive, with a target value of  $1 \text{ ng m}^{-3}$  being placed on the allowable annual average concentration of BaP which is the chosen 'marker' PAH at any given monitoring station (Brown and Brown, 2012). Contribution of PAH sources to the atmosphere will depend on a number of factors, including the emission rate of sources, geography and local weather conditions. PAHs with two or three benzene rings exist in the vapour phase, while PAHs with more than five rings are primarily in the particulate phase; the latter then move into the soil, water, sediments and vegetation through dry or wet deposition. Atmospheric PAHs are preferentially bound to small particles. PAHs should not be present in the air, at all or their concentrations should be at the lowest possible. Yet more than 500 PAHs have been identified in the air however routine measurements include only up to 20 PAHs. BaP is the most commonly measured PAH and it is used as an indicator of carcinogenic hazard in polluted environments (Šišović *et al.*, 2012).

### 2.1.2 PAHs in Sediments and Water

PAHs are introduced to aquatic environments from a variety of anthropogenic sources. Marine environment, particularly in areas like harbours, marinas, dockyards, estuaries and other shallow coastal areas are exposed to anthropogenic inputs of PAHs from petroleum products, accidental oil spills, discharge from routine tanker operations, municipal and urban runoff, off-shore oil exploitation, natural gas flaring and natural oil seeps. Hence marine sediments are one of the most important reservoirs of PAHs (Sojinua *et al.*, 2009, Barakat *et al.*, 2011a, Barakat *et al.*, 2011b). PAHs, because of their low solubility, high hydrophobicity and their strong affinity for organic carbon in particulate matter entering the aquatic environment preferentially adsorb onto particulates and finally accumulate in the sediments. (Karlsson and Viklander, 2008, Barakat *et al.*, 2011a). Therefore while their concentration in water is generally low concentrations of PAHs in river sediments are generally much higher. Atmospheric deposition is considered to be an important route of PAHs to surface waters. PAHs are transported through the atmosphere over long distances entering into the aquatic environment by wet and dry deposition. The most important processes contributing to the degradation of PAHs in water are photo-oxidation, chemical oxidation and bio-degradation by aquatic micro organisms. In sediments, microbial metabolism is the major process for degradation of PAHs. Degradation of PAHs in sediments is generally slow, particularly for the higher molecular weight PAHs and when sediments are anaerobic. It is conceivable that re-suspension of sediments during the flood season and from dredging activities may release PAHs back into the overlaying water. In addition, PAHs are accumulated in aquatic plants, fish, invertebrates and many animals.

Some are able to metabolize and eliminate these compounds (Nagy *et al.*, 2012). Table 2.1 shows the concentration of PAHs in sediments from various studies.

**Table 2. 1: PAHs Concentration in Some Sediment Samples**

<b>Location</b>	<b>Number of PAHs Determined</b>	<b>Concentration (ng/g)</b>	<b>Source</b>
Arcachon Bay	16	32–4120	Baumard <i>et al.</i> , (1998)
Port Vendres, France	14	145–6940	Baumard <i>et al.</i> , (1998 )
Biscay Bay	15	20–5159	Tronczynsky <i>et al.</i> , (2004)
Spain Barcelona harbour	14	1740–8420	Baumard <i>et al.</i> , (1998 )
Santander Bay	16	20–344,600	Viguri <i>et al.</i> , (2002)
Barcelona harbour	16	300–10,320	Martínez-Lladó <i>et al.</i> , (2007)
Urdaibai Estuary	16	0.7–136.5	Cortazar <i>et al.</i> , (2008)
Santander Bay	16	nd – 4900	Antizar-Ladislao (2009)
Italy Chioggia, Ancona	16	24.1–455.5	Magi <i>et al.</i> , ( 2002)
Coastal areas	16	2875–26,247	Bertolotto <i>et al.</i> , (2003 )
Porto Torres, Sardinia	16	70–1210	De Luca <i>et al.</i> , (2004)
Olbia harbor, Sardinia	16	160–770	De Luca <i>et al.</i> , (2005 )
Naples harbour	16	9–31,774	Sprovieri <i>et al.</i> , (2007)
Marine protected areas	16	0.71 – 1550	Perra <i>et al.</i> , ( 2011)
Croatia Rijeka Bay area	16	32–13,681	Alebic-Juretic (2011)
Turkey Black Sea coast	17	10–530	Readman <i>et al.</i> , (2002)
Morocco Mediterranean coast	16	10–550	Pavoni <i>et al.</i> , (2001)
Egypt Mediterranean coast	16	88–6338	El Nemr <i>et al.</i> , (2007 )
Abu Qir Bay, Egypt	16	n.d.-2660	Khairy <i>et al.</i> , (2009)
Mediterranean Coastal Eygpt	16	3.5–14,100	Barakat <i>et al.</i> , (2011b)
Lagos Lagoon	16	880 -1000	Oluseyi (2009)

### **2.1.3 PAHs in Soil**

Soil is a vital natural resource on which human lives depend (Maliszewska-Kordybach *et al.*, 2009) and it is also the main sink for most organic contaminants (Maliszewska-Kordybach *et al.*, 2012). With the rapid development of economies, modernization of industry and urbanization, pollutants such as heavy metals, PAHs, pesticides, veterinary drugs, feed additives and other emerging pollutants have been released into the soils through many paths (Cao *et al.*, 2012).

Atmospheric deposition also contributes to high amounts of PAHs in soils. Other sources include disposal of waste oils, crankcase oil, runoffs, tyre shredding, accidental fuel spills and leakages as well as industrial wastewaters. PAHs emitted into the atmosphere are largely adsorbed on particulate matter in the atmosphere and are deposited into aquatic and soil environments by fallout (wet in form of rain and snow or dry deposition processes such as dry particles or gases from atmospheric turbulence, molecular diffusion or gravitational settling) (Lau *et al.*, 2012), a process which subsequently leads to water and soil pollution. The lipophilic/hydrophobic nature of PAHs and the physico-chemical properties of soils make PAHs more of a contaminant of soil rather than water. Consequently, soils and sediments act as strong environmental sinks for PAHs as they become rapidly adsorbed onto soil particles, in particular to the humic materials associated with soil organic matter (Alcántara *et al.*, 2009, Maliszewska-Kordybach *et al.*, 2012).

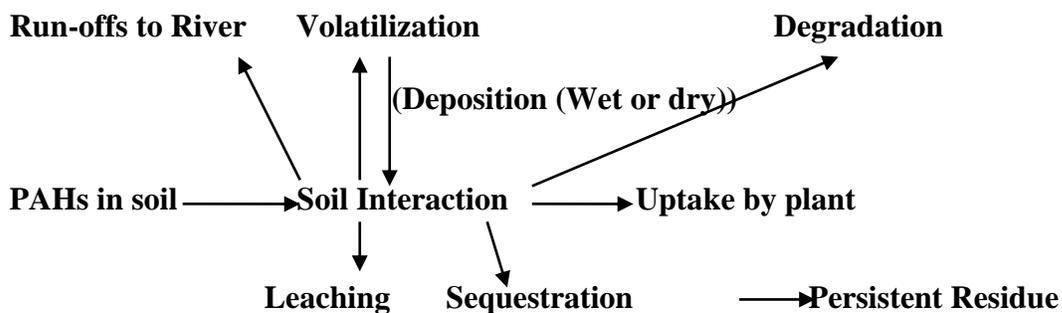
The amount of PAH adsorption has been found to be dependent on organic carbon, hydrophobicity of soil organics and length of time for which the PAH has been resident in soil. Soil texture also plays a role in soil adsorption of PAHs. In fact, it has been reported that higher PAH recovery efficiencies were obtained from coarse-textured soils, whereas recovery was lower in fine-textured soils due to the increase of soil-reactive surfaces which facilitate PAH adsorption (Conte *et al.*, 2001). The effect of adsorption generally increases as the number of benzene rings in the PAH-molecule increases (Khan *et al.*, 2008a). The half-life of PAHs in soil (time after which half of the initial PAH amount will undergo degradation) has been found to vary from around a dozen to several hundred days, to even several years (Kaszubkiewicz *et al.*, 2010).

PAHs in soil can be dispersed as runoff and street dust. Hence soil is both a sink and a source of PAHs through volatilization of PAHs and street dust (Netto *et al.*, 2006). Street dust which is also soils can be considered as sink, but is also a secondary source for several substance classes, including PAHs to the atmosphere and other environmental compartments (Netto *et al.*, 2006, Jiang *et al.*, 2011). The levels of PAHs in soils in urban areas are approximately a factor of ten times higher than those in rural areas. PAH concentrations in the immediate surroundings of point sources are usually increased compared to other places. For example, high concentrations in soils have been found close to a large scale chemical fire involving ten thousand tonnes of polypropylene, soils close to highways and complex road intercepts have higher concentrations of PAHs similar to soils in urban areas (Bakker *et al.*, 2000, Khan *et al.*, 2005).

It was reported that PAHs concentrations in soil were associated significantly with the corresponding levels in air, house dust, urban street dust (Essumang *et al.*, 2011, Okedeyi *et al.*, 2012). Soils ingested by people, especially children, are another exposure route. Outdoors hand-to-mouth activities, the exposure to skins and the inhalation of polluted dust are the main routes of PAHs to man. Generally, the different intake pathways concurrently contribute to an overall exposure, but to variable extents dependent on individual activities and on age-specific behaviours and is an important human exposure pathway of contaminants, particularly relevant for children playing in contaminated sites due to their hand to mouth activities (Khan *et al.*, 2008b, Okedeyi *et al.*, 2012). In estimating risk to children, the US EPA (USEPA, 2008a, USEPA, 2008b) assumes that most children ingest relatively small quantities of soil, e.g., <100 mg/day. Children may also deliberately ingest larger quantities of soil (of the order of 1000–5000 mg/day or more) either occasional or in pica behaviour (Vasiluk *et al.*, 2011).

Therefore, PAHs concentrations in soil may provide important information on the environmental pollution, urban atmosphere environmental quality and which will be helpful to the future in the development of study areas and human health (Dai *et al.*, 2008). PAHs concentrations in the environment also serve as a good indicator of the level of environmental pollution by human activities. It can provide information on regional pollution sources, the long-range transport of PAHs, the rates of pollutant retention and their ultimate destination (Essumang *et al.*, 2011). It was reported that typical concentrations of

PAHs in soil without anthropogenic pollution were 1 -10 ng/g (Lv *et al.*, 2010). In the meantime, PAHs have been detected around the world even at soil sites which are located far from industrial activity such as the Polar Regions; anthropogenic PAHs could only have reached it by long-range transport according to the “global distillation” hypothesis. In many tropical soils, naphthalene, phenanthrene, and perylene are most abundant and are present at concentrations comparable to those in temperate soils, whereas the sum of PAH concentrations is lower than in temperate soils (Wilcke, 2007). Based on the soil contamination information available, bodies such as the Department of Environment and Environment Agencies have set levels of acceptable contamination depending on the end use of the land (Smith *et al.*, 2006). A pictorial illustration of the fate of PAHs in soils is as seen in Figure 2. 3. Some studies on PAHs in soils have been carried out and their findings are as shown in Table 2.2.



**Figure 2.4 Fate of PAHs in the soil (modified diagram from Stokes *et al.*, 2006)**

**Table 2. 2: A Review of PAH Levels in Soils from Different Places**

Location	Levels (ng/g dw)	Number of PAHs analysed	Source	Reference
Linz (Austria)	1450	18	Industrial area	Weiss <i>et al.</i> , (1994)
W' Macedonia (Greece)	55.2–495	16	Lignite-fired power plants	Stalikas <i>et al.</i> , (1997)
Kohtla-Järve (Estonia)	12,390 ± 9810	16	Oil-shale thermal treatment industry, power station and traffic	Trapido (1999)
Tallinn (Estonia)	2200 ± 1396	12	Urban soils	Trapido (1999)
Harjumaa (Estonia)	232 ± 153	12	Rural soil	Trapido (1999)
Zelzate (Belgium)	300,000	7	50 m from an oil refinery	Bakker <i>et al.</i> , (2000)
Zelzate (Belgium)	3000–14,000	7	1.3–4.2 km from an oil refinery	Bakker <i>et al.</i> , (2000)
Ilawa Glowna (Poland)	383.7	14	Control	Malawska and Wiołkomirski (2001)
New Orleans (United States)	3731	16	Urban soils	Mielke <i>et al.</i> , (2001)
Five cities (Tallinn, Helsinki, Vilnius, Chicago and London)	1092	16	Urban soils	Saltiene <i>et al.</i> , (2002)
Novi Sad (Serbia and Montenegro)	47,870	16	Oil refinery (after Kosovo war)	Skrbic and Miljevic (2002)
Novi Sad (Serbia and Montenegro)	4650	16	Oil refinery (after Kosovo war)	Skrbic and Miljevic (2002)
Tokushima (Japan)	610.6	13	Urban soils	Yang <i>et al.</i> , (2002)
Five sites in Korea	49.4	16	Control unburnt soil	Kim <i>et al.</i> , (2003)
Korea	158a	16	Agricultural soils	Nam <i>et al.</i> , (2003)
Tianjin (China)	818.2 ± 796.2	16	Industrial area	Wang <i>et al.</i> , (2003)
Tarragona County (Spain)	58±44	16	Industrial area	Nadal <i>et al.</i> , (2004)
Tarragona County (Spain)	179 ± 141	16	Residential area	Nadal <i>et al.</i> , (2004)
Tarragona County (Spain)	28 ± 22	16	Unpolluted soil	Nadal <i>et al.</i> , (2004)
Chang-Zhu-Tan (China)	7,538	16	Street dust	Long <i>et al.</i> , (2012)
Changsha (China)	6,539	16	Street dust	Long <i>et al.</i> , (2012)
Zhuzhou (China)	6,953	16	Street dust	Long <i>et al.</i> , (2012)
Xiangtan (China)	13,527	16	Street dust	Long <i>et al.</i> , (2012)
Urban dust (UK)	590-46700	16	Street dust	Lorenzi <i>et al.</i> , (2011)
Urban dust (, UK)	900-140, 400	16	Former Industrial Site for Tar, Pitch, Naphtha, Creosol, Benzol and Cyanide Distillation	Lorenzi <i>et al.</i> , (2012)

#### 2.1.4 PAHs in Plants

Some studies have shown the uptake of PAHs by plants (Kipopoulou *et al.*, 1999, Meudec *et al.*, 2005, Vácha *et al.*, 2010, Ashraf *et al.*, 2013) and have reported that PAHs can be taken up by plants leaves through air deposition or by partitioning from PAH contaminated soil to the roots.

The two generic uptake pathways are the soil–root–shoot pathway and the air–leaf pathway (Zhang and Wang, 2011). The soil–root–shoot pathway is hypothesized to occur via partitioning from pore water into lipids and then uptake via transpiration due to the high lipophilicity of PAHs. They are absorbed on leaves cuticle and passed through by solubilisation in waxes or absorbed on root cortical zones and absorbed on roots cell. While mainly low molecular weight PAHs are able to migrate to shoots, the higher molecular weight PAHs are strongly adsorbed on the root epidermis. The transfer of PAHs in the xylem also correlates with the lipophilic ability of the PAHs (Watts *et al.*, 2006, Wang *et al.*, 2011b). The amount of PAH uptake varies significantly and appears to be a function of many factors, including plant species, initial soil concentrations and microbial population (Watts *et al.*, 2006). Soil root pathway can reduce PAHs in soils and water and is the major pathway of PAH entry into plants (Kang *et al.*, 2010). They do so by accumulation, detoxification or metabolizing organic contaminants using four techniques such as 1) phyto-degradation or phyto-transformation, (2) phytoextraction or phyto accumulation, (3) phyto-volatilization and (4) rhizosphere biodegradation (Yasseen, 2014).

Typical responses of plants to PAHs are poor germination and growth (Hong *et al.*, 2009). In a study by Watts *et al.*, (2006), PAH concentrations were measured in *Spartina alterniflora* plants grown in pots of contaminated sediment. The study looked at plants grown in native sediment at a marsh contaminated with up to 900 µg/g sum PAHs and from plants grown in uncontaminated (control) sediment. PAH compounds were detected at up to 43 µg/g dry weight in the root tissue of plants grown in pots of contaminated soil. PAH compounds were detected in the leaves of plants grown in pots of contaminated soil at levels up to 0.2 µg/g. Concentrations less than 0.004 µg/g were detected in the leaves of plants grown at a reference site. Since diet is believed to be one of the major sources of human exposure to PAHs (Tao *et al.*, 2004, Wang *et al.*, 2010, Wang *et al.*, 2011b), the presence of PAHs in vegetables constitute a major concern to scientists and local authorities as to how and to what extent PAHs are accumulated in vegetable produced in contaminated area (Tao *et al.*, 2004, Ashraf and Salam, 2012). Table 2.3 is a review of PAH concentrations in some vegetables and plants.

**Table 2. 3: A Review of PAH Levels Detected in Some Plants**

PAHs	China		Saudi Arabia	Pakistan	Germany	France
Source	Market	Farm lands	Market	farmland	Manufacturers	heavy N°6 fuel oil contaminated soil
Medium	Vegetables: 9 Different types 4 PAHs studied (average) (µg/kg)	11 vegetable species, 16 USEPA PAHs (µg/kg)	Leafy (spinach) (µg/kg)	Leafy (spinach) (µg/kg)	48 dried Tea Samples (green, black, white and mate)15 SCF PAHs (µg/kg)	<i>Salicornia fragilis</i> (Glasswort) Shoot. 16 PAHs (µg/kg)
Naphthalene						52130 ± 2285

Acenaphthylene						5395 ± 315
Acenaphthene						25630 ± 1575
Fluorene						40610 ± 535
Phenanthrene						165940 ± 9045
Anthracene	6460		2.85±1.39	3.17±2.15		26170 ± 595
Fluoranthene	4050					14220 ± 910
Pyrene						83910 ± 2700
Benzo(a)anthracene	3330		1.09±0.76	1.79±1.24		37550 ± 610
Chrysene						51700 ± 1410
Benzo(b)fluoranthene				0.89±0.54		8640 ± 920
Benzo(k)Fluoranthene		Nd	0.66±0.21	0.87±0.33		5760 ± 410
Benzo(a)pyrene	4500	ND	2.12±1.20	2.45±1.12		22795 ± 1025
Dibenzo(a, h)anthracene			1.7±0.75	nd		3445 ± 845
Benzo(g,h,i)perylene		ND	0.40±0.29	0.57±0.38		4620 ± 585
Indeno(1,2,3,c)pyrene		ND				8055 ± 790
Sum PAHs		7.0 - 5353 l			14 - 2,662 l	548.3 ± 14.9
Reference	(Zhong and Wang, 2002)	(Mo <i>et al.</i> , 2009)	(Ashraf and Salam, 2012)	(Ashraf <i>et al.</i> , 2013)	(Ziegenhals <i>et al.</i> , 2008)	(Xu <i>et al.</i> , 2007)

(Zhong and Wang, 2002, Ashraf and Salam, 2012, Ashraf *et al.*, 2013 masses are based on fresh weight)

Phytoremediation is also based on plant uptake of contaminants. It is defined as the use of plants to remove, destroy or sequester hazardous substances from soil environment while it can be applied to both organic and inorganic pollutants, present in soil substrates. However, there is a growing interest in broadening this technology to remove/degrade organic pollutants in the environment. Organic contaminants like PAHs are stabilized within a soil matrix, taken up by plants and transformed or stored in a non-phytotoxic form (Martí-Cid *et al.*, 2008, Vácha *et al.*, 2010).

## 2.2 PAHs IN FOOD MATERIALS

Humans are exposed to complex mixtures of substances through the diet, with some of them having negative impacts on consumer's health. Among potential dietary chemical hazards, PAHs contribute more than 70% of the sum exposure (McGrath *et al.*, 2007, Martorell *et al.*, 2010). While for non-smokers the major route of exposure is consumption of food, for smokers the contribution from smoking may be significant. Contamination of PAHs was often found in various food categories in substantial quantities, depending on the mode of cooking, preservation and storage (Ashraf *et al.*, 2013, EFSA, 2012). Since PAHs have been found in different food products, such as dairy products, vegetables, fruits, oils, coffee, tea, cereals and smoked meat, the analysis of PAHs in food is a matter of concern (Plaza-Bolanos *et al.*, 2010). Food items and products could be contaminated by soils, polluted air and water (environmental sources) (WHO, 2005, Alexander *et al.*, 2008). Some aquatic food products, such as fish, can be exposed to PAHs present in water and sediments. The PAH content greatly depends on the ability of the aquatic organisms to metabolize them (Plaza-Bolanos *et al.*, 2010). On the other hand, PAHs are also found in foods as a result of certain industrial and home cooking/ food processing methods or practices such as smoke curing, broiling, roasting and grilling over open fires or charcoal which permit the direct contact between food and combustion products (Alexander *et al.*, 2008). For example, PAHs have been found in meat or fish smoked at high temperatures (Diletti *et al.*, 2005). Furthermore, in the food processing industry, food additives such as smoke flavouring products,

lubricants, solvents, propellants, glazing agents and protective coatings contribute to contamination of food items by PAHs (Loague and Crowin, 2005).

It has been found that raw foods do not usually contain high levels of PAHs. However, PAHs in uncooked food, such as vegetables, seeds and grains have been found to accumulate on the waxy surface of many vegetables and fruits. In areas remote from urban or industrial activities, the levels of PAHs found in unprocessed foods reflect the background contamination, which originates from long distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires. Nevertheless, other studies have shown the possibility of vegetables taking PAHs from soil and water and metabolizing them. Another example of possible PAH contamination in food is due to traffic, i.e. crops or livestock close to urban roads could be exposed to PAHs and nitro-PAHs (combustion derived derivatives from parent PAHs). In the neighborhood of industrial areas or along highways, the contamination of vegetation can be ten-fold higher than in rural areas (Larsson and Sahlberg, 1982). PAHs also get into food through food processing methods such as smoking and roasting. Table 2.4 shows a review of some studies done on roasted foods.

**Table 2.4: Concentrations of PAHs Found in Some Smoked and Roasted Foods**

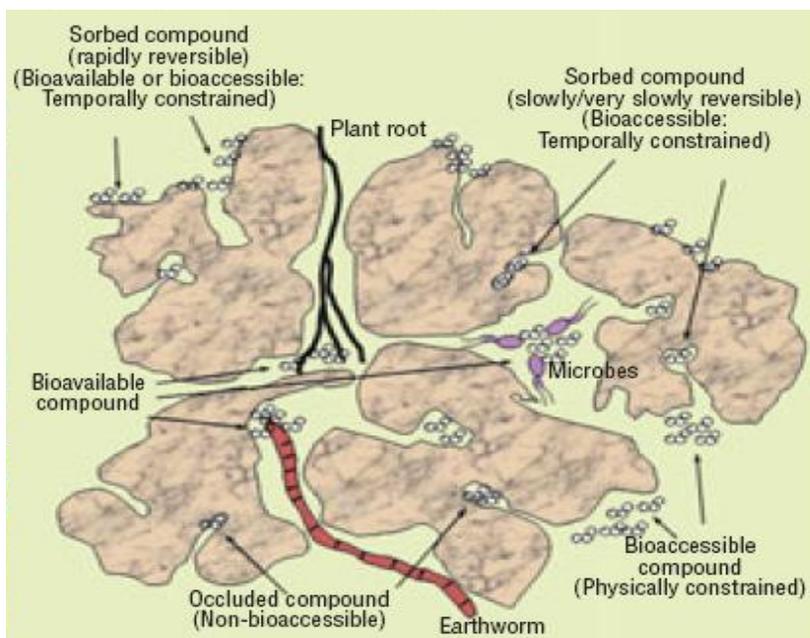
Roasted food samples	PAHs analyzed	Sum of PAHs before Smoking	Sum of PAHs in roasted foods(ng/g)	Reference
Ayam bakar (Malaysian smoked food)	FLT, , BbF, BaP,		28.9 - 51.1	Farhadian <i>et al.</i> , (2010)
Ikan bakar (Malaysian smoked food)	FLT, , BbF, BaP,		9.36 -15.0	Farhadian <i>et al.</i> , (2010)
Beef Satay (Malaysian smoked food)	FLT, , BbF, BaP,		81.0 -132	Farhadian <i>et al.</i> , (2010)
Chicken Satay (Malaysian smoked food)	FIT, BbF, BaP,		143-37.6	Farhadian <i>et al.</i> , (2010)
Beef Kebab (Malaysian smoked food)	FLT, BbF, BaP,		9.36 -15.0	Farhadian <i>et al.</i> , (2010)
Chicken Kebab (Malaysian smoked food)	FLT, BbF, BaP,		8.23 - 20.4	Farhadian <i>et al.</i> , (2010)
Grilled Chicken	FLT, BbF, BaP,		4.63 – 12.4	Farhadian <i>et al.</i> , (2010)
Tandoori Chicken	FLT, BbF, BaP,		4.65 -9.66	Farhadian <i>et al.</i> , (2010)
Oven grilled Chicken	FLT, BbF, BaP,		3.51 -7.14	Farhadian <i>et al.</i> , (2010)
Beef ham	16 USEPA PAHs	0.4	1.5	Djinovic <i>et al.</i> , (2008)
Pork ham	16 USEPA PAHs	0.9	2.6	Djinovic <i>et al.</i> , (2008)
Bacon without skin	16 USEPA PAHs	0.6	4.7	Djinovic <i>et al.</i> , (2008)
Bacon with skin	16 USEPA PAHs	0.7	2	Djinovic <i>et al.</i> , (2008)
Cajna sausage	16 USEPA PAHs	3.5	3.9	Djinovic <i>et al.</i> , (2008)
Sremska sausage	16 USEPA PAHs	2.0	2.7	Djinovic <i>et al.</i> , (2008)
Roasted Ground Cofee	BaP	-	100 - 500	Garcia-Falcon <i>et al.</i> , (2005)
Chouriço de Carne Sausages, (Portugal traditional smoked	BaP		5350	Santos <i>et al.</i> , (2012)

food)				
Moura Sausage (Portugal traditional smoked food)	BaP		5870	Santos <i>et al.</i> , (2012)
Salpicão Sausage (Portugal traditional smoked food)	BaP		4510	(Santos <i>et al.</i> , 2012)

Djinovic *et al.*, (2008) smoked their samples for three days and analysed them with a gas chromatograph linked to a mass spectrometer while Farhadian *et al.*, (2010), bought their samples from restaurants and analysed with a high pressure liquid chromatograph – fluorescence detector. The process involved in making the sausages in the study by Santos *et al.*, (2012) involved smoking for 2, 2 and 6 days respectively. BbF: Benzo(b)fluoranthene, BaP: benzo[a]pyrene, FLT: Fluoranthene.

### 2.3 BIOAVAILABILITY AND BIOACCESSIBILITY OF PAHs

The dictionary meaning of bioavailability is the degree to which a drug or other substance becomes available to the target tissue after administration (Dorland, 2012). It is also defined as the fraction of an ingested dose that crosses the gastro intestinal epithelium and becomes available for distribution to internal target tissues and organs (Denys *et al.*, 2012). In pharmacology and toxicology, the term relates to the systemic availability of a xenobiotic after intravenous or oral dosing (Wilkinson, 1997, Oomen *et al.*, 2003). Bioavailability is a term that describes the portion of the substance that is absorbed from the intestinal lumen to the bloodstream. Some of the substances can precipitate, decompose, or change chemically into another more or less toxic form (Marques *et al.*, 2011).



**Figure 2. 5: Bioavailable and Bioaccessible Contaminant in Soil (Semple *et al.*, 2004)**

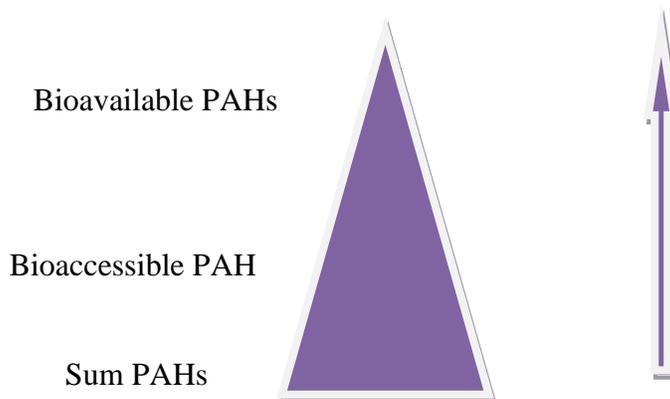
The National Research Council (UK) do not have a definition for bioavailability but rather defines the bioavailability processes as the individual physical, chemical, and biological interactions that determine the exposure of organisms to chemicals associated with soils and sediments (Ehlers and Luthy, 2003). Environmental scientists have tried to adapt this usage when considering human exposure to soil borne contaminants suggesting that the bioavailable portion is the amount of compound that is removed from soil through desorption processes under physiological conditions, which is transferred to the circulatory system (Semple *et al.*, 2001, Semple *et al.*, 2004) (Figure 2.4). Intawongse and Dean (2006) defined ‘bioavailability’ as the extent, to which a chemical absorbed by a living organism reaches the systemic circulation. They also said the part of a contaminant from the readily mobilized soil environment that is taken up by plant roots is the bioavailable fraction. Stokes

*et al.*, (2006), identifying the confusion and lack of clarity among environmental scientists discussing 'bioavailability' proposed that bioavailability represents, at a given time, the fraction of a chemical that is freely available to cross an organism's (cellular) membrane of microbes, plant or animal. It is also the extent to which a substance is absorbed and circulated in the body of the organism (Semple *et al.*, 2004).

Bioaccessibility encompasses what is actually bioavailable plus what is potentially bio available (Stokes *et al.*, 2006). Bioaccessibility sometimes called "bioaccessible fraction" is the fraction of the sum amount of a chemical present in ingested food, water, or ingested soil and sediment particles, that at maximum can be released during digestion. More specifically, the bioaccessible fraction may also be regarded as the fraction that, after ingestion, may be mobilized into the gut fluids (chyme). This fraction is considered to represent the maximum amount of contaminant available for intestinal absorption. Bioaccessible contaminants can subsequently be absorbed, in other words, transported across the intestinal wall and transferred into the blood or lymph stream (Denys *et al.*, 2012, Pelfrêne *et al.*, 2012). In relation to soil risk assessment, polycyclic aromatic hydrocarbons, released from ingested soil during digestion are defined as the bioaccessible fraction.

The Environment Agency in this premise of soil risk assessment defined the bioaccessible fraction as "The proportion of a chemical released from soil following ingestion and digestion, and entering into solution, is referred to as the bioaccessible fraction" (Chen *et al.*, 2013). The bioaccessible fraction is free to be absorbed through the small intestine into the

lymphatic or blood system via the portal vein (Armstrong *et al.*, 2007). The relationship between bioavailable and bioaccessible PAHs can be illustrated as shown in Figure 2.5



**Figure 2. 6: Representation of Bioavailable and Bioaccessible PAH in a System**

The bioaccessibility of PAHs in soils is influenced by aging, weathering, microbial action, methylation/hydroxylation, adsorption / desorption hysteresis, ultra-violet light interaction and organic carbon quality and quantity. The soil organic carbon-water partition coefficients ( $K_{oc}$ ) for PAHs vary depending on the size of associated soil particles with the highest values in silt (fine particles) followed by sand and clay (Dandie *et al.*, 2010). PAHs in soils undergo a weathering process such that the lighter chain fractions are removed (primarily by volatilization). Heavier fractions bind more readily to the soil organic matter and remain behind in the top soil horizon. As the mixture of PAHs age, bioaccessibility changes as the fraction remaining bind more tightly. Aging reduces the bioaccessibility/bioavailability of PAHs in soils. Chemicals in soils age by becoming incorporated inside the crystal lattice structure of the soil particle, or partitioning onto organic matter or soil nanopores, so they are no longer available for uptake (Juhasz *et al.*, 2007, Grimmera *et al.*, 1983).

Consideration of bioaccessibility/ bioavailability is important during risk assessment given that the mobility and the subsequent availability of contaminants are inversely related to risk-based cleanup levels. This means that the risk-based cleanup level of a contaminant increases with its decreasing bioavailability (Wan-ling *et al.*, 2011, Rostami and Juhasz, 2011, Zia *et al.*, 2011). Bioavailability and bioaccessibility are complex issues that determine whether or not adverse effects are to be expected when organisms or plants are exposed to contaminants (Peijnenburg and Jager, 2003). Bioavailability is often used as the key indicator of potential risk that chemicals pose to environment and human health (Stokes *et al.*, 2006). Information on contaminant bioaccessibility has been recognized by researchers, legislators and regulators as a decision-support tool for contaminated land assessment and has been an object of interest and discussion at both national and international levels (Latawiec *et al.*, 2010, Stokes *et al.*, 2006). A sustainable, proportionate and risk-based approach to contaminated land management has been adopted by contaminated land regimes throughout the world (Latawiec *et al.*, 2010 ). Thus measuring the sum concentration of organic contaminants present at contaminated sites may lead to over conservative risk assessments as only the bioavailable fractions can cause toxic effects. Recently, approaches for ecological risk assessment have been developed where bioavailability data, obtained from the results of bioassays are used. Since the total amount of a contaminant does not always reflect the amount that is available to the body and only a fraction of the contaminant may be bioavailable after oral exposure to exert its toxic action. There is a demand for tools that enable the estimation of internal exposure of contaminants

caused by mouthing of toys, soils especially by children and adults (Brandon *et al.*, 2006) and food consumed by man.

## **2.4 BIOAVAILABILITY MODELS**

Several physical, chemical, and biological techniques have been proposed for estimating the bioavailability of PAHs and other hydrophobic organic chemicals in contaminated soils and food matrices. Assays for estimating chemical bioavailability have been introduced with several targeted endpoints: microbial degradation, uptake by higher plants and soil fauna, and toxicity to organisms (Farhadian *et al.*, 2010). They can be broadly be classified as *in vivo* and *in vitro* models as seen in Figure 2.7

### **2.4.1 *In vivo* Bioavailability/ Bioaccessibility models**

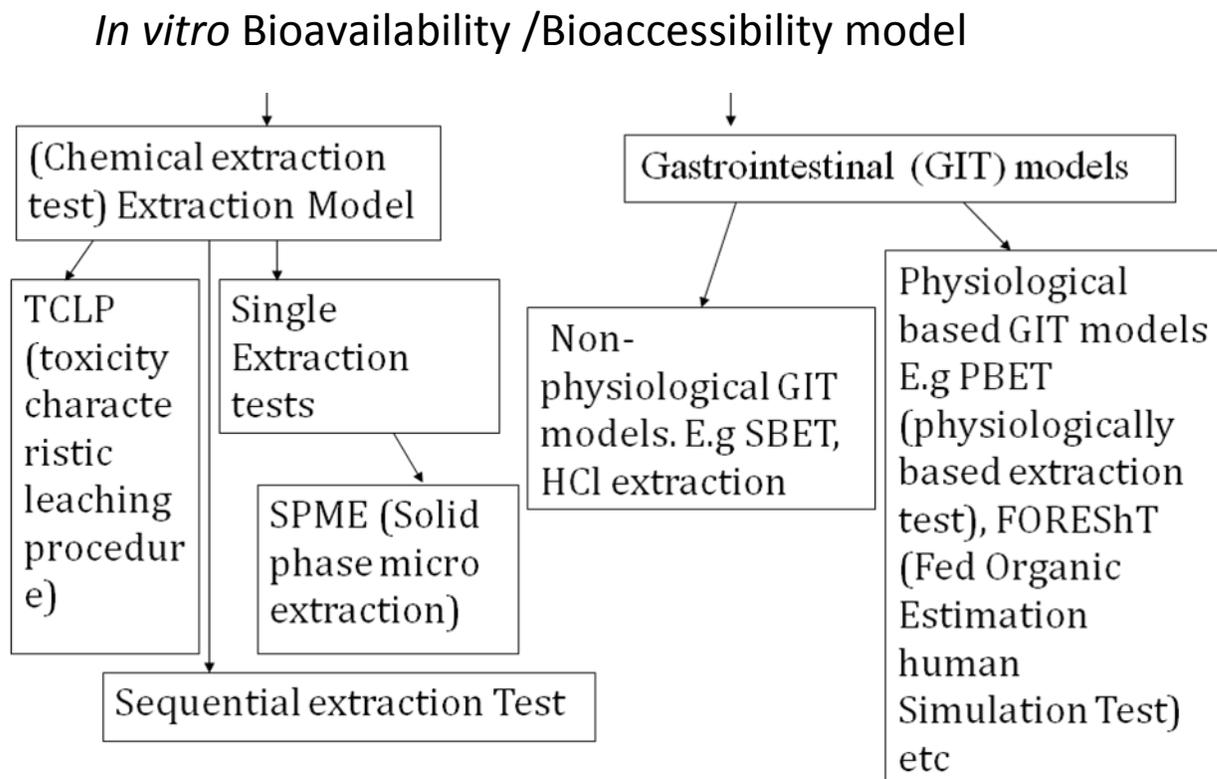
*In vivo* (Latin for “within the living”) refers to experimentation using living organism as opposed to a partial or dead organism (Jacob, 1996). Therefore, the use of animals in bioavailability studies are called *in-vivo* models. A variety of animal assays have been developed to determine bioavailability of contaminant. Bioavailability of contaminated soils for human health risk assessment have been done using rodents, rabbits, dogs, primates and swine to quantify the amount of contaminant that enters systemic circulation following administration of contaminated soil and food (Zia *et al.*, 2011). Earth worms have also been used for PAHs from soils (Van de Wiele *et al.*, 2004, Sun and Li, 2005, Li *et al.*, 2007, Farhadian *et al.*, 2010, Gomez-Eyles *et al.*, 2011).

*In vivo* bioavailability studies involve dosing the animals intravenously or orally and monitoring the contaminant level in blood, urine, fatty tissue and faeces (Ko *et al.*, 2000, Grimmera *et al.*, 1983, Lodovici *et al.*, 2004, Rhodes *et al.*, 2005). Immature (juvenile) swine are considered remarkably similar to humans especially children in terms of their metabolism, their body size, weight and bone to weight ratio is similar to young children, the age group at greatest risk. Although primates are the first choice for bioavailability studies due to their close relatedness to man, few studies have been undertaken with them. This due to the prohibitive costs associated with the primate animal model (Juhasz *et al.*, 2007). Animal testing is the standard for determining dietary uptake efficiency of nutrients or xenobiotics (Brandon *et al.*, 2006); however, trials using animals for measurements of contaminant bioavailability are expensive (about \$30,000 per test soil with swine), time consuming and subject to ethical considerations (Zia *et al.*, 2011). Hence, researchers have developed *in vitro* models as alternative. *In vitro* models, focuses on bioaccessibility instead of bioavailability. It is a cheap and reproducible tool to investigate the bioaccessibility of PAHs in soil and food (Oomen *et al.*, 2003).

#### **2.4.2 *In vitro* Bioavailability/ Bioaccessibility Models**

*In vitro*, a Latin word for ‘within the glass’ refers to techniques, processes or reactions taking place in a test tube, culture dish or elsewhere outside a living organism (Jacob, 1996). *In vitro* assays overcome the time and expense limitations of *in vivo* studies thereby providing a surrogate measurement of bioavailability that is quick and inexpensive compared to animal. Several *in vitro* methods have been developed. Researchers have

shown that the *in vitro* study results can be simple, rapid and low in cost. It may provide insights not achievable in whole animal studies (Intawongse and Dean, 2006). *In vitro* bioaccessibility extractions now allow for sufficient number of samples to more fully characterize the contaminant (Zia *et al.*, 2011). Types of *In vitro* bioaccessibility models can be divided into two categories: those using chemical extraction tests that equate the ‘easily extractable contaminants’ usually for metals at low pH conditions, with those that are likely to be bioaccessible; and gastro or gastrointestinal analogue tests/models which attempt to mimic the biochemical conditions in the human/animal gastrointestinal tract as shown in Figure 2.7.



**Figure 2.7: *In vitro*-Bioavailability/Bioaccessibility Models**

### 2.4.2.1 Chemical extraction tests model

Mild extraction test for bioaccessibility of contaminants to plant and man often called chemical extraction test generally falls into various categories (Sprovieri *et al.*, 2007):

i) Single extraction tests that simulate the leaching of potentially toxic contaminants (metals) from soil or waste by rainwater or landfill leachate. pH of solutions usually employed are between 2.88 and 4.93 described in the USEPA methods for soil or waste piles by rain or landfill leachate as in USEPA method 1311 Toxicity Characteristic Leaching Procedure (TCLP) (Smith *et al.*, 2011).

ii) Single extraction tests that are designed to determine the phytoavailable fraction of an inorganic or organic contaminants within the soil.

iii) Multiple extraction tests also called sequential extraction tests are designed to either extract specific physico-chemical bioavailable soil contaminant phases and to determine the distribution of metals within the soil. The distribution of the metals is methodologically defined in five categories: exchangeable, carbonates, reducible, oxidizable, and residual (Sprovieri *et al.*, 2007).

To assess the bioavailability from contaminated soil to a crop plant/man, using mild extraction (single extraction tests), the selection of an extractant which simulates the available fraction of the element is of importance (Intawongse and Dean, 2006). Sequential mild extraction methods include extraction using alcohols, hexane/water, supercritical fluids (carbon dioxide), aqueous hydroxypropyl-beta-cyclodextrin extraction, polymeric

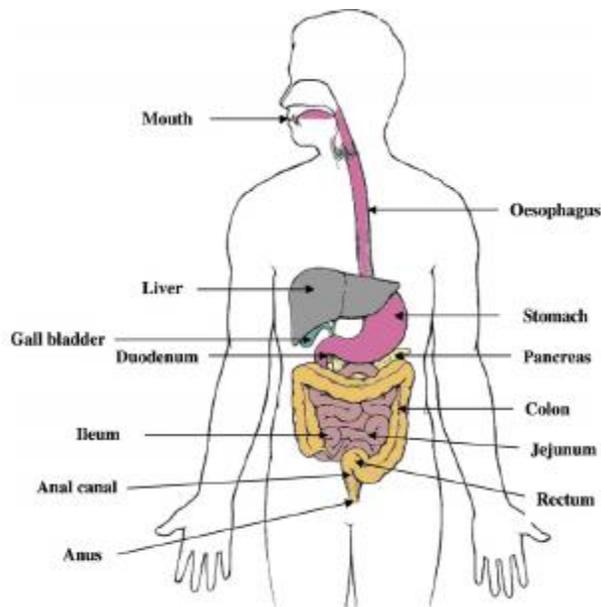
TENAX beads extraction, and poly(dimethylsiloxane)-coated solid-phase micro extraction have been used (Farhadian *et al.*, 2010).

Though mild extraction methods were developed for metal studies, a correlation relationship study of pyrene extractability by butanol and mild supercritical fluid extraction (SFE) with bioavailability to earthworm uptake for aged soils has been carried out. Both butanol and mild supercritical fluid extraction (SFE) were suitable with mild SFE being a better approach to predict the bioavailability of organic chemicals in field soils compared to butanol extraction (Sun and Li, 2005). The approach of mild extraction bio-accessibility methods is not necessarily to mimic a human's gastro intestinal tract, but to solubilise trace component using "simple" solutions that yield a high correlation with the bioavailable fraction in the soil. This approach uses the measured bioaccessibility to estimate bioavailability using mathematical relationships gleaned from *in vivo* studies. In theory, if a mathematical relationship exists between *in vitro* and *in vivo* trials results, then the fluid composition of the *in vitro* test does not necessarily need to be a close mimic of a GI tract (Zia *et al.*, 2011). Some extractions are simply buffered acid at stomach pH (Pies *et al.*, 2008). USDE (2008) used solid phase micro extraction (SPME) in the assessment of bioavailability of PAHs. Generally the mild extractant has limited applicability for *in vitro* estimations and it is better used in the prediction of leaching characteristics of contaminants (especially in organics) from waste materials (Ng *et al.*, 2010). Although the results of these tests give a broad idea of easily mobilised contaminants, the extraction conditions and the leaching reagents are not representative of those found in the human gastrointestinal tract

and therefore their use in assessing risk to human health is very limited (Sprovieri *et al.*, 2007).

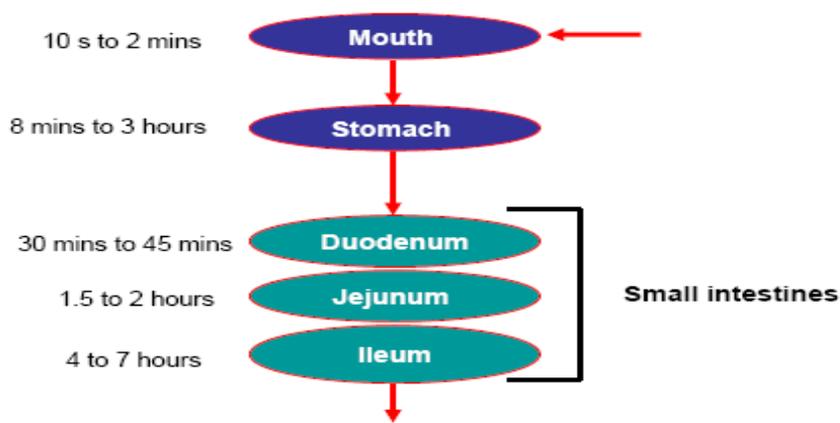
#### 2.4.2.2 Gastrointestinal models

Information from *in vivo* studies can be difficult to interpret due to physiological discrepancies between humans and the experimental animals adopted. Such problems have led to the development of *in vitro* systems based on human gastrointestinal (GI) tract processing of food in different compartments of the guts (as shown in Figures 2.8 and 2.9). The process consists of ingestion, mastication, deglutition, digestion, absorption, peristalsis and defecation.



**Figure 2. 8: The human Digestive System (Dean and Ma, 2007)**

Bioaccessibility techniques that measure the fraction of a contaminant which is solubilised from a sample under simulated gastrointestinal conditions (stomach and small intestinal pH and chemistry, soil-to-solution ratio, stomach mixing, and stomach emptying rates, food retention time in guts as in Figures 2.8 and 2.9) using simulated parameters representative of the human digestive tract (Khan *et al.*, 2008b) are called the gastrointestinal model/test.



**Figure 2.9: Physiology of the human gut (Rhodes *et al.*, 2005).**

Variety of *in vitro* GI methods have been developed and proposed, These tests involve a wide range of pH (1.07 to 7.5), different sample size fractions and different sample to solution ratio (Oomen *et al.*, 2003, Skupinska *et al.*, 2004, Rhodes *et al.*, 2005).

Gastrointestinal models are of two types: the non-physiological and physiological based gastrointestinal models. Non-physiologically based tests mimic the gut conditions like pH, retention and mixing without the use of enzymes or physiological fluids. Examples of non-physiological based gastrointestinal models include hydrochloric acid extractions, relative bioaccessibility leaching procedure (RBALP) or simple bioaccessibility extraction test

(SBET), the Solubility Bioavailability Research Consortium procedure (SBRC), and variants of these tests (Readman *et al.*, 2002). Physiologically based extraction gastrointestinal models/tests attempt to simulate the physiological fluids and conditions of the digestive tract. This is usually achieved through the employment of digestive enzymes. Examples of the physiologically based extraction gastrointestinal models/tests include physiologically based extraction test (PBET), the *in vitro* gastrointestinal method [IVG], the Netherlands National Institute for Public Health and the Environment (Dutch:Rijksinstituut voor Volksgezondheid en Milieu or simply RIVM) model (RIVM model), the unified barge method [UBM], the DIN 1738 (DIN) model, the TNO Gastrointestinal Model (TIM) model, Simulator of Human Intestinal Microbial Ecosystem (SHIME) model, and their modifications, either in dynamic or static modes (Readman *et al.*, 2002, C.I.E.H, 2009).

- **The physiologically based extraction test (PBET):** This is an *in vitro* test system for predicting the bioavailability of metals from a solid matrix and incorporates gastrointestinal tract parameters representative of a human (Tilston *et al.*, 2011). PBET is usually in a fasted state. This is achieved by the use of mouth, stomach and intestinal digestive juices to extract the contaminant without food. The PBET was designed in United Kingdom by British geological survey (C.I.E.H, 2009) based on simulating the paediatric gastrointestinal tract for a child of 2-3 years old. This age group was chosen because it is believed to be at greatest risk from accidental soil ingestion (Srogi, 2007). This test is essentially a two stage sequential extraction using various enzymes to simulate both the gastric and small intestine compartments with extraction carried out at 37°C. Initially Ruby and his team in 1993 introduced a chemical extraction method to

estimate the bioavailability of soil lead which was well correlated with bioavailability measured from rabbits, and this extraction result was labelled “bioaccessibility” to make clear it was not a biological measurement of bioavailability. Within the next few years, they extended the development of their earlier bioaccessibility extraction method and called it the Physiologically - Based Extraction Test (PBET) (Oomen *et al.*, 2003). The modified test was more complicated, but seemed well correlated with results of available feeding studies. After this development, there were some studies along similar lines by several researchers. They tried to see if some chemicals similar to gastric juice/GI secretions could simulate living animal digestion behaviour for measurement of Pb (and other metals) entry to the systemic circulation (Intawongse and Dean, 2006, Tilston *et al.*, 2011). For lead (Pb), the results of the PBET linearly correlated with results from a Sprague-Dawley rat model. For arsenic (As), the results of the PBET are over predicting bioavailability study results in rabbit and primate models (2–11% difference between *in vitro* and *in vivo* results, depending on the animal model) (Tilston *et al.*, 2011).

USEPA (2000) applied PBET to dust samples collected from various domestic and working settings to establish bioaccessible concentrations limits of metals (Al, Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sn, U, Zn) in the indoor environment. They found that with the exception of Ca, Cd, and Zn in the stomach phase, mean bioaccessibilities (relative to respective sum metal concentrations) were less than 50%. For a given metal, bioaccessibility in either phase was variable among samples but, in many cases,

displayed an inverse dependence on sum concentration. They suggested that, to a good approximation, variations in both metal contamination and accessibility in the indoor environment arise from variable proportions of metal-rich particulates of low digestibility. Compared with accessibility in the stomach, accessibility in the more alkaline, carbonate rich intestine was either lower (Al, Ca, Cd, Mn, Ni, Sn, Pb, Zn), similar (Co, Cu, Fe) or greater (Cr, U). All these observations were attributed to precipitation and/or re-absorption in the intestine, stabilization by complexation, or anion-like absorption of negatively charged polyatomic species respectively.

In another study by Turner and Ip (2007) using PBET they showed that the bioavailability of soil contaminants varies between site and type of matrix. Their study demonstrated that assuming 100% bioavailability of arsenic (As) and lead (Pb) from soils and mine waste materials overestimates the risk associated with human exposure. In *in vitro* systems, the simulated bioavailability of a contaminant is referred to as the “bioaccessibility” and is used as an alternative quantitative indicator for *in vivo* derived bioavailability estimates. Significant correlation was observed between bioaccessibility values from PBET, and bioavailability values generated for both rats and cattle, hence they demonstrated the potential to utilize PBET as a relatively inexpensive alternative to *in vivo* models for bioavailability assessment.

- **Colon extended PBET (CE-PBET):** This is a modification of PBET approach. In this model, the colon phase of PEBT was extended to 8 h since food stays longer in the

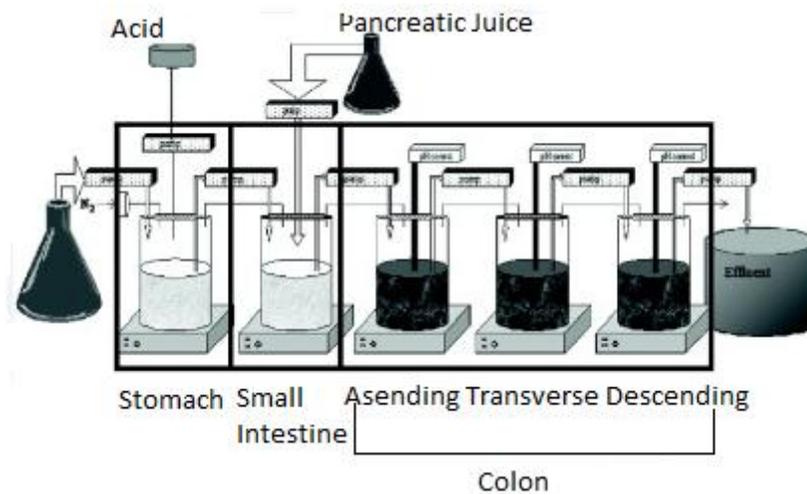
Colon compared to any other part of the digestive tract (Eisler, 1987). Though PBET is primarily for the study of inorganic contaminants and in fasted state, Tilston *et al.*, (2011) in their CE-PBET added a carbohydrate-rich medium (fed state) and used CE-PBET to demonstrate increased assessments of soil-bound PAH bioaccessibility by up to 50% in laboratory soils and a factor of 4 in field soils. They attributed increase in bioaccessibility to a combination of the additional extraction time and the presence of carbohydrates in the colon compartment, both of which favour PAH desorption from soil. They proposed that future assessments of the bioaccessibility of organic pollutants in soils using physiologically based extraction tests should have a colon compartment as in CE-PBET. Later, in 2012, CE-PBET in fed state was applied for the first time to study the bioaccessibility of brominated flame retardants (BFRs) from the 3 main GIT compartments (stomach, small intestine and colon) following ingestion of indoor dust. Results revealed the bioaccessibility of  $\gamma$ -HBCD, a- and b-isomers (flame retardants) were 72%, 92% and 80% respectively Mohamed *et al.*, (2012).

- **Simplified bioaccessibility extraction test (SBET):** SBET is a simplified form of the PBET extraction procedure developed specifically for lead. The stomach phase of this technique has been shown to correlate well for lead in a series of young swine studies conducted by USEPA region VIII and the University of Missouri. The development of the SBET was in response to a request by the USEPA region VIII and the need for other US laboratories to be able to use and apply simple bioaccessibility testing regimes (Sprovieri *et al.*, 2007). Although it mimics only the stomach of humans (CCME,

2010). However, Smith *et al.*, (2011) included an intestinal phase by using acetate buffer and raising the pH to 6.5. Juhasz *et al.*, (2007) also carried out a study using the SBET on arsenic and found correlations with *in vivo* studies. They also suggested that As bioavailability (*in vitro*) may be estimated using the less expensive, rapid *in vitro* chemical extraction method (SBET) to predict As exposure in human health risk assessment. SBET entails mixing of about 0.5g of soil with 50 mL of glycine (0.4 M) at pH = 1.5 adjusted with HCl for 1 h at 37<sup>0</sup>C. The combined supernatant centrifuged and filtered using Whatman paper number 2 to get extracts for analysis of the metal content (Readman *et al.*, 2002, Skupinska *et al.*, 2004). Skupinska *et al.*, (2004) also employed this model in their study of phosphorus-induced Pb immobilization in Pb-contaminated soils study.

- **Relative bioaccessibility leaching procedure (RBALP):** RBALP is a method originally developed for lead in soil by John Drexler (University of Colorado, US) (Oomen *et al.*, 2006) and adopted by Solubility Bioaccessibility and Research Consortium (SBRC) US. This method is sometimes referred to the SBRC method (Readman *et al.*, 2002). This method employs a pH relevant to the physiology of the stomach using glycine buffer as the extraction medium (Nogueroles, 2007). The RBALP was initially called SBET (Pu *et al.*, 2004).
- **Simulator of human intestinal microbial ecosystem (SHIME):** The SHIME is a unique scientifically validated dynamic model of the complete gastrointestinal tract to

study physicochemical, enzymatic and microbial parameters in the gastrointestinal tract in a controlled *in vitro* setting. The model consists of five reactors which sequentially simulate the stomach (acid conditions and pepsin digestion), small intestine (digestive processes) and the 3 regions of the large intestine, i.e. the ascending, transverse and descending colon (microbial processes) as in Figure 2.10. Careful control of the environmental parameters in these reactors allows for obtaining the complex and stable microbial communities which are highly similar in both structure and function to the microbial community in the different regions of the human colon. This method is PBET plus microbial degradation during gastrointestinal digestion (Van de Wiele *et al.*, 2004).



**Figure 2.10: A Schematic Diagram of SHIME (Kemperman *et al.*, 2013)**

Nutritional status and gastrointestinal microbes effect on arsenic bioaccessibility from soils and mine tailings in the simulator of the human intestinal microbial ecosystem was investigated by Brandt and Watson (2003) using the SHIME model. They found that

microbial activity increased arsenic bioaccessibility relative to sterile conditions from four of five samples under fasted conditions and three of the five samples under fed conditions and suggested that *in vitro* gastrointestinal (GI) models operated under fed conditions and with microbes provides a more conservative estimate of *in vitro* bioaccessibility. They also found that for some samples, the arsenic bioaccessibility in the SHIME colon (with microbial activity) was equivalent to values observed in a separate physiologically based extraction test under small intestinal conditions (without microbial activity). Their study suggested that the incorporation of microbial activity into *in vitro* GI models does not necessarily make estimates of arsenic bioaccessibility more protective than those generated using *In vitro* models that do not include microbial activity.

In another study by Stalikas *et al.*, (1997) to assess bioaccessibility using the SHIME method, PAHs release in the stomach and small intestine compartments of the SHIME was low (8%). In fact, PAH release in the SHIME was lower from the <45mg size fraction despite the fact that this fraction had higher levels of PAHs than the bulk soil. They postulated that this occurs because PAHs adsorbed to soil did not reach equilibrium with the small intestinal fluid. In contrast, PAH release in the colonic compartment of the SHIME reached equilibrium and was linked to soil concentration. They estimated incremental lifetime cancer risk and found it significantly greater for the <45 mm soil fraction compared to the bulk fraction. When bioaccessible PAH concentrations in a simulated small intestine were used in the risk assessment

calculations, cancer risk was slightly lower in the <45 mm soil fraction for these soils. Their results highlighted the significance of using a small soil size fraction for contaminated site human health risk assessment.

- In vitro* gastrointestinal method (IVG):** Like PBET it employs simulated gastric solution but unlike the PBET method, the simulated gastric solution is prepared in a 0.15 M sodium chloride matrix, uses different concentrations of reagents and a lower pH (pH 1.8 for the stomach phase and 5.5 for the intestine phase) (Figure 2.11). In addition to these differences, simulated food was added to the initial gastric solution in the form of dough (200 g of wet feed termed “dough”) (Sprovieri *et al.*, 2007). IVG has been employed by Saravanabhavan *et al.*, (2007) for Pb, As, and Cd bioavailability study, by Jiang *et al.*, (2009) in the study of As bioavailability and (Planas *et al.*, 2006) in the study of Pb bioavailability.

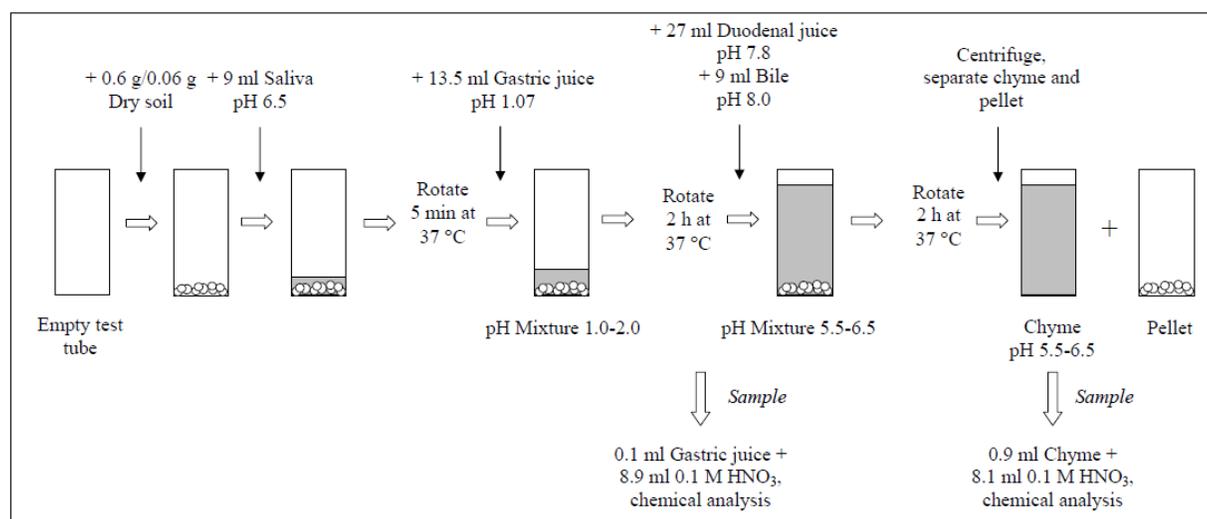


Figure 2. 11: A Schematic Diagram of the Fasted IVG (Oomen *et al.*, 2006)

- **German DIN 19738 (DIN) method:** DIN was designed by the University of Bochum, Germany as a system for mobilising pollutants into synthetic digestive juices from contaminated matrices. This method has been validated (partially) for organic and inorganic contaminants (Sprovieri *et al.*, 2007) and comparison of DIN method with other bioaccessibility methods by C.I.E.H (2009) has been carried out. The synthetic juices used, contain both inorganic and organic chemical components. At 37 °C, the sample is placed in gastric juice of pH  $2.0 \pm 0.3$  for two hours, followed by a 6-hour small intestine phase (pH  $7.5 \pm 0.3$ ). Nitrogen is sometimes used to create anaerobic conditions but is optional (Sprovieri *et al.*, 2007).
- **RIVM *In vitro* digestion model:** is an *in vitro* digestion model designed by the National Institute of Public Health and the Environment (RIVM) in the Netherlands. This is a three-stage sequential extraction method using a five minute saliva phase at pH 6.5 the least important stage, followed by a two hour stomach extraction at pH 1.07 and another two hour small intestine extract at pH 5.5 (Oomen *et al.*, 2003).
- **TNO simulated gastro-intestinal tract model (TIM)** is a multi-compartmental, dynamic, computer-controlled system that simulates the human upper GI tract which was developed at TNO Nutrition and Food Research at Zeist in the Netherlands. It was initially developed for studies of pollutants in soils but has now been used for studies on availability/absorption of minerals, vitamins and food mutagens in humans; the survival of ingested bacteria and yeasts (Dickinson *et al.*, 2012, Barker *et al.*, 2014, Krul *et al.*,

2000). It has also found application in drug industry for studying bioavailability and accessibility of drugs (Barker *et al.*, 2014). The method developed by TNO Nutrition group, is a complex *In vitro* test system involving a number of gastrointestinal solutions. It mimics the transit through the guts, the pH of the stomach and intestine, secretion of digestive juices over time. The system is usually dynamic. It involves mathematical modelling of gastric and intestinal delivery with power exponential equations on computers for controlling meal transit through the system (Van de Wiele *et al.*, 2007). Methods to understand and predict the oral bioavailability of drug products are a prioritized research area within the pharmaceutical industry.

- ***In vitro* model of gastrointestinal digestion** is a GI model that includes sorption to human enterocytes caco-2 (human colorectal carcinoma cell line) (Vasiluk *et al.*, 2008, Wan-ling *et al.*, 2011). The Caco-2 cell model is the best characterized among intestinal cell culture model because of its transport properties of in pharmaceutical where it is used (Environment Agency, 2009, Vasiluk *et al.*, 2011).
- ***In vitro* model of gastrointestinal digestion followed by sorption to a surrogate membrane, ethylene vinyl acetate thin film** (Samsøe-Petersen *et al.*, 2002).
- **Unified BARGE method (UBM):** This bio-accessibility test has been developed by the Bio-accessibility Research Group of Europe (BARGE) for assessing inorganic

contaminants and is known as the Unified BARGE method (UBM). This progression in 2005 took the form of combining the collective efforts of researches from its member to evaluate *in vitro* methods already in existence. The method originally developed by National Institute for Public Health and Environment (RIVM) of Dutch as in Oomen *et al.*, (2003). This was considered to be the most representative of the physiochemical conditions in the human GI tract. Modifications were made to the RIVM methodology to ensure adequate conservatism and that the *In vitro* test was robust and applicable to the local geological conditions in a range of different countries, It was tested and validated against the animal method and a standard operating procedure (SOP) was made. The UBM procedure is as seen in Figure 2.11. This has now been extensively used in bioavailability study of potentially toxic metals (Denys *et al.*, 2006, Gomez-Eyles *et al.*, 2011, Denys *et al.*, 2012, Pelfrêne *et al.*, 2012).

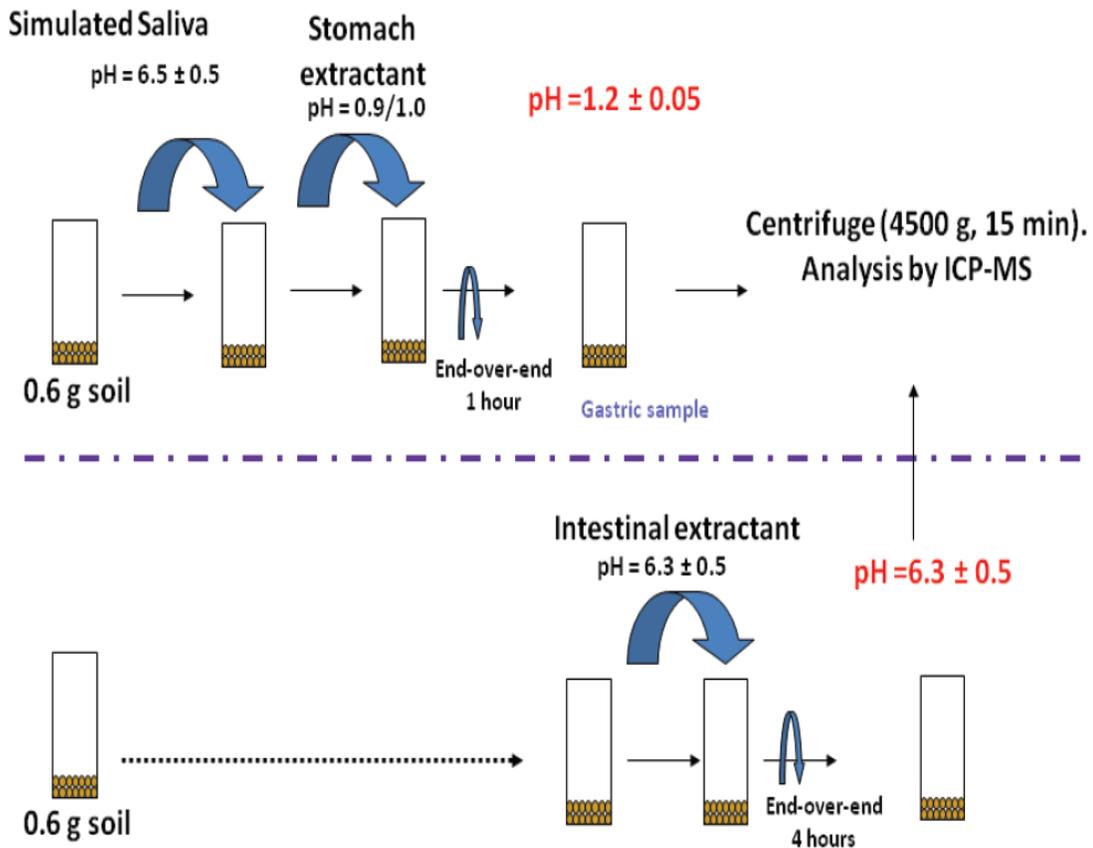
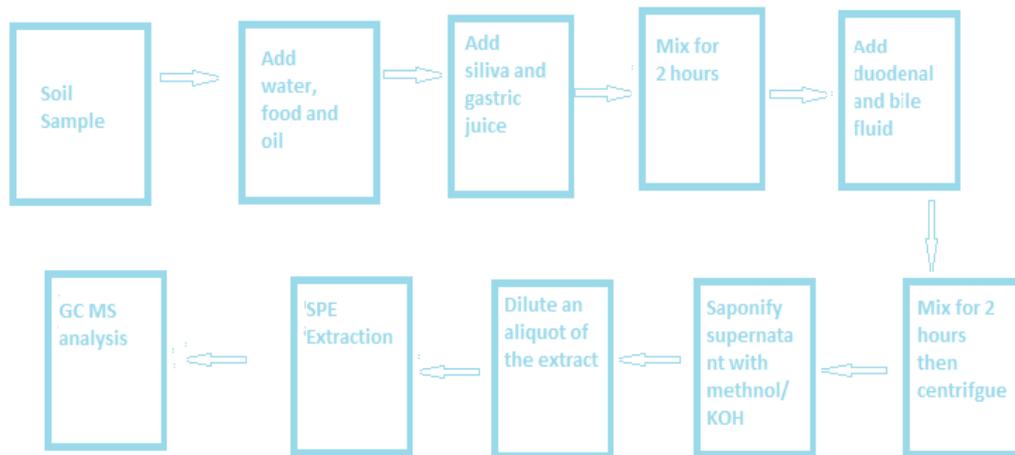


Figure 2. 12: Schematic diagram of UBM methodology (Nogueroles, 2007).

- Fed organic estimation human simulation test (FOREhST):** Members of BARGE who originally developed the UBM which is fasted state protocol for As and Pb (inorganic contaminants) proposed a standardised bioaccessibility test for PAHs (organic contaminants) in soils, referred to as the FOREhST method. As part of the extraction test, pure sunflower oil and an ‘organic’ creamy porridge infant food are included. They added these ingredients because of their similarity to the macronutrient composition of the average diet of a 4–6 years old child in the UK. The food constituents added to the *in vitro* gastrointestinal extraction test considerably complicates the related analytical

methodology required to isolate the PAHs. As a result of the complication caused by food addition to enzymatic juices of the guts, additional steps of isolation and clean-up (e.g. saponification, solid phase extraction) were included and compared to a fasted version of the extraction test (UBM) as shown in Figure 2.12 (Cave *et al.*, 2010, Lorenzi *et al.*, 2012).



**Figure 2. 13: Schematic Diagram of FOREShT Methodology.**

A summary/ comparison of the various GIT models is as shown in Table 2.5.

**Table 2. 5: A Comparison and Summary of the Various GIT Models.**

Method	PBET	CE-PBET	SBRC (SBET /RBALP)	IVG	RIVM	DIN	SHIME	TIM	UBM	FORES <sub>h</sub> T
<b>Segments included</b>	Stomach, intestine	Stomach, intestine	Stomach, intestine rarely	Stomach or intestine	Saliva, Stomach and intestine	Stomach and intestine. Saliva optional	Stomach and small intestine, colon optional	Saliva, Stomach and intestine	Saliva, Stomach and or intestine	Saliva, Stomach and intestine
<b>Fasted or Fed</b>	Fasted	Fed	Fasted	Fasted or fed	Fasted or fed	Fasted or fed	Fasted or fed	Fasted	Fasted	Fed
<b>Principles in relation to <i>In vitro</i> bioavailability</b>	Simplified based upon human physiology	Simplified based upon human physiology	Simple buffered aiming at robust worst case	Simplified based upon human physiology	Corresponding to human physiology	Corresponding to human physiology	Corresponding to human physiology	Dynamic simulation of the human physiology	Simplified based upon human physiology	Simplified based upon human physiology
<b>Mechanism of operation/ conditioning</b>	End over end rotation at 30± 2 rpm, 37 ± 2 <sup>o</sup> C	End over end rotation at 30± 2 rpm, 37 ± 2 <sup>o</sup> C	End over end rotation at 30± 2 rpm, 37 ± 2 <sup>o</sup> C	-	End over end rotation at 55rpm, 37 <sup>o</sup> C ± 2	Agitator at 200 rpm, 37 <sup>o</sup> C ± 2	Mechanical stirring 150 rpm, 37 ± 2 <sup>o</sup> C	Peristaltic movements	End over end rotation at 30± 2 rpm, 37 ± 2 <sup>o</sup> C	End over end rotation at 30± 2 rpm, 37 ± 2 <sup>o</sup> C
<b>Mass dried Soil/ soild matrice used</b>	0.6g	0.6g	1g	0.6g	0.6g	2g	10g	10g	0.6g	0.3g
<b>pH of Siliva (s), Stomach (G) and intestinal (I) solutions</b>	S-Not applicable, G-2.5, I- Small intestine	S-Not applicable, G-2.5, I- Small intestine:7.0 I- Colon:6.5	S-Not applicable, G-1.5, I- Not applicable	S-6.8 ±2, G-0.9 to 1.3±2, I - duodenum- 8.1±2,I	S-Not applicable, G- 1.1, I- (bile Juice)- 8 (Chyme pH mixture -5.5)	S-Not applicable, G-2, I- 7.5(mixture in the intestine)	s-Not applicable, G-4.0	S-5, G- Initially 5 but decreases to 3.5, 2.5, 2, after 30, 60 and 90 minutes	S-6.5, G- 0.9 to 1, I - duodenum- 7.4±2,I-Bile I-Juice- 8.0±2	S-6.5, G- 0.9 to 1, I - duodenum- 7.4±2,I-Bile I-Juice-8.0±2

				<b>-Bile I- Juice- 8.2+2</b>				respectively, <b>I- duodenum- 6.5, I- Jejunum- 6.8, I- Ileum- 7.2</b>		
<b>Length of incubation time in Saliva(s), Stomach (G) and intestinal (I) compartment</b>	S-Not applicable, <b>G-1h, I- Not applicable</b>	S-Not applicable, <b>G-h, I- Small intestine:4 h</b>	S-Not applicable, <b>G-1h, I- Not applicable</b>	S- 5mins, <b>G-2h, I- 2h</b>	<b>S-5mins, G- 2h, I-2h</b>	S-Not applicable, <b>G- 2h, I-6h</b>	S-Not applicable, <b>G-3h, I-5h</b>	S-5mins, <b>G- Gradual secretion of content at 0.5 ml/min, I-secretion of intestinal fluids at 1 ml /mins</b>		<b>S-5 mins, G- 2h, I-2h</b>
<b>Enzymatic composition of saliva (s), gastric Juice (G) and intestinal juice (I)</b>	S-Not applicable, <b>G-Pepsin, I:Pancreatin, bile salt, bile salt</b>	S-Not applicable, <b>G-Pepsin, I-small intestine:P ancreatin, bile salt I- colon: bile salt</b>	S-Not applicable, <b>G-None, I- Not applicable</b>	<b>S-α- Amylase G-</b>	<b>S-, G-Pepsin, mucin, bovine serum albumin bovine origin, I- Lipase, Pancreatine, bovine serum albumin (Enzyme source : Borvine)</b>	S-Not applicable, <b>G-Pepsin, mucin, I- trypsin, Pancreatine ( Enzyme source:Porcine)</b>	S-Not applicable, <b>G- Pectin, Mucin, cellobiose, proteose peptone, starch, I – Pancreatine (Enzyme origin : borvine)</b>	<b>S-, G-Lipase, Pepsin, I – Pancreatine ( Enzyme source:Porcine)</b>	<b>S-α- Amylase G-</b>	<b>S-α- Amylase G-</b>

<b>Contaminants tested and validated against <i>In vitro</i> model</b>	Lead, arsenic, (inorganics )	Lead, arsenic, (inorganics ) and PAHs( usually in the fed state), Flame retardants	Lead, Arsenic, (Inorganics )	Lead, arsenic, Cadmium (Inorganics)	Lead, Arsenic, Cadmium from soil, ochratoxin A, aflatoxin B1 from food, and phthalate from PVC	Lead, Cadmium, Nickel , (inorganics)P AHs, Organics	Lead, PAHs	Lead	Inorganic( leads, Cadmium, Arsenic ,et.c) PAHs(note fed version used here)	PAHs, Pesticides, Flame retardants (organics )
<b>Contaminated Matrix Organic contaminants studied</b>		OECD standard soil artificially spiked with PAHs. For flame retardant, dust from UK homes were analysed.	Soil from a former railway soils, dip sites, mines	Soils from 18 hazardous sites in USA			Soil contaminated by at atmospheric deposition. Sum PAHs ;49±1.5mg/kg, Organic matter content ;3.3%, sand ;94%,silt ;2%, Clay; 2%.pH;6.5		Soil was from a manufacturing gas plant, mine waste, households. Soil properties were TOC;0.5-1.7%,Clay; 8-36%,pH;4.6-7.4	Soil was from a former industrial site Designated as contaminated site by BGS
<b>Bioaccessibility values for PAHs</b>		Bioaccessible PAHs ranged from 7% to 90%. Bioaccessible flame retardant ranged from 12% to 92%	As bioavailability <i>In vitro</i> study ranged between 6.9% to 74.7% and these values correlated to SBET <i>in vivo</i>	Pb bioavailability ranged from 0.56 to 32%			% bioaccessibility for sum PAHs ranged from 0.1 to 1.4%		Benzo(a)pyrene bioaccessibility ranged from 2.16% and Dibenz(a,h)anthracene ranged from 11-21%	Bioavailable PAHs ranged from 12% to 155%

			study by $r^2=0.92$							
<b>Reference</b>	(Abrahams <i>et al.</i> , 2006, Dandie <i>et al.</i> , 2010)	(Tilston <i>et al.</i> , 2011, Mohamed <i>et al.</i> , 2012)	(Oomen <i>et al.</i> , 2006, Smith <i>et al.</i> , 2011)	(Fuller and Leadbe ater, 1935, Versant voort <i>et al.</i> , 2005, Dandie <i>et al.</i> , 2010)	(Oomen <i>et al.</i> , 2006, Lorenzi <i>et al.</i> , 2012)	(Gron, 2005, Juhasz <i>et al.</i> , 2007)	(Gron, 2005, Juhasz <i>et al.</i> , 2007, Van de Wiele <i>et al.</i> , 2007, Van de Wiele <i>et al.</i> , 2004, Ng <i>et al.</i> , 2010, Lorenzi <i>et al.</i> , 2012)	(Gron, 2005, Juhasz <i>et al.</i> , 2007)	(Mohamed <i>et al.</i> , 2012)	(Lorenzi <i>et al.</i> , 2012)

## 2.5 A REVIEW OF RISK ASSESSMENT AND BIOACCESSIBILITY STUDIES OF PAHS IN SOILS

Risk assessment of polycyclic aromatic hydrocarbons (PAHs) in soils and sediments has been discussed by Davoli *et al.*, (2010), Chen and Chen (2011), Yang *et al.*, (2012). Yang *et al.*, (2012) in their study of polycyclic aromatic hydrocarbons (PAHs) concentrations in agricultural soils from the Huanghuai plain, China, found the sum concentrations of 16 PAHs in soils varied from 15.7 to 1247.6 mg/kg. The seven carcinogenic PAHs accounted for 10.6 – 75.9 % of the sum PAH concentration and contributed more to sum BaP<sub>eq</sub> concentrations. Sediment samples collected from some locations of Kaohsiung Harbor, Taiwan and analyzed for polycyclic aromatic hydrocarbons (PAHs) had sum PAH concentrations which varied from 472 to 16,201 ng/g dry weight. BaP<sub>eq</sub> concentrations of the soil samples also varied from 55 to 1964 ng/g. Higher sum BaP<sub>eq</sub> values were found at industrial zone docks (from 1404 to 1964 ng/g) compared to other zones. Concentration values of PAHs in some of these sites especially at the industrial docks zone when compared with the US Sediment Quality Guidelines (SQGs), exceeded the effects range low (ERL), and could thus cause acute biological damage (Chen and Chen, 2011).

Davoli *et al.*, (2010) also assessed the health risk of dioxins, furans and PAHs from landfills to people living near it based on population exposure, for both children and adults. Hazard index, (HI) is largely below the values accepted from the WHO, US EPA and national legislation. The incremental probability of developing cancer over lifetime, based on a reasonable maximum exposure to PAHs present in Lisbon residential soils, was  $9.0 \times 10^{-6}$  and  $2.4 \times 10^{-6}$  for an

occupational exposure, both were slightly higher than the target risk of  $1 \times 10^{-6}$ . Similarly, the mutagenic risk of PAHs in Lisbon was  $3.3 \times 10^{-5}$  for residential soils and  $1.8 \times 10^{-6}$  in recreational areas (Cachada *et al.*, 2012).

Lorenzi *et al.*, (2012) in their study of the sum and bioaccessible concentration of 16 polycyclic aromatic hydrocarbons (PAHs) in soil from a former industrial facility found the typical sum concentrations across the sampling sites ranged from 1.5 mg/kg for acenaphthylene up to 243 mg/kg for fluoranthene. The oral bioaccessibility of PAHs in soil was assessed using the FOREhST method. The oral bioaccessibility data indicated that fluorene, phenanthrene, chrysene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene had the highest percentage bioaccessible fraction while the other PAHs had lower percentage bioaccessible fractions (ranging from 35 to 59 %). Significantly lower bioaccessibilities were determined for naphthalene. The sum PAH concentrations at the site was compared with generic assessment criteria (GAC) using the residential land use scenario (with plant uptake at 6 % soil organic matter). Concentrations of seven of the PAHs investigated within the soils could lead to an unacceptable risk to human health at this site. Bioavailable PAHs from soils in other studies have been found to vary between 10 % and 60 % for soil containing sum PAHs between 10,000 and 300,000 ng/g (Cave *et al.*, 2010), 0.1 to 1.4 % (Van de Wiele *et al.*, 2004), 1 to 3 % in aged soils crude oil contaminated soil (Kogel-Knabner *et al.*, 2000) and 0.5 to 2 % gastro intestinal solubility (Holman *et al.*, 2002).

## 2.6 ANALYSIS OF PAHs

Analytical procedures have several steps: sampling, sample preparation, separation, quantification, statistical evaluation, decision and action (Pawliszyn, 1997, Khan *et al.*, 2005). Sampling involves investigation, ensuring representative samples are obtained etc. Sample preparation includes washing, shredding, and sieving depending on the type of sample. Separation is usually to isolate and clean up the analyte of interest as instruments cannot handle dirty mixed or dirty extract. Quantification is determining of the amount or concentration of analyte. Usually this involves instruments like the gas chromatograph, ultraviolet spectrometer and liquid chromatograph. Statistical evaluation provides estimates and adds meaning to data generated. The data generated indicates certain decisions which might lead to taking new samples for further research. Analytical steps follow one after the other and not simultaneously and the next one cannot commence until the preceding step finishes as shown in Figure 2.10. Therefore the slowest step determines the overall speed of the analytical process and error in a preceding step is usually carried over to the next step (Pawliszyn, 1997, Khan *et al.*, 2005).

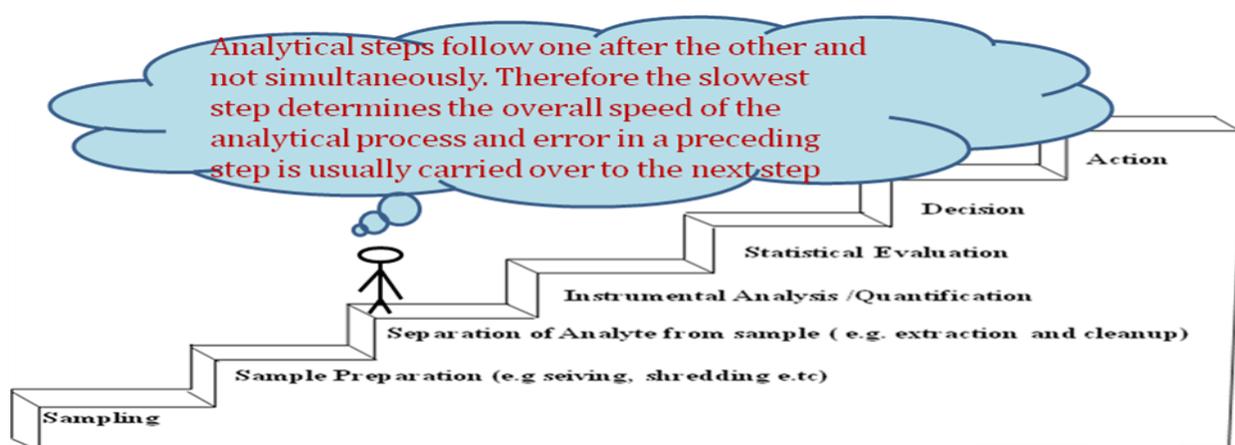


Figure 2.14: Steps in Analytical Process

### **2.5.1 Extraction of PAHs**

Reliable trace-level analysis begins with the quantitative extraction of the analytes from the sample matrix in a manner which is compatible with the rest of the analytical procedure (Shu *et al.*, 2000). The extraction and analysis of PAHs from soils (natural soils, contaminated soils, and soils before and after remediation by various techniques) and other environmental matrices, such as sediments, dust, plant tissues and tar particles, are an essential step in research. Mechanical shaking or stirring, Soxhlet extraction and ultrasonic extraction are the most commonly used methods for extracting PAHs from soils and other solid environmental materials (Song *et al.*, 2002).

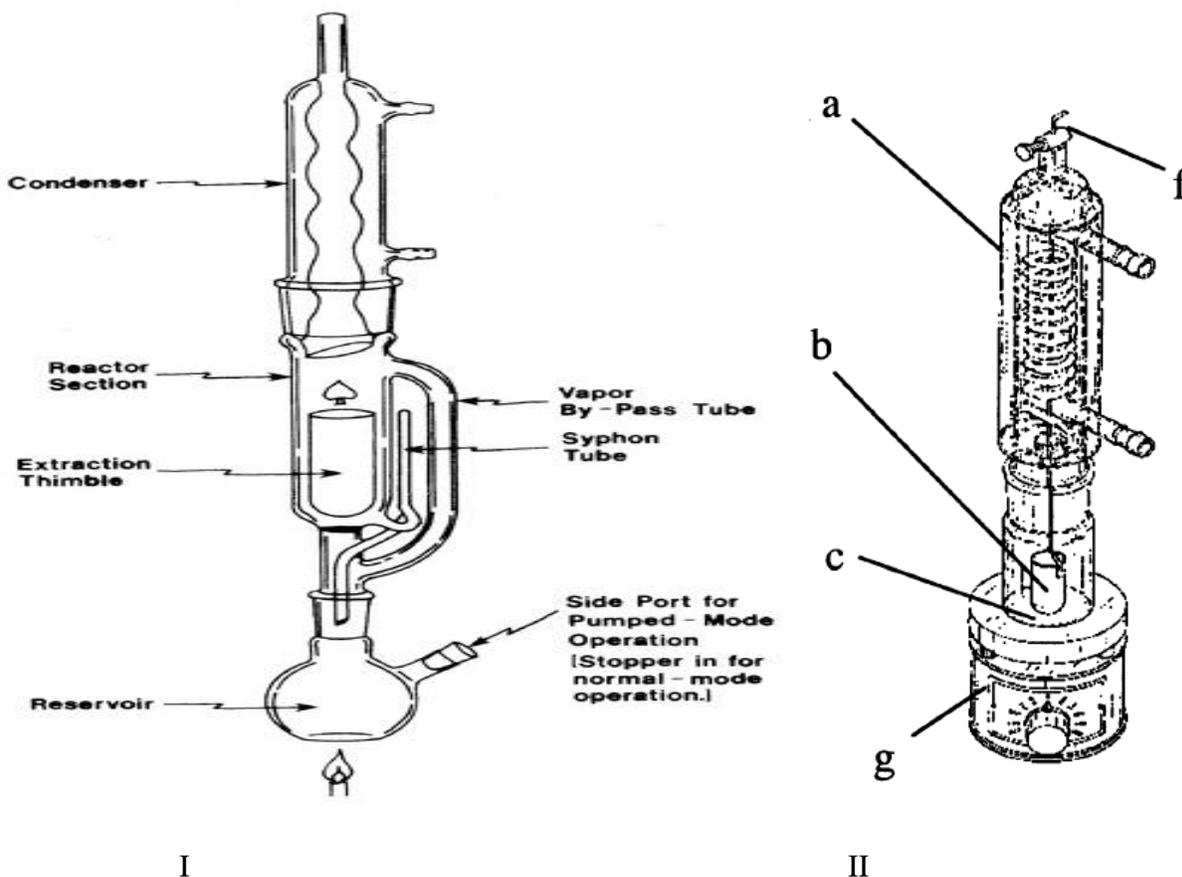
#### **2.6.1.1 Soxhlet extraction**

Soxhlet as shown in Figure 2.15, has been around for over 100 years, having been invented by Franz von Soxhlet in 1879 and has for many years been the standard method for preparing solvent extracts of solid matrices containing PAHs (Sun *et al.*, 1998, Diletti *et al.*, 2005, Environment Agency, 2002). However, it is tedious as the extraction time is long. Basically, in the Soxhlet extraction technique, the solid sample is placed into an extraction thimble which is then extracted using an appropriate solvent via the reflux cycle. Once the solvent is boiled, the vapour passes through a bypass arm into the condenser, where it condenses and drips back onto the solvent in the thimble. As the solvent reaches the top of the siphon arm, the solvent and the extract are siphoned back onto the lower flask whereby the solvent re-boils, and the cycle is repeated until all the sample is completely extracted into the lower flask (Shu *et al.*, 2000 , Environment Agency, 2002). The main disadvantage of this extraction process is the use of large

volumes of solvent, which can be more than 150 ml and up to 500 ml (Shu *et al.*, 2000 ) for the extraction of PAHs from about 10 g of soil sample. In addition, the method is labour intensive and time consuming, as the solvent has to be refluxed up to 4 to 48 h (Schuwab *et al.*, 1999, Shu *et al.*, 2000 ) to achieve considerable extraction efficiencies. A quarter to one third of bulk organic matter is removed during extraction. Chromatograms of extracts produced via Soxhlet using gas chromatogram mass spectrometer and gas chromatogram – flame ionization detector yielded more artefact peaks with branched alkane “humps,” demonstrating that compounds such as n-alkanes and humic substances other than PAHs are co-extracted using the Soxhlet technique (Lau *et al.*, 2010). Other drawbacks of using the Soxhlet apparatus include the likelihood of sample carryover, the need to fractionise extracts to avoid heavy contamination of GC injection port and the unfeasibility of re-dissolving dried Soxhlet extracts (Diletti *et al.*, 2005).

Nonetheless, the Soxhlet extraction is still used because of its comparative extraction results despite the nature of matrix extract. Not only does the Soxhlet extraction yield similar results with methods such as the supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), and ultrasonic methods, but the results also show small variations with low relative standard deviations (Lau *et al.*, 2010). The efficiency of the Soxhlet extraction increases with molecular weight, reaching an efficiency range of 84 to 100% for PAHs with more than 4 rings. To further improve the Soxhlet extraction technique, Edward Randall in 1974, patented the automated Soxhlet extraction method in 1974 (Environment Agency, 2002, Lau *et al.*, 2010) (Figure 2.15-11). This is a two-step procedure which combines

boiling and rinsing such that the sum extraction time is reduced while the evaporated solvent condenses rapidly for reuse, reducing the amount of sum solvent required. In this improved technology, the extraction thimble is initially lowered directly into the flask containing the boiling solvent to remove residual extractable material while the extractable materials pass readily from the sample and dissolve into the solvent simultaneously. The level of solvent is then reduced to a level below the extraction thimble such that the configuration mimics the traditional Soxhlet extractor whereby the PAH is extracted by refluxing condensed solvent and collected in the solvent below the extraction thimble. With this improvisation, the PAH extraction efficiencies and precisions were statistically improved (Wightman and Dayton, 1982) and Automated Soxhlet extraction is approved by the U.S. Environmental Protection Agency (EPA) for the extraction of organic analytes from soil, sediment, sludge, and waste solids (Environment Agency, 2002).



**Figure 2. 15: I- A Soxhlet Extractor, II- A Randall Extraction Apparatus (a) Condenser (b) sample Thimble (c) Solvent Flask (d) Siphon Tube (e) Solvent Vapor Tube (f) Thimble Positioning Mechanism (g) Heater (not shown on the Soxhlet). In the original Randall Method, the Thimble is Positioned by use of the Slide Rod (f). Lowering the Thimble (b) into the Boiling Solvent for the Boiling step, then Raising it out of the Solvent for the Rinsing Step. In Both Stages, Condensed Solvent is Flowing Continuously Through the Sample and Thimble Back into the Boiling Solvent (Wightman and Dayton, 1982).**

### 2.6.1.2 Microwave assisted extraction

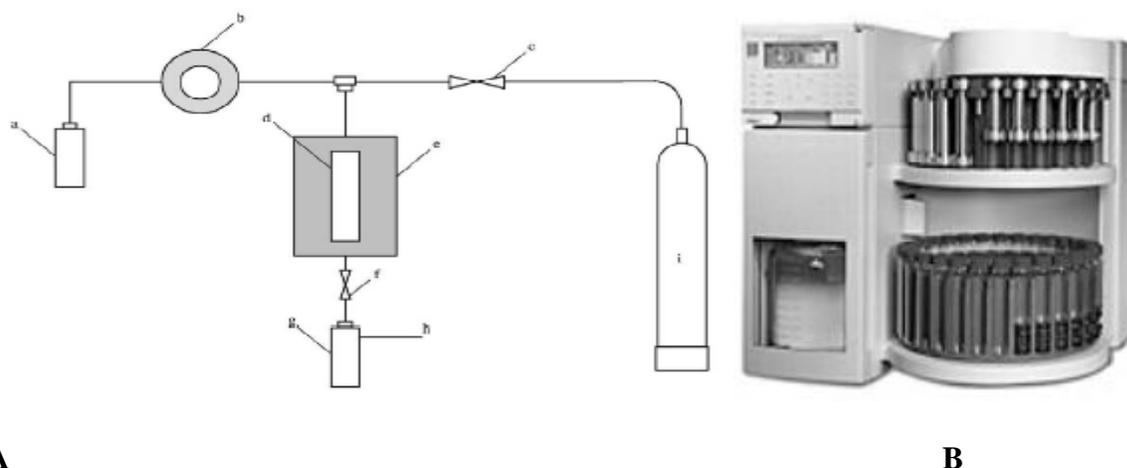
Microwave assisted extraction (MAE), first reported in 1995 was considered as an appreciable technique (Letellier *et al.*, 1999). The main advantages are low temperature requirement, high extraction rate, automation and the possibility of simultaneously extracting different types of

samples. MAE utilizing microwave energy to cause rotation of molecules with permanent dipole and disorganised movement, leading to rapid heating of the solvent and pressure, has been developed (Letellier *et al.*, 1999) and has now been found to be good alternative to Soxhlet for extraction of PAHs from environmental matrices (soils, sediments, air particles, biological organisms, plant and food) with advantages of security and easy manipulation with a reduction of solvent volume and extraction time. Microwave irradiation is often used instead of conventional heating because the solvent is rapidly heated in a reproducible way. Moreover, the selective interaction with polar molecules allows local heating and an improvement of extraction efficiency. MAE for PAHs has been published (Sun *et al.*, 1998, Lang *et al.*, 2000). Considering the safety, the temperature and the pressure inside the extraction vessel should be controlled (Szolar *et al.*, 2004).

### **2.6.1.3 Pressurized solvent extraction**

Pressurised Liquid Extraction is also known as accelerated solvent extraction (ASE) is like Soxhlet extraction, the only difference being the use of solvents that are raised to the near-supercritical region, where they show better extraction properties. At high temperature, the rate of extraction increases because the viscosity and the surface tension of the solvent drop, while its solubility and diffusion rate into the sample increase. Pressure keeps the solvent below its boiling point and forces its penetration into the pores of the sample. The combination of high temperature and pressure results in better extraction efficiency, so minimizing solvent use and expediting the extraction process. The time required for extraction is practically independent of the sample mass and the efficiency of extraction is mainly dependent on temperature. The

sample is loaded in a stainless steel extraction cell into which solvent is pumped and brought to a specified temperature and pressure. The temperature is normally kept between 80 and 200 °C pressure ranges between 10 and 20 MPa. Conditions are kept constant for some minutes in order to permit the static transfer of analytes from the sample into the solvent. The extract is pushed into the collection vial by a second aliquot of solvent inserted into the extraction cell and this second aliquot is then collected into the same vial by pushing it with an inert gas flow. The whole procedure takes about 15–20 minutes (Buldini *et al.*, 2002). It has the advantage of reducing solvent consumption and extraction time although it has a disadvantage of having to use expensive specialized equipment in order to comply with safety precautions related to the high pressure and superheated solvent. PAHs and isomer ratios from biomass burning emissions were extracted by ASE in a study by Rajput *et al.*, (2011). In another study by Wu *et al.*, (2006), ASE was employed in the extraction of PAHs in samples for particle size distributions in Tianjin, China and Zhou *et al.*, (2005) has also developed a micro pressurized liquid extraction device for the determination of PAHs on PM<sub>10</sub> samples. A schematic representation of PLE is as seen in Figure 2.16.



**Figure 2. 16: A -Schematic Representation of PLE Apparatus: a) Solvent Supply b) Pump c) Purge Valve d) Extraction Cell e) Furnace f) Static Value g) Collection Vial (h) Filter and (i) Dual High-Pressure Piston Pump (Buldini *et al.*, 2002). B-A picture of PLE.**

#### 2.6.1.4 Mechanical shaking extraction

This simple, low-cost method uses agitation or mixing action to extract the PAHs from samples in a shake-flask placed onto a rotary shaker, or with a magnetic stirrer submersed into the flask directly. Although it is an easy handling method with minimal glassware and smaller volumes of extraction solvent, this method has not been as widely used as the Soxhlet and sonication due to the lower extraction efficiency and unsatisfactory quantitative results. Schwab *et al.*, (1999) and Zhou *et al.*, (2005) reported that this method was comparable to the Soxhlet technique. However, results obtained using mechanical shaking showed larger variations and less selectivity. Comparable results were only attainable with long shaking times to extend the contact time with solvent (Lau *et al.*, 2010, Oluseyi *et al.*, 2011). Mechanical shaking involves

placing a mixture of sample and appropriate solvent in a glass ware to extract PAHs. The extract is usually left for hours, solvent decanted and cleaned up for analysis.

#### **2.6.1.5 Ultrasonic extraction**

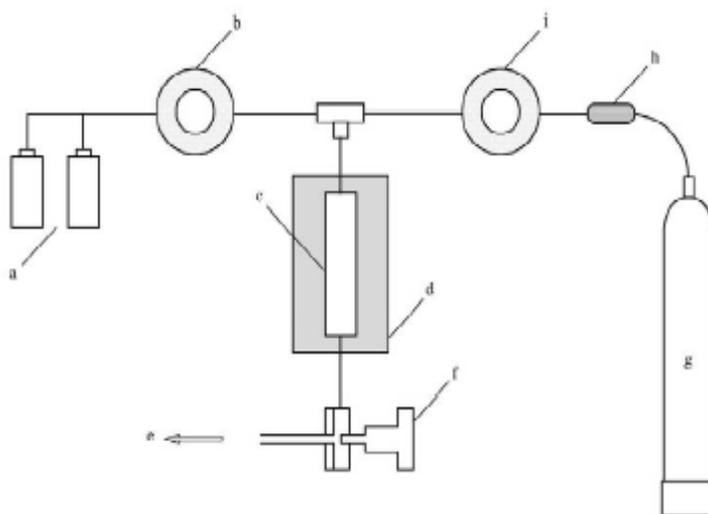
Ultrasonic radiation sometimes called sonication is a powerful technique as it facilitates and accelerates operations such as the extraction of organic and inorganic compounds in solid and liquid samples. This technique which makes use of a mechanical acoustic energy of ultrasonic waves with at least a frequency of 16 kHz (low frequency) in fluid, in the form of a shearing action giving rise to rapid compression and rarefaction of fluid movement (Khan *et al.*, 2005). These movements results in the cavitation phenomenon, that is, the reoccurring formation and collapse of microbubbles (Lau *et al.*, 2010). Ultrasonic agitation can be performed either by immersing a sonicator transducer (ultrasonic horn) into the sample solvent mixture or placing the sample solvent mixture directly into a sonication bath. A piezoelectric ceramic attached either to the ultrasonic horn or the wall of the sonication bath generates the ultrasound. This is a simple method and in many cases, ultrasound extraction is an expeditious, a relatively low cost method, rapid and efficient alternative to conventional extraction techniques to Soxhlet and in some cases, even to supercritical fluid and MAS extraction, as demonstrated by application to both organic and inorganic analytes in a wide variety of samples (Priego-López and Luque-de-Castro, 2003, Wheatley and Sadhra, 2004, Oluseyi *et al.*, 2011). Studies have shown that ultrasonic extraction techniques yield comparable or even greater quantities (Song *et al.*, 2002, Lau *et al.*, 2010) of PAHs than other techniques of extraction. Depending on the contaminants and matrix, sonication can have the advantage of faster extraction times and usually provide,

using small volumes of organic solvent without the need of elaborate glassware and instrumentation. In addition, the equipment for ultrasonication is very simple, and it is easy to operate (Priego-López and Luque-de-Castro, 2003).

### **2.6.1.6 Supercritical fluid extraction**

This technique resembles Soxhlet extraction but the solvent used is a supercritical fluid (Lang *et al.*, 2000). Examples of supercritical fluids used include water, ammonia, nitrous oxide and carbon dioxide. Supercritical fluids above their critical temperature and pressure, provides unusual combination of properties including liquid-like density, low viscosity, high diffusivity and zero surface tension enabling them through solids but dissolve analytes like liquids, so that they cause the conditions that are similar to those in Soxhlet extraction. Nitrous oxide is a good supercritical fluid but is flammable, ammonia is a good supercritical fluid but it is reactive in nature. Water has also been considered as the extraction fluid. However, the use of supercritical water is limited because of the high temperature (>374 °C) and pressure (>218 atm) requirements that creates a highly corrosive environment (Lau *et al.*, 2010). Carbon dioxide which has a supercritical temperature and pressure of 31 °C and 74 bar respectively is an excellent alternative to the potentially hazardous and expensive solvents used in Soxhlet. Extraction by supercritical fluid can be performed in static, dynamic or re-circulating mode (Pawliszyn, 1997, Kataoka *et al.*, 2000, Buldini *et al.*, 2002). When performing static extraction, the cell is filled with the supercritical fluid pressurised and allowed to equilibrate. In the dynamic mode, the supercritical fluid is passed through the extraction cell continuously. In the

re-circulating mode, the same fluid is repeatedly pumped through the sample and, after the required number of cycles; it is pumped out to the collection vial. The high rate of penetration of the supercritical fluid into the sample permits fast back diffusion of analyte, thus reducing extraction time. The complete step is performed in less than 30 minutes (Szolar *et al.*, 2004). Although supercritical fluid extraction has many advantages over other methods for sample preparation, it requires complicated equipment and which sometimes costs more (Buldini *et al.*, 2002, Lang *et al.*, 2000). A schematic representation of SFE is as seen in Figure 2. 17.

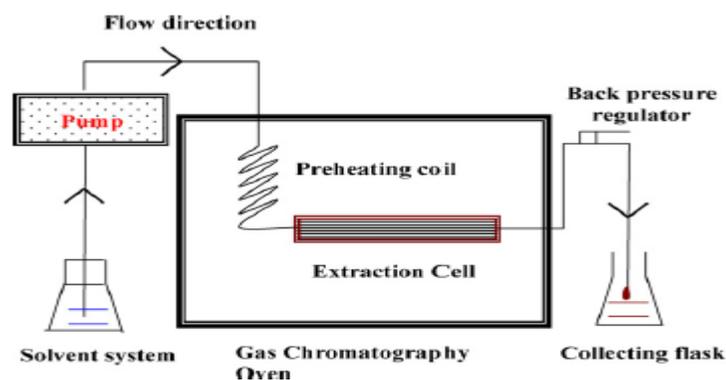


**Figure 2. 17: Schematic of SFE Equipment: (a) Modifier Supply, (b) Pump, (c) Extraction Cell, (d) Furnace, (e) to Collection, (f) Flow Restrictor, (g) Fluid Supply, (h) Filter and (i) Dual High-Pressure Piston Pump (Buldini *et al.*, 2002).**

### 2.6.1.7 Pressurized hot water extraction

Pressurized hot water (PHW) has been extensively used to replace organic solvents in extraction processes. The term, “PHW” is used to denote the region of condensed phase of water between the temperature range from 100°C (boiling point of water) to 374°C (critical point of water).

Other common terms such as “superheated water”, “near critical water”, “subcritical water”, “high temperature extraction” and “extraction using hot compressed water” have also been used. In the case of PHW, the density of water remains almost constant over this range of temperature so that the pressure effect on the properties of water is minimal (Teo *et al.*, 2010). It is used in place of supercritical water extract due to the limitation posed by supercritical water. The altered physico-chemical properties of pressurised hot water can be exploited in the extraction of organics from solid samples. As the temperature of water is raised from 100°C to near the critical temperature under pressure, the hydrogen bonding network of water molecules weakens resulting in a lower dielectric constant and simultaneously decreasing of its polarity. Pressure has to be high enough to keep the water in liquid state. Thus, subcritical water becomes more hydrophobic and organic-like than ambient water, promoting miscibility of light hydrocarbons with water. In contrast to SF extraction which extracts mostly non-polar organic compounds, PHW extraction has been applied for extraction of many organic compounds including PAHs (Khan *et al.*, 2005). It has been reported that PHW gives better preference to more polar organic analytes (Khan *et al.*, 2005, Teo *et al.*, 2010), therefore providing a higher extraction efficiency of PAHs with less or almost no extraction of other alkanes. Wet oxidation or PHW combined with oxidation using oxidising agents such as air, oxygen, or hydrogen peroxide was reported to remobilise bound organic residues, providing a higher extraction capability. PHW extraction is gaining increasing applications as a green extraction solvent. Depending on temperature, it can be very effective to selectively extract a variety of polar or non-polar organic compounds from many different matrices (Fornari *et al.*, 2011). An example of the PHWE is shown in Figure 2.18.

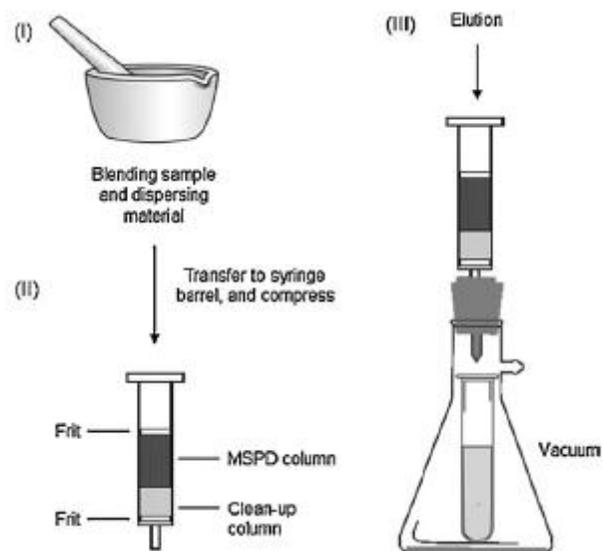


**Figure 2. 18: A schematic Diagram of a Laboratory Assembled PHWE System (Teo *et al.*, 2010)**

### **2.6.1.8 Matrix solid-phase dispersion**

Matrix solid-phase dispersion (MSPD) is a simple and cheap sample preparation procedure allowing for the reduction of organic solvent consumption, exclusion of sample component degradation, improvement of extraction efficiency and selectivity, elimination of additional sample clean-up and pre-concentration step before chromatographic analysis (Dawidowicz and Rado, 2010 , Barker, 2007). The main steps in MSPD extraction procedure are as described in Figure 2.19.

- (I) The sample is blended with the dispersant material in a mortar with a pestle.
- (II) The homogenized powder is transferred in a solid-phase extraction cartridge and compressed.
- (III) Elution with a suitable solvent or solvent mixture is performed by the aid of a slight vacuum



**Figure 2. 19: The MSPD Process**

## 2.6.2 Clean up of extracts

Different approaches have been employed to cleanup and further concentrate the extracts to isolate PAHs from interfering matrix substances. The approaches employed include the use of sorbent, membrane, liquid–liquid extraction (LLE) or liquid-phase microextraction (Xu and Lee, 2008) and gel permeation chromatography (GPC) (Alexander *et al.*, 2008, Diletti *et al.*, 2005).

### 2.6.2.1 Sorbent extraction

The use of a solid adsorbent material to clean-up or extract analytes from a solution was developed in the 1980s and is now widely applied to many matrices. A sorbent with strong affinity towards some target analytes will retain and concentrate those compounds from the sample solution. Many sorbents are specifically suited for the extraction of different analytes with various degrees of selectivity. Appropriate sorbents are usually loaded onto long columns.

The columns are usually prepared by slurry packing the adsorbents. On loading the sample onto the column, the samples are eluted (washed) with a solvent, the fraction containing the non-polar co-extractives was discarded and PAHs are then extracted with the appropriate solvent (Smith *et al.*, 2006). Solid phase extraction (SPE) and the solid phase micro extraction (SPME) are common examples of the sorbent technique (Buldini *et al.*, 2002, Pawliszyn, 1997). SPE has been successfully used instead of packed chromatographic columns (Barranco *et al.*, 2003).

- **Solid-phase extraction**

Solid-phase extraction (SPE) is method that uses a solid-phase and a liquid-phase to isolate analyte from a solution into a small volume. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantify the amount of analyte(s) in the sample (Sun *et al.*, 1998, Marce and Borrull, 2000, Kouzayha *et al.*, 2011, Oluseyi *et al.*, 2011,). The general procedure is to load a solution onto the SPE phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube. The principle of SPE is similar to that of liquid-liquid extraction (LLE), involving a partitioning of solutes between two phases. However, instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. Solid-phase extractions use the same type of stationary phases as are used in liquid chromatography columns. The stationary phase is contained in a glass or plastic column above a frit or glass wool. There are two types of stationary phases used in liquid chromatography.

**-Reversed-phase** involves a polar or moderately polar sample matrix (mobile phase) and a non-polar stationary phase. The analyte of interest is typically mid- to non-polar. Retention of organic analytes from polar solutions (e.g. water) onto these SPE materials is due primarily to the attractive forces between the carbon-hydrogen bonds in the analyte and the functional groups on the sorbent surface. These non-polar non-polar attractive forces are commonly called vander Waals forces or dispersion forces. A non-polar solvent, which can disrupt the forces between the sorbent and compound, is used to elute an adsorbed compound from a reversed phase SPE tube or disk. The following materials are used as reversed phase: carbon-based media, polymer-based media, polymer-coated and bonded silica media. Carbon-based media consist of graphitic, non-porous carbon with a high attraction for organic polar and non-polar compounds from both polar and non-polar matrices. Retention of analytes is based primarily on the analyte's structure, rather than on interactions of functional groups on the analyte with the sorbent surface. Polymer-based sorbents are styrene/divinylbenzene materials. It is used for retaining hydrophobic compounds which contain some hydrophilic functionality, especially aromatics. Elution steps can be done with mid- and non-polar solvents, because the polymeric packing is stable in almost all matrices. Polymer-coated and bonded silica media is hydrophobic-bonded silica that is coated with a hydrophilic polymer. The pores in the polymer allow small, hydrophobic organic compounds of interest (e.g. drugs) to reach the bonded silica surface, while large interfering compounds (e.g. proteins) are shielded from the bonded silica by the polymer and are flushed through the SPE tube.

**-Normal-phase** involves a polar analyte, a mid- to non-polar matrix (e.g. acetone, chlorinated solvents and hexane) and a polar stationary phase. Retention of an analyte under normal phase conditions is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. These include hydrogen bonding,  $\pi$ - $\pi$  interactions, among others. A compound adsorbed by these mechanisms is eluted by passing a solvent that disrupts the binding mechanism, usually a solvent that is more polar than the sample's matrix. The bonded silicas have short alkyl chains with polar functional groups bonded to the surface. These silicas, because of their polar functional groups, are much more hydrophilic relatively to the bonded reversed phase silicas. As with typical normal phase silicas, these sorbents can be used to adsorb polar compounds from non-polar matrices.

The polar adsorption material is modified silica gel commonly used as the base of all of the bonded phases. The functional groups that are involved in the adsorption of compounds from non-polar matrices are the free hydroxyl group on the surface of the silica particles. That may be used to adsorb polar compounds from non-polar matrices with subsequent elution of the compounds in an organic solvent more polar than the original sample matrix.

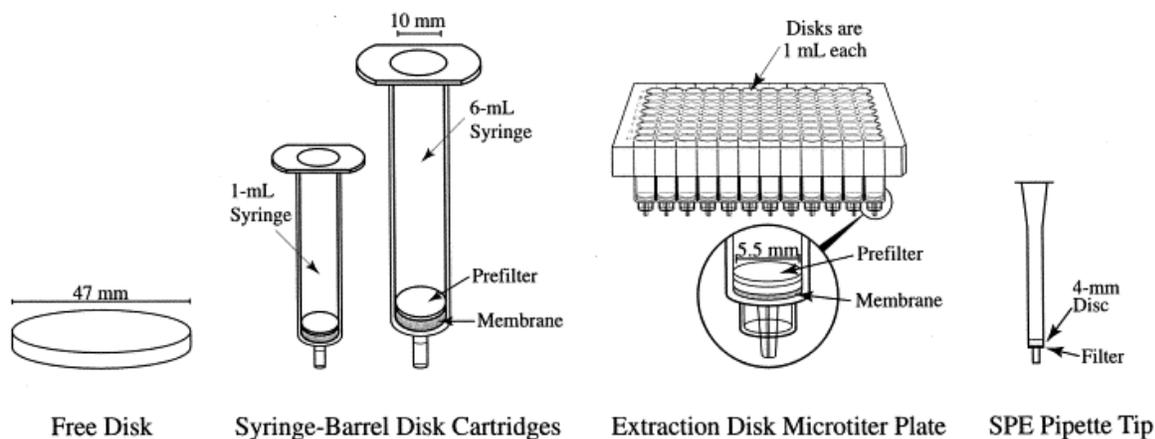
**-Ion exchange** sorbents can be used for compounds that are in a solution. Anionic (negatively charged) compounds can be isolated on an aliphatic quaternary amine group that is bonded to the silica surface. Cationic (positively charged) compounds are isolated by using the silica with aliphatic sulfonic acid groups that are bonded to the surface. The primary retention mechanism of the compound is based mainly on the electrostatic attraction of the charged functional group in the compound to the charged group that is bonded to the silica surface. pH plays significant

role in the use of ion exchange in SPE. Solutions used in SPE procedures have a very broad pH range. Silica-based packings usually have a stable pH range of 2 to 7.5 and at pH levels above and below this range, the bonded phase can be hydrolyzed and cleaved off the silica surface, or the silica itself can be dissolved in SPE, however, the solutions usually have a contact with the sorbent for a short period of time. The fact that SPE cartridges are disposable, and are meant to be used only once, allows one to use any pH to optimize retention or elution of analytes.

In SPE technique four types of sorbent formats exist: free disks (which are generally 47 mm in diameter or the standard filtration size), disks in syringe barrels-cartridge (which vary in size from microsized disks in 1 ml syringes to a 6 ml syringe), a 96-well microtiter plate configuration that uses the 1-ml disk, and the SPE pipette tip (Żwir-Ferenc and Biziuk, 2006).

SPE is used alone or in combination with other techniques. For instance, in the determination of PAHs in solid samples such as soil, sludge, sediment or tissue, SPE has been used after Soxhlet extraction, ultrasonic extraction or accelerated solvent extraction. Compared to the column chromatography clean up technique, SPE consumes fewer amounts of toxic solvents which is of a great advantage from the environmental sustainability standpoint (Oluseyi *et al.*, 2011) as well as saving time from parking long column. Since PAHs have low polarity SPE of pollutants from samples is usually carried out on a bonded octadecyl-silica stationary phase (Sun *et al.*, 1998, Marce and Borrull, 2000). SPE has been shown by Martín *et al.*, (2011) to give better recoveries of analytes than other clean-up or pre-concentration methods. They come in different formats as shown in Figure 2.20.

Limitations include blockage of the pores by solid and oily components of the extract so that it becomes overloaded. Hence variation sets in and can bring about poor reproducibility in some instances. These limitations lead to the development of solid phase micro extraction (SPME).

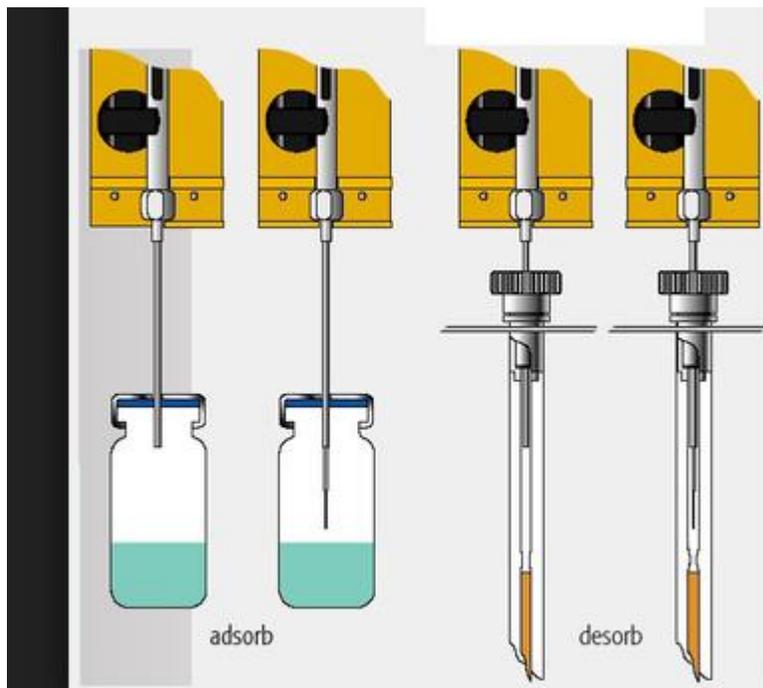


**Figure 2. 20: SPE Formats**

### 2.6.2.2 Solid phase micro extraction (SPME)

This is a technique in which the adsorbent is permanently attached to the fiber fixed to a syringe. This addresses the limitation SPE may pose and allows for re-use. The miniature nature allows for rapid mass transfer during extraction and desorption. The use of solid phase micro extraction (SPME) involves two steps. In the first step, the coated fibre is exposed to the sample extract or headspace. This causes target partition from sample matrix to the fibre. In the next stage, the fibre bearing concentrated analytes is transferred to an instrument (Usually the Gas Chromatograph) for desorption and quantification of analyte (Pawliszyn, 1997) as shown in Figure 2.21. SPME also has some disadvantages, including fiber fragility, cost and problems with analyte carryover (Xu and Lee, 2008). SPME has been used successfully to analyse a variety of HOCs in various samples including organophosphorus pesticides (Beltran *et al.*, 1998)

substituted benzene compounds (Arthur *et al.*, 1992). Published results suggest that quantitative extraction of a variety of PAHs from environmental samples is possible (Doong *et al.*, 2000, Havenga and Rohwer, 2000, Cheng *et al.*, 2013).



**Figure 2. 21: Solid Phase Micro Extraction Process**

### **2.6.2.3 Membrane extraction**

The selective nature of membranes has made them a unique alternative to solvent extraction for sample clean up, especially if coupled with chromatographic techniques. The relative sizes of different molecules largely determine the permeation selectivity of a membrane, in the absence of strong specific interactions. The main advantages over solvent extraction are the use of high ratio between surface area and volume, the lack of emulsions and no phase separation step. The process is the result of differences in the transport rates of the species through the membrane

interface: the separation is achieved when some species are transported to a greater extent than others. The forces that are able to generate transport through membranes are directly related to differences in pressure and concentration. Membranes are usually made of synthetic polymeric materials, although natural substances, such as cellulose, or inorganic materials, such as glass fibres or alumina, are also used. First of all, the membrane serves to retain molecules larger than a critical, material-related size. The maximum size of a solute that can pass through the membrane is called the cut-off value and it is normally given as the molecular weight of the smallest compound of which more than 90% is retained (MWCO, molecular weight hydro cut-off in Daltons units) (Pawliszyn, 1997, Buldini *et al.*, 2002). Calimag-Williams *et al.*, (2011) in the analysis of monohydroxy metabolites of polycyclic aromatic hydrocarbons used octadecyl extraction membranes.

#### **2.6.2.4 Gel permeable chromatography**

Gel permeation chromatography (GPC) is a size-exclusion clean-up procedure that readily separates high molecular weight interfering molecules from sample extracts. The procedure uses organic solvents and a porous hydrophobic gel (primarily a cross linked divinylbenzene-styrene copolymer) that readily separates large molecular weight molecules from the smaller molecular weight analytes of interest. GPC cleanup is recommended for the elimination of lipids, polymers, copolymers, proteins, natural resins, cellular components and other high molecular weight compounds (example PAHs) from a sample extract (USEPA, 2007b). GPC as a technique has been used for the cleanup of a variety of PAHs from environmental samples

(Dusek *et al.*, 2002, Fromberg *et al.*, 2007).

#### **2.6.2.5 Liquid - liquid extraction**

Liquid-liquid extraction is the simplest form of the extraction and purification of analytes from liquid samples and extracts. It is based on the tendency of an analyte to prefer one solvent over another immiscible solvent. Log P, the partitioning coefficient is the ratio of concentration a species in an organic solvent to water but the often-quoted Log P is that of octanol- water. Log-P values of a number of PAHs have been calculated. The Large volumes of high-purity solvents used in this technique make it expensive. The disposal of these solvents, also incur additional cost and negative effects on the environment (Feilden, 2011).

In this technique, solvent or a combination of solvents are used to remove analytes from extracts. It has been employed in PAHs clean up (Oluseyi, 2009, Dusek *et al.*, 2002). Usually this method employs the use of separating funnel. Liquid liquid extraction as a cleanup technique is very useful for separating analytes from interferences by partitioning the sample between these two immiscible liquids or phases (Feilden, 2011). Typically, one phase is usually aqueous (often the denser or heavier phase) and the second phase is usually an organic solvent (usually the lighter phase). Hydrophilic compounds prefer the polar aqueous phase. Węgrzyn *et al.*, (2006) and Luks-Betlej (1997) in their study of PAHs used liquid liquid extraction as the cleaning up technique in sample preparation.

### **2.6.3 Instrumental Analysis of PAHs**

The instrumental analysis of low level PAHs require a method that can identify the compounds and quantify them. The determination of PAHs is carried out by chromatographic techniques. Chromatography is the most powerful and widely-used separation technique for complex mixtures. All chromatographic methods use a *stationary phase* (solid or liquid) and a *mobile phase* or *eluent* (gas or liquid) that carries the analyte through the *column*. The nature of the mobile phase determines the category of chromatography:

- 1) Liquid chromatography (LC) uses a liquid mobile phase
- 2) Gas chromatography (GC) uses a gaseous mobile phase

In terms of chromatography, gas chromatograph-mass spectrometer (GC-MS) and liquid chromatograph-mass spectrometer (LC-MS) are appropriate and are the state of the art technologies to accomplish this purpose (Zou *et al.*, 2003, Mahindrakar *et al.*, 2011). Earlier HPLC with an ultraviolet (UV) or a photo-diode array (PDA) detector and GC with a flame ionisation detector (FID) were techniques often applied but were limited by poorer selectivity and sensitivity (Alexander *et al.*, 2008) though they are still employed techniques in the determination of PAHs (Sanchez-Brunete *et al.*, 2006, Danyi *et al.*, 2009, Ma *et al.*, 2010,).

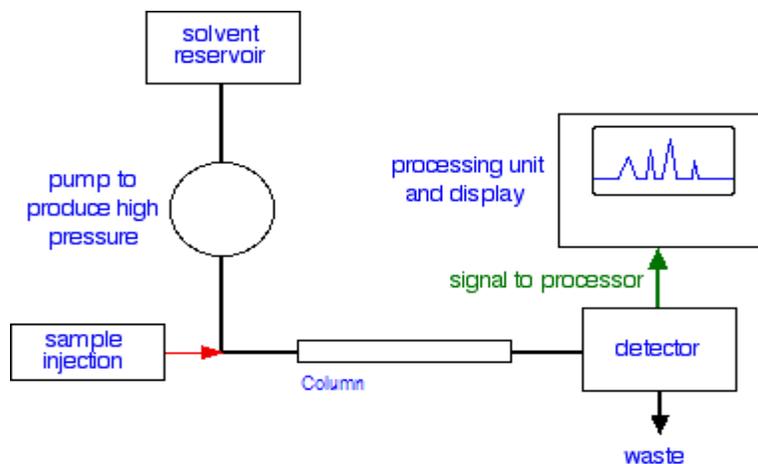
#### **2.6.3.1 High performance liquid chromatograph (HPLC)**

The advantages of HPLC sometimes called liquid chromatograph (LC) in the fractionation of complex PAH mixtures are un-doubtful and some useful methods have been validated. For example, the US EPA recommended analytical procedures are documented in EPA Methods 550.1 (drinking water), 610 (waste water), 8310 (solid waste) and TO-13 (air) (Voelker *et al.*,

2013). All these methods are based on LC with ultraviolet (UV) absorption, fluorescence detection and mass spectrometer. Reversed-phase liquid chromatography (RP-LC) is widely used for analysis of the main 16 PAHs because of its great efficiency of separation (Sun *et al.*, 1998, McMaster and McMaster, 1998). HPLC which is a type of chromatography employs a liquid mobile phase and a very finely divided stationary phase or a liquid stationary phase held on a finely divided packing material (Figure 2.22). The mobile phase used for the 16 EPA PAH separation is usually acetonitrile (ACN) or methanol gradient in water, starting from 50-60% and linearly increasing to 100% of ACN/methanol. The use of ACN enables an easier optimization of the elution parameters and a faster analysis, but good performance may be obtained also using MeOH, paying attention to a change in the elution order between dibenzo (ah) anthracene (DhA) and benzo (ghi) perylene (BgP). The use of methanol allows for the reduction of analyses costs (since ACN has become very expensive in recent years) (Purcaro *et al.*, 2012).

To obtain satisfactory flow rates, the liquid is usually highly pressurised. Analysis of anything in HPLC is achievable if it can be dissolved. If it can be dissolved then it can be separated in HPLC. Insoluble compounds are usually converted to their derivatives which are soluble (Skoog *et al.*, 2005 ). In the analysis of PAHs, binary gradient elution (gradient elution is the technique of using a mixture of more than two eluents as mobile phase) is usually employed to ensure proper separation. Manual or auto sampler can be employed. Thermostated (10-30 °C) reverse phase columns (usually an octadecylsilane (C<sub>18</sub>) of 15- 25 cm (the longer the better) with inner diameter 4.6 mm or less (the smaller the better) and particle size of 5 µm or less (the smaller the better) (Khan *et al.*, 2005)) is preferred. For the detection of PAHs, the more sensitive

and selective fluorescence or mass spectrometric detectors are preferred to the UV detector or diode array detectors (Khan *et al.*, 2005, Purcaro *et al.*, 2012).

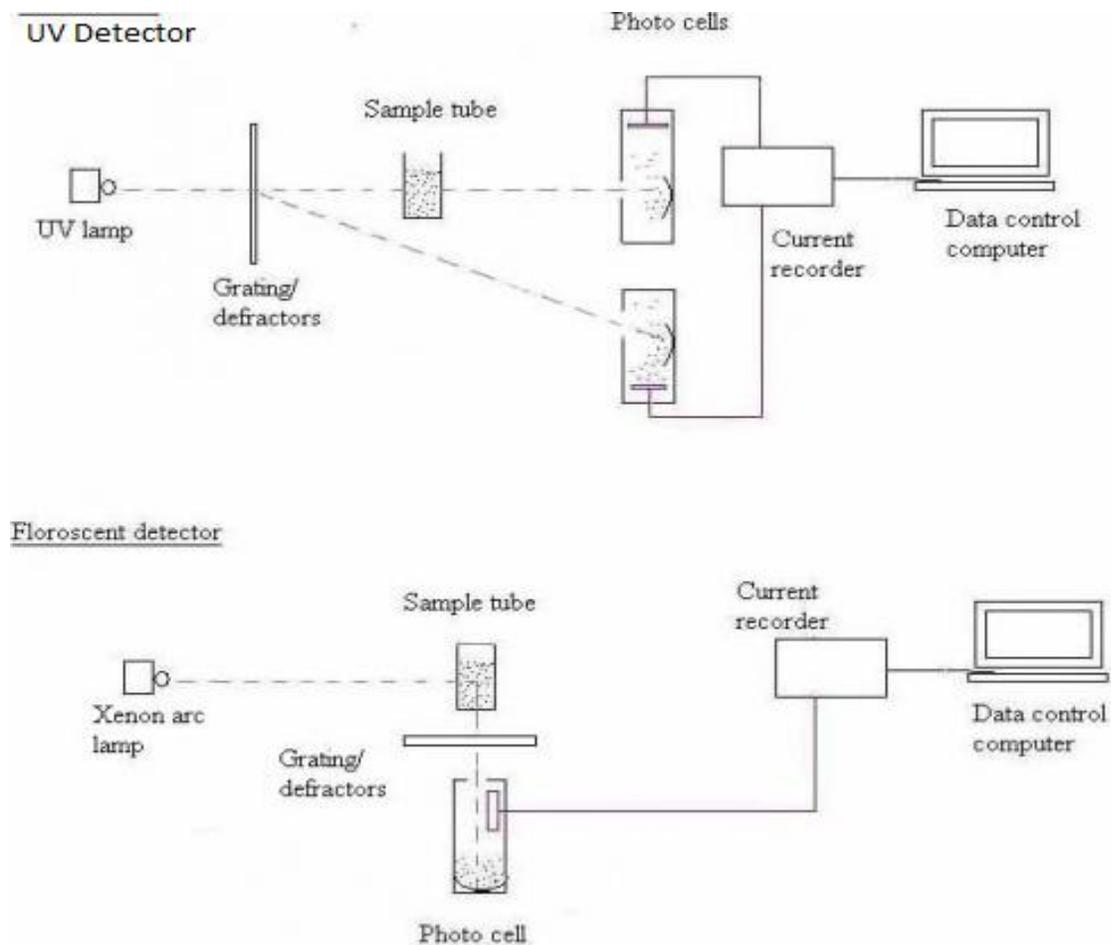


**Figure 2. 22: A Schematic Diagram of a HPLC**

### 2.6.3.2 HPLC detectors used for PAH analysis

The energies corresponding to the electronic transitions in PAH molecules are determined by their  $\pi$ -electrons, thus, the electronic spectra (UV absorbance and fluorescence) of PAHs are usually more featured than other organic species. The details of the electronic transitions are governed by the size and shape of the PAH compounds. Determination of PAHs by LC is commonly carried out by using Ultraviolet (UV) detection (Sun *et al.*, 2004, Sun *et al.*, 1998, Cloarec *et al.*, 2002, Dost and İdeli, 2012) (Figure 2.23). However, it is well known that UV detection shows a number of disadvantages, such as selectivity problems and sensitivity limitations, and it cannot discriminate matrix interferences, especially in complex matrices (Khan *et al.*, 2005). The selectivity of UV can be slightly improved with diode array detector (DAD) by on-line acquisition of UV spectra. However, UV is still being used for analysis of

PAHs because of its availability and PAH(s) standard availability (Dost and İdeli, 2012). On the contrary fluorescence detector (FLD) is more selective and sensitive than UV detection, because it operates on variable excitation and emission wavelengths as illustrated in the diagram in Figure 2.23.



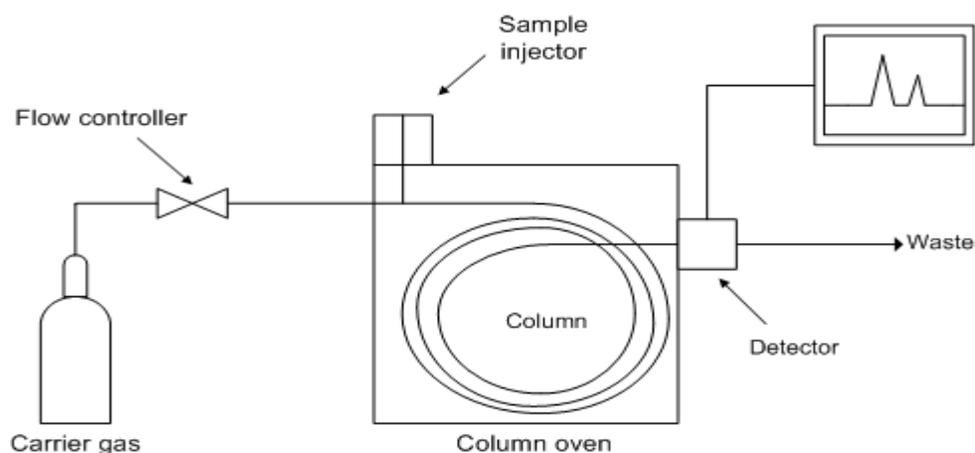
**Figure 2. 23: Schematic diagrams of UV and Fluorescence detectors**

Liquid chromatograph connected to a fluorescence detector has been extensively applied for the determination of PAHs in very different matrices, since it is cheap and simple, in comparison to other detection systems like the mass spectrometer. Anthracene and perylene are best measured

by FLD due to their selective and sensitive fluorescence characteristics. Despite the improvement in comparison to UV detection, FLD still show a lack of selectivity especially due to the presence of alkylated PAHs. Alkylated PAHs are considered the main impurities of PAH fractions especially in PAHs from petrogenic sources. These compounds show similar fluorescence responses to the unsubstituted PAHs with FLD. Another disadvantage is the impossibility of using certain isotopically labelled compounds because FLD cannot distinguish between PAH isotopes and the native PAHs. The combination of UV and FLD in some analyses was an attempt to correct the limitation of both UV and FLD though the selectivity problem was not solved. As an alternative, to LC-FLD, benzo[b]chrysene or deuterated compounds, which can be chromatographically separated on MS was developed to overcome these limitations of FLD and UV (Plaza-Bolaños *et al.*, 2010). However, its application has been limited by cost (Plaza-Bolaños *et al.*, 2010, Stołyhwo and Sikorski, 2005).

### **2.6.3.3 Gas chromatographs**

Capillary columns used in GC analysis of PAHs have efficiency of the order of 60,000 plates/30 m (ID 0.25 mm) (Stołyhwo and Sikorski, 2005). Thus the components of natural extracts can be better separated by GC (Figure 2.24) than by a HPLC.



**Figure 2. 24: A schematic diagram of a GC**

If the system of “cool on column” injection is used, over 100 peaks can be separated including alkylated PAHs and several other interfering compounds. Hyphenation to another GC will even further increase resolution leading to more peaks. In recent years, comprehensive two-dimensional GC (2D GC or GC×GC) has received increased attention for the analysis of complex samples (Versantvoort *et al.*, 2005, Papadopoulos *et al.*, 2011). This large number of peaks in extracts products which can be detected by the FID (Saari *et al.*, 2007), but cannot be identified by their retention times only, without further, often multistage clean-up procedures to remove interfering compounds. Therefore, mass spectrometry (MS) is preferred for identification and quantification of PAHs. MS has been used in the determination of PAHs in smoke and smoked products, soil extract, plant extract, fish among other (Fernandes *et al.*, 2009, Ghasemzadeh-Mohammadi *et al.*, 2012, Kumar and Kothiyal, 2012). By using the selected ion mode (SIM) technique, the individual PAHs can be identified at concentrations at least 100 times lower than is possible by HPLC and GC FID. GC/MS methods have been successfully

applied to determine 16 PAHs. Examples include the USEPA TO-13A (USEPA, 1999) and Stołyhwo and Sikorski (2005) methods.

In analysis of PAHs by GC, column properties are important. Usually, the column has a minimum length of 25 m although 30 m is preferred, maximum inner diameter of 0.25 mm, film thickness between 0.2  $\mu\text{m}$  and 0.4  $\mu\text{m}$  (although the thinner the better). Stationary phase of a wide range is employed from non-polar to slightly polar stationary phases. However, 5 % phenyl-95 % methylpolysiloxane is preferred (e.g. typical HP-5 or HP-5ms) (Plaza-Bolaños *et al.*, 2010). Helium is the carrier gas usually used especially for GC/MS. However, when using columns with very small inner diameters, the use of hydrogen is essential. Auto samplers are usually employed for sample injection and the two systems commonly used are splitless or split. A frequently used detector for PAHs analysis is a flame ionization detector or a MS detector. The use of a FID is also possible, but since the selectivity of the FID is low for PAHs, it is not recommended (Khan *et al.*, 2005, Stołyhwo and Sikorski, 2005, Purcaro *et al.*, 2012). It is also subject to background interferences (Purcaro *et al.*, 2012).

MS detectors for PAHs are normally used in the Selected Ion Monitoring (SIM) mode (Purcaro *et al.*, 2012). Electron impact ionization (EI) may be used as the ionization method. The selectivity of a mass spectrometric detector is excellent and the chromatographic noise of a standard is similar to that of a sample. However, major drawbacks are the matrix-dependent response and the convex calibration curves that both often occur and make quantification difficult (Khan *et al.*, 2005). Another mode of PAH identification is the full-scan MS. It operates

with less sensitivity to SIM (Zeigler *et al.*, 2008 ) but is also a powerful analytical tool. The individual PAH are identified by comparing the retention time of the PAH in a sample with that of the respective compound in a standard solution analysed under the same conditions. When chromatograms are processed using automated integrators, the baseline is not always set correctly and always needs visual inspection. The sensitivity of the LC-MS and GC-MS can be increased by hyphenation of their detector to MS. Hyphenation of an LC to an LC-MS and a GC to another GC-MS has also shown to increase sensitivity and even resolution. Some very recent techniques such as

- UHPLC $\times$ UHPLC-MS (Uliyanchenko *et al.*, 2012),
- LC $\times$ LC-MS $\times$ MS (Klavins *et al.*, 2014)
- GC $\times$ GC-MS (Xu *et al.*, 2003)
- LC $\times$ LC-MS (Jandera, 2012)
- GC  $\times$  GC-TOF (time of flight) MS, HR(high resolution) GC  $\times$  HRMS, HRLC  $\times$  HRMS, GC-Q (quadrupole)  $\times$  Q  $\times$  QMS (Garrido-Frenich *et al.*, 2005) are now being developed and some have been employed in organic pollutants (PAH) analysis to increase sensitivity and resolution from complex matrices.

These techniques have been used in the analyses of PAHs and a review reported of methods and instrumentations for analyses of PAHs is as shown in Table 2.6.

**Table 2.6: A Review of PAHs Analyses Methods**

<b>Sample</b>	<b>Reference</b>	<b>Extraction</b>	<b>Clean-up</b>	<b>Instrumental Analysis</b>
Soil	Cao <i>et al.</i> , (2012)	ultrasonic extraction <b>Mass of soil:</b> 5 g <b>Volume of solvent:</b> 25 ml dichloromethane <b>Duration:</b> 2hrs at 30°C <b>Extract concentration:</b> rotary evaporator( extract dried), 1.0 ml acetone to reconstitute.	pipette column equipped with 1.0 g of silica gel (70–230 mesh, Sigma) <b>Eluent:</b> dichloromethane and n-hexane (1:1) <b>Concentration:</b> Collected extract was dried via nitrogen stream and reconstituted in 1.0 ml with acetonitrile for analysis of PAHs.	LC Florescence
Soil	Essumang <i>et al.</i> , (2011)	ultrasonic extraction <b>Mass of soil:</b> 10 g <b>Volume of Solvent:</b> 50 ml dichloromethane <b>Duration:</b> 24hrs at Room temp <b>Extract concentration:</b> rotary evaporator (extract dried)	C18 solid phase extraction and PTFE filters were employed in cleaning up of samples. <b>Eluent:</b> Dichloromethane was <b>Concentration:</b> Collected extract was dried via nitrogen stream reconstituted 3 mL with cyclohexane for analysis of the PAHs.	analyzed using Varian 8200 Cx Auto Sampler and Varian 3800 GC coupled with Varian Saturn 2000 GC/MS/MS with ion trap detector (TCD).
Soil	Gomez-Eyles <i>et al.</i> , (2011)	Mechanical Shaking (using an orbital shaker) <b>Mass of soil:</b> 4 g <b>Volume of Solvent:</b> 10 ml (1:1) Acetone : Hexane <b>Duration:</b> 2hrs <b>Extract concentration:</b> rotary evaporator (extract dried)	Clean up was via sedimentation for 30mins, After which an aliquot was dried with sodium sulphate for analysis	GC-MS
Soil	Kim <i>et al.</i> , (2011)	accelerated solvent extraction (ASE) <b>Mass of soil:</b> 20 g <b>Volume of Solvent:</b> 200 ml	and cleaned up on packed column of 10g activated silica gel and 1 g of sodium sulfate and 10 g of <b>Eluent:</b> 200 mL of dichloromethane : hexane	gas chromatograph/ion trap mass spectrometer (PolarisQ, ThermoQuest, San

		(1:1) Dichloromethane : acetone <b>Extract concentration:</b> nitrogen evaporator (Turbo Vap-LV, Zymark, Sparta, NJ, USA) to 1 mL	(1:9) <b>Concentration:</b> samples were dried by a rotary evaporator and reconstituted in with 50 µL of toluene before injection.	with a DB-5MS column (60 m, 0.25 mm ID, 0.25 µm film thickness).
Lamp black samples	Hong <i>et al.</i> , (2009)	Ultrasonic extraction (EPA Method 3550B) Sequentially <b>Volume of Solvent</b> 3 vol of 40 mL hexane:acetone mixture (50:50) <b>Duration:</b> 6 min (pulsing for 15 s on and 15s off) The combine extract from the sequential extraction was concentrated and exchanged into cyclohexane.	Cleanup (EPA Method 3630C) was on a silica gel column/ cartridges. With cartridges, a vacuum manifold was required to obtain reproducible results. The collected fractions	A gas chromatograph (Agilent model 6890) with a fused silica capillary column (HP-5, 30 m 0.25 mm i.d.) and a flame ionization detector was used for analysis based on EPA Method 8100 for PAHs
Soil	Maliszewska-Kordybach <i>et al.</i> , (2012)	Accelerated Solvent Extraction with dichloromethane as extracting solvent.	Clean up of the concentrated extracts was by employing activated silica gel.	GC-MS system (Agilent 6890N gas chromatograph with an Agilent 5973 network mass spectrometer and Agilent 7683B series autosampler) using DB-5 MS+DG fused silica capillary column

Soil	Okedeyi <i>et al.</i> , (2012)	<p>Soxhlet extraction  <b>Mass of soil:</b> 10 g of sample  <b>Solvent</b> (1:1) hexane: acetone  <b>Duration:</b> 6 hrs.  <b>Extract concentration:</b>  Rotary evaporator was used to pre-concentrate sample to 2 ml and further concentration was via a vacuum rotary evaporator</p>	<p>Clean up was by silica gel column,  <b>Eluent:</b> Acetone  <b>Concentration:</b> Gentle stream of nitrogen was employed.</p>	DB-1HT 30 m× 0.25 mm i.d. with 0.1 µm film thickness, GC-MS
Soil	Oleszczuk and Pranagal (2007)	<p>ultrasonic extraction  <b>Mass of soil:</b> 40g  <b>Volume of Solvent:</b> 40 ml dichloromethane in two batches.  <b>Temperature of extraction:</b> ≤35°C.  <b>Extract concentration:</b> was carried out by centrifuging the extract and drying the extract using a rotary vacuum evaporator at 40 °C. The residues were re-dissolved in 4mL of acetonitrile–water mixture</p>	<p>Clean up was by solid phase extraction (SPE) using C18 Octadecyl columns</p>	LC
Soil	Oleszczuk and Baran (2004)	<p>ultrasonic extraction.  <b>Solvent:</b> Dichloromethane  <b>Duration of Extraction:</b> 90 mins.  <b>Extract concentration:</b> was carried out by centrifuging</p>	<p>Clean up was carried out as a comparative study for various cartridges and they are: silica gel (3 mL, 500 mg), C8-octyl (endcapped, 3 mL, 500 mg), C18-octadecyl (end-capped, 3 mL, 500 mg), C18 PP (PolarPlus, octadecyl, non end-capped, 3 mL,</p>	LC

		<p>the extract and drying the extract using a rotary vacuum evaporator at 40 °C. The residues were re-dissolved in 4mL of acetonitrile : water mixture (1:1) C8, C18 for SPE clean up or 4 ml of acetonitrile for silica gel cleanups</p>	<p>500 mg), phenyl (C6H5, 3 mL, 500 mg), cyano (3 mL, 500 mg). The Cartridges were conditioned with 1 × 3mL of methanol, 2 x 3mL of water: 2- propanol (9:1). The 4mL of the reconstituted sample extract was loaded onto the conditioned SPE cartridges, dried in light vacuum (5 min). These Cartridges were washed with 1×3mL of methanol:water (1:1), vacuum dried (5 mins) and PAHs were slowly eluted by 2 × 1.5mL of acetonitrile. For Silica gel cartridges, conditioning was with <i>n</i>-hexane (2×1 mL) and PAHs elution was by dichloromethane : <i>n</i>-hexane (1:1). The various elutes collected were evaporated to near dryness with the aid of a rotary vacuum evaporator at 30 °C then reconstituted in 2mL of acetonitrile for instrumental analysis</p>	
Water	Ma <i>et al.</i> , (2010)		<p>SPE cartridges were used. They were conditioned with 10mL <i>n</i>-hexane, 10mL methanol ( this remove air and leach impurity), and with 10mL ultrapure water (this equilibrates the phase). 500mL of sample was made to pass through the cartridge. The cartridges were dried under vacuum for 30mins and PAHs were eluted by 15mL <i>n</i>-hexane. The final extracts were condensed to dryness by the use of a gentle flow of nitrogen at room temperature. The residue was dissolved with 1mL <i>n</i>-hexane for GC-MS analysis</p>	GC–MS analysis.

#### 2.6.3.4 Comparison of HPLC and GC analysis of PAHs

Peak separations of samples in HPLC analysis are often incomplete, therefore the use of peak heights is recommended for quantification. However when using GC techniques, either peak heights or peak areas can be used. GC–MS the main alternative to LC–FLD has been applied to all kinds of sample analyses and methods based on GC–MS are more frequently reported in bibliography (Khan *et al.*, 2005). This because GC in particular has a higher peak capacity, and efficiency compared to LCs due to the column length differences while the LC is said to be more selective (Purcaro *et al.*, 2012). The column length usually employed in GC analysis of PAHs from environmental or food extracts is 30 m to 50 m fused-silica capillary column as against that used in PAHs analysis by HPLC of maximum column length of 15 cm to 30 cm. Efficiency is a quantitative parameter while selectivity is more qualitative parameter. High column efficiency has the advantage of analysing complex mixtures while column selectivity has the advantage of separating isomeric compounds (Skoog *et al.*, 2005 ) but using GC or LC, the retention times should be reproducible to within  $\pm 0.05$  minutes, and additionally there are deuterated analogues of many of the parent compounds present for comparative purposes. For HPLC, reproducibility of retention times may be less good, but should certainly be within  $\pm 1$  minute (Khan *et al.*, 2005). Compared with GC method, HPLC, especially reverse phase HPLC (RP-HPLC), can analyze the compounds without considering volatility and molecular weight, whereas with GC volatility is critical (Li-bin *et al.*, 2007, Phillips *et al.*, 1996). The main drawback of LC is that of greater susceptibility to matrix interferences in comparison

with GC-MS. However, analytical results by HPLC were comparable to GC-MS. Therefore, for many laboratories, HPLC is a useful alternative to GC-MS (Phillips *et al.*, 1996).

## **2.7 STANDARD METHODS AND QUALITY ASSURANCE**

Quality assurance (QA) according to IUPAC technical report published, involves all those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy a given requirement for quality (Thompson and Wood, 1995). A QA is the complete set of measures a laboratory must undertake to ensure that it can always achieve high-quality data. This include the use of validation and/or standardized methods, method optimization (under method development), effective internal quality control (IQC) procedures (use of reference materials (RMs), control charts, among others. Analytical method validation forms the first level of QA in the laboratory (Taverniers *et al.*, 2004)

Prior to running a series of samples and standards, as a measure of quality control, the equilibration of the GC or HPLC is important. In addition, standards used for multilevel calibration are required to be regularly distributed over the sample series such that matrix-and non matrix- containing injections alternate. A sample series includes:

- A procedural blank
- A laboratory reference material

- At least five standards for calibration and the use of R-Squared co-efficient ( $r^2$ ) value are necessary for assessing linearity
- One standard that has been treated similarly to the samples (recovery determination).  
Limit of blank (LoB), Limit of detection (LoD), and Limit of quantization (LoQ) for individual compounds is required at this stage (Khan *et al.*, 2005). LoB, LoD, LoQ are terms used to describe the smallest concentration of an analyte that can be reliably measured by an analytical procedure.
- LoB is the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested.
- LoD is the lowest analyte concentration likely to be reliably distinguished from the LoB and at which detection is feasible. LoD is also three times standard deviation of the blank signal and can be calculated as the concentration at which baseline noise to signals is three at the expected retention time for individual target PAHs (Zou *et al.*, 2003, USEPA, 2007c, Al-Rashdan *et al.*, 2010).
- LoQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met. The LoQ may be equivalent to the LoD or it could be at a much higher concentration. Typically, LoQ will be found at a higher concentration than LoD (Armbruster and Pry, 2008). It is also defined as the concentration leading to a signal-to-noise ratio of 10 (Al-Rashdan *et al.*, 2010)

## 2.8 CHEMOMETRICS AND MODELLING

As complexity, variety, growing importance of quality and uncertainty mark today's chemistry, a statistical approach to experimental design is almost inevitable. Therefore the use of mathematical and statistical multivariate analytical tools for data analysis is called 'Chemometrics'. Experimental design and techniques are becoming increasingly widespread in analytical chemistry and other sciences. Multivariate designs and multiple regression analysis, which allow the simultaneous study of several control variables, are faster to implement and more cost-effective than traditional univariate approaches. Several experimental design models exist that reduce the number of experiments and that can be used in different cases (Ferreira *et al.*, 2004). Chemometrics is now being applied in recent studies on PAHs (Kitti *et al.*, 2003, Tobiszewski *et al.*, 2010, Oluseyi *et al.*, 2011 Thavamani *et al.*, 2012).

## CHAPTER THREE

### 3.0 METHODOLOGY

This chapter describes the methodologies and chemicals used in this study. Section 3.1 contains the determination of PAHs in some locally consumed smoked fishes in Nigeria while section 3.2 contains the quantification of PAHs and proximate analysis of roasted plant foods. Section 3.3 describes the optimization of extraction/clean up and method development for the analyses of 16 priority USEPA and alkylated PAHs by GC-MS. The last section, (section 3.5) describes the analysis of sum and bioaccessible PAHs in soil. The three types of PAHs were analysed in this study namely: the 16 priority PAHs (uPAHs), six deuterium labelled PAHs mix ((dPAHs, internal standards) (d10-ancanthene, d12-chrysene, d8-naphthalene, d12-perylene, d10-phenanthrene, d4-1,4-dichlorobenzene of 2000 µg/mL, dichloromethane: benzene)) and the alkylated PAHs of (C0 – C4 alkyl PAHs) (RPAHs).

### 3.1 DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOME LOCALLY CONSUMED SMOKED FISHES IN LAGOS, NIGERIA

#### 3.1.1 Fish Samples Smoking and Preparations

Three species of locally consumed fresh fish samples were used in this study and they were bought from a fish-market in Ijora area of Lagos (Nigeria). They were *Arius heude loti* also known as cat-fish (called 'aro' in Yoruba), *Cynoglossus senegalensis* also known as sole (called 'abo' in Yoruba), and *Haake sp* also known as fresh stock fish (called 'Panla' in Yoruba). They were weighed and their lengths were taken using

calibrated weighing balance and ruler. Some of the fishes were smoked while other fresh samples were homogenised using a blender and dried in an oven for 2 days at low temperature of about 40 °C. The weighed fishes were smoked using three African traditional processes of smoking: firewood, charcoal and sawdust methods. The fishes were smoked for about 8 h at high temperature and a thermometer was used to take the temperature of the smoking process. The smoking process involved placing a piece of cardboard over the fishes to cover the fishes during the process. The piece of cardboard traps the smoke to enable it act directly on the fish samples. The smoked fishes were further dried in an oven at low temperature of 40 °C for four days to ensure that the fish samples were properly dried. The smoked dried fishes were homogenised using a mortar and pestle and were wrapped in such a way that water will not get into it 4 °C prior to extraction and analyses.

### **3.1.2 Chemical Analysis of Fish Samples for Fat (oil) Content**

2 g of each homogenized dried fish samples were used for fat and oil determination. The fish samples were placed in a Soxhlet extractor and a 100 ml of 1:1 mixture of chloroform and methanol was used to extract the fat for 2 h. The extract was poured into weighed crucibles and dried in a fume cupboard. The difference in weight between the empty crucible and the extract contained crucible was taken as the fat content.

### 3.1.3 PAH Determination in Fish Samples

For the determination of the PAHs content, 5 g of each type of smoked dried fishes were weighed into amber glass bottles and extracted sequentially by ultrasonication using 25 ml of n-hexane for 1 h. The supernatant of the extracts were decanted into a vial and 15 ml of fresh solvent was added for another hour of ultrasonication. The process was repeated with another 10 ml of fresh solvent for an hour. The combined extracts (50 ml) were centrifuged at 2500 rpm for 10 min and the supernatant decanted (Garcia-Falcon *et al.*, 1996). The supernatant was cleaned-up using the Whatman nylon filter membrane. Further clean-up was done using the solid phase extraction (SPE) cartridges. The sorbent of the SPE cartridges were first conditioned with n-hexane, after which the filtered extracts were loaded on to the cartridges, the analytes were eluted with dichloromethane. The volume of the dichloromethane was blown down to dryness, reconstituted in 200  $\mu$ l of acetonitrile and analysed by HPLC for PAHs. The quantification of PAHs was performed using an Agilent 1100 model HPLC system with a quaternary pump, vacuum degasser, a temperature controlled column oven and a UV diode-array detector. Separation of the PAHs was performed on a monomeric type octadecyl silica column, Supelcosil LC PAH 2 cm  $\times$  4.6 mm i.d containing 5  $\mu$ m particles at ambient temperature ( $25 \pm 1$  °C) at a flow rate 1.0 ml/min. Gradient elution using acetonitrile and water was employed (60:40 to 0:100). Peak detection and integration of data was carried out using chemstation software series. External calibration was carried out using mixed PAH standards. From the chromatogram, the retention times of the standards were used for the identification and quantification of

the individual PAHs. A standard mixture of the USEPA 16 priority PAHs and 2 PAHs derivatives (2000 µg/ml, dichloromethane: benzene): naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-c,d)pyrene was obtained from SUPELCO, Bellefonte, PA, USA. Appropriate working dilutions of the standard solution with HPLC grade acetonitrile were made. All other solvents used were of high purity analytical grade.

### **3.1.4 Risk Assessment of Smoked Fish**

The comparison of the levels in food samples with limits and guideline values was used as one approach to risk assessment. A value of 5 µg/kg, for BaP concentration has been set in the European Union as limit for smoked fish and meat (Wenzl *et al.*, 2006). The Mean Daily intake (MDI) approach was also employed for the risk assessment of smoked fishes.

$$\text{MDI} = [\text{EC} \times \text{IR}]$$

Where BW= Body weight of adult ((70 kg) ATSDR, 2005)

IR= Fish (smoked fish) ingestion rate for adult (25 g/day)

EC= Experimental concentration (µg/g).

For the calculations, fish consumption rate was set at 68.5 g/day from the annual per capita fish consumption of 25 kg for Nigeria (Nkpa *et al.*, 2013, Olaji *et al.*, 2014). Akinyele (2009) and Samuel *et al.*, (2010) who studied the fish consumption pattern in Nigeria found more fresh fishes were consumed compared to processed fishes. Samuel

*et al.*, (2010) found that 36.6 % of of the sum per capita per annum consumption of fishes were processed (smoked and salted/sun-dried processed fish). Using the 36.6 % as the percentage of smoked fishes consumed, 36.5 % of 68.5 g/day will be 25 g/day.

## **3.2 QUANTIFICATION OF PAHs AND PROXIMATE ANALYSIS OF ROASTED PLANT FOODS**

### **3.2.1 Sampling and Processing**

Samples of corn (*Zea mays*), yam (*Dioscorea Sagittifolia*) ripe and unripe plantain (*Musa paradisiaca*) were bought from the Yaba market in Lagos State, South West Nigeria. Raw yam samples were peeled, cut into one quarter inches thick and roasted using the open flame roasting method, where a metal grid was placed over a pot of lighted charcoal and the samples were roasted with occasional fanning. This was used to regulate the flame with temperature between 140 and 200 °C. The corn and plantain samples were roasted in a similar way. Raw and roasted food samples were ground, blended and stored in the refrigerator at 4 °C prior to analyses. Proximate analyses of the samples were carried out using the method of Association of Official Analytical Chemist (AOAC, 1990, Oleszczuk and Baran, 2005) Analysis for PAHs in roasted food was carried out using the HPLC-UV.

### **3.2.2 Extraction of PAHs in Roasted food**

PAHs in roasted and raw foods viz: yam, maize, ripe and unripe plantains were extracted by solvent extraction using ultrasonication. 5 g each of the food samples were

weighed into amber glass bottles and extracted sequentially using ultrasonication using 25 ml of n-hexane for one hour. After ultrasonication the supernatant of the extracts were decanted into a vial and 15 ml of fresh solvent added for another one hour of ultrasonication. The process was repeated with another 10 ml of fresh solvent for one hour. The combined extracts (50 ml) were centrifuged at 2500 rpm for 10 min and the supernatant decanted. Clean-up was done using the solid phase extraction (SPE) cartridges. The supernatant was further cleaned-up using the Whatman nylon filter membrane and the solid phase extraction supelco C18 (SPE) cartridges. The sorbent of the SPE cartridges were first conditioned, after which the filtered extracts were loaded on to the cartridges. The analytes were eluted with dichloromethane. The volume of the dichloromethane was blown down to dryness and extract was reconstituted in acetonitrile and the extracts analysed for the 16 USEPA PAHs using the HPLC with a UV detector.

### **3.2.3 Instrumental (HPLC-UV) Analysis of PAH(s) Extract**

After the solvent extraction of the PAHs from the food samples, analysis using the Agilent 1100 model HPLC system coupled to a variable wavelength UV detector was carried out. Separation of the PAHs was performed on an octadecyl silica column, Supelcosil LC-PAH column at ambient temperature ( $25 \pm 1^\circ\text{C}$ ) with a flow rate of 1.0 ml/min. Gradient elution using acetonitrile and water was employed (60:40 to 0:100). Peak detection and integration of data was carried out using Chemstation software series. External calibration was carried out using mixed PAH standards purchased from

Sigma Aldrich. From the chromatogram, the retention times of the standards were used for the identification and quantitation of the individual PAHs.

### **3.2.4 Proximate Analysis of Roasted Foods**

Micro-kjeldahl method as described by AOAC (1990) was employed to determine the nitrogen content. The crude protein was calculated based on nitrogen conversion factor of 6.25. Moisture content, Crude lipid (Soxhlet extraction), crude fibre and ash contents (gravimetric) were determined based on methods described by the Association of Official Analytical Chemist method (AOAC, 1990, Oleszczuk and Baran, 2005) and the summary of these methods are follows:

#### **3.2.4.1 Determination of moisture content of roasted foods**

The moisture content of the various food samples was determined on drying at 100 °C in an oven until a constant weight was attained. The difference in initial and final weight of sample was expressed as percentage moisture

#### **3.2.4.2 Determination of crude protein in roasted foods by micro kjeldahl method**

10 g of each of the samples were treated with 10 ml of concentrated H<sub>2</sub>SO<sub>4</sub> in a heating tube. 5 g of potassium sulphate catalyst and one glass bead was added to the tube and mixture heated inside a fume cupboard with initial low then high temperature till a clear solution was obtained. From this point, the sample was heated for one more hour to ensure complete breakdown of organics. This whole digest was transferred to a flat bottom flask, to which was added 100 ml of water and 70 ml of 50 % NaOH solution (this was added excess to ensure that all the nitrogen was distilled and the reaction in

H<sub>2</sub>SO<sub>4</sub> is fully quenched). The mixture was distilled to give a distillate that was collected from a receiver suspended below the surface of a 50 ml 4 % boric acid solution to which 3 drops of methyl red indicator was added. The distillate was titrated with the standardised 0.1 N hydrochloric acid until the first appearance of the pink colour. The sample was duplicated and the average value taken. The Nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein content.

This is given as percentage Nitrogen =  $\frac{(S-B) \times N \text{ Normality of HCl} \times 0.014 \times D \times 100}{\text{Weight of the sample} \times \text{Volume taken for distillation}}$

Where

S = Sample titration reading

B = Blank titration reading

D = Dilution of sample after digestion

0.014 = Milli equivalent weight of Nitrogen

#### **3.2.4.3 Determination of crude lipid content in roasted foods.**

10 g of the food sample and 120 ml chloroform: methanol (1:1) were placed in a thimble fitted to a clean round bottom flask and heated under reflux on a heating mantle/ Soxhlet set up for five hours. The extract in the round bottom flask was concentrated in the flask to about 30 ml after the set up was cooled and thimble removed. The distilled solvent in the upper chamber of the Soxhlet extractor was collected before it is returned to flask. The cooled concentrated extract in the flask was poured into a weighed 50 ml beaker, and the beaker rinsed. After a constant weight was obtained from further concentration, crude lipid was calculated.

$$\% \text{ Crude lipid Content} = \frac{W_2 - W_1 \times 100}{W_3}$$

**Where**

W<sub>1</sub> = weight of the beaker

W<sub>2</sub> = weight of the beaker and extracted fat

W<sub>3</sub> = weight of the sample

**3.2.4.4 Determination of crude fibre in roasted foods**

2 g sample and 200 ml of 1.25 % of H<sub>2</sub>SO<sub>4</sub> were boiled in a 500 ml flat bottom flask for 30 minutes. The residue was separated using a Buchner funnel and residue was further boiled in NaOH (200 ml) for 30 minutes. The new mixture was filtered using a Buchner funnel and washed with alcohol twice. The residue obtained was put in a weighed clean dry crucible and dried in an oven at 105 °C till constant weight was obtained.

$$\% \text{ Crude Fibre} = \frac{W_1 - W_2 \times 100}{W_3}$$

**Where**

W<sub>1</sub> = weight of crucible + crude fibre

W<sub>2</sub> = weight of crucible

W<sub>3</sub> = weight of original sample

**3.2.4.5 Determination of ash content in roasted foods**

Ten grams of each of the samples was weighed into crucible, heated in an oven at 100 °C for 3 h. Thereafter the sample was transferred into a muffle furnace and heated at 550 °C for 6 h until the colour turned white (hence free of carbon and organics). The sample

was cooled in a desiccator to room temperature and reweighed immediately. Residual ash was calculated as ash content.

$$\text{Percentage Ash} = \frac{\text{Weight of Ash} \times 100}{\text{Weight of sample taken}}$$

#### **3.2.4.6 Determination of percentage carbohydrate in roasted foods**

Percentage carbohydrate was calculated as 100 - (% moisture + % protein + % fat + % ash +% crude fibre). Calculated energy was calculated from the sum crude carbohydrate as follows: Sum crude carbohydrates (%) = 100 – (Crude protein + Crude lipid + Crude fibre + Ash) (Muller and Tobin, 1980). While gross energy values were calculated based on the formula: Gross energy (kJ/100 g) = (protein x 16.7) + (lipid x 37.7) + (carbohydrates x 16.7) (Ekanayake *et al.*, 1999).

#### **3.2.4.7 Determination of mineral content in roasted foods**

The levels of the macro minerals viz., copper, iron, magnesium, manganese and zinc were determined after digestion of samples with aqua regia and analysed using (Analyst Perkin Elmer 200-(2) ) flame atomic absorption spectrometer (AOAC, 1990).

#### **3.2.5 Statistical Analysis and Risk Assessment of PAHs in Roasted Foods**

The t-test was employed to ascertain the difference between raw and roasted food samples for proximate composition, mineral components and PAHs. Source groupings and the association of the parameters were determined using principal component analysis (PCA) and applying varimax with Kaiser Normalization rotation method to

facilitate easier interpretation of data. Principal components having eigenvalues >1 of the complete data set were retained. Factor analysis in this study was carried out using the statistical analysis SPSS 15.0 software package.

The comparison with limits and guideline values and BaP equivalence (BaP<sub>eq</sub>) approach were employed for assessing the risk associated with the samples. Health-risk posed by the exposure of 16 USEPA priority PAHs is based on carcinogenic potency relative to BaP (Tsai *et al.*, 2001). Hence, the toxicities of other PAHs are determined in relation to BaP and expressed as toxicity equivalence factor (TEF). TEF is used to determine BaP equivalent dose (BaP<sub>eq</sub>). The TEFs developed by Nisbet and LaGoy (1992) were used in this study because they were reported to be a better set of indicators by Xia *et al.*, (2010) and Boström *et al.*, (2002) as shown in Table 2.1. BaP<sub>eq</sub> dose was calculated as follows:

BaP<sub>eq</sub> dose (μg/g) = TEF x concentration (μg/g) (Huang *et al.*, 2005),

Sum BaP<sub>eq</sub> dose (μg/g) = ∑ (TEF x concentration (μg/g))

**Table 3. 1: TEF Values for Individual PAHs**

<b>Compound</b>	<b>TEF Value</b>	<b>Source</b>
NAP	0.001	(Nyarko <i>et al.</i> , 2011, Nisbet and LaGoy, 1992)
ACY	0.001	(Nisbet and LaGoy, 1992, Boström <i>et al.</i> , 2002, ATSDR, 1995)
ACP	0.001	(Nisbet and LaGoy, 1992, Boström <i>et al.</i> , 2002, ATSDR, 1995)
FLR	0.001	(Nisbet and LaGoy, 1992)
PHE	0.001	(Nisbet and LaGoy, 1992, Boström <i>et al.</i> , 2002, ATSDR, 1995)
ANT	0.01	(Nisbet and LaGoy, 1992)
FLT	0.001	(Nisbet and LaGoy, 1992)
PYR	0.001	(Nisbet and LaGoy, 1992, Boström <i>et al.</i> , 2002, ATSDR, 1995)
BaA	0.1	(Huang <i>et al.</i> , 2005, Nisbet and LaGoy, 1992)
CHR	0.01	(Nisbet and LaGoy, 1992)
BbF	0.1	(Nisbet and LaGoy, 1992)
BkF	0.1	(Huang <i>et al.</i> , 2005, Nisbet and LaGoy, 1992)
BaP	1	(Nisbet and LaGoy, 1992, Boström <i>et al.</i> , 2002, ATSDR, 1995)
DaH	1	(Huang <i>et al.</i> , 2005, Nisbet and LaGoy, 1992, Boström <i>et al.</i> , 2002)
BgP	0.01	(Boström <i>et al.</i> , 2002, Nisbet and LaGoy, 1992)
IcP	0.1	(Huang <i>et al.</i> , 2005, Nisbet and LaGoy, 1992)

### **3.3 OPTIMIZATION OF EXTRACTION/CLEAN UP AND METHOD DEVELOPMENT FOR THE ANALYSES OF 16 PRIORITY USEPA AND ALKYLATED PAHs BY GC-MS**

#### **3.3.1 GC-MS Analyses of PAHs**

Agilent 6890N GC system furnished with an auto sampler (Agilent 7683 injector series) was coupled to a 5973 Network mass selective detector (GC-MS) (based on a quadruple mass separator) was used to run PAH, Alkylated PAHs and deuteriated PAHs (internal standards). A J&W Scientific HP-5MS UI silica fused capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) was used with helium as the carrier gas at a constant flow rate of 1.3 ml/min. Splitless injection of 2 µl of the sample extract

was automatically done by an injector (injector 7683 series) on the instrument using a 10 µl syringe. Purge flow to split vent during the splitless mode was 60 ml/sec of ultra pure helium (99.999 %) at 2 mins before reverting to gas saver mode of 20 ml/min and split delay was 3.50 mins. Solvent delay of 6 minutes was used. Syringe wash programme was made up of two post injection solvent washes, one sample pre injection wash and two pre injection pressure purges to ensure there was no carry over effect from one sample to the other. The injector and auxiliary temperatures were 290 °C and 300 °C respectively. The electron impact energy was set at 70 eV. Ion source temperature was 250 °C. The mass spectrum was scanned from 50 to 500 Da using a temperature programme for the GC oven of 50 °C with a hold for 3.20 mins then ramped to 300 °C at a rate of 20 °C/min. Optimisation of the programme was achieved by varying the ramp rate (°C/min) and the hold time (mins). Individual PAHs, internal standard and methylated PAHs were identified based on their masses and retention times. Based on the identified masses and retention time of the PAHs (from the scan mode), they were grouped for single ion monitoring (SIM) method for quantitative analysis of PAHs.

### **3.3.2 GC-MS Method Performance and Calibration**

The PAHs were quantified by internal standard method which relies on the recovery of internal standard in the calibration standards/ samples prepared. A standard mixture of the USEPA 16 priority PAHs (uPAH) + 2 methylated PAHs (2000 µg/mL, dichloromethane: benzene) and 6-deuterium labelled PAHs mix ((dPAHs) (d10-

ancenaphthene, d12-chrysene, d8-naphthalene, d12-perylene, d10-phenanthrene, d4-1,4 dichlorobenzene of 2000 µg/mL, dichloromethane: benzene)) were purchased from SUPELCO, Bellefonte, PA, USA. A custom Mix of C0 – C4 alkyl PAHs (RPAHs) was purchased from GREYHOUND chromatography and Allied Chemicals, UK ((RPAHs) (200 µg/mL, toluene)). Dilutions of the standard solutions with HPLC grade Hexane were made. Different concentrations of PAH standards ranging from 0 ng/ml (calibration blank of 250 ng/ml internal standard and no PAH) to 10,000 ng/ml (all the calibration standards had concentration of 250 ng/ml for internal standard) were prepared to check for the linearity and machine working range. Ten points were used within this range. All working standard solutions were prepared daily in *n*-hexane.

The system conditions were optimised for all the analytes by varying the temperature programme. When this was verified, the Limit of Detection and Limit of Quantification were determined for each of the 16 PAHs. Based on the observed linearity range, 6 points (calibration points) were chosen for subsequent calibration. The precision was improved by making up to three replicate injections for each of the concentration levels,

### **3.3.3 Reproducibility**

400 µg/ml was run five times using the optimised chromatographic conditions. Verification of calibration was carried out at periodic intervals and when there were variances, recalibration was done. Calibration verification for linear calibrations was

done by calculating the percent drift or the percent difference of the instrument response between the initial calibration and each subsequent analysis of the verification standard. The equation below was used to calculate % drift as follows;

$$\% \text{ Drift} = \frac{\text{Calculated concentration} - \text{Theoretical concentration}}{\text{Theoretical concentration}} \times 100\%$$

### **3.3.4 Selection of Solvent for the Extraction of PAHs from Certified Reference Material (CRM)**

Different organic solvents were tested for extraction of the PAHs from CRM using ultrasonication method of extraction in order to compare their extraction efficiencies. Solvents such as acetone, hexane, ethyl acetate, and acetone: hexane, methanol and dichloromethane were studied for the extraction procedures of PAHs from certified reference soil materials (CRM 172 and 115). 0.5 g of certified reference materials were weighed into amber glass bottles and extracted sequentially by ultrasonication using 5 ml of solvent (ethyl acetate, methanol, acetone, n-hexane or n-hexane: acetone) for an hour. After ultrasonication the supernatant of the extracts were decanted into a vial and 3 ml of fresh solvent was added and ultrasonicated for another one hour of ultrasonication. The process was repeated with a fresh 2 ml of solvent for one hour. The combined extracts (10 ml) was spiked with 25  $\mu$ l of 10 ppm dPAH, 50  $\mu$ l of nonane and concentrated with a stream of nitrogen gas in a turbovap sample concentrator evaporator system. Concentrated extract clean-up was done using the C<sub>18</sub> solid phase extraction (SPE) cartridges.

### **3.3.5 SPE Clean-up of CRM Extracts by Improved SPE Procedure**

CRM extracts were cleaned-up using a 12-port vacuum manifold from SUPELCO to which bond elute LC-18 SPE 5 ml cartridge of 200 mg sorbents were assembled. The SPE cartridges were conditioned with 5 ml of dichloromethane (DCM), 5 ml of methanol, 5 ml of methanol:water (1:1), 5 ml water, 5 ml of 40 % of the extracting solvent in water, 5 ml of extracting solvent to prepare the SPE column for the clean up process. The concentrated extract was loaded and aspirated through the cartridge under gentle vacuum at <2 ml/min flow rate and then eluted by 5 x 1 ml of DCM:hexane at 1 ml/min flow rate (each time the sorbents were soaked for 10 minutes with the elution solution before the elution). The eluates were collected into a 20 ml vial and concentrated under a gentle nitrogen stream using the sample concentrator and reconstituted in 1 ml of hexane. Great care was taken so that the surface of the sorbent in the cartridge was not dry during the conditioning and loading of the sample extract. The method used was adapted from Marce and Borrull (2000) and Oluseyi *et al.*, (2011). The optimised extraction, clean up procedure and GC-MS method (as in section **3.3.1. and 3.3.4**) was used for analysis of subsequent samples in this study.

### 3.4: ANALYSIS OF SUM AND BIOACCESSIBLE PAHs IN SOIL

#### 3.4.1 Sampling

Composite surface soil (depth 0-10 cm) samples were obtained from different sampling points in Lagos area, Nigeria and labelled A to L as shown in Table 3.2. The samples were air dried for 4 days in the dark at ambient room temperature  $\leq 32^{\circ}\text{C}$  and sieved using 2 mm sized sieve. These samples were stored in amber bottles sealed with aluminium covers and frozen till analyses.

**Table 3. 2 : Sampling Points for This Study and their Co-ordinates**

Sample ID	Location	Co-ordinates
A	Dump site near Onike canal	N 06 X 30' 42.11" E 003 X 23' 15.5 "
B	A farm in Lagos	N 06 X 34' 44. 7" E 003 X 24' 57.2 "
C	Depot and loading point for Used oil (Black oil), Iganmu, Orile	N 06 X 30' 56.31'' E 003 X 23' 58.5''
D	Busy road side, Akoka	N 04 X 30' 46.7" E 003 X 29' 21.3 "
E	Oando premium motor spirit and kerosine depot Apapa	N 06 X 27' 31.0" E 003 X 21' 36.2"
F	Dumpsite in University of Lagos	N 06 X 30' 51.7" E 003 X 23' 32.4 "
G	Coconut Island premium motor spirit and kerosine Depot	N 06 X 26' 26.25" E 003 X 19' 49.5"
H	Road side	N 06 X 30' 40.1" E 009 X 20' 21.9 "
I	Trailer Park/ Mechanic workshop Ibafo	N 06 X 43' 44.4" E 003 X 24' 57.2 "
J	Car Park Akoka	N 05 X 30' 44.0" E 003 X 23' 23.1 "
K	Control (forest soil)	Outside Lagos in Ogun state, Nigeria
L	Mechanic workshop in Onike	N 06 X 30' 42.1" E 003 X 23' 15.3 "

### **3.4.2 Physico Chemical Analysis of Soil Samples**

#### **3.4.2.1 Determination of particle size distribution in soil samples**

The particle size distribution of soils (A to L) were determined using the wet sieving and sedimentation as described by The British Standard Method for soils (Lorenzi, 2011, Yunker *et al.*, 2002). Soils samples were dried at 105 °C for one day and allowed to cool. 100 g each of the cooled, dreied samples were soaked with 2 g/L sodium hexa meta phosphate at a level just enough to cover it and left for one hour to stand while stirring intermittently. The soils were washed in clean water using the 63µm aperture sieve until nothing passed through it and dried till it attained a constant weight. Each of the dried soil residues were passed through fitted test sieves of aperture sizes of 75mm, 63mm, 50mm, 37.5 mm, 28 mm, 20 mm, 14 mm, 10 mm, 6.3 mm, 5 mm, 3.35 mm, 2 mm, 1.16 mm, 600 µm, 425 µm, 300 µm, 212 µm, 150 µm, 63 µm. Masses retained in the sieves were weighed and used for calculations. Calculated values include percentage retained and percentage that passed through the various apertures. On plotting these values on a particle size distribution chart from BSI the various particle sizes were obtained and classified into gravel, sand, silt and stones.

Sedimentation was used to determine sizes below 63 µm. 50g of dried soil was added to 250 mL of distilled water and 100mL of sodium hexametaphosphate ((NaPO<sub>3</sub>)<sub>6</sub>) (a deflocculating agent, which aided the separation of clays into individual particles) after which, the mixture was stirred thoroughly. This mixture was further shaken using a milk shake mixer for 5 minutes and poured into a 1000 mL beaker to sit overnight to

allow for chemical deflocculation. The mixture was poured into a 1000 ml measuring cylinder and made up to mark with distilled water and allowed to settle. A control of distilled water in a 1000 ml measuring cylinder was also set up and temperature was taken. Using hydrometers the specific gravity of the mixture was measured by taking the reading at the top of the meniscus. The measuring cylinder was closed up with its lid and the content shaken by inverting the cylinder repeatedly for one minute. Immediately this was done and returned to the table, a stop watch was turned on and the specific density was taken for the sample and control. The hydrometer readings were taken at 45sec, 1min, 90 sec, 3 min, 5 min, 10 min, 15 min, 30 min, 45 min, 1 hr, 2 hr, 4 hr, 6 hr, 1 day, 2 day. Control temperatures and specific gravity readings were periodically read during the analysis, especially as the time intervals are spread further apart. The values from the experiments were plotted on a particle size distribution chart from BSI to determine what the various particle sizes were.



**Figure 3. 1: Sedimentation Experiment to Determine Soil Sizes Below 63  $\mu\text{m}$**

#### **3.4.3.2 Preparation of soil for physico chemical and PAH analyses**

Soil samples for physico chemical and PAH analyses were air dried. The dried soil was then ground with pestle and mortar to breakdown the aggregate and passed through an aluminum sieve of 2 mm mesh. Soil samples larger than 2 mm were discarded. The 2 mm soil samples were then stored in polythene bags for physico chemical analysis and in amber bottles for PAHs analysis.

#### **3.4.2.3 Determination of pH (Electrometric method) of soil samples**

The pH of each sample was determined by adding 10 ml of 0.01 mol/L CaCl<sub>2</sub> solution to 5 g (1:2, soil: solutions). The resulting mixture was allowed to stand for 30 minutes with occasional stirring with a glass rod. The electrode of the pH meter was inserted into the party settled suspension and the pH was measured electronically with a Mettler Toledo Seven Easy pH Meter. The pH meter was calibrated with pH 7 and pH 4 buffer solution prior to measurement.

#### **3.4.2.4 Determination of total organic carbon and total organic matter in soil samples**

The sum organic carbon (TOC) and sum organic matter (TOM) of soil samples were determined by the Walkley-Black Titrimetric Method (Environment Agency, 2009). 0.1- 10.5 g of soil sample was weighed into an Erlenmeyer flask. To this, 10ml of (0.167 M) K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 20 ml concentrated H<sub>2</sub>SO<sub>4</sub> added. The mixture was gently

swirled until soil and reagents were properly mixed and were allowed to stand for 30 minutes after which 100ml of distilled water was added. The content was titrated against standardized (0.5 M/0.4 N) Ferrous Sulphate solution to a reddish brown end point using ferroin as the indicator. The TOC was subsequently calculated.

$$\% \text{TOC} = \frac{(A - B) \times M \times 0.003 \times 100 \times f}{\text{g of air-dried soil}}$$

Where

f = correction factor=1.33

B= volume of sample

A= volume of blank

%TOM was determined by multiplying % TOC by 1.724 (Phongoudome *et al.*, 2012).

#### **3.4.2.5 Determination of oil and grease in soil samples**

5g of soil sample was weighed and 20 ml of acetone: n-hexane (1:1) was added to weighed sample. This sample was swirled, ultra-sonicated for 20 mins at room temperature, allowed to settle for 5minutes and decanted into a pre-weighed dish in a fume cupboard. This extract was allowed to evaporate in a fume cupboard at room temperature, dried and the difference was calculated (Hong *et al.*, 2003). This procedure was repeated with blanks and subtracted from sample. The oil and grease was expressed in percentage.

### **3.4.5 Extraction, clean-up and analysis of PAHs from Soil Samples.**

0.5–5 g of soil was weighed into an amber glass bottles and extracted sequentially by ultrasonication using Hexane: acetone (1:1). The optimised extraction, clean up procedure and GC-MS method (as in section **3.3.1. and 3.3.4**) were used for analysis of soil samples.

### **3.4.6 Bioaccessible PAHs from Soil to Human Guts**

Bioavailability of PAHs from soil to human guts was done using FOREhST of Barge as described by Cave *et al.*, (2010) and Lorenzi *et al.*, (2012) with modification of the clean up stage.

#### **3.4.6.1 Preparation of the gastrointestinal fluids**

1) *Simulated saliva fluid* was prepared by adding 145 mg of amylase, 50.0 mg mucin and 15.0 mg uric acid to a 2 L HDPE screw top bottle first. Then, 896 mg of KCl, 888 mg NaH<sub>2</sub>PO<sub>4</sub>, 200 mg KSCN, 570 mg Na<sub>2</sub>SO<sub>4</sub>, 298 mg NaCl and 1.80 mL of 1.0 M HCl were added into a 500 mL volume container and made up to the mark with water (*inorganic saliva components*). To another 500 mL volumetric flask, 200 mg of urea was added and made up to mark with water (*organic saliva components*). The 500 mL of inorganic and 500 mL of organic saliva components were simultaneously poured in to the 2 L HDPE screw top bottle. The entire content of the screw top bottle was shaken thoroughly and the pH of the solution (gastric simulated saliva fluid) measured and was found to be within the range  $6.5 \pm 0.5$ .

2) *Simulated gastric fluid* was prepared by first adding 1000 mg of bovine serum albumin, 3000 mg mucin and 1000 mg pepsin to a 2 L HDPE screw top bottle. Then, 824 mg of KCl, 266 mg NaH<sub>2</sub>PO<sub>4</sub>, 400 mg CaCl<sub>2</sub>, 306 mg NH<sub>4</sub>Cl, 2752 mg NaCl and 8.30 mL of 37% HCl were added separately into a 500 mL container and made up to the mark with water (*inorganic gastric components*). The organic phase was prepared in another 500 mL volumetric flask and the component were 650 mg glucose, 20.0 mg glucuronic acid, 85.0 mg urea and 330 mg glucosamine hydrochloride and water which was used to make up the mark. Then, the contents of the two 500 mL volumetric flasks with inorganic and organic components were poured simultaneously in to the 2 L HDPE screw top bottle. The entire content of the screw top bottle was shaken thoroughly and the pH of the solution (gastric simulated fluid) measured. The pH was found to be within the range 0.9-1.0. The pH of the combination of mixed saliva fluid (1 mL) and gastric fluid (1.5 mL) was checked and was in the range of pH 1.2 –1.4.

3) *Simulated duodenal fluid* was prepared by weighing 200 mg of CaCl<sub>2</sub>, 1000 mg bovine serum albumin, 3000 mg pancreatin and 500 mg lipase into a 2 L HDPE screw top bottle. Then, 564 mg of KCl, 80 mg KH<sub>2</sub>PO<sub>4</sub>, 50.0 mg MgCl<sub>2</sub>, 5607 mg NaHCO<sub>3</sub>, 7012 mg NaCl and 180 µL of 37% HCl were separately added into a 500 mL volumetric flask and made up to the mark with water (*inorganic duodenal components*). Into a second 500 mL volume container was added 100 mg urea and made up to the mark with water (*organic duodenal components*). Then the content of the two 500 ml

volumetric flasks i. e the inorganic and organic duodenal components was simultaneously poured into the 2 L HDPE screw top bottle. The bottle with the simulated duodenal fluid thoroughly shaken and the pH of the solution (simulated duodenal fluid) was measured. The pH was within the range of  $7.4 \pm 0.2$ .

4) *Simulated bile fluid* was prepared by adding 222 mg of  $\text{CaCl}_2$ , 1800 mg bovine serum albumin and 6000 mg bile to an empty 2 L HDPE screw top bottle. Then into a 500 ml volumetric flask were 376 mg of KCl, 5785 mg  $\text{NaHCO}_3$ , 5259 mg NaCl and 180  $\mu\text{L}$  of 37 % HCl. Water was added to make up the mark (*inorganic bile components*). 250 mg urea was weighed into another 500 ml volume flask and made up to the mark with water (*organic bile components*). The 500 mL inorganic and 500 mL organic bile components were simultaneously poured into the 2 L HDPE screw top bottle to simulate the bile fluid. The simulated bile fluid in the screw top bottle was shaken and allowed to stand for approximately 1 h at room temperature, to ensure complete dissolution of reagents. This solution measured for pH and was found to be within the range  $8.0 \pm 0.2$ . The pH of the combination of saliva fluid (1.0 mL), gastric fluid (1.5 mL), 3.0 mL duodenal fluid and 1.0 mL bile fluid was checked to be within the range  $6.3 \pm 0.5$ .

#### **3.4.6.2 Preparation and extraction of samples using FOREShT of the BARGE**

FOREShT method adapted from Cave *et al.*, (2010) and Lorenzi *et al.*, (2012) was used in the preparation and extraction of bioavailable PAHs from soils.

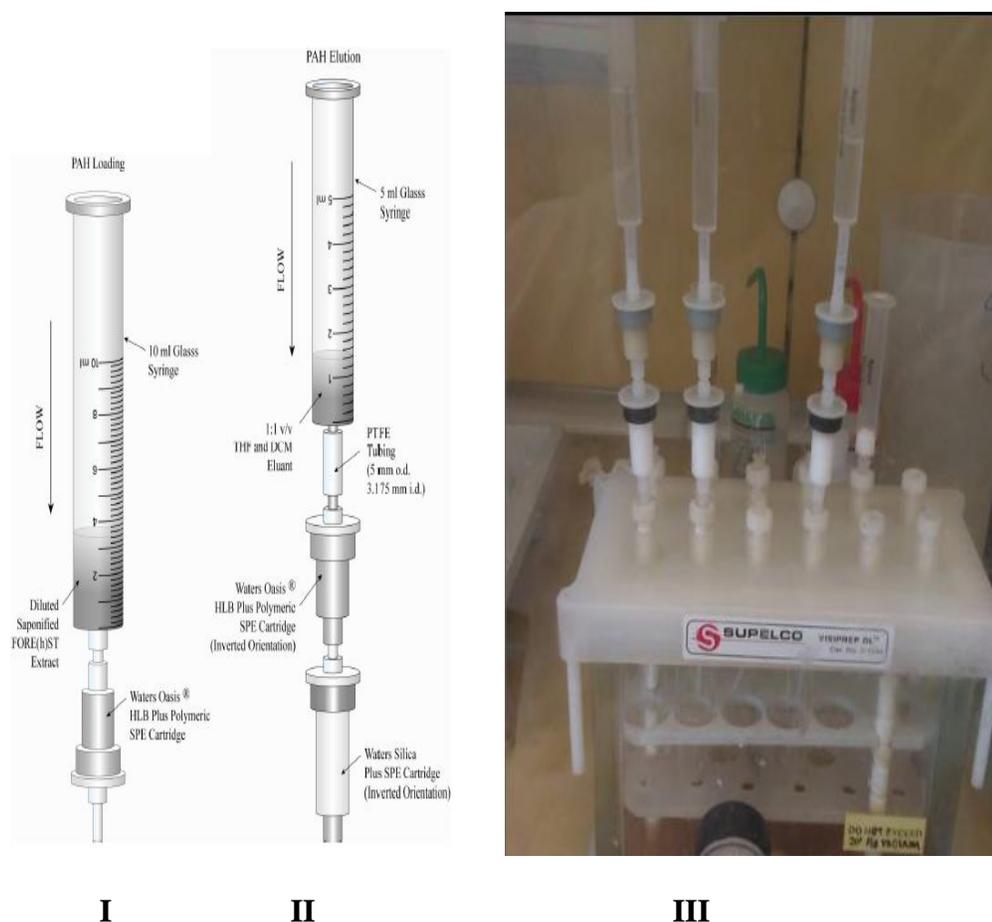
For blank study, 0.813 g of food (HIPP organic cream porridge (HIPP UK Ltd, Berkshire UK)), 2.45 ml of distilled water and 50 µl of oil (sunflower oil (ASDA stores limited, Leeds, UK)) were placed into a 40 mL glass amber screw vials with 4.5 mL of **simulated saliva fluid** . This mixture was shaken for 5 minutes. To this mixture, was added 9 mL of **simulated gastric fluid** added. At that stage the pH of each soil suspension was checked (the pH should be maintained between 1.2 -1.7). The new mixture was shaken on an end-over-end shaker maintained at  $37 \pm 2$  °C, 30 rpm for 2 h. After the mixture of **simulated saliva**, sample, food and **simulated gastric fluid** 9.0 mL of simulated duodenal fluid and 4.5 mL of simulated bile fluid were added by manually shaking the soil-fluid mixture in the screw-cap vessel. The pH of the resultant mixture was adjusted to  $6.3 \pm 0.5$ , by the dropwise addition of 37 % HCl, 1 M or 10 M NaOH, as required. The resultant mixture was again shaken on an end-over-end shaker maintained at  $37 \pm 2$  °C for 2 h as in Figure 3.2.



**Figure 3.2: Extraction of Bio- accessible PAHs End-over-end shaker**

Then, the sample suspension was removed, measured and maintained at a pH between  $6.3 \pm 0.5$  using HCL or NaOH solution. The resultant mixture was centrifuged at 3000 rpm for 5 mins. 1.0 mL of supernatant was removed into a heat resistant glass vial

for saponification. **Saponification** of the extract was done by adding 3 ml of KOH in methanol (5.6M KOH) and heated at 100 °C for 1 h. After cooling, 6ml of water was added to the saponified extract and cleaned up on a preconditioned waters oasis HLB cartridge as described in **section 3.2.5**. A repeat was done using C18 SPE cartridges in place of HLB and silica cartridge. Another repeat was done by using all the FOREShT extract (30 ml) for saponification (3 ml KOH) and diluting with water to a volume of 36 ml. The set up for the HLB cartridge was as in Cave *et al.*, (2010) (Figure 3.3).



**Figure 3.3: I- Experimental Set up for Clean- up of PAHs using HLB Cartridge in the Pre-Conditioning and Separation of PAHs Stage. II - Experimental set up of HLB Cartridge in the Elution of Separated PAHs Stage (Cave *et al.*, 2010). III-The laboratory set up Showing the HLB Cartridge on the SPE Manifold Attached to a Pump.**

The elution was done by passing 5 ml dichloromethane through cartridges holding cleaned analytes. Eluents were collected in a vial, concentrated using a gentle stream nitrogen gas and reconstituted in n-hexane. Hexane reconstituted eluents were analysed by the GC-MS method (as described in section **3.3.1. and 3.3.4**). The whole process was repeated for recovery. Optimised condition was used in the extraction of bioaccessible PAHs from 0.3 g of soil samples.

#### **3.4.7 Risk Analyses Studies of PAHs in the Soil Samples.**

Health-risk posed by the exposure of 16 USEPA priority PAHs is based on carcinogenic potency relative to BaP (Tsai *et al.*, 2001). TEFs developed by Nisbet and LaGoy (1992) were used in this study because, they were suggested to be a better set by Xia *et al.*, (2010) and Boström *et al.*, (2002). BaP<sub>eq</sub> dose was calculated as follows:

$$\text{BaP}_{\text{eq}} \text{ dose } (\mu\text{g/g}) = \text{TEF} \times \text{Concentration } (\mu\text{g/g}) \text{ (Huang } et al., 2005)$$

$$\text{Sum BaP}_{\text{eq}} \text{ dose } (\mu\text{g/g}) = \sum (\text{TEF} \times \text{Concentration } (\mu\text{g/g}))$$

Exposure route for PAHs from soil to man include dermal, inhalation and ingestion. However, the key exposure route was found to be ingestion route in a study by Zhong *et al.*, (2013). Soil ingestion can occur by the inadvertent ingestion of soil on hands or food items, mouthing of objects, or through intentional ingestion of soil. The risk associated with the workers on site based on involuntary ingestion can be estimated (Lorenzi *et al.*, 2011). The estimated daily intake dose (D), another risk assessment approach is an estimation of PAHs consumed due to involuntary consumption of soils

(Davoli *et al.*, 2010, Lorenzi *et al.*, 2011). It seeks to estimate daily exposure to PAHs.

It was calculated as follows:

Dose (D) of PAH ingested was estimated based on daily exposure as

$$D (\mu\text{g}/\text{kg}^{-1}\text{day}^{-1}) = [\text{EC} \times \text{SIR} \times \text{AF}]/\text{BW}$$

The annual daily dose exposure **Da** also called **the average life time daily exposure** was calculated by introducing the **EF (exposure frequency)** value.

$$D_a (\mu\text{g}/\text{kg}^{-1}\text{day}^{-1}) = [\text{EC} \times \text{SIR} \times \text{EF} \times \text{AF}]/\text{BW} \text{ (ATSDR, 2005, ODH, 2011)}$$

Where: AF = Bioavailability factor (bioaccessibility factor) ((1=100 %) is usually assumed to be 1 except where stringent experiments are conducted to get the value) (ATSDR, 2005).

BW= Body weight of adult 70 kg (ATSDR, 2005)

SIR= Soil ingestion rate for adult (0.1 g/day) (Environment Agency, 2009)

EF (days/year) = (F X ED)/AT. In many instances, the exposure factor (EF) is usually 1 representing a daily exposure to the contaminant. However, some exposure may occur on an intermittent or irregular basis. For these kinds of exposures, an EF can be calculated to average the dose over the exposure interval (ATSDR, 2005).

EC=Exposure concentration of PAHs ( $\mu\text{g}/\text{g}$ ),

F = frequency of exposure (days/year) 246 day a year (365- (15 public holidays in Nigeria and 2 weekend days a week x 52 weeks in a year) in Nigeria considering the public holiday and weekends only. The nature of work done on these sites is unstructured so no leave was considered in this assumption.

AT=Average time (ED x 365 days/year)

ED= Exposure duration (years) (40 years)(an adult person does the works 8 hours per day for 40 years (25 to 65 year of age))

Using the above parameters EF value of 0.67 was derived and Da, an estimate of annual occupational exposure for an adult on these sites was calculated.

The mean dietary intake (MDI) also called average daily intake was calculated from the D as MDI (mg/day) = D\*BW= [EC X SIR] (Ding *et al.*, 2013, Lorenzi *et al.*, 2011).

Cancer risk is estimated as

ER = CSF x dose (ODH, 2011)

Where, ER = estimated theoretical cancer risk

CSF = cancer slope factor (mg/[kgday])<sup>-1</sup>(7.3 (mg/[kgday])<sup>-1</sup>)(Nyarko *et al.*, 2011, ODH, 2011).

Dose = estimated exposure dose (calculated using the BaP<sub>eq</sub> concentration into the Da equation) (mg/[kgday])

### **3.4.8 PAH Uptake by Planted Vegetables (Bioavailable PAHs in Plants)**

#### **3.4.8.1 Soil sample preparation for planting**

Soil C, E, G, K were used for planting. These soils were classified as heavily contaminated and contaminated soils except K which served as the control. The soils were prepared for planting by removing large stone with the help of a farm sieve.

Fertilizer (pink granular free flowing N.P.K called 15-15-15 ) was added to the different soils in the ratio of 600 g to 40 Kg and stored in the dark (cold) for 4 days. Soils C and K were mixed in the ratio of 1:9, 1:3, 1:1 and 3:1 and 1:0. The mixing of soils in different ratios was to ensure growth of plants in this study. This was repeated for E and K, G and K. 1.4 kg of soil and their mixes were bagged for planting in perforated black special planting nylons ready for planting in the green house. K not mixed with other soils served as control. Each soil mixture was sampled and kept at -20<sup>0</sup>C for analysis before planting commenced.

#### **3.4.8.2 Seed sourcing and planting**

Seeds of *Corchorus Olitorius* (*Ewedu* in yoruba), *Celosia Argentea* (*Soko* in yoruba), *Amaranthus cruentus L* (*Grain Amaranthus, Tete* in yoruba) were sourced from National Horticultural Research Institute Nigeria (NIHORT) while *Basella Alba* (*Amunu tutu* in yoruba, White Spinash), *Lactuca Sativa* (Lettuce), *Allium Ascalonicum* (Spring Onions, *Alubasa elewe* in yoruba), *Talinum Triangulare* (Water leaf), *Telfairia Occidentale* (*Ugwu* in yoruba), were sourced from commercial farms in Lagos.

These seeds were pre-germinated and nursed using compost nursery in a green house at the Botanical garden of the University of Lagos. At the stage of 5 to 6 seed leaves (after two weeks), seedlings were taken to the Department of Botany, University of Lagos for species confirmation and identification.

The seedlings were transferred to bagged soils at the Botanical garden of the University of Lagos to grow till maturation (based on the recommendation at the seed sourcing) time according to their species. They were each planted for 7 weeks, harvested washed and half of the harvest was preserved in foil papers and frozen at  $-53^{\circ}\text{C}$  for PAH analysis and bioavailability study while the other half was preserved by cutting the aerial part of the plant and subsequently packed in aluminium foils. They were dried at room temperature and shredded with a domestic shredder. Finally, samples were kept in a double layered aluminium foil, packed in labelled plastic bags, and stored at  $0^{\circ}\text{C}$  temperature until analyses in an attempt to protect them from solar light (Nadal *et al.*, 2004).



**Plate 3.1: Picture of the Plants growing in the green house**

#### **3.4.8.3 Extraction, clean-up and analysis of PAHs in Plants.**

0.5-5 g of soil and plant samples were weighed into amber glass bottles and extracted sequentially by ultrasonication using hexane: acetone (1:1). The optimised extraction, clean up procedure and GC-MS analysis method (as described in section **3.3.1. and 3.3.4**) were used for analysis of soil samples.

## **CHAPTER FOUR**

### **4.0 RESULTS AND DISCUSSION**

This chapter describes the results of analyses and risk of PAHs in the various matrices (smoked fishes, roasted foods, soil and plants) used for this study. Sections 4.1 and 4.2 showed the results, assessments and discussion on the effects of smoking methods on the levels of PAHs in some locally consumed smoked fishes and roasted foods in Nigeria. Section 4.3 contains results and discussion on the optimisation, method development, extraction, clean up and analysis of PAHs by GC-MS. The assessment of alkylated polycyclic aromatic hydrocarbons and risk assessment of polycyclic aromatic hydrocarbons in soils at different anthropogenic sites around Lagos, Nigeria were also analysed. The results and discussion are in section 4.4 while section 4.5 contains the results and discussion on the PAH uptake by some local edible plants in South West Nigeria from potted soils (bioavailable PAHs in plants).

### **4.1 EFFECTS OF THE METHODS OF SMOKING ON THE LEVELS OF PAHS IN SOME LOCALLY CONSUMED FISHES IN NIGERIA**

#### **4.1.1 Length, Weight and Fat (Oil) Content of Fishes and the smoking conditions Used for this Study**

The average length of the fresh fish samples studied ranged from 24.0 to 38.5 cm. The wet weight of the fish samples ranged from 74.49 to 525 g while the dry weight of the fish samples ranged from 16.06 to 139.23 g, with the cat fish having the highest

average weight. The sizes of the fishes showed they were matured fishes. Of the three species of fishes studied the cat fish (*Arius Heude loti*) had the highest average oil content of 48.94 mg/g, while *Haake* had the lowest oil content of 2.34 mg/g. Table 4.1 shows the sizes, weight and fat content of the fish samples studied.

**Table 4. 1: Length, Weight and Fat Content of Fishes used for this Study.**

Common name of fish	Scientific name	Length (cm)	Wet weight (g)	Dry weight(g)	Fat content (mg/g)
Cat fish	<i>Arius heude loti</i>	36.8 -38.5	450 – 525	107.65-139.23	48.94± 0.45
Sole	<i>Cynoglossus senegalensis</i>	24.0 -26.7	74.49 -80.85	16.06 -19.98	3.43 ±.40.00
Fresh stock fish	<i>Haake sp</i>	27.0 -28.0	89 .08 99.57	19.86 - 21.72	2.34±0.50

The fishes were smoked employing the traditional smoking methods using charcoal, firewood and sawdust respectively as the source of fuel. The charcoal had the highest temperature of 250 °C, followed by the firewood (200 °C) and sawdust (120 °C) as presented in Table 4.2. The smoking took place at low to moderate temperatures. Incomplete combustion of wood, charcoal and fuels generally lead to the formation of PAHs. They are formed at high temperature (e.g 700 °C), pyrolysis of organic materials by low to moderate temperature (e.g 100 – 150 °C) and diagenesis of organic materials by microorganisms (Neff, 1985). The levels of PAH in smoke depends on heat source (coal, wood, gas among others), temperature, flame intensity in flame combustion, particulate material generated during combustion among others (Muthumbi *et al.*, 2003, Rey-Salgueiro *et al.*, 2004, Garcia-Falcon and Simal-Gandara, 2005, Wretling *et al.*, 2010). The PAHs studied can be classified as those from pyrolysis of organic materials

at moderately high temperature. Generally it was observed that at high temperatures less smoke was produced and at lower temperatures more smoke was produced during the smoking process.

**Table 4. 2: Temperature of Fish Smoking Processes.**

<b>Types of smoking</b>	<b>Temperature of the smoking process (°C)</b>	<b>Observations made during the smoking process.</b>
Charcoal	250	Least amount of smoke was produced
Firewood	200	Moderate amount of smoke was produced
Sawdust	120	Highest amount of smoke was produced

#### **4.1.2 PAHs Content in Fishes Processed by Different Methods**

Various smoking treatments were given to the *Arius heude loti* as shown in Table 4.3. The sum concentration of PAHs in the oven dried method gave the least value, having a concentration of 333 µg/kg. Sawdust smoked fish had the highest concentration of 2058 µg/kg, while firewood and charcoal smoked *Arius heude loti* had concentrations of 1321 and 1137 µg/kg respectively. Most of the individual PAHs were not detected in the oven dried fish samples. This could be as a result of the method not involving the use of smoke. Some PAHs were not detected in *Arius heude loti* smoked fishes. Benzo (a) pyrene for example was not detected in all the samples. The concentration of sum PAHs in the fishes were 2,058 µg/kg, 1,321 µg/kg, 1,137 µg/kg and 333 µg/kg for the sawdust smoked, firewood smoked, charcoal smoked and oven dried methods

respectively. The level of sum PAHs in the smoked fishes was higher than those in the oven dried fishes. The concentrations of PAHs, in smoked fishes using four different smoking methods were found to vary with the heat source. This agrees with the findings of some researchers who studied the effects of cooking methods on foods and found that PAHs concentrations in food varied with cooking methods (Muthumbi *et al.*, 2003, Rey-Salgueiro *et al.*, 2004).

**Table 4.3: PAHs Concentration ( $\mu\text{g}/\text{kg}$ ) of *Arius heude loti* (Cat fish) Processed by Different smoking Methods**

PAHs	Sawdust	Fire wood	Charcoal	Oven dried
Naphthalene	124	109	ND	ND
Acenaphthylene	321	269	99.5	27.2
Acenaphthene	514	23.9	543	ND
Fluorene	ND	802	143	132
Phenanthrene	30.7	ND	ND	ND
Anthracene	188	30	35.5	ND
Fluoranthene	329	17.9	82.9	68.2
Pyrene	247	43.1	ND	ND
Benzo(a)anthracene	ND	ND	ND	20.4
Chrysene	23.1	5.5	ND	8.9
Benzo(b)Fluoranthene	ND	ND	ND	ND
Benzo(k)fluoranthene	18.5	ND	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND
Dibenzo(a,h)anthracene	216	19.7	233	50.9
Benzo(g,h,i)perylene	ND	ND	ND	ND
Indeno(1,2,3,c)pyrene	47.7	2.2	ND	25
Sum PAHs	2058	1321	1137	331

\*ND-Not detected  $\leq 0.2 \mu\text{g}/\text{kg}$

Table 4.4 shows the results of the concentration of individual and sum PAHs found in *Cynoglossus senegalensis* from the various smoking procedures. The oven dried

method gave the least concentration of sum PAHs. Most of the individual PAHs were not detected except for acenaphthylene, benzo(a)anthracene, chrysene, benzo(a)pyrene and indeno(1,2,3-c,d). However the smoked fishes had higher concentration of the individual and sum PAHs. The sawdust smoked *Cynoglossus senegalensis* had the highest levels of the sum PAHs, followed by the fire wood and charcoal. This was similar to the findings for *Arius heude loti* in Table 4.3.

**Table 4.4: Concentration ( $\mu\text{g}/\text{kg}$ ) of PAHs in *Cynoglossus senegalensis*\_(Sole) Processed by Different Smoking Methods.**

PAHs	Sawdust	Fire wood	Charcoal	Oven dried
Naphthalene	236	ND	ND	ND
Acenaphthylene	ND	528	12.4	18.3
Acenaphthene	630	300	98.6	ND
Fluorene	ND	142	ND	ND
Phenanthrene	ND	ND	12.0	ND
Anthracene	15.3	21.8	ND	ND
Fluoranthene	68.7	88.5	5.4	ND
Pyrene	442	78.3	30.1	ND
Benzo(a)anthracene	ND	36.1	ND	5.3
Chrysene	3.6	17.1	ND	2.5
Benzo(b)Fluoranthene	ND	ND	ND	ND
Benzo(k)fluoranthene	ND	ND	4.9	ND
Benzo(a)pyrene	ND	ND	ND	5.6
Dibenzo(a,h)anthracene	ND	ND	ND	ND
Benzo(g,h,i)perylene	ND	10.9	ND	ND
Indeno(1,2,3-c,d)pyrene	ND	34.7	13.4	14.4
Sum PAHs	1395	1258	177	46.1

\*ND-Not detected  $\leq 0.2 \mu\text{g}/\text{kg}$

Various smoking methods were applied to the *Haake* and the results of PAHs in the samples are shown in Table 4.5. The oven dried fish which can be regarded as the

control since smoking was not carried out on it. The smoked fishes had more PAHs identified and at higher levels. Benzo(a)pyrene was not detected in the oven dried and smoked fishes. The sum PAHs level in the fishes were 856µg/kg, 781µg/kg, 121µg/kg and 37.9µg/kg for the sawdust smoked, firewood smoked, charcoal smoked and oven dried respectively. Like the other fish sample type, the sum PAHs in the smoked *Haake* compared with the oven dried *Haake* were higher.

**Table 4.5: PAHs Concentration (µg/kg) Found in *Haake. sp.* Processed by Different Smoking Methods.**

PAHs	Sawdust	Fire wood	Charcoal	Oven dried
Naphthalene	34.7	14.2	96.3	ND
Acenaphthylene	631	42.9	24.5	6.1
Acenaphthene	119	11.0	ND	ND
Fluorene	38.7	29	ND	3.2
Phenanthrene	29.1	22.8	ND	12.4
Anthracene	4.0	60.9	ND	11.0
Fluoranthene	ND	281	ND	0.5
Pyrene	ND	81.1	ND	4.7
Benzo(a)anthracene	ND	ND	ND	ND
Chrysene	ND	55.1	ND	ND
Benzo(b)Fluoranthene	ND	ND	ND	ND
Benzo(k)fluoranthene	ND	16.0	ND	ND
Benzo(a)pyrene	ND	ND	ND	ND
Dibenzo(a,h)anthracene	ND	82.6	ND	ND
Benzo(g,h,i)perylene	64.1	41.5	ND	ND
Indeno(1,2,3,c)pyrene	ND	43.1	ND	ND
Sum PAHs	856	781	121	37.9

\*ND-Not detected  $\leq 0.2$  µg/kg

The values of the sum PAHs showed that the *Arius heude loti* had highest level of PAHs followed by the *Cynoglossus senegalensis*, then *Haake.Sp.* The difference might be related to the oil/fat content in the fishes. *Arius heude loti* had the highest oil content

(48.94 mg/g) of the three fishes investigated followed by *Cynoglossus senegalensis*, (3.43 mg/g) and *Haake.Sp* (2.34 mg/g). Hence there was a correlation between the oil/fat content and sum PAHs content in the fish samples. Some authors have determined the effects of various processing methods such as steaming, roasting, smoking, charcoal grilling, etc. on foods (Garcia-Falcon *et al.*, 1996, Chen and Lin, 1997, Wu *et al.*, 1997, Mottier *et al.*, 2000, Chen and Chen, 2001, Duedahl-Olesen *et al.*, 2006, Rey-Salgueiro *et al.*, 2009,). All these mentioned authors found a link between food fat and PAH concentrations. They observed that the highest PAH concentrations were generated during grilling or barbecue through pyrolysis and during charbroiling of meat and other products, both deposition and penetration of smoke components into foods occur. The hypothesis is that melted fat from the heated food drips onto the hot coals and is pyrolyzed, giving rise to PAHs generation, which are then deposited on the meat surface as the smoke rises. The result in this study is similar to their findings. Biological membranes particularly in fishes are composed of lipids (oils). PAHs have strong affinity for oils (lipophilic). It has been suggested that the larger the lipid content of the biological membrane, the higher is the rate of uptake of pollutants (Hamelink and Spacie, 1977).

#### **4.1.4 Risk Assessment Studies of PAHs in Fishes Smoked by Different Methods**

BaP a bio marker of PAHs and one of the best characterised PAHs may be bio transformed in humans and animals to numerous phase 1 metabolites (Rey-Salgueiro *et al.*, 2009). 3,4-benzopyrene, found in smoked products, serves as an indicator of the

possible presence of other polycyclic aromatic hydrocarbons (PAH) and has been used repeatedly as a quantitative index of chemical carcinogens in foods. The BaP level found in the smoked fish samples in this study were lower than legislated in the European regulatory maximum level for smoked meat and fishes which is 5 µg/kg (Wenzl *et al.*, 2006) except for the oven dried *Cynoglossus senegalensis* (Sole) (5.6 µg/kg).

In addition, the potential health risk from smoked fishes can be assessed by determining the concentration of each individual PAH. Smoked fish ingestion rate has been calculated as 25g per day (Fish consumption rate was set at 68.5 g/day from the annual per capita fish consumption of 25 kg for Nigeria (Nkpa *et al.*, 2013, Olaji *et al.*, 2014) but more fresh fishes are consumed compared to processed fishes in Nigeria (Samuel *et al.*, 2010, Akinyele, 2009) as shown in section 3.1.4. Based on this assumption, the MDI of individual PAH ingested from smoked fishes were calculated for this study for an adult (of body weight 70 kg) in Nigeria. Comparison of MDIs for the smoked fishes with oral MDIs for PAHs in food as given by Nathaniel *et al.*, (2009) and Lorenzi *et al.*, (2011) were carried out and the results are as shown in Tables 4.6, 4.7 and 4.8. Smoked *Arius heude loti* (Catfish) (Table 4.6) had some MDI of its individual PAHs exceeding the oral MDI for food indicating some risks associated ingestion of these fishes based on this estimate. More of the lower weight molecular PAHs compared to the higher molecular weight (PAHs with 5 or 6 rings) in Smoked *Arius heude loti* (Catfish) had their MDI exceeding that of food. Less risk is associated with the lower

molecular weight PAHs. Ajiboye *et al.*, (2011) stated that many lower molecular weight PAHs may not be carcinogenic but may act as synergists to cancer occurrence. The four, five and six membered ring PAHs appear to be more carcinogenic than PAHs with smaller or larger ring systems and highly angular configurations tend to be more carcinogenic than linear ring systems (Neff, 1985). Sum MDI for sawdust smoked *Arius heude loti* (Catfish) 34.4µg/day > Sum MDI for fire wood smoked *Arius heude loti* (Catfish) 31.4µg/day> Sum MDI for charcoal smoked *Arius heude loti* (Catfish) 4.42 µg/day> Sum MDI for Oven dried *Arius heude loti* (Catfish) 1.15 µg/day. The sum MDI for the other two fish species studied showed similar trends. The order was consistently sawdust method > firewood method > charcoal method > oven dried method. Sawdust smoked fishes consistently gave higher sum MDI values compared to other types of smoked fishes. This suggests that more risk is therefore associated with the sawdust smoked fishes compared to the firewood smoked compared and more risk is associated with the firewood smoked fishes compared to the charcoal smoked fishes. However, the value of MDI in this study requires further confirmation by using data from bioavailability/ bio accessibility studies (*in vitro* or *in vivo*) such as FOREShT.

**Table 4.6: MDI Concentration for PAHs in Smoked *Arius heude loti* (Catfish) ( $\mu\text{g}/\text{day}$ )**

PAHs	Sawdust	Fire wood	Charcoal	Oven dried	<sup>a</sup> Oral MDI food
Naphthalene	3.10	2.71	0.00	0.00	7.00
Acenaphthylene	<b>8.02</b>	<b>6.72</b>	<b>2.49</b>	<b>0.68</b>	0.14
Acenaphthene	<b>12.84</b>	0.60	<b>13.56</b>	0.00	0.98
Fluorene	0.00	<b>20.04</b>	<b>3.57</b>	<b>3.31</b>	0.59
Phenanthrene	0.77	0.00	0.00	0.00	1.54
Anthracene	<b>4.69</b>	<b>0.75</b>	<b>0.89</b>	0.00	0.08
Fluoranthene	<b>8.23</b>	<b>0.45</b>	<b>2.07</b>	<b>1.71</b>	0.35
Pyrene	<b>6.17</b>	<b>1.08</b>	0.00	0.00	0.35
Benzo(a)anthracene	0.01	0.00	0.00	0.51	0.06 (0.05) <sup>b</sup>
Chrysene	<b>0.58</b>	<b>0.14</b>	0.00	<b>0.22</b>	0.11
Benzo(b)Fluoranthene	0.00	0.00	0.00	0.00	0.11
Benzo(k)fluoranthene	<b>0.46</b>	0.00	0.00	0.00	0.09
Benzo(a)pyrene	0.00	0.00	0.00	0.00	0.11
Dibenzo(a,h)anthracene	<b>5.41</b>	<b>0.49</b>	<b>5.83</b>	<b>1.27</b>	0.10
Benzo(g,h,i)perylene	0.00	0.00	0.00	0.00	0.04
Indeno(1,2,3,c)pyrene	<b>1.19</b>	0.00	0.00	<b>0.63</b>	0.06
Sum MDI of PAHs	51.46	32.97	28.41	8.32	

<sup>a</sup>Oral mean daily intake threshold for PAHs in food (oral MDI) (Lorenzi *et al.*, 2011), <sup>b</sup> Alternative measure of oral MDI (Falco *et al.*, 2003). Where a value of 0.00  $\mu\text{g}/\text{day}$  MDI for individual PAH, means that the concentration MDI of that PAH is equal or less than 2.1  $\mu\text{g}/\text{kg}$ . The bold values are values greater than the PAH MDI for food.

**Table 4. 7: MDI for PAHs in Smoked *Cynoglossus senegalensis* (Sole) (µg/day)**

PAHs	Sawdust	Fire wood	Charcoal	Oven dried	<sup>a</sup> Oral MDI food
Naphthalene	5.90	0.00	0.00	0.00	7.00
Acenaphthylene	0.00	<b>13.20</b>	<b>0.31</b>	<b>0.46</b>	0.14
Acenaphthene	<b>15.74</b>	<b>7.51</b>	<b>2.47</b>	0.00	0.98
Fluorene	<b>0.00</b>	<b>3.54</b>	0.00	0.00	0.59
Phenanthrene	0.00	0.00	0.30	0.00	1.54
Anthracene	<b>0.38</b>	<b>0.55</b>	0.00	0.00	0.08
Fluoranthene	<b>1.72</b>	<b>2.21</b>	0.14	0.00	0.35
Pyrene	<b>11.05</b>	<b>1.96</b>	<b>0.75</b>	0.00	0.35
Benzo(a)anthracene	0.00	<b>0.90</b>	0.00	0.13	0.06(0.05) <sup>b</sup>
Chrysene	0.09	<b>0.43</b>	0.00	0.06	0.11
Benzo(b)Fluoranthene	0.00	0.00	0.00	0.00	0.11
Benzo(k)fluoranthene	0.00	0.00	<b>0.12</b>	0.00	0.09
Benzo(a)pyrene	0.00	0.00	0.00	0.14	0.11
Dibenzo(a,h)anthracene	0.00	0.00	0.00	0.00	0.10
Benzo(g,h,i)perylene	0.00	<b>0.27</b>	0.00	0.00	0.04
Indeno(1,2,3,c)pyrene	0.00	<b>0.87</b>	<b>0.34</b>	<b>0.36</b>	0.06
Sum MDI of PAHs	34.88	31.44	4.42	1.15	

<sup>a</sup>Oral mean daily intake threshold for PAHs in food (oral MDI) (Lorenzi *et al.*, 2011), <sup>b</sup> Alternative measure of oral MDI (Falco *et al.*, 2003). Where a value of 0.00 µg/day MDI for individual PAH, means that the concentration MDI of that PAH is equal or less than 2.1 µg/kg. The bold values are values greater than the PAH MDI for food.

**Table 4.8: MDI for PAHs in Smoked *Haake Sp* (Fresh stock fish, *Panla*) ( $\mu\text{g/day}$ )**

PAHs	Sawdust	Fire wood	Charcoal	Oven dried	<sup>a</sup> Oral MDI food
Naphthalene	0.87	0.36	2.41	0.00	7
Acenaphthylene	<b>15.78</b>	<b>1.07</b>	<b>0.61</b>	<b>0.15</b>	0.14
Acenaphthene	<b>2.97</b>	0.28	0.00	0.00	0.98
Fluorene	<b>0.97</b>	<b>0.73</b>	0.00	0.08	0.59
Phenanthrene	0.73	0.57	0.00	0.31	1.54
Anthracene	0.10	<b>1.52</b>	0.00	0.28	0.08
Fluoranthene	0.00	<b>7.02</b>	0.00	0.01	0.35
Pyrene	0.00	<b>2.03</b>	0.00	0.12	0.35
Benzo(a)anthracene	0.00	0.00	0.00	0.00	0.06(0.05) <sup>b</sup>
Chrysene	0.00	<b>1.38</b>	0.00	0.00	0.11
Benzo(b)Fluoranthene	0.00	0.00	0.00	0.00	0.11
Benzo(k)fluoranthene	0.00	<b>0.40</b>	0.00	0.00	0.09
Benzo(a)pyrene	0.00	0.00	0.00	0.00	0.11
Dibenzo(a,h)anthracene	0.00	<b>2.07</b>	0.00	0.00	0.1
Benzo(g,h,i)perylene	<b>1.60</b>	<b>1.04</b>	0.00	0.00	0.04
Indeno(1,2,3,c)pyrene	0.00	<b>1.08</b>	0.00	0.00	0.06
Sum MDI of PAHs	23.01	19.52	3.02	0.95	

<sup>a</sup>Oral mean daily intake threshold for PAHs in food (oral MDI) (Lorenzi *et al.*, 2011), <sup>b</sup> Alternative measure of oral MDI (Falco *et al.*, 2003). Where a value of 0.00  $\mu\text{g/day}$  MDI for individual PAH, means that the concentration MDI of that PAH is equal or less than 2.1  $\mu\text{g/kg}$ . The bold values are values greater than the PAH MDI for food. The bold values are values greater than the PAH MDI for food.

## 4.2 PROXIMATE ANALYSIS AND CONCENTRATION OF PAHs IN ROASTED FOODS

### 4.2.1. Proximate Analysis of Raw and Roasted Foods

Proximate analysis (which includes percentage crude fibre, percentage protein, percentage carbohydrate, percentage water, percentage ash, macro nutrients) was

carried out on the roasted and raw food samples selected for this study and the results are as shown Table 4.9.

**Table 4.9: Proximate Analysis of Raw and Roasted Food samples**

	<b>% Crude Fibre</b>	<b>% Protein</b>	<b>% Carbohydrate</b>	<b>% Moisture</b>	<b>% Crude Fat</b>	<b>% Ash</b>
<b>Raw Unripe Plantain</b>	11.83	3.88	40.13	41.93	1.12	1.12
<b>Roasted Unripe Plantain</b>	2.32	3.15	49.22	39.57	4.23	1.52
<b>Raw Maize Corn</b>	1.58	5.81	35.93	50.70	4.75	1.23
<b>Roasted Maize Corn</b>	0.82	4.73	48.03	38.84	6.07	1.51
<b>Raw Ripe Plantain</b>	12.95	8.27	55.52	18.70	2.80	1.76
<b>Roasted Ripe Plantain</b>	7.11	5.25	51.12	15.39	19.56	1.57
<b>Raw Yam</b>	14.21	4.94	47.37	32.95	1.40	1.90
<b>Roasted Yam</b>	10.07	4.91	43.95	30.63	1.20	2.59

All the raw food samples had higher percentage of crude fibre than the roasted samples. Raw yam had the highest percentage crude fibre of 14.21 %, followed by raw ripe plantain with a composition of 12.95 %, while raw unripe plantain and raw maize corn had percentage crude fibre compositions of 11.83 % and 1.58 % respectively. The percentage compositions of crude fibre in the roasted food samples ranged between 0.82 % - 10.07 %, with roasted maize corn having the least percentage composition. Percentage composition of protein and carbohydrate were also found to be higher in the raw food samples than in the roasted food samples. The proximate analysis results for roasted maize and yam were comparable with that of similar studies on proximate composition of street snacks purchased from selected motor parks in Lagos, Nigeria.

The fibre content of roasted yam was found to be 8.49 g/100 g and the carbohydrate content of roasted plantain was found to be 61.58 g/100 g (Oboh and Ogbebor, 2010).

There was no significant difference between the ash content of the raw and roasted food samples. The ash content which is the inorganic residue remaining after the organic matter has been burnt away is a very useful parameter in assessing the quality of edible material (Ojokoh and Gabriel, 2010). Moisture content in the roasted sample was less than in the raw sample samples as seen in Table 4.9. This is obviously due to the drying effect of the open flame roasting method. Macro nutrients in the raw and roasted maize, yam and plantain samples showed they were all rich in copper, manganese, magnesium, iron and zinc with the values shown in Table 4.10.

**Table 4.10 : Mineral Composition (mg/kg) of Raw and Roasted Food Samples**

	Cu	Mn	Mg	Fe	Zn
Raw Unripe Plantain	10.65	2.40	1.95	28.30	1.05
Roasted Unripe Plantain	14.30	3.80	3.40	5.05	3.85
Raw Maize Corn	12.45	0.70	4.05	6.85	6.20
Roasted Maize Corn	12.20	0.80	4.7	3.15	9.60
Raw Ripe plantain	20.05	2.05	4.4	10.70	3.60
Roasted Ripe plantain	7.05	3.20	2.2	16.20	3.70
Raw Yam	17.30	1.00	4.95	16.10	1.30
Roasted Yam	20.60	2.45	1.15	4.90	5.60

#### 4.2.2 PAHs in Roasted Foods

PAHs in roasted and raw ripe and unripe plantain, yam, and corn were extracted by ultrasonic extraction and initially cleaned up with nylon filter membrane. Analysis of these extract revealed an unresolved chromatogram. Unresolved Chromatogram must have been due to very high organic content as a result, the use of column clean up was revisited. A more intensive conditioning and column preparation step was used according to clean up the roasted food extracts before analysis the results shown in Table 4.11. Levels of PAHs in roasted samples were found to be higher than in the raw samples. However, roasted plantain was found to have the highest level of sum PAHs with a concentration of 40,330  $\mu\text{g}/\text{kg}$ .

Sum PAH concentrations of raw food samples had a range of 19  $\mu\text{g}/\text{kg}$  in raw unripe plantain to 3,170  $\mu\text{g}/\text{kg}$  in raw corn. As expected, higher concentrations were found in roasted food samples, with roasted yam having a concentration of 3,620  $\mu\text{g}/\text{kg}$  and roasted ripe plantain having a value of 40,330  $\mu\text{g}/\text{kg}$  for sum PAHs. It was also found that the unripe food samples whether they were raw or roasted had lower concentrations of sum PAHs than the ripe food samples. Raw unripe plantain had a concentration of 190  $\mu\text{g}/\text{kg}$  of sum PAHs while the ripe raw plantain had a concentration of 350  $\mu\text{g}/\text{kg}$ . Similar trend was also observed in the roasted unripe plantain, sum PAHs in roasted unripe plantain was found to be 10,280  $\mu\text{g}/\text{kg}$  while the roasted ripe plantain had a concentration of 40,330  $\mu\text{g}/\text{kg}$ . Individual concentrations of the carcinogenic PAHs (benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene,

and indeno(1,2,3-c,d)pyrene) ranged from non-detectable in several of the food samples to 4,530 µg/kg in roasted ripe plantain.

**Table 4. 11: Concentration (µg/kg) of PAHs in Raw and Roasted foods**

	<b>PAHs</b>	<b>ROUP</b>	<b>RAUP</b>	<b>ROC</b>	<b>RAC</b>	<b>RORP</b>	<b>RARP</b>	<b>ROY</b>	<b>RAY</b>
1	Naphthalene	ND	ND	2730	2630	4490	ND	620	ND
2	Acenaphthylene	500	ND	1700	ND	3010	ND	ND	ND
3	Acenaphthene	ND	ND	5120	ND	1700	ND	ND	ND
4	Fluorene	780	ND	850	ND	4940	ND	1510	120
5	Phenanthrene	260	ND	160	ND	2250	140	140	ND
6	Anthracene	660	110	ND	ND	7560	50	350	80
7	Fluoranthene	670	ND	610	ND	5550	ND	ND	ND
8	Pyrene	650	ND	350	ND	1340	110	450	130
9	Benzo(a)anthracene	610	ND	70	ND	4530	500	160	ND
10	Chrysene	610	ND	50	ND	2770	ND	70	ND
11	Benzo(b)fluoranthene	1310	80	370	ND	1100	ND	310	0.20
12	Benzo(k)fluoranthene	670	ND	190	ND	ND	ND	ND	ND
13	Benzo(a)pyrene	ND	ND	90	ND	ND	ND	ND	ND
14	Dibenz(a,h)anthracene	2400	ND	1560	540	540	ND	ND	ND
15	Benzo(g,h,i)perylene	560	ND	ND	ND	ND	ND	ND	ND
16	Indeno(1,2,3,c)pyrene	600	ND	ND	ND	550	ND	ND	ND
	<b>SumPAHs</b>	<b>10280</b>	<b>190</b>	<b>13850</b>	<b>3170</b>	<b>40330</b>	<b>350</b>	<b>3620</b>	<b>530</b>

Key: ROUP - Roasted unripe plantain, RAUP - Raw unripe plantain, ROC - Roasted corn, RAC - Raw Corn, RORP – Roasted ripe plantain, RARP – Raw ripe plantain, ROY – Roasted yam, RAY – Raw yam. Limit of quantitation is 10 µg/kg.

#### 4.2.3 Risk Assessment of PAHs in Roasted Foods

There is no limit yet for PAHs in foods such as plantain, maize and yam but the result showed that BaP, a bio-indicator, was not detected in the raw and roasted samples except in the roasted maize. BaP is the most studied carcinogenic polycyclic aromatic hydrocarbon and one of the most potent and it is often used as a toxicological prototype

or surrogate for all carcinogenic polycyclic aromatic hydrocarbons (Collins *et al.*, 1991). The EU has established a maximum permissible level of 5µg/kg for BaP in smoked meat and smoked meat products although a legal limit of 1 µg/ kg had previously been adopted by some European countries. The EU has also set a maximum limit for BaP present in foodstuffs as a result of the use of smoking-flavour agents at 0.03 µg/kg (Lorenzo *et al.*, 2011).

Out of all the known potentially carcinogenic PAHs, BaP is the best characterized, most potent of the carcinogenic PAH compounds and is the only PAH for which its toxicological potency factor labelled (Huang *et al.*, 2005, NDEP, 2009). The carcinogenic potency approach for calculating the potential health risks for PAHs with the characteristic “bay-K region,” a structural distinction that confers carcinogenic properties to BaP and the other carcinogenic PAHs was employed. Hence, the toxicities of other PAHs are determined in relation to BaP, expressed as TEF was used to determine BaP equivalent dose (BaP<sub>eq</sub>). The sum BaP<sub>eq</sub> dose for each roasted food was also calculated using the concentrations of PAHs found in the sample (Table 4.11) and the BaP<sub>eq</sub> is as shown in Table 4.12. Though BaP was not detected in the raw and roasted samples except in the roasted maize, the sum BaP<sub>eq</sub> in the samples had values between 6 and 2740 µg/kg. The BaP<sub>eq</sub> showed that more risk is associated with roasted food compared with raw foods.

**Table 4.12: BaP<sub>eq</sub> concentration (µg/kg) and Sum BaP<sub>eq</sub> Concentration (µg/kg) for the PAHs in Roasted and Raw foods.**

<b>Compound</b>	<b>ROUP</b>	<b>RAUP</b>	<b>ROC</b>	<b>RAC</b>	<b>RORP</b>	<b>RARP</b>	<b>ROY</b>	<b>RAY</b>
Naphthalene	0.0	0.0	3.0	3.0	4.0	0.0	1.0	0.0
Acenaphthylene	1.0	0.0	2.0	0.0	3.0	0.0	0.0	0.0
Acenaphthene	0.0	0.0	5.0	0.0	2.0	0.0	0.0	0.0
Fluorene	1.0	0.0	1.0	0.0	5.0	0.0	2.0	0.0
Phenanthrene	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Anthracene	7.0	1.1	0.0	0.0	76.0	1.0	4.0	1.0
Fluoranthene	1.0	0.0	1.0	0.0	6.0	0.0	0.0	0.0
Pyrene	1.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0
Benzo(a)anthracene	61.0	0.0	0.007	0.0	453	5.0	16.0	0.0
Chrysene	6.0	0.0	1.0	0.0	28.0	0.0	1.0	0.0
Benzo(b)Fluoranthene	131	8.0	37.0	0.0	110	0.0	31.0	20.0
Benzo(k)Fluoranthene	67.0	0.0	19.0	0.0	0.0	0.0	0.0	0.0
Benzo(a)pyrene	0.0	0.0	90.0	0.0	0.0	0.0	0.0	0.0
Ideno(1,2,3-c-d)pyrene	2400	0.0	1560	540	540	0.0	0.0	0.0
Dibenzo(a,h)anthracene	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Benzo(g,h,i)perylene	60	0.0	0.0	0.0	55.0	0.0	0.0	0.0
<b>Sum BaP<sub>eq</sub> dose</b>	<b>2740</b>	<b>9.0</b>	<b>1725</b>	<b>543</b>	<b>1285</b>	<b>6.0</b>	<b>54.0</b>	<b>21.0</b>

Key: ROUP - Roasted unripe plantain, RAUP - Raw unripe plantain, ROC - Roasted corn, RAC - Raw Corn, RORP – Roasted ripe plantain, RARP – Raw ripe plantain, ROY – Roasted yam, RAY – Raw yam. A BaP of 0.0 means the concentration of the PAHs was < 10 µg/kg.

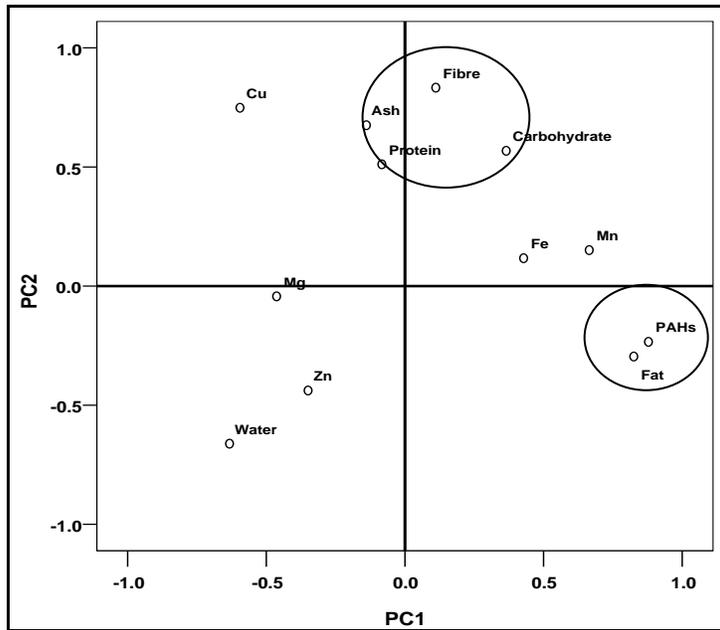
#### **4.2.4 Chemometric Study of Relationships Between PAHs in Roasted Foods and Their Nutritional Properties.**

Chemometrics is the use of mathematical and statistical multivariate analytical tools for data analysis. To understand the relationship between the various data from this study, chemometry was used. The data were subjected to principal component analysis (PCA) (15.0 software package) to elucidate the correlation between the various parameters in both the raw and roasted food samples. Eigen values (Table 4.13) were used to

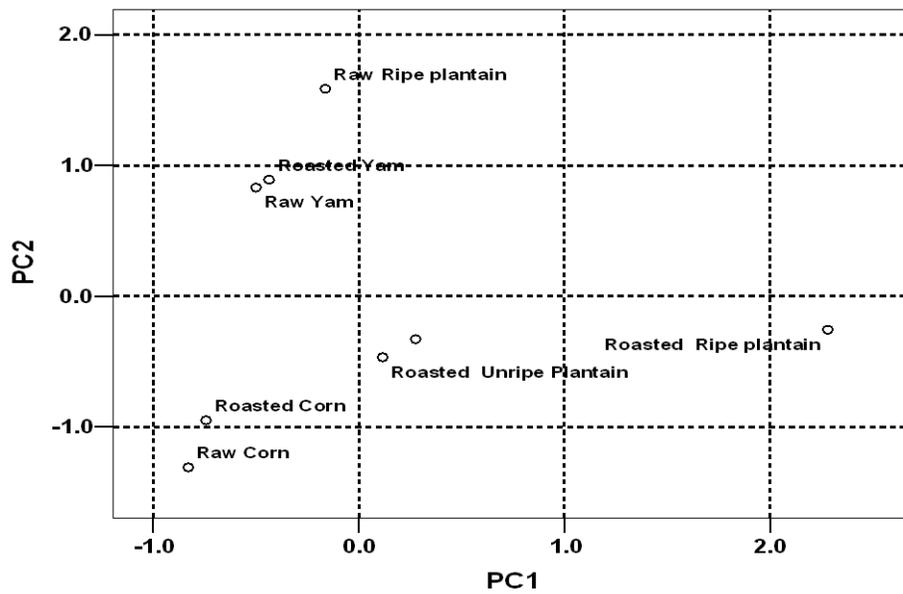
determine the number of principal components that should be retained for further study. For both raw and roasted food samples, the first two principal components had eigen values greater than 1 which explained 54% of the variance. PC1 accounts for about 28% of the variation in the data while PC2 accounts for 28% of the variation. The loadings for PC1 suggest that it is correlated with sum of the PAHs and percentage crude fat content in the roasted food samples, but to a lesser degree, the concentrations of iron and manganese. PC2 was found to be strongly correlated with percentage fibre, ash, protein, carbohydrate and copper. The rest of the PCs do not describe as much variation in the data analysis. This is clearly illustrated in Figure 4.1. The score plot (Figure 4.2), shows that roasted ripe plantain has a high correlation with PC1 which in turn is correlated with high sum PAHs and percentage fat content. The high fat content of roasted ripe plantain (19.56%) probably suggests the increase in the amount of sum PAHs that can be absorbed by the food sample on roasting.

**Table 4.13: Eigen Values of Factor for Proximate Composition, Macronutrients and Sum PAHs in the Food Samples**

Component	Initial Eigen values		
	Sum	% of variance	Cumulative %
1	3.341	27.841	27.841
2	3.104	25.863	53.704



**Figure 4.1: Plots of loadings of proximate composition, macronutrients and sum PAHs in the food samples**



**Figure 4.2: Plots of scores for proximate composition, macronutrients and sum PAHs in the food samples.**

### **4.3 OPTIMIZATION AND METHOD DEVELOPMENT FOR EXTRACTION, CLEAN UP AND ANALYSIS OF PAHS BY GC-MS.**

#### **4.3.1 Optimization of the GC-MS for Analysis of PAHs.**

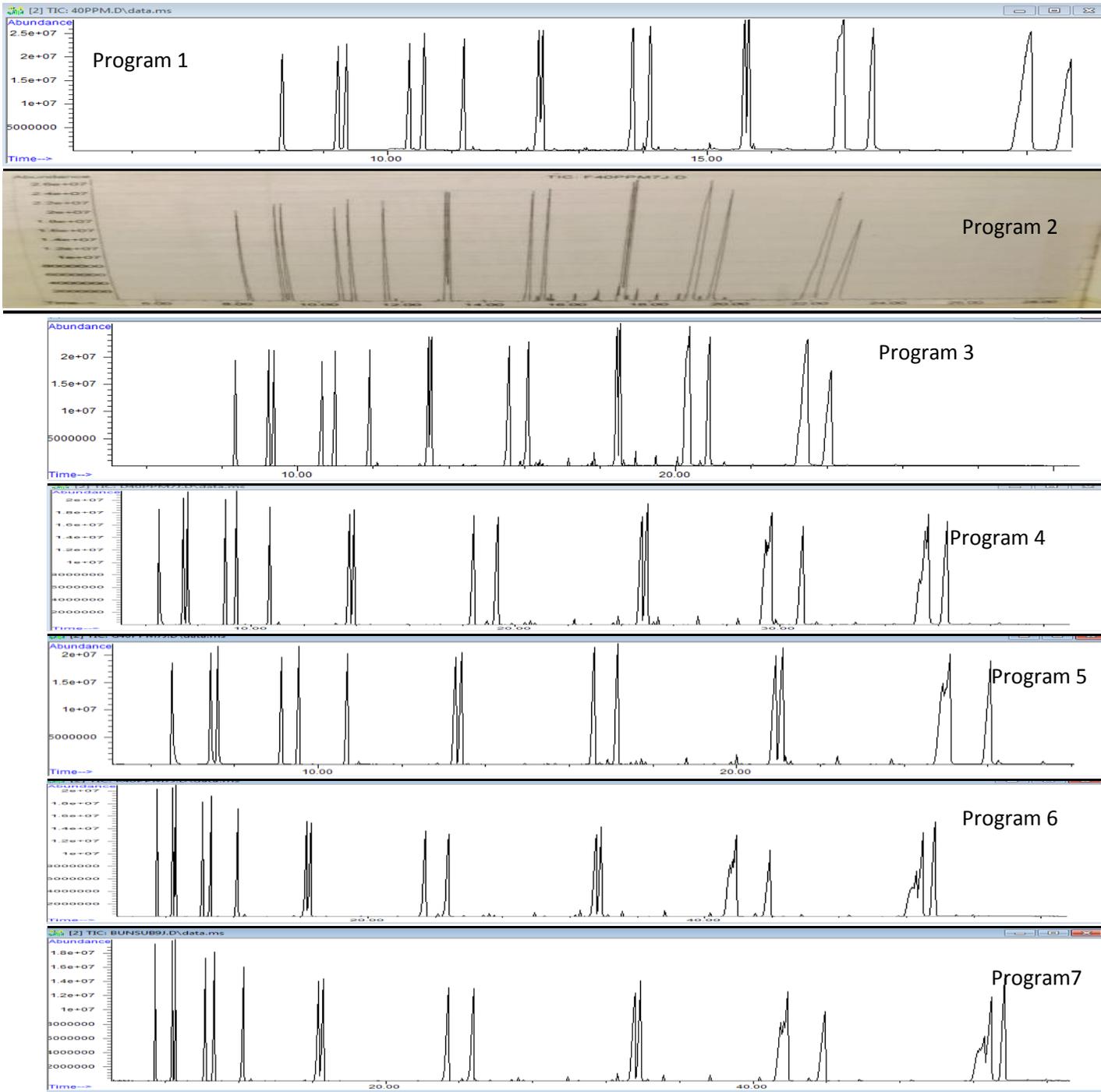
To carry out temperature programming optimisation for the GC method, PAH standard mix Supelco 47543-U (16 USEPA + 2 methylated PAHs ) was diluted to 40 µg/ml from 2000 µg/ml and scanned on different runs on the GC-MS (HP6890N/5973MSD) using the different temperature ramps outlined in Table 4.14. Figure 4.3 shows the derived chromatograms from the various runs from varying temperature programs. The chromatogram obtained from program 1 showed 16 of the 18 peaks expected with the 16<sup>th</sup> peak not completely resolved. The chromatogram of the second program was similar to the first but its 16<sup>th</sup> peak was completely resolved. The resolution of the chromatogram from the third program was similar to the second but poorer in program 4. Temperature ramp was slower and resolution of the chromatogram was gradually improved. Splitting of 2 peaks began to occur and 16 peaks with two partially splitted peaks were observed. The chromatogram obtained from the fifth program, was worse. Program six and seven were carried out with the aim of increasing the resolution and program seven was chosen as the optimum program in the scan mode and the ion chromatograms. Program 6 and 7 are similar except that the 320°C was removed in the latter to save the column as 320 °C was near its temperature limit (325 °C at isothermal and 350 °C in temperature programme) and (since at from calculation, the retention time at which the column is 320 °C shows nothing was eluting). The ion chromatograms

(Figure 4.4 and 4.5) and ion spectrum (Figure 4.6) were inspected and the m/z of abundant ions were noted.

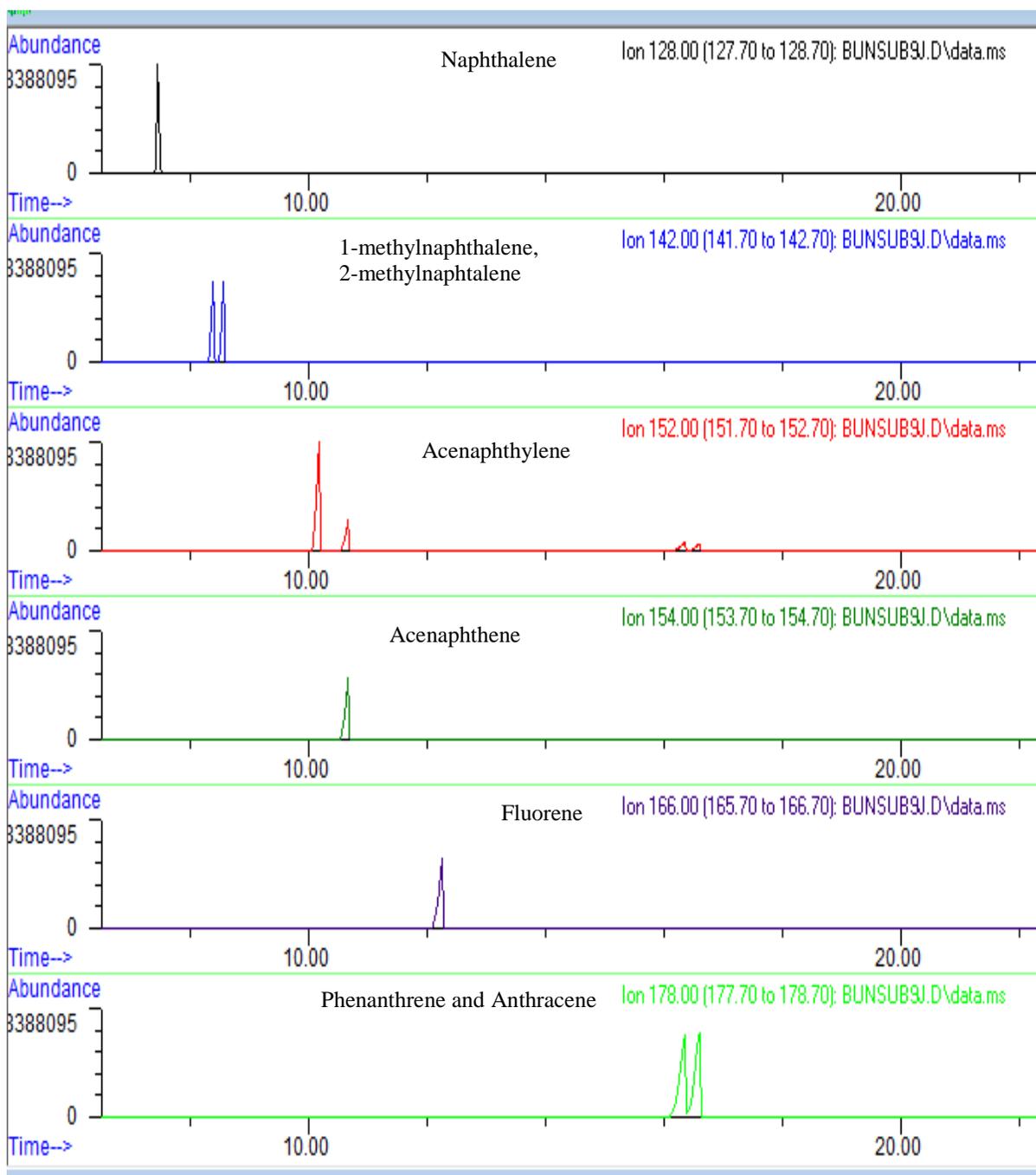
**Table 4.14: GC-MS Temperature Programs**

<b>Program 1</b>				
<b>Ramp</b>	<b>Ramp Rate °C/mins</b>	<b>Temp °C</b>	<b>Hold (mins)</b>	<b>Run Time (mins)</b>
Initial		50.00	3.20	3.20
Ramp 1	20.00	300.00	5.00	20.70
Ramp 2	0.00			
<b>Program 2</b>				
<b>Ramp</b>	<b>Ramp Rate °C/mins</b>	<b>Temp °C</b>	<b>Hold (mins)</b>	<b>Run Time (mins)</b>
Initial		50.00	3.20	3.20
Ramp 1	20.00	168.00	1.00	10.10
Ramp 2	20.00	231.00	1.00	14.25
Ramp 3	20.00	294.00	1.00	18.40
Ramp 4	70.00	300.00	10	28.70
<b>Program 3</b>				
<b>Ramp</b>	<b>Ramp Rate °C/mins</b>	<b>Temp °C</b>	<b>Hold (mins)</b>	<b>Run Time (mins)</b>
Initial		50.00	3.20	3.20
Ramp 1	20.00	168.00	1.50	10.60
Ramp 2	20.00	231.00	2.00	15.75
Ramp 3	20.00	294.00	1.50	20.40
Ramp 4	70.00	300.00	10.00	30.70
<b>Program 4</b>				
<b>Ramp</b>	<b>Ramp Rate °C/mins</b>	<b>Temp °C</b>	<b>Hold (mins)</b>	<b>Run Time (mins)</b>
Initial		70.00	3.20	3.20
Ramp 1	30.00	150.00	0.00	5.87
Ramp 2	5.00	296.00	0.00	35.07
Ramp 3	70.00	300.00	5.00	40.12
	0.00			
<b>Program 5</b>				
<b>Ramp</b>	<b>Ramp Rate °C/mins</b>	<b>Temp °C</b>	<b>Hold (mins)</b>	<b>Run Time (mins)</b>
Initial		70.00	3.20	3.20
Ramp 1	30.00	150.00	0.00	5.87
Ramp 2	5.00	157.00	1.00	8.27
Ramp 3	10.00	185.00	1.00	12.07

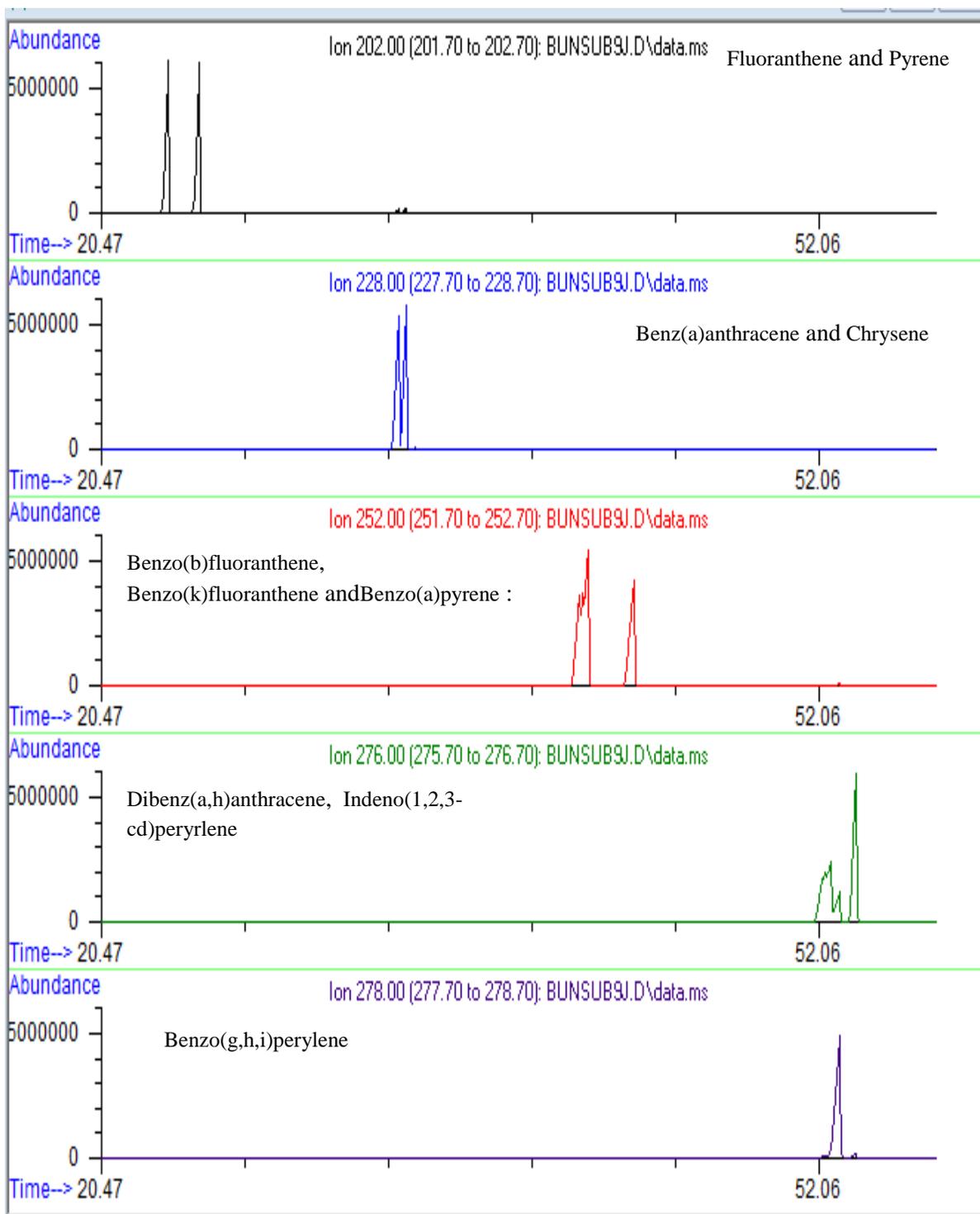
Ramp 4	10.00	240.00	1.00	18.57
Ramp 5	5.00	265.00	1.00	24.57
Ramp 6	10.00	290.00	1.00	28.07
Post		300	8.00	36.07
<b>Program 6</b>				
<b>Ramp</b>	<b>Ramp Rate °C/mins</b>	<b>Temp °C</b>	<b>Hold (mins)</b>	<b>Run Time (mins)</b>
Initial		50.00	3.20	3.20
Ramp 1	30.00	150.00	0.00	6.53
Ramp 2	3.00	238.00	0.00	35.87
Ramp 3	2.00	272.00	0.00	52.87
Ramp 4	70.00	300.00	2.73	56.00
Ramp 5	70.00	320.00	0.00	56.28
Ramp 6	70.00	300.00	5.00	61.57
<b>Program 7</b>				
<b>Ramp</b>	<b>Ramp Rate °C/mins</b>	<b>Temp °C</b>	<b>Hold (mins)</b>	<b>Run Time (mins)</b>
Initial		50.00	3.20	3.20
Ramp 1	30.00	150.00	0.00	6.53
Ramp 2	3.00	238.00	0.00	35.87
Ramp 3	2.00	272.00	0.00	52.87
Ramp 4	70.00	300.00	2.73	56.00
Post	0.00	315.00	1.47	



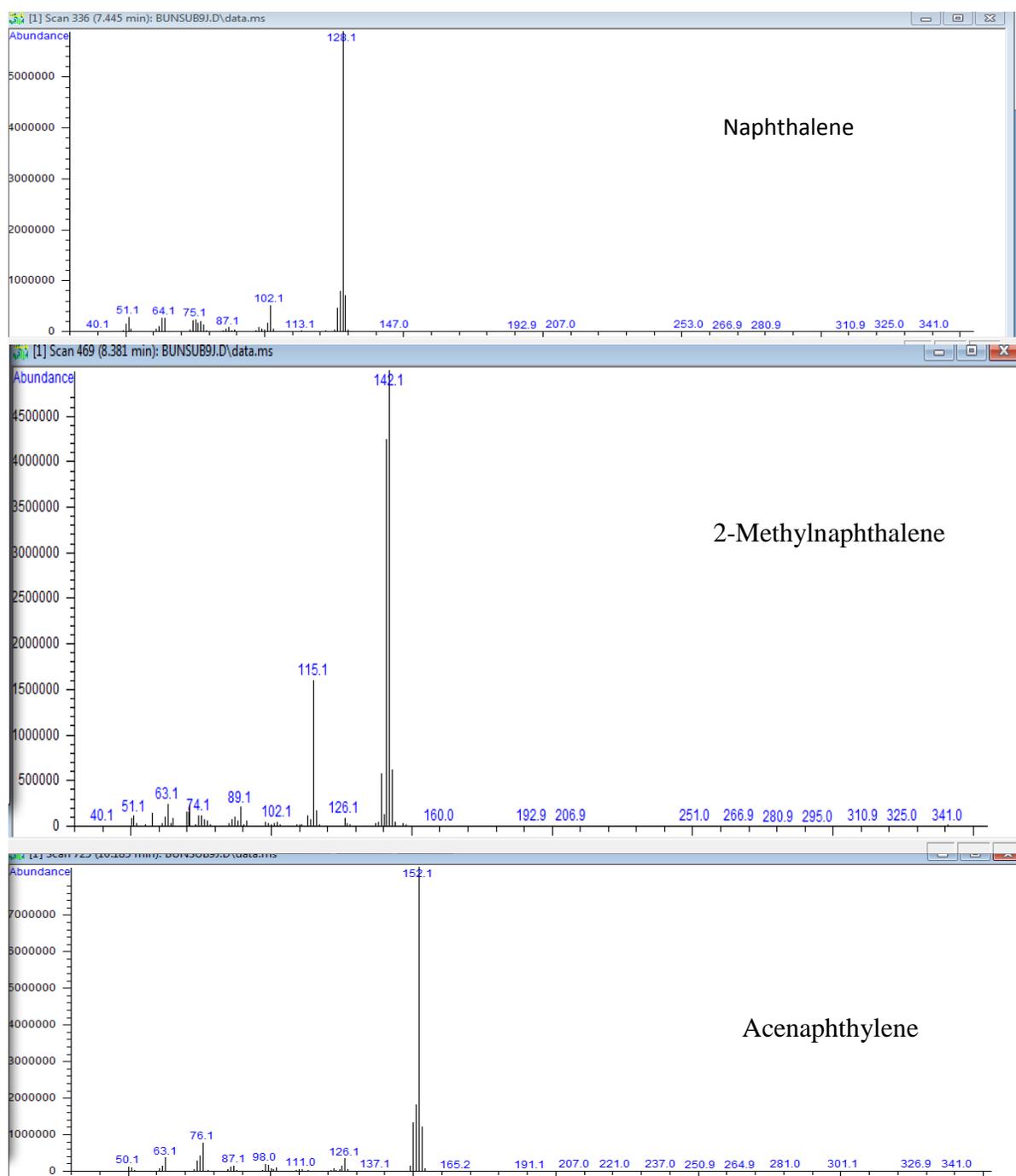
**Figure 4. 3: Chromatograms from the Various Programs**



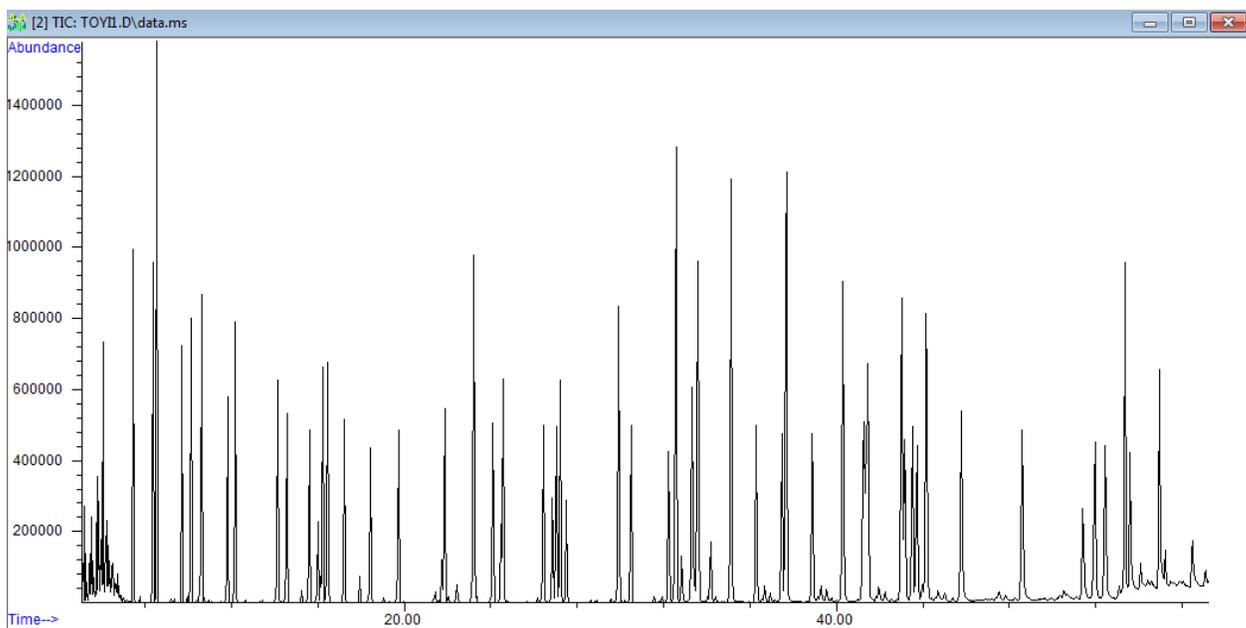
**Figure 4. 4:** Ion chromatogram from program 7 for Naphthalene, 1-Methylnaphthalene, 2-Methyl naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene and Fluoranthene



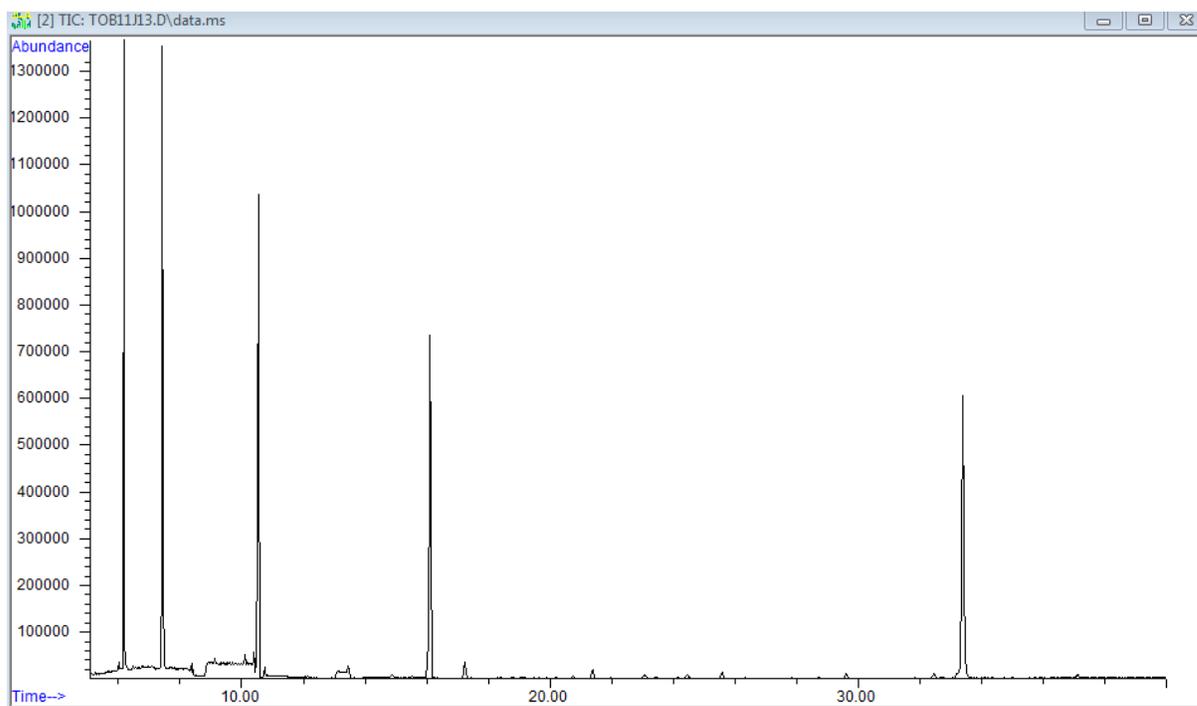
**Figure 4. 5: Ion Chromatogram from Program 7 Fluoranthene, Pyrene, Benz(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Indeno(1,2,3-cd)perylene, Dibenz(a,h)anthracene, Benzo(g,h,i)perylene**



**Figure 4.6: Some Spectrum from Program 7's Chromatogram used to Identify Target Ions and Qualitative Ions for Single Ion Monitoring (SIM) Mode Set-up.**



**Figure 4.7: 2µl/ml Mix of All Three Standard (uPAHs, dPAHs and RPAHs) in Scan Mode**



**Figure 4.8: 2ppm snapshot dPAHs in scan mode**

Applying program seven, GC runs of dPAHs, RPAHs and mixture of the three standards (dPAHs, RPAHs, and uPAHs) were carried out. From the different chromatograms (for example Figure 4.7 and 4.8) and spectrum (example of such spectrum is as in Figure 4.6) the target ions (in most cases molecular ion) and identification (qualifying ions) were identified as in Tables 4.15, 4.16, 4.17 and 4.18 for individual compound based on their abundance and properties and were used in setting up the single ion monitoring (SIM) mode of operation.

**Table 4.15: Observed Retention Time, Target Ion and Identifying ions of Compound in Scan Mode Used to Set up the SIM Mode for uPAHs**

S/N	Retention Time (mins)	Compound	M <sup>+</sup>	Other target ions (abundant ions) in Spectra
1	7.529	Naphthalene	128	126,127
2	8.38	2-Methylnaphthalene	142	141,115
3	8.550	1-Methylnaphthalene	142	141,115
4	10.163	Acenaphthylene	152	150, 76
5	10.586	Acenaphthene	153	154, 152
6	12.227	Fluorene	166	165,163
7	16.326	Phenanthrene	178	176, 152
8	16.615	Anthracene	178	176, 152
9	23.448	Fluoranthene	202	200, 201
10	24.539	Pyrene	202	200, 201
11	33.795	Benzo(a)Anthracene	228	226,113
12	33.844	Chrysene	228	226,113
13	41.20	Benzo(b)Fluoranthene	252	126, 250
14	41.895	Benzo(k)Fluoranthene	252	126, 250
15	43.833	Benzo(a)pyrene	252	126,250
16	52.04	Ideno(1,2,3-c-d)pyrene	276	274,138
17	53.09	Dibenzo(a,h)anthracene	278	276,139
18	53.676	Benzo(g,h,i)perylene	276	126, 250

**Table 4.16: Observed Retention Time, Target Ion and Identifying Ions of Compound in Scan Mode Used to Set up the SIM Mode for RPAHs**

S/N	Retention Time (mins)	Compound	M <sup>+</sup>	Other target ions (abundant ions) in Spectra
1	8.38	2-Methylnaphthalene	142	141,115,
2	8.56	1,3-Dimethylnaphthalene	156	141,115,
3	11.54	2,3,5-Trimethylnaphthalene	170	155,169,153
4	14.05	1,4,6,7, Tetramethylnaphthalene	184	183,169
5	14.601	1-methylfluorene	180	165,179
6	15.5	Dibenzothiophene	184	139,183
7	15.930	9-n-propylfluorene	208	165,179
8	17.179	1,7-Dimethylfluorene	194	179,178
9	18.47	2-Methyldibenzothiophene	198	197
10	19.651	1-Methylphenanthrene	192	191,189
11	21.91	3,6-Dimethylphenanthrene	206	191,189
12	23.80	1,2-Dimethyldibenzothiophene	212	197
13	24.138	2,4,7-Trimethyldibenzothiophene	226	211
14	26.490	2-Methylfluoranthene	216	215, 108
15	27.131	1,2,6-Trimethylphenanthrene	220	205,189
16	30.540	1,2,6,9-Tetramethylphenanthrene	234	219,204
17	34.640	Triphenylene+ Teracene	228	113,226
18	37.56	1-Methylchrysene	242	241,239
19	38.831	6-Ethylchrysene	256	241,239
20	43.60	6-n-Butylchrysene	284	126,250
21	43.670	Benzo(e)pyrene	252	241,239
22	44.244	Benzo(j)Fluoranthene +1,3,6 – TrimethylChrysene	270 + 252	250,126
23	44.360	Perylene,	270	252,250

**Table 4.17: Observed retention time, target ion and identifying ions of compound in scan mode used to set up the SIM mode for dPAHs.**

S/N	Retention Time (mins)	Compound	M <sup>+</sup>	Other target ions (abundant ions) in Spectra (Confirmatory ion)
1	6.17	1,4-dibenzobenzene-D4	150	152,115
2	7.20	Naphthalene-D8	136	128,108
3	10.50	Acenaphthene-D10	164	162,160
4	16.17	Phenanthrene-D10	188	94,
5	33.10	Chrysene-D12	240	120,236
6	44.311	Perylene-D12	264	260,265

**Table 4.18: Observed Retention Time, Target Ion and Identifying Ions of Compound in Scan Mode Generated from Running Mixes of UPAHs, RPAHs and UPAHs and used to set up the SIM Mode for all Three Types of PAHs in this Study.**

S/N	Retention Time (mins)	Compound	M <sup>+</sup>	Other target ions (abundant ions) in Spectra
1	6.17	1,4-dibenzobenzene-D4	150	152,115
2	7.35	Naphthalene-D8	136	,108, 68
3	7.529	Naphthalene	128	64, 108
4	8.38	2-Methylnaphthalene	142	141,115,
5	8.56	1-Methylnaphthalene	142	141,115,
6	9.65	1,3-Dimethylnaphthalene	156	141,115
7	10.073	Acenaphthylene	152	150, 76
8	10.480	Acenaphthene-D10	164	162,160
9	10.593	Acenaphthene	153	154, 152
10	11.54	2,3,5-Trimethylnaphthalene	170	155,169,153
11	12.129	Fluorene	166	165,163
12	14.05	1,4,6,7-Tetramethylnaphthalene	184	183,169
13	14.2	1-methylfluorene	180	165,179
14	15.5	Dibenzothiophene	184	139,183
15	15.930	9-n-propylfluorene	208	165,179
16	16.17	Phenanthrene-D10	188	94,178
17	16.30	Phenanthrene	178	176, 152
18	16.51	Anthracene	178	176, 152
19	17.272	1,7-Dimethylfluorene	194	179,178
20	18.468	2-Methyldibenzothiophene	198	197

21	19.70	1-Methylphenanthrene	192	191,189
22	21.968	3,6-Dimethylphenanthrene	206	191,189
23	23.80	1,2-Dimethyldibenzothiophene	212	197
24	23.145	Fluoranthene	202	200, 101
25	24.138	2,4,7-Trimethyldibenzothiophene	226	211
26	24.603	Pyrene	202	200, 101
25	26.490	2-Methylfluoranthene	216	215, 108
26	27.096	1,2,6-Trimethylphenanthrene	220	205,189
27	30.540	1,2,6,9-Tetramethylphenanthrene	234	219,204
28	33.414	Benzo(a)anthracene	228	226,113
29	33.527	Chrysene-D12	240	236,120
30	34.640	Triphenylene+ Tetracene	228	113,226
31	33.844	Chrysene	228	226,113
32	37.56	1-Methylchrysene	242	241,239
33	38.831	6-Ethylchrysene	256	241,239
34	41.20	Benzo(b)Fluoranthene	252	126, 250
35	41.409	Benzo(k)Fluoranthene	252	126, 250
36	43.60	6-n-Butylchrysene	284	241,239
37	43.68	Benzo(e)pyrene	252	250,125
38	44.29,	Benzo(a)pyrene	252	126,250
39	44.311	Perylene-D12	264	260,
40	44.360	Perylene	252	126,250
41	44.207	Benzo(j)Fluoranthene +1,3,6 – TrimethylChrysene	270	252,250
42	52.101	Ideno(1,2,3-c-d)pyrene	276	274,138
43	53.594	Dibenzo(a,h)anthracene	278	276,139
44	53.432	Benzo(g,h,i)perylene	276	274,138

Target ions and identifier ions identified from the spectrum of peaks in chromatograms obtained from running dPAH, RPAHs and dPAHs singularly and in mixtures as in Tables 4.15, 4.16, 4.17 and 4.18 were grouped. Grouped ions are as in Tables 4.19, 4.20 and 4.21. Three SIM modes were set up. The first to SIM was set to acquire data for uPAHs and dPAHs. The second was set to acquired data for all the PAHs (dPAHs, RPAH and UPAH) and a third SIM was set to acquire data for uPAHs and dPAHs only.

**Table 4. 19: SIM to Parameters Used to Quantify Unsubstituted PAHs (USEPA 16 priority PAHs and Internal Standard (dPAHs) was Set up**

<b>Group</b>	<b>Ion</b>	<b>Dwell time (msec)</b>	<b>Start time (mins)</b>
1	102,115,128,136,150,152	50	0.00
2	150,152,153,164	50	8.00
3	94,163, 165,166,176,178, 188	50	12.00
4	101,200,202	50	22.00
5	113,120,226,228,236,240	50	31.50
6	126,250,252,260,264,265	50	40.00
7	138,274,276,278	50	50.00

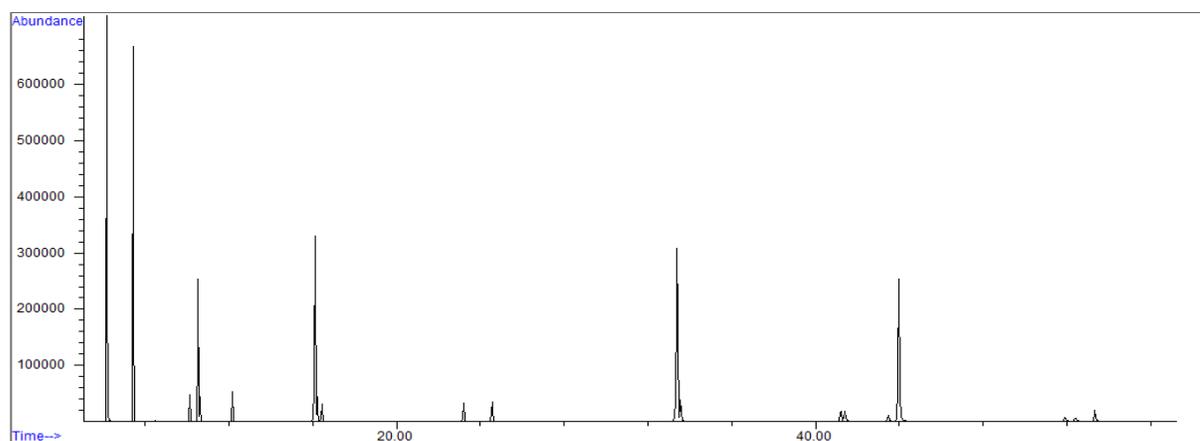
**Table 4.20: SIM Parameters Used to Acquire ions for Quantification of the Three types of PAHs (dPAHs, RPAHs and uPAHs)**

<b>Group</b>	<b>Ion</b>	<b>Dwell time(msec)</b>	<b>Start time(mins)</b>
1	108,128,136,150	50	0.00
2	141,142,150,152,153,154,156,164, 166,170	50	8.00
3	178,180,184,188,192,194,198,206, 208,	50	13.50
4	101,200,202,212,216,220,226	50	21.50
5	120,226,228,234,240,242,256	50	31.50
6	126,250,252,260,264,265,270,280	50	40.00
7	138,274,276,278	50	50.00

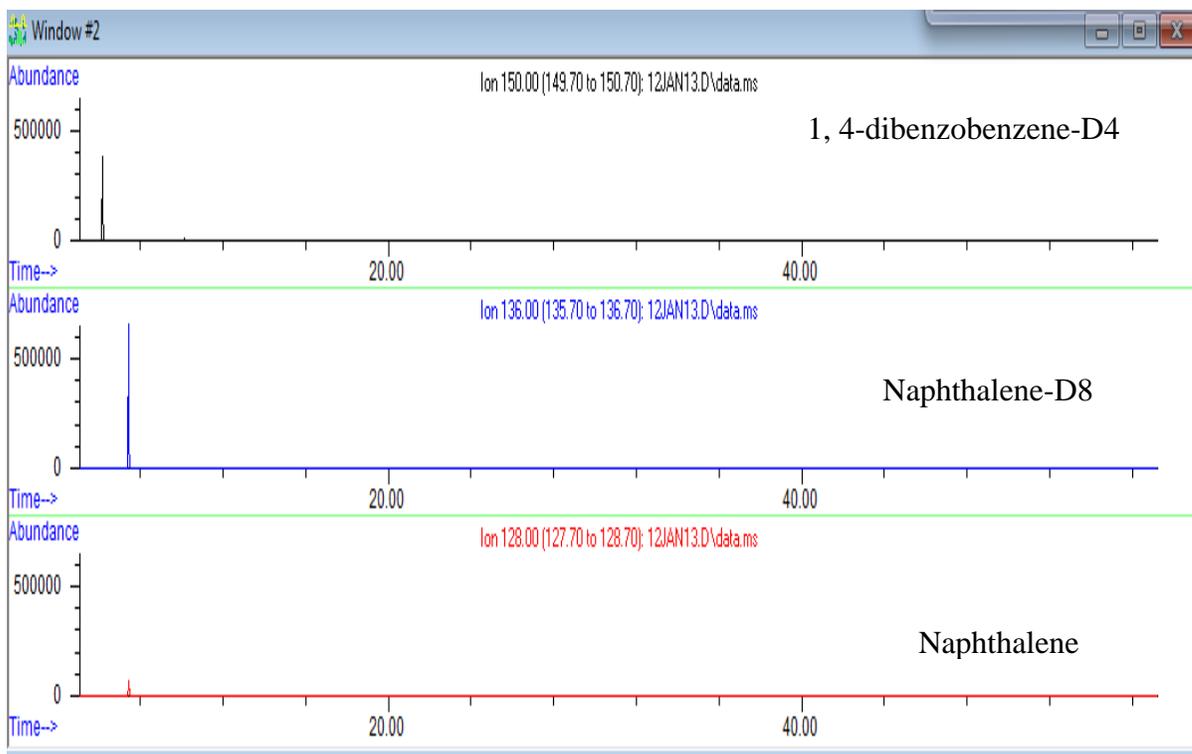
**Table 4. 21: SIM Parameters Used to Acquire ions for Quantification of Only dPAHs and RPAHs**

Group	Ion	Dwell time (msec)	Start time(mins)
1	108,136,150	50	0.00
2	141,142,150,156,164, 170	50	8.00
3	180,184,188,192,194,198,206, 208,	50	13.50
4	101,200,212,216,220,226	50	21.50
5	120,226,234,240,242,256	50	31.50
6	126,250,252, 260,264,265,270,280	50	40.00

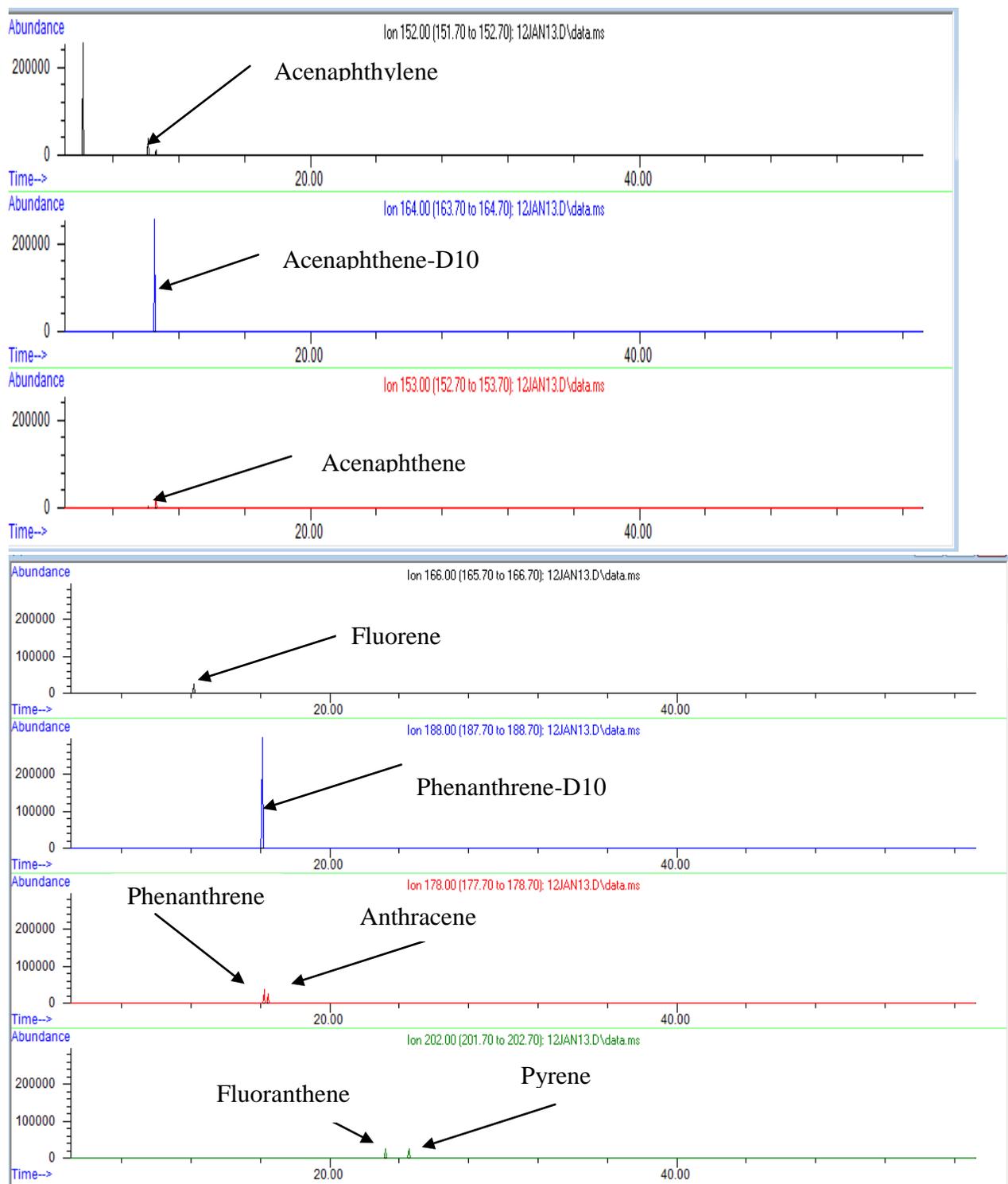
The resulting Chromatograms from SIM run were more selective and sensitive compared with the earlier runs in scan mode as in Figure 4.3 to 4.4 (for higher conc). An example of the enhanced selectivity and sensitivity is as seen in Figure 4.9, Figure 4.14 and the ion Chromatograms (Figures 4.10, 4.11, 4.12 and 4.13).



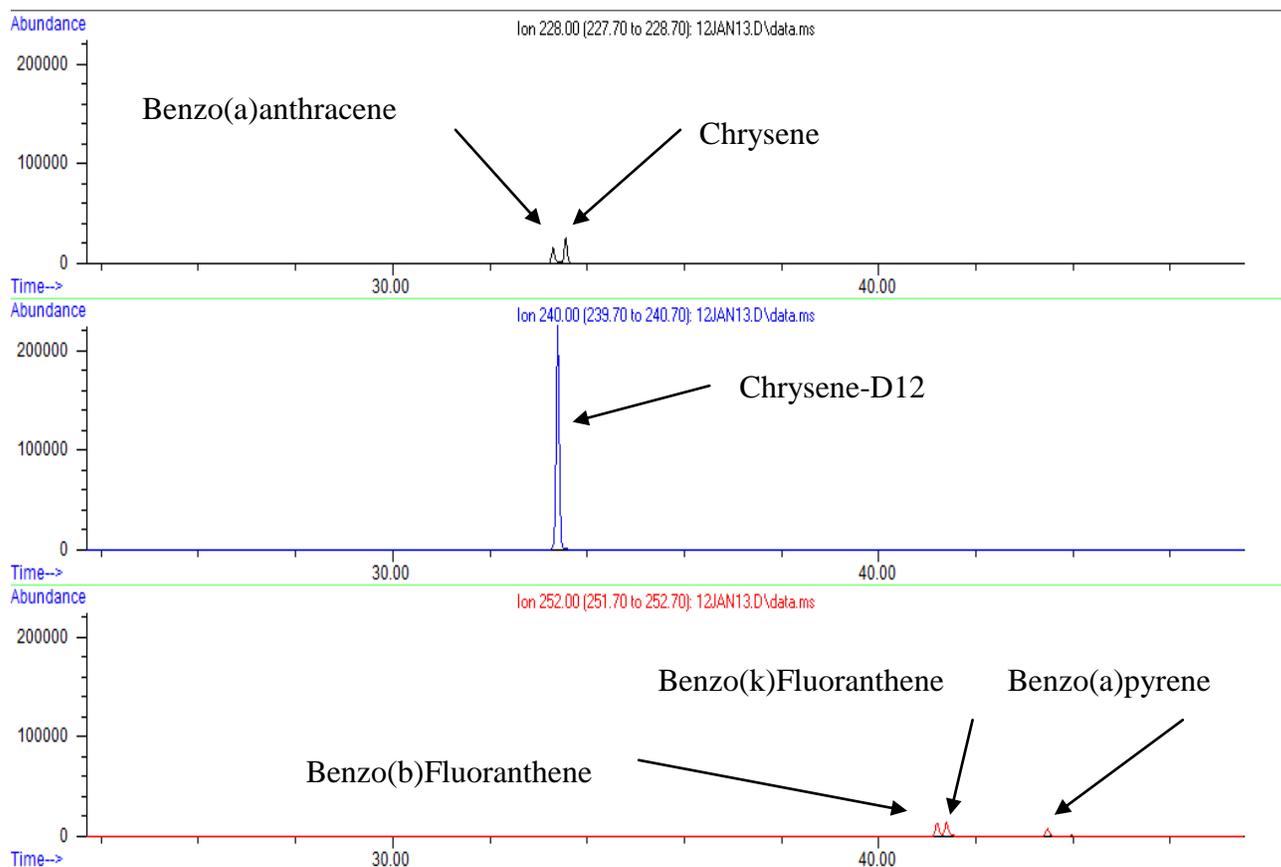
**Figure 4.9: A Chromatogram of uPAHs (1ppm) and dPAH (10ppm) from SIM run**



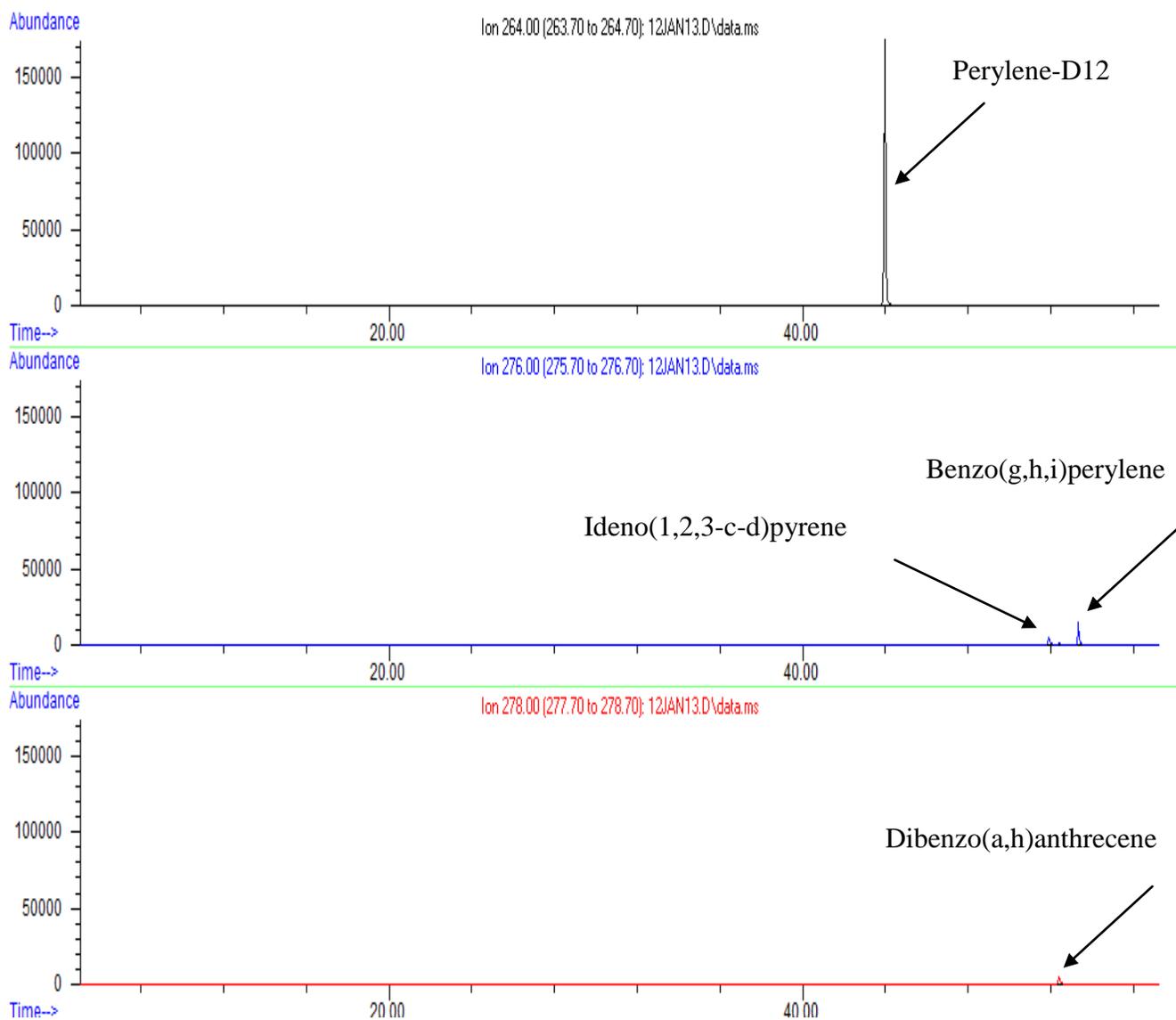
**Figure 4.10: Ion Chromatogram of Figure 4.8 for 1, 4-dibenzobenzene-D4, Naphthalene-D8, Naphthalene [A Chromatogram of uPAHs (1 ppm) and dPAH (10 ppm) from SIM run]**



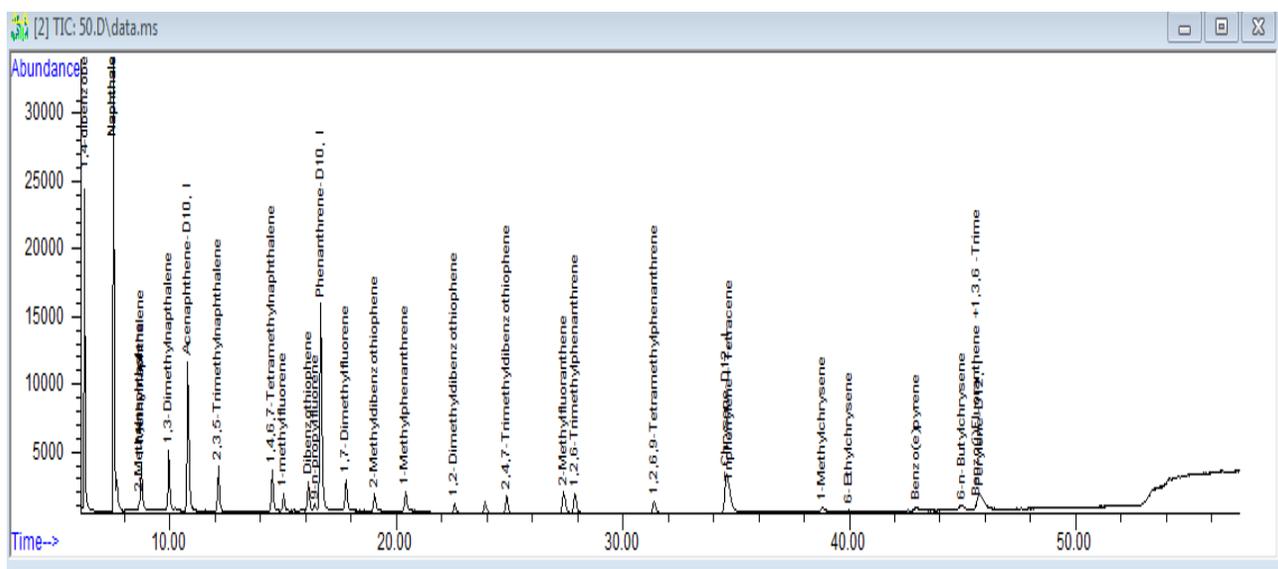
**Figure 4.11: Ion Chromatogram of Figure 4.8 for acenaphthylene, acenaphthene-d10, acenaphthene, fluorene, phenanthrene-d10, anthracene, phenanthrene, anthracene, fluoranthene and pyrene [Chromatogram of uPAHs and dPAH ( 1 ppm and 10 ppm respectively)from SIM run]**



**Figure 4.12: Ion Chromatogram of Figure 4.8 for Benzo(a)anthracene, Chrysene, Chrysene-D12, Benzo(b)Fluoranthene, Benzo(k)Fluoranthene and Benzo(a)pyrene [Chromatogram of uPAHs and dPAH (1 ppm and 10 ppm respectively)from SIM run]**



**Figure 4.13: Ion Chromatogram of Figure 4.8 for perylene-d12, benzo(g,h,i)perylene, ideno(1,2,3-c-d)pyrene and ideno(1,2,3-c-d)pyrene [Chromatogram of uPAHs and dPAH (1 ppm and 10 ppm respectively) from SIM run].**



**Figure 4.14: Representative chromatograms for RPAHs standard in SIM mode**

### 4.3.2 Calibration, Performance and Reproducibility of GC – MS

Based on the optimised SIM programs, linearity range for each PAH was determined by preparing standard solutions for 10 point calibration ranging from 0  $\mu\text{g/ml}$  (calibration blank) to 10,000  $\mu\text{g/ml}$  where every standard had dPAH mix of 250  $\mu\text{g/ml}$ . Plots of the response ratio against concentration ratio of uPAH : dPAH were drawn for each PAH and the linearity range was determined by drawing a line to point deviation from the straight line. Subsequent calibrations were done using 6 point calibration within the determined linearity range. The instrumental limits of detection (LODs) were calculated by a signal-to-noise ratio of 3 from the direct injection of the least concentrated PAH standard and a signal-to-noise ratio 10 for limit of quantisation (LOQ) (Ratola *et al.*, 2011, Oleszczuk and Baran, 2004, Shrivastava and Gupta, 2011) and the results are as shown in Table 4.22 and Table 4.23.

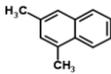
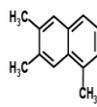
**Table 4.22: LOQ, LOD, Calibration Range and Equation for PAH Quantitation**

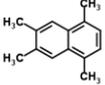
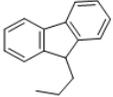
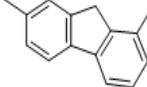
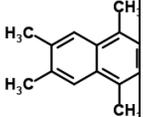
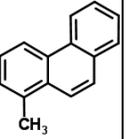
SN	Compound	MS target Ion for Quantitation (M <sup>+</sup> )	Group based on Retention Time and ion in Table 4.11	R <sup>2</sup> Value	Calibration Range ng/ml	LOQ ng/ml	LOD ng/ml	Calibration line equation (mx+C=y) x is response ratio and y conc ratio
1	NAP	128	Group 1(6mins)	0.995	0-5000	38.8	11.6	1.9051x-0.1569=y
2	ACY	152	Group 1 (6mins)	0.994	0- 5000	3.8	1.2	1.8371x-0.06028=y
3	ACP	153*	Group 2 (8mins)	0.995	0-4950	88.6	26.6	2.7729x-1.2049=y
4	FLR	166	Group 2 (8mins)	0.995	0-4980	44.4	13.3	8.7574x-3.7387=y
5	PHE	178	Group 3 (12mins)	0.995	0-4990	12.0	3.6	1.1554E1+2.2025=y
6	ANT	178	Group 3 (12mins)	0.996	0-5000	12.00	3.6	1.0142E1-0.4297=y
7	FLT	202	Group 4 (22mins)	0.995	0-4970	90.0	27.0	8.5591x-1.5146=y
8	PYR	202	Group 4 (22mins)	0.999	0- 4950	90.0	27.0	8.2281x-1.3204=y
9	BaA	228	Group5 (31.5mins)	0.996	0-4900	36.1	10.8	1.7149x-0.2471=y
10	CHR	228	Group5 (31.5mins)	0.999	0-5000	16.3	4.9	1.9085x-0.0803=y
11	BbF	252	Group6 (40mins)	0.996	0-4890	141.2	42.4	1.3997x-0.0986=y
12	BkF	252	Group6 (40mins)	0.997	0-4800	120.0	36.0	1.3062E3x-1.61219E5=y
13	BaP	252	Group6 (40mins)	0.995	0-4900	11 4.3	34.3	8.9807E2x-1.1181E5=y

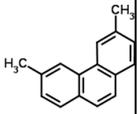
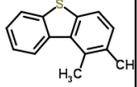
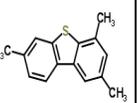
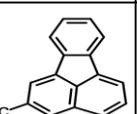
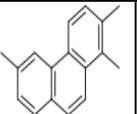
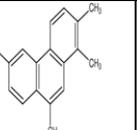
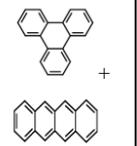
14	DaH	276	Group7 (50mins)	0.998	0-4890	200.0	60.0	0.8849x-0.0059=y
15	BgP	278	Group7 (50mins)	0.998	0-4800	187.6	s	0.6948x-0.2826=y
16	IcP	276	Group7 (50mins)	0.997	0-4900	50.0	15.0	1.4624x+0.0600=y

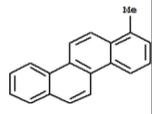
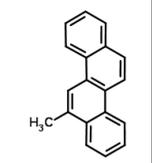
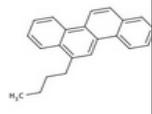
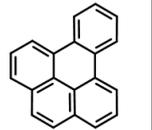
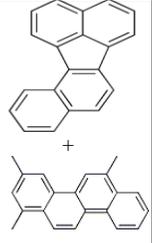
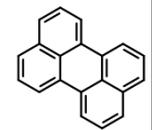
Naphthalene: NAP; Acenaphthylene: ACY; Acenaphthene: ACP; Fluorene: FLR; Phenanthrene: PHE; Anthracene: ANT ; Fluoranthene ; FLT ; Pyrene : PYR ; : CHR ; Benzo(b)fluoranthene : BbF ; Benzo(k)fluoranthene : BkF ; Benzo(a)pyrene : BaP ; Indeno(1,2,3-cd)perylene : IcP ; Dibenz(a,h)anthracene : DaH ; Benzo(g,h,i)perylene : BgP \*Except for Acenaphthene whose M<sup>+</sup> is 154 but had its most abundant ion as 153.

**Table 4.23 : LOQ, LOD, Calibration Range and Equation for RPAH Quantitation**

SN	PAH Structure	Empirical Formulae	Compound	MS target Ion for Quantitation (M <sup>+</sup> )	R <sup>2</sup> Value	Calibration Range ng/ml	LOQ ng/ml	LOD ng/ml	Calibration line equation (mx+C=y) x is response ratio and y conc ratio
1		C <sub>11</sub> H <sub>10</sub>	1-Methylnaphthalene	142 (Group 2, 8mins)	0.9984	0-5000	38.8	11.6	0.005830x - 0.001033 = y
2		C <sub>12</sub> H <sub>12</sub>	1,3-Dimethylnaphthalene	156 (Group 2, 8mins)	0.9981	0- 5000	3.8	1.2	0.6912x-2.214=y
3		C <sub>13</sub> H <sub>14</sub>	2,3,5-Trimethylnaphthalene	170 (Group 2, 8mins)	0.9948	0-5000	88.6	26.6	1.037x - 0.5750 = y

4		$C_{14}H_{16}$	1,4,6,7, Tetramethylnaphthalene	184(Group 3, 8mins)	0.995	0 - 5000	44.4	13.3	$1.242x - 0.6830 = y$
5		$C_{14}H_{12}$	1-methylfluorene	180 (Group 3, 8mins)	0.994	0-5000	12.0	3.6	$0.8985x - 0.5608 = y$
6		$C_{12}H_8S$	Dibenzothiophene	184 (Group 3, 8mins)	0.995	0-5000	12.00	3.6	$1.820x - 1.046 = y$
7		$C_{16}H_{16}$	9-n-propylfluorene	208 (Group 3, 8mins)	0.994	0-5000	90.0	27.0	$0.2446x - 0.1549 = y$
8		$C_{15}H_{14}$	1,7-Dimethylfluorene	194 (Group 3, 8mins)	0.994	0- 5000	90.0	27.0	$0.6039x - 0.3808 = y$
9		$C_{14}H_{16}$	2- Methyldibenzothiophen e	198 (Group 3, 8mins)	0.995	0-4900	36.1	10.8	$0.7635x - 0.4529 = y$
10		$C_{15}H_{12}$	1-Methylphenanthrene	192(Group 3, 8mins)	0.995	0-5000	16.3	4.9	$0.9066x - 0.989 = y$

11		$C_{16}H_{14}$	3,6-Dimethylphenanthrene	206 (Group 3, 8mins)	0.994	0-5000	141.2	42.4	$0.006740x - 0.005981 = y$
12		$C_{14}H_{12}S$	1,2-Dimethyldibenzothiophene	212 (Group 4, 21.5mins)	0.994	0-5000	120.0	36.0	$0.2245x - 0.0925 = y$
13		$C_{15}H_{14}S$	2,4,7-Trimethyldibenzothiophene	226 (Group 4, 21.5mins)	0.993	0-7000	114.3	4.3	$0.4649x - 0.3768 = y$
14		$C_{17}H_{12}$	2-Methylfluoranthene	216 (Group 4, 21.5mins)	0.993	0-5000	200.0	60.0	$0.9512x - 0.727 = y$
15		$C_{17}H_{16}$	1,2,6-Trimethylphenanthrene	220 (Group 4, 21.5mins)	0.992	200-4800	187.6	56.3	$0.777x - 0.6011 = y$
16		$C_{22}H_{12}$	1,2,6,9-Tetramethylphenanthrene	234 (Group 5, 30mins)	0.993	0-5000	181.1	54.8	$0.0197x + 0.0002 = y$
17		$C_{18}H_{12}$ + $C_{18}H_{12}$	Triphenylene+ Teracene	228 (Group 5, 30mins)	0.983	0-5000	92.00	27.62	$1.699x - 0.5518 = y$

18		$C_{19}H_{14}$	1-Methylchrysene	242 (Group 5, 30mins)	0.992	0-7000	29.40	8.86	$1.048x - 1.192 = y$
19		$C_{19}H_{14}$	6-Ethylchrysene	256 (Group 5, 30mins)	0.992	0-7000	18.97	5.69	$0.3850x - 0.5275 = y$
20		$C_{22}H_{20}$	6-n-Butylchrysene	284 (Group 6, 40mins)	0.992	0-7000	10.07	3.06	$0.4530x - 0.5517 = y$
21		$C_{20}H_{12}$	Benzo(e)pyrene	252 (Group 6, 40mins)	0.996	0-5000	16.39	4.92	$0.9567x - 1.046 = y$
22		$C_{20}H_{12} + C_{21}H_{18}$	Benzo(j)fluoranthene + 1,3,6-Trimethylchrysene	252 + 270 (Group 6, 40mins)	0.989	0-5000	88.49	26.58	$0.7423x - 0.6438 = y$
23		$C_{20}H_{12}$	Perylene	270 (Group 6, 40mins)	0.990	0-2000	6.00	1.80	$0.155x + 0.07547 = y$

Reproducibility is a measure of drift was calculated for replicate runs of 400µg/ml on the GC- MS was determined and Table 4.2 shows the % drift.

**Table 4.24 : Percentage drift of each PAHs in 400 µg/ml PAH standard**

<b>Compound</b>	<b>Calculated Conc</b>	<b>% Drift</b>
Naphthalene	345.9	13.525
Acenaphthylene	346.5	13.375
Acenaphthene	353.6	11.6
Fluorene	353.28	11.68
Phenanthrene	356.4	10.9
Anthracene	353.2	11.7
Fluoranthene	359.6	10.1
Pyrene	360.2	9.95
Benzo(a)Anthracene	373.7	6.575
Chrysene	414.3	3.575
Benzo(b)Fluoranthene	379.6	5.1
Benzo(k)Fluoranthene	369.7	7.575
Benzo(a)pyrene	382.6	4.35
Ideno(1,2,3-c-d)pyrene	412	3
Dibenzo(a,h)anthrecene	409	2.25
Benzo(g,h,i)perylene	392.6	1.85

#### **4.3.3 Selection of Solvent for the Extraction of PAHs and Quality Control**

Certified reference materials (CRM) are used in a laboratory to assist with the method development, validation of accurate methods of analysis, ensuring traceable measurement results at a working level and within uncertainty range. CRMs are also used to verify that test methods in current use are performing according to validated performance levels at that time. Hence they act as quality control and quality assurance (QA&QC) tools for the

analyst/laboratory's day to day activity. They also provide a check on the calibration of the method (Emons *et al.*, 2006). Organic solvents are used in the extraction of PAHs. In this study, CRM 172-100 g, a certified reference material for sandy loam soil purchased from Sigma Aldrich was extracted with seven different HPLC grade solvents. The selected solvents for this study were from solvents usually employed in extraction studies of PAHs such as acetone (Song *et al.*, 2002), hexane (Wang *et al.*, 2010), ethyl acetate (Vasilieva *et al.*, 2012, Sanchez-Brunete *et al.*, 2006), acetone: hexane (Luo *et al.*, 2012), dichloromethane (Yang *et al.*, 2002), acetonitrile and propan-2-ol (Khodadoust *et al.*, 2000) were compared and the results are as shown in Figure 4.15.

The polarity of the selected solvents, ranged from polar organic solvent (acetonitrile, acetone) to mildly polar organic solvents (propan-2-ol, dichloromethane) to non polar organic solvents (hexane). The solvent miscibility with water ranged between miscible (acetone, propan- 2-ol and acetonitrile) and immiscible (ethyl acetate, n-hexane, dichloromethane). Extracts were cleaned up as in section 3.2.5 and analysed on the GC-MS (HP6890N / 5973MSD) by the optimised SIM method for the 16 USEPA priority PAHs. N-hexane: acetone (1:1) consistently gave (better) results between the prediction interval. In this study, n-hexane: acetone (1:1) was therefore the preferred extraction solvent. The performance of n-hexane : acetone may be due to synergy between the acetone which breaks up solid aggregates and allows intensive contact between particles (Lau *et al.*, 2010) and extreme solubility of PAHs in n-hexane being a non-polar solvent. It has been noted that mixtures of non polar and polar solvents are more efficient than single solvents when

extracting analytes with a wide range of polarities (Ajibola *et al.*, 2012, Lau *et al.*, 2010, Song *et al.*, 2002). In extraction of compounds, the polarity of the solvent should be close to the target compound (Ajibola *et al.*, 2012) based on theory of like dissolves like, the polarity of solvent with respect to the polarity of PAH contaminants also plays a role in determining the extent of solubility, medium polar to non polar organic solvents are ideal for PAHs extraction (Lau *et al.*, 2010).

This finding was similar to the findings of Pena *et al.*, (2007) (compared hexane, acetone, acetonitile, acetone: n-hexane (1:1)), Shu and Lai (2001) (compared cyclohexane–acetone (1:1), hexane–acetone (1:1)) and dichloromethane), Ajibola *et al.*, (2012) (who compared acetone:dichloromethane, acetone: dichloromethane, Acetone:n-Hexane, dichloromethane:n-hexane). In the comparative solvent study of PAHs by Zuloaga *et al.*, (2000), acetone, dichloromethane, acetonitrile, acetone-dichloromethane (1:1), acetone-iso-hexane (1:1), iso-hexane, methanol and toluene were used to extract PAHs from the CRM 524. The Iso hexane an isomer of n-hexane, with acetone to form a mixture was the preferred extraction solvent when recoveries were compared. However in another study by USEPA (2009), ethyl acetate a mild polar, non-toxic organic solvent was said to be comparable to n-hexane:acetone mixture and was also the preferred extraction solvent compared to n-hexane, acetone, n-hexane: acetone(1:1), n-hexane:ethyl acetate (1:1) in another study by (Senar *et al.*, 2011).

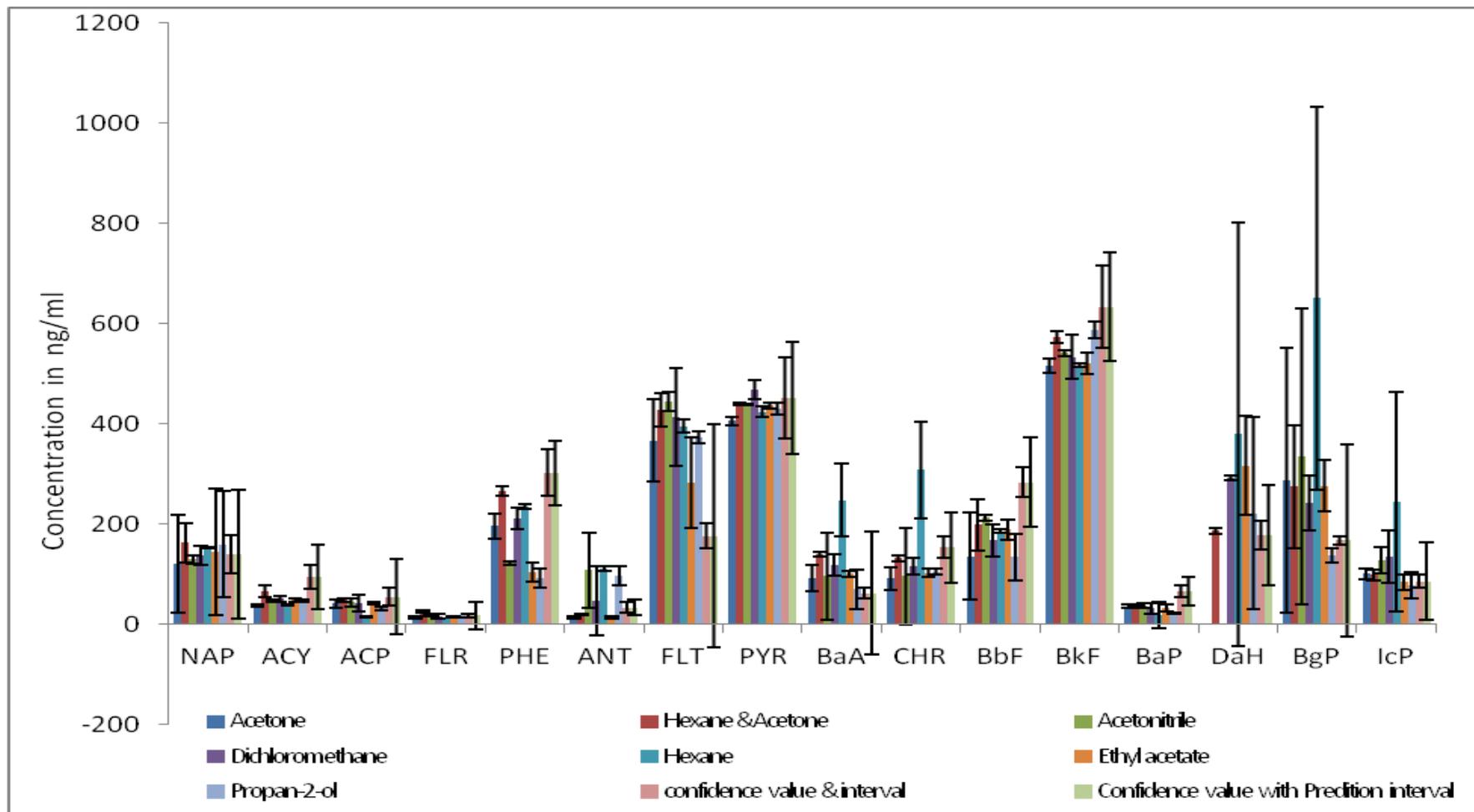
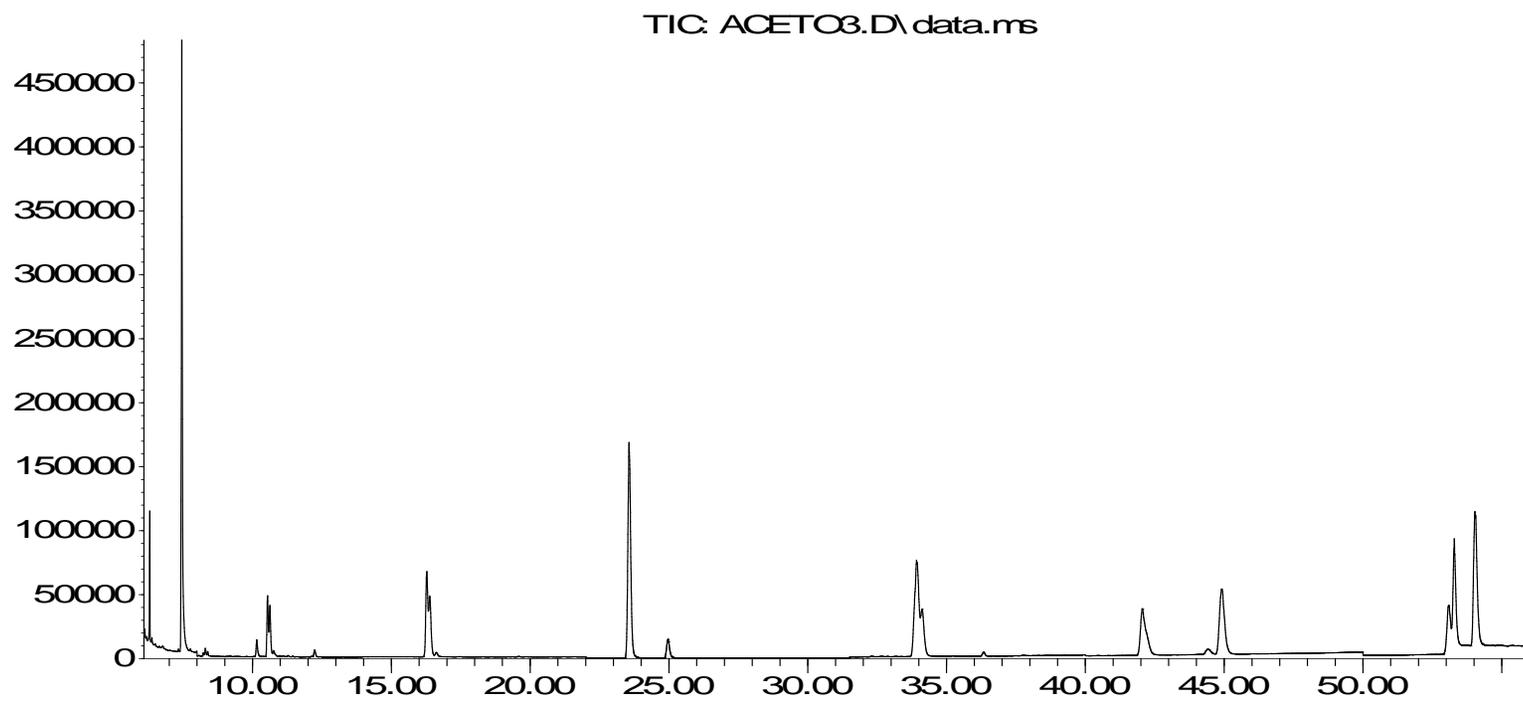


Figure 4.15: Concentration of PAHs in CRM using different solvents

Abundance



Time→

Figure 4.16: A representative Chromatogram of one of the CRM Extracts

#### **4.4. ASSESSMENT OF ALKYLATED POLYCYCLIC AROMATIC HYDROCARBONS AND RISK ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOILS AT DIFFERENT ANTHROPOGENIC SITES AROUND LAGOS, NIGERIA.**

This section describes the results for analyses and risk associated with PAHs in soil. The Risk associated with the concentrations in soils were assessed using approaches such as the MDI, BaP<sub>eq</sub>, ER, and invitro bioaccessibility and their results and discussions are as shown in sections 4.4.1 to 4.4.2

##### **4.4.1 Properties of Soils from Different Anthropogenic Sites in Lagos and its Environment**

The soils were characterized for particle size by the BS:ISO-11277:2009 method and the results are as in Table 4.15. The content of clay fraction in all soil samples varied within the range of 4 to 22 %. The content of silt fractions (0.05 - 0.002 mm) for all the samples under analysis varied within the range of 0.04 % to 25.36 %. The highest fraction in the soil formations investigated in this study was that of sand fraction (2.0-0.05 mm), which fell within the range of 49.71 % to 88.48.1 % (Table 4.25).

For the soil samples analyzed, soil pH values measured in 0.01 M CaCl<sub>2</sub> ranged from 6.8 to 8.6. pH is an important soil property since it has been found to influence other soil properties (e.g. organic content) which directly influences PAHs sorption and bioavailability (Pignatello, 1998) and lowering of acidity in soil can stimulate the activity of heterotrophic microorganisms which, at the same time, can create a more favourable condition for the

development of the bacteria that play a decisive role in PAH degradation (Antizar-Ladislao, 2009). In terms of soil reaction classification, the studied sites can be said to range from weakly acidic, neutral to weakly basic. Sum organic content determined by the Walkley-Black titrimetric method varied from 0.036 % to 4.5 % and the gravimetrically determined percentage oil and grease varied from 0.131% to 9.345%.

**Table 4. 25: Soil Physico Chemical Parameters for Soils from Different Sites.**

Sample ID	Location	Grain particles size analysis				Physico chemical parameter			Description of soil sample
		Gravel (%)	Sand (%)	Silt (%)	Clay (%)	TOC (%)	pH	Oil & Grease (%)	Physical appearance
A	Dump site near Onike canal	8.30	50.20	20.40	21.10	3.570	8.1	1.86	Dark, grey,sandy, clayery soil
B	A farm in univriy of Lagos	2.90	55.90	25.36	15.36	1.366	7.9	0.40	Brown, grey, sandy, silty soil
C	Depot and loading point for Used oil (Black oil) Iganmu, Orile	28.52	66.50	0.06	4.92	1.732	8.0	4.92	Dark black,oily,sandy, Gravelish soil
D	Busy Road side Akoka	6.67	88.48	0.04	4.81	1.610	8.1	0.18	Brown, sandy, soil
E	Premium Motor Spirit and Kerosine Oando Depot Apapa	13.79	74.67	6.37	11.54	2.232	7.3	1.41	Dark brown, silty soil
F	Dumpsite in Akoka	1.77	71.63	24.60	4.00	2.765	8.1	2.29	Dark sandy, silty soil
G	Premium Motor Spirit and Kerosine Depot Coconut Island	4.99	84.73	0.08	10.20	2.399	7.1	3.96	Black, oily, sandy clayery soil
H	Road side in lagos	16.86	71.17	2.63	09.34	0.493	7.77	0.13	Sandy, gravelish soil
I	Trailer Park/ Mechanic workshop Ibafo	0.31	71.17	16.52	12.00	1.844	8.6	9.35	Greyish, sandy, silty soil
J	Car Park Akoka	5.85	80.78	0.07	13.30	0.359	7.7	0.18	light redish brownish sandy soil
K	Control (forest soil)	1.25	49.71	29.01	20.03	4.500	6.8	0.18	Dark brown, sand silty soil
L	Mechanic workshop in Onike	8.00	50.50	19.40	22.10	3.600	8.1	1.92	Dark black,oily, sandy clayery soil

#### **4.4.2 PAHs and RPAHs of soils from different anthropogenic sites in Lagos and its Environment**

The extraction and GC-MS analysis methods used was able to separate all of the standard PAHs. The calibration curves were linear and gave good regression ( $r^2$ ) values of 0.98 or higher for all the individual PAHs and RPAHs as earlier shown in Tables 4.22 and 4.23 (pages 194 and 196 respectively) respectively. The representative chromatograms for RPAHs and PAHs standards are as shown on Figures 4.9 and 4.14. Tables 4.26 and 4.27, show the results for analysis of PAHs and RPAHs from composite soil samples obtained from the selected sites of various anthropogenic activities in Lagos, Nigeria. The physico chemical properties of these soils are as shown in Table 4.25. The concentration of individual RPAHs and sum RPAHs were generally higher than the individual 16 priority PAHs and sum PAHs. Sum PAH ranged from 207 to 253,922 ng/g as shown in Table 4.26. These concentrations are similar to those reported by other studies undertaken at related field sites elsewhere in the world as shown on Table 2.2 (section 2.1.3). Wang *et al.*, (2012) reported that the PAHs concentration in surface dusts from various sources varied greatly and was related to the anthropogenic activities undertaken in the areas. In this study, the site with the highest concentration of sum PAH was found at the petroleum product depot on Coconut Island Lagos (site G). At this sampling site, transfer of petroleum products from tanks to kegs and smaller containers occur regularly as well as cleaning of trailer tanks. A stench of petroleum was evident during sampling.

The concentrations of the sum PAHs (Table 4.26) decreased from Coconut Island fuel depot (G) to forest soil (K) [Coconut Island fuel depot (G) > black oil depot Orile (C) > mechanics workshop Lagos (L) > large dump site in Akoka (F) > small dump site in Onike (A) > a road side near a dump site (H) > fuel depot area Apapa (E) > trailer park Ibafo (I) > farm land in Lagos (B) > a road side (D) > car park Akoka (J) > forest soil (K)]. Petroleum handling activities [PAHs are known to be constituents of petroleum and its product (Pampanin and Sydnes, 2013, Pavlova and Ivanova, 2003)] on these sites may probably be the reason for the high concentrations of PAHs in G, C and L compared to other sites. The activities on these sites were such that a lot of petroleum related products like engine oil, kerosene, and gasoline were used and spilled on the ground. At the mechanic workshop where soil L was sampled, during servicing of cars, spent engine oil was spilled on the bare soil as there was no structured way of disposing the used oil. Sample C was a composite soil sample from a used oil depot where dirty oils (black in colour) were stored in drums which often leaks to the surrounding. Sample E though sourced from a location similar to G (where petroleum products were used and handled), the sum PAHs was low. This may be explained by the fact that this place is sparingly used for this purpose because it was an illegal location for sales and buying of petroleum products and it was located outside the depot. Samples A and F are both dump sites samples. The dumpsite where sample F was sampled is an older and bigger dumpsite. Sum PAH concentration values for these dumpsites were 11,851 ng/g and 21,435 ng/g respectively. These values were higher than the values for sum PAHs concentration of 4341 ng/g, 5,926 ng/g and 2,502 ng/g by Nduka *et al.*, (2013) for other dumpsites. In another study of illegal waste dumps and its

surroundings, values that ranged from 21,000 ng/g to 59,000 ng/g were recorded for sum PAH (Kaszubkiewicz *et al.*, 2010). The method of waste reduction at these dumpsites during this study was open burning.

Two vehicle park samples J and I gave sum PAHs of 216 ng/g and 689 ng/g respectively. The difference in sum PAH may be due to the type of vehicular activity. In the vehicle park where J was sampled, cars were the predominant vehicles parked there and small vehicles are usually fuelled by gasoline but in the park where I was sampled, trailers and lorries were the predominant vehicle type parked there. Trailers and Lorries are known to run on diesel. Emissions from diesel engines are more visible than emissions from petrol engines. Due to the nature of combustion in diesel engines high particulate emissions occur (Ali *et al.*, 2012) and PAHs because of their strong affinity for organic carbon in particulate matter are known to preferentially adsorb onto particulates which finally accumulate in the sediments or soils. (Barakat *et al.*, 2011a, Karlsson and Viklander, 2008). Large concentration difference for 14 individual PAHs from diesel and gasoline exhaust samples were observed by Pohjola *et al.*, (2004). Higher concentration of 14 PAHs, were found in diesel than in gasoline extracts.

Sample H and D were both road soil samples but showed a wide variation in sum PAHs concentration. Sample D sourced from a main road had lower concentration amount of sum PAHs compared to H. This may be explained by its proximity to a dumpsite where open burning is employed as a waste reduction technique.

#### **4.4.2.1: Source/ chemical fingerprinting of PAHs in soil samples sites of different activities in Lagos and its Environment.**

Sum PAHs in this study ranged from 207 to 253,922 ng/g which shows that anthropogenic activities contributed to PAHs present in the soils sampled since the 1-10 ng/g range which is typical of endogenous sum PAH in soil (resulting from plant synthesis and natural fires) was exceeded as suggested by Edward (1987), Wilcke (2000), Abbas and Barck (2005). The sum of concentration of the 2-3 ringed PAHs were generally higher compared to the sum of concentration of the 4, 5 and 6 ringed PAHs (Boehm, 2006) except for samples B, F, H, L as seen in Table 4.26. This showed the dominance of petrogenic contribution (since petrogenic PAHs is usually dominated by lower ringed systems) (Pampanin and Sydnese, 2013, Man *et al.*, 2013) as source for all the samples. Higher sum of 4-6 ring PAHs indicate pyrogenic source. For samples B, C, F, H and L, 4, 5 and 6 ringed PAHs were higher in concentration (pyrogenic PAHs dominated). The petrogenic source can be traced to the activities on these sites. The 4 and 5 ringed PAHs (pyrogenic PAHs) which dominated sample B can be traced to the type of farming practice currently practiced 'bush burning' before tilling the soil for planting. Fires and smokes, from burning of vegetation for agricultural purposes, and bush fires release large amounts of PAHs into the environment (Essumang *et al.*, 2006, Djinovic *et al.*, 2008, Ferguson, 2010). Road soil H which was dominated by 4-5 ringed PAHs may be due to combustion from exhaust of vehicles plying on the road (Man *et al.*, 2013) as well as its proximity to a dumpsite where burning of refuse takes place. The predominance of 4-5 ringed PAHs

(pyrogenic PAHs) in sample F (also a dump site sample) may be due to open burning that is practiced there. The predominance of pyrogenic PAHs in sample C may be because these oils were used oil and must have been impacted by combustion of fuels from the engines they were used in.

Sample A, from a dumpsite where open burning was also employed but had its PAHs dominated by the two and three ringed PAHs can be termed petrolytic sourced PAHs. This may be due to the type of waste on this site since it was a dumpsite for a mechanic workshop where car parts, spent lubricating and engine oils were disposed. The sum concentration for the 2-3 ringed PAHs in the samples were generally higher compared to the sum concentration of the 4, 5 and 6 ringed PAHs (Boehm, 2006) except for samples B, C, F, H and L (Table 4.26). These showed the dominance of petrogenic contribution (since petrogenic PAHs are usually dominated by lower ringed systems) (Man *et al.*, 2013, Pampanin and Sydnese, 2013) as source for all the samples except for samples B, C, F, H and L (where pyrogenic PAHs dominated). The petrogenic source can be traced to the activities on these sites.

**Table 4.26: Concentration (ng/g) of Sixteen Priority PAHs Found in the Twelve Composite Soil Samples Collected in the Lagos area, Nigeria.**

		A	B	C	D	E	F	G	H	I	J	K	L
No of rings	PAHs	Dump site	Farm land	Black oil depot	road side	Depot oando	Dumpsite Akoka	Fuel depot coconut	Road side	Trailer Park Ibafo	Car park Akoka	forest soil	Mechanic workshop
<b>2-3 rings</b>	<b>NAP</b>	6531	139	675	113	105	733	1070	161	308	86.8	105	2274
	<b>ACY</b>	79.4	4.76	2540	4.7	25.29	47.02	8353	108	2.93	1.01	2.4	3843
	<b>ACP</b>	51.11	ND	128	4.22	43.07	31.35	640	125	4.33	0.32	2.25	122
	<b>FLR</b>	142	1.6	752	8.36	58.4	67.59	1855	40.92	22.68	2.74	2.54	652
	<b>PHE</b>	962	21.79	17970	23.65	83.47	181.7	86179	408	107	8.66	1.97	17078
	<b>ANT</b>	935	6.7	2898	24.65	4.81	34.08	84837	111	56.05	4.46	3.57	3945
<b>Sum</b>		<b>8701</b>	<b>174</b>	<b>24963</b>	<b>179</b>	<b>320</b>	<b>1095</b>	<b>182934</b>	<b>954</b>	<b>501</b>	<b>104</b>	<b>118</b>	<b>27914</b>
<b>4 rings</b>	<b>FLT</b>	63	37.4	4022	28.13	13.69	54.19	2585	320	28.72	9.53	2.69	5663
	<b>PYR</b>	242	33.13	10703	16.43	30.71	50.71	44834	246	23.19	8.27	3.5	6927
	<b>BaA</b>	216	10.38	6840	6.99	35.98	2500	2054	232	7.43	3.3	2.47	7053
	<b>CHR</b>	320	14.08	20108	54.01	77.88	15119	11217	353	35.18	3.83	4.43	3982
<b>Sum</b>		<b>841</b>	<b>94.99</b>	<b>41673</b>	<b>106</b>	<b>158</b>	<b>17724</b>	<b>60690</b>	<b>1151</b>	<b>94.52</b>	<b>24.93</b>	<b>13.09</b>	<b>23625</b>
<b>5 rings</b>	<b>BbF</b>	20.95	55.11	8038	26.43	28.82	20.49	ND	381	17.83	17.02	15.59	7453
	<b>BkF</b>	28.51	68.18	10320	28.06	35.07	28.92	ND	492	23.04	22.74	21.77	10202
	<b>BaP</b>	903	17.59	3297	8.2	8.15	1184	ND	143	12.32	6.06	4.31	1295
	<b>DaH</b>	209	67.81	10043	49.65	76.62	425	10049	330	28.54	24.08	23.41	10993
<b>Sum</b>		<b>1161</b>	<b>209</b>	<b>31698</b>	<b>112</b>	<b>149</b>	<b>1658</b>	<b>10049</b>	<b>1346</b>	<b>82</b>	<b>70</b>	<b>65</b>	<b>29943</b>
<b>6 rings</b>	<b>IcP</b>	34.35	14.35	5789	36.67	68.71	87.46	ND	657	10.43	10.80	7.91	4655
	<b>BgP</b>	1113	9.29	318	9.70	6.12	871	249	ND	1.29	6.75	2.79	236
<b>Sum</b>		<b>1147</b>	<b>23.64</b>	<b>6107</b>	<b>46.37</b>	<b>74.83</b>	<b>958</b>	<b>249</b>	<b>657</b>	<b>11.72</b>	<b>16.55</b>	<b>10.70</b>	<b>4891</b>
	<b>Sum PAHs</b>	<b>11851</b>	<b>501</b>	<b>104443</b>	<b>442</b>	<b>701</b>	<b>21435</b>	<b>253922</b>	<b>4106</b>	<b>689</b>	<b>216</b>	<b>207</b>	<b>86373</b>

Sum PAHs <sub>10</sub>	11106	339	72237	333	439	20793	188191	2877	589	163	157	56383
Target val	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000
Interv val	40,000	4000	40,000	40,000	40,000	40,000	40,000	40,000	40,000	40,000	40,000	40,000

Naphthalene: NAP; Acenaphthylene: ACY; Acenaphthene: ACP; Fluorene: FLR; Phenanthrene: PHE; Anthracene: ANT ; Fluoranthene ; FLT ; Pyrene : PYR ; Benz(a)anthracene : BaA ; Chrysene : CHR ; Benzo(b)fluoranthene : BbF ; Benzo(k)fluoranthene : BkF ; Benzo(a)pyrene : BaP ; Indeno(1,2,3-cd)perylene : IcP ; Dibenz(a,h)anthracene : DhA ; Benzo(g,h,i)perylene. Where ND is  $\leq 0.03 \mu\text{g/g}$  for ACY,  $\leq 0.04 \mu\text{g/g}$  for FLR,  $\leq 0.02 \mu\text{g/g}$  for PHE,  $\leq 0.03 \mu\text{g/g}$  for ANT,  $\leq 40 \text{ ng/g}$  for FLT,  $\leq 30 \text{ ng/g}$  for PYR,  $\leq 10 \text{ ng/g}$  for BaA,  $\leq 10 \text{ ng/g}$ , for CHR,  $\leq 20 \text{ ng/g}$ , for BbF,  $\leq 20 \text{ ng/g}$ , for BkF,  $\leq 20 \text{ ng/g}$ , for BaP,  $\leq 40 \text{ ng/g}$  for DaH,  $\leq 10 \text{ ng/g}$  for BgP,  $\leq 10 \text{ ng/g}$  for IcP. Sum PAHs<sub>10</sub> - Sum of 10 PAHs = sum of NAP, PHE, ANT, FLT, BaA, CHR, BkF, BaP, IcP and BgP. Target val – The ‘New Dutch List’ target value for the sum of 10 PAHs = sum of NAP, PHE, ANT, FLT, BaA, CHR, BkF, BaP, IcP and BgP. Interv val- The ‘New Dutch List’ intervention value for the sum of 10 PAHs = sum of NAP, PHE, ANT, FLT, BaA, CHR, BkF, BaP, IcP and BgP.

**Table 4.27: Concentration (ng/g) of Alkylated PAHs Found in the Twelve Composite Soil Samples Collected in the Lagos Area, Nigeria.**

	A	B	C	D	E	F	G	H	I	J	K	L
	Dumpsite Akoka	Farm land	Black oil depot	Busy road	Depot oando	Dump site	Petrol depot coconut	Road side	Car Park Ibafo	Car park Akoka	forest soil	Mechanic workshop
1-MNAP	68144	557	31604	527	21004	4380	42752	971	527	178	935	11433
1,3-DMNAP	5645	340	27470	601	15346	18359	50874	1045	601	142	538	5454
2,3,5-TIMNAP	ND	251	4499	310	2322	7328	506	471	310	202	299	205639
1,4,6,7-TEMNAP	46569	164	152	218	1787	4444	4453	221	218	181	146	27626
1-MFLR	468400	179	4023	238	1031	3022	158	303	238	192	167	251502
DBTIP	272	160	147	171	367	1073	154	213	171	173	147	4185
9-n-PFLR	12758	81	169	161	172	259	165	294	161	162	ND	1212
1,7-DMFLR	1758	180	1206840	203	3288	211941	424	291	203	199	161	19770
2-MDBTIP	2595	154	294263	187	10153	93191	302	247	187	161	148	17200

1-MPHE	Nd	205	43811	239	20062	515494	5353	315	239	256	153	66073
3,6-DMPHE	144243	370	503281	1106	9349	10365	7836980	274	1106	262	478	17277
1,2-DMDBTIP	42264	116	4126	130	457	2084	1102	187	130	122	111	2379
2,4,7-TIMDBTIP	3305	Nd	346679	Nd	966	13829	356	Nd	Nd	Nd	Nd	3545
2-MFLT	452	199	681	228	213	478	3657	225	228	208	191	17927
1,2,6-TIMPHE	2644	199	Nd	241	6599	46710	91891	203	241	205	194	2702
1,2,6,9-TEMPHE	ND	ND	ND	ND	ND	44	ND	ND	ND	ND	ND	ND
TIPHY+ TEC	ND	ND	138	18	210	27090	ND	1610 9	18	Nd	Nd	60156
1-MCHR	688	294	4276	325	330	559	169293	1603	325	303	284	652
6-ECHR	Nd	Nd	18849	354	175	10561	ND	730	354	ND	ND	ND
BeP	1165	411	969	484	444	31556	551	8183	484	541	281	815
6-n-BCHR	409	155	599	305	305	994	589	362	305	310	0	758
BjF +1,3,6 – TIMCHR	349	228	6947	243	218	883	822	235	243	239	217	1208
PRL	847	419	2346	196	236	651	668	264	196	642	196	1030
<b>Sum RPAH</b>	<b>802508</b>	<b>4661</b>	<b>2501868</b>	<b>6485</b>	<b>95035</b>	<b>1005294</b>	<b>8211049</b>	<b>3274</b> <b>7</b>	<b>6485</b>	<b>4677</b>	<b>4646</b>	<b>718542</b>

1-Methylnaphthalene: 1-MNAP; 1,3-Dimethylnaphthalene: 1,3-DMNAP; 2,3,5-Trimethylnaphthalene: 2,3,5-TIMNAP; 1,4,6,7, Tetramethylnaphthalene: 1,4,6,7-TEMNAP; 1-methylfluorene: 1-MFLR; Dibenzothiophene: DBTIP; 9-n-propylfluorene: 9-n-PFLR; 1,7-Dimethylfluorene: 1,7-DMFLR; 2-Methyldibenzothiophene: 2-MDBTIP; 1-Methylphenanthrene: 1-MPHE; 3,6-Dimethylphenanthrene: 3,6-DMPHE; 1,2-Dimethyldibenzothiophene: 1,2-DMDBTIP; 2,4,7-Trimethyldibenzothiophene: 2,4,7-TIMDBTIP; 2-Methylfluoranthene: 2-MFLT; 1,2,6-Trimethylphenanthrene: 1,2,6-TIMPHE; 1,2,6,9-Tetramethylphenanthrene: 1,2,6,9-TEMPHE; Triphenylene+ Teracene: TIPHY+ TEC; 1-Methylchrysene: 1-MCHR; 6-Ethylchrysene: 6-ECHR; 6-n-Butylchrysene: 6-n-BCHR; Benzo(e)pyrene: BeP; Benzo(j)Fluoranthene + 1,3,6 –TrimethylChrysene: BjF +1,3,6 –TIMCHR; Perylene : PRL. Where ND is  $\leq 30$  ng/g

#### **4.4.3: Assessment of PAHs and RPAHs in Soils from Sites of Different Anthropogenic Activities in Lagos and its Environment**

Risk assessment of associated PAHs concentrations in soils were based on the total PAH concentration (determined from the exhaustive technique: Ultrasonication).

##### **4.4.3.1 Comparison of PAHs in soils from sites of different anthropogenic activities in Lagos and its environment with limits / guideline values established by legislations**

Maliszewska-Kordybach *et al.*, (2008) and Yang *et al.*, (2012) classified contamination levels in soils based on sum PAHs. Soils with sum PAHs greater than 1000 ng/g were classified as heavily contaminated soils. Soils with sum PAHs between 600 ng/g and 1000 ng/g were classified as contaminated soils, weakly contaminated soil were soils with sum PAHs between 600 ng/g and 200 ng/g and soils with sum PAHs below 200 ng/g were classified as not contaminated soils. Based on their classification, results in Table 4.26 showed that soils from sites A, C, F, G, H, L were heavily contaminated, E, I were contaminated, D, B J and K were weakly contaminated.

Since there is no soil standard for PAHs in soils and sediments in Nigeria, ‘The New Dutch list’ a set of standards developed in Dutch was used in the study. ‘The New Dutch List’ has a Target Value of 1000 ng/g and an intervention value of 40000 ng/g for the sum of 10 PAHs (summation of the concentrations of Naphthalene, Phenanthrene, Anthracene: Fluoranthene, Benz(a)anthracene, Chrysene,

Benzo(k)fluoranthene, Benzo(a)pyrene, Indeno(1,2,3-cd)perylene, Benzo(g,h,i)perylene) (Sum PAHs<sub>10</sub>) (Man *et al.*, 2013, Leung *et al.*, 2006). The target value indicates the benchmark for quality in the long term. At the target level; compounds and/or elements are known or assumed not to affect the natural properties of the soil. The intervention value is the maximum tolerable concentration above which remediation is required and If exceeded, entails serious potential risk to biota and the functional properties of the soil (VROM, 1994), Soil/sediment values in the New Dutch list are expressed as the concentration in a standard soil (10 % organic matter and 25 % clay). No soil correction value is required to correct soils with organic matter content of up to 10 % (VROM, 2000, Smreczak *et al.*, 2008,). Hence, the Dutch limit values were applied without any correction in the assessment of soil A to L because none of the soil organic matter content exceeded 10 % as shown in Table 4.25. Sum PAHs<sub>10</sub> in soils from sites A, C, F, G, H and L already classified as heavily contaminated sites in this study exceeded the 1000 ng/g ‘new Dutch list’ target value hence possess serious risk. However, only C, G, L exceeded the ‘new Dutch list intervention level of 40,000 ng/g (Table 4.26). Since the intervention value is the maximum tolerable concentration above which remediation is required and if exceeded, entails serious potential risk to biota and the functional properties of the soil (VROM, 1994), remediation is required for these sites (G,C, L). The soil samples B, D, E, I, J, and K already classified as contaminated and weakly contaminated in this study, were within the target value of the ‘new Dutch list’ for Sum PAHs<sub>10</sub>.

#### 4.4.3.2: Potency factors approach in potential health risks assessment of PAHs in soils from different anthropogenic sites in Lagos and its environment

TEF value of each PAH was used to determine BaP<sub>eq</sub> dose. For each soil, sum BaP<sub>eq</sub> dose was calculated using the concentrations of PAHs found in the sample (Table 4.28) was calculated using the concentrations of PAHs detected in the soil samples. Sum BaP<sub>eq</sub> dose of soil samples from the six sampling sites classified as 'highly contaminated sites' (A, C, F, G, H and L) in this study were also observed to have the higher values compared to the other samples. The values were 1.174 µg/g, 6.709 µg/g, 2.034 µg/g, 11.363 µg/g, 0.655 µg/g and 15.343 µg/g for A, C, F, G, H and L. Sum BaP<sub>eq</sub> dose of 0.892 µg/g was calculated for the roadside soil of Shanghai, China (Jiang *et al.*, 2009), 1.009 µg/g in the traffic soil from Delhi India, 0.048 µg/g rural soil from Delhi India (Agarwal, 2009), 0.650 µg/g, for surface soils of Agra, India (Masih and Taneja, 2006) and 0.124 µg/g for soil from Tarragona, Spain (Nadal *et al.*, 2004). In this study values of 0.6552 µg/g and 0.0687 µg/g were calculated for road soils. Sum BaP<sub>eq</sub> dose for different sampling sites in this study had values between 0.0327 µg/g (K, Forest soil) to 16.7087 µg/g (C, the lubricating oil depot soil). Sum BaP<sub>eq</sub> dose order for sites in this study was C > G > L > F > A > H > E > D > B > I > J > K which was different from the order for sum PAH concentrations (G > C > L > F > A > H > E > I > B > D > J > K). The soil with the highest concentration of PAHs in this study G (253,922 ng/g) did not have the highest sum BaP<sub>eq</sub>. The higher value of BaP<sub>eq</sub> for sample C compared to sample G implies more risk is associated with sample C where black oils were handled for sale and storage.

**Table 4. 28 : Results of BaP<sub>eq</sub> (µg/g) and Sum BaP<sub>eq</sub> (µg/g) of the PAHs in the Sample**

<b>Compound</b>	<b>A</b>	<b>B</b>	<b>C</b>	<b>D</b>	<b>E</b>	<b>F</b>	<b>G</b>	<b>H</b>	<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>
NAP	0.0065	0.0001	0.0007	0.0001	0.0001	0.0007	0.0011	0.0002	0.0003	0.0001	0.0001	0.0023
ACY	0.0001	0.0000	0.0025	0.0000	0.0000	0.0000	0.0084	0.0001	0.0000	0.0000	0.0000	0.0038
ACP	0.0001	0.0000	0.0001	0.0000	0.0000	0.0000	0.0006	0.0001	0.0000	0.0000	0.0000	0.0001
FLR	0.0001	0.0000	0.0008	0.0000	0.0001	0.0001	0.0019	0.0000	0.0000	0.0000	0.0000	0.0007
PHE	0.0010	0.0000	0.0180	0.0000	0.0001	0.0002	0.0862	0.0004	0.0001	0.0000	0.0000	0.0171
ANT	0.0094	0.0001	0.0290	0.0002	0.0000	0.0003	0.8484	0.0011	0.0006	0.0000	0.0000	0.0395
FLT	0.0001	0.0000	0.0040	0.0000	0.0000	0.0001	0.0026	0.0003	0.0000	0.0000	0.0000	0.0057
PYR	0.0002	0.0000	0.0107	0.0000	0.0000	0.0001	0.0448	0.0002	0.0000	0.0000	0.0000	0.0069
BaA	0.0216	0.0010	0.6840	0.0007	0.0036	0.2500	0.2054	0.0232	0.0007	0.0003	0.0002	0.7053
CHR	0.0032	0.0001	0.2011	0.0005	0.0008	0.1512	0.1122	0.0035	0.0004	0.0000	0.0000	0.0398
BbF	0.0021	0.0055	0.8038	0.0026	0.0029	0.0020	0.0000	0.0381	0.0018	0.0017	0.0016	0.7453
BkF	0.0029	0.0068	1.0320	0.0028	0.0035	0.0029	0.0000	0.0492	0.0023	0.0023	0.0022	1.0202
BaP	0.9030	0.0176	3.2970	0.0082	0.0082	1.1840	0.0000	0.1430	0.0123	0.0061	0.0043	1.2950
DaH	0.2090	0.0678	10.0430	0.0497	0.0766	0.4250	10.0490	0.3300	0.0285	0.0241	0.0234	10.9930
BgP	0.0111	0.0001	0.0032	0.0001	0.0001	0.0087	0.0025	0.0000	0.0000	0.0001	0.0000	0.0024
IcP	0.0034	0.0014	0.5789	0.0037	0.0069	0.0087	0.0000	0.0657	0.0010	0.0011	0.0008	0.4655
<b>sum BaP<sub>eq</sub></b>	<b>1.1737</b>	<b>0.1007</b>	<b>16.7087</b>	<b>0.0687</b>	<b>0.1029</b>	<b>2.0341</b>	<b>11.3629</b>	<b>0.6552</b>	<b>0.0482</b>	<b>0.0358</b>	<b>0.0327</b>	<b>15.3425</b>

Naphthalene: NAP; Acenaphthylene: ACY; Acenaphthene: ACP; Fluorene: FLR; Phenanthrene: PHE; Anthracene: ANT ; Fluoranthene ; FLT ; Pyrene : PYR ; Benz(a)anthracene : BaA ; Chrysene : CHR ; Benzo(b)fluoranthene : BbF ; Benzo(k)fluoranthene : BkF ; Benzo(a)pyrene : BaP ; Indeno(1,2,3-cd)perylene : IcP ; Dibenz(a,h)anthracene : DhA ; Benzo(g,h,i)perylene. A BaP<sub>eq</sub> of 0.00 µg/g, means that the concentration of a particular PAHs in table 1, is equal to 0.00 µg/g of BaP in terms of toxicity.

#### **4.4.3.3: Risk Assessment of PAHs consumed due to involuntary consumption of soils from different anthropogenic sites (Based on sum PAH concentration)**

Exposure route for PAHs from soil to man include dermal, inhalation and ingestion. However, the key exposure route was found to be ingestion route in a study by Zhong *et al.*, (2013). Soil ingestion can occur by the inadvertent ingestion of soil on hands or food items, mouthing of objects, or through intentional ingestion of soil. The risk associated with the workers on site based on involuntary ingestion can be estimated (Lorenzi *et al.*, 2011). The potential health risk from soil can be assessed by determining the concentration of each individual PAH if 0.1 g of dust was ingested by an adult of BW (70 kg) (the average daily intake also called estimated mean daily intake (MDI)) and comparing the values with the MDI of food as given by Nathaniel *et al.*, 2009 as cited in Lorenzi *et al.*, (2011). Soil ingestion rate for involuntary ingestion of soil by adult has been set as 0.1 g/day (Environment Agency, 2009). Based on this, MDI of individual PAH in soils samples were calculated. MDI values obtained were compared with oral MDIs for food. Results in this study showed that all individual PAHs in samples B, D, E, F, H, I, J and K were less than the oral MDI oral for food. However, some individual PAHs in samples A, C, G, and L exceeded the recommended MDI value (Table 4.29), indicating some risk associated with activities on these sites based on this estimate. However, the value of MDI for the exposure group in this study requires further confirmation by using data from bioavailability studies (*In vitro* or *In vivo*) such as FOREShT.

Ohio Department of Health (ODH, 2011), Fromberg *et al.*, (2007), Ding *et al.*, (2013) estimated the cancer risk using the cancer Slope Factor of  $7.3 \text{ (mg/[kgday])}^{-1}$ . ER, Da and  $D_{a(\text{BaP}_{\text{eq}})}$  were estimated based on the sum PAH concentration in Table 4.26 and results are

as shown in Table 4.28. For an adult of 70 Kg working on sites A, B, C, D, E, F, G, H, I, J, K and L respectively ERs of  $8.2 \times 10^{-6}$ ,  $7.1 \times 10^{-7}$ ,  $1.2 \times 10^{-4}$ ,  $4.9 \times 10^{-7}$ ,  $7.3 \times 10^{-7}$ ,  $1.4 \times 10^{-5}$ ,  $7.9 \times 10^{-5}$ ,  $4.6 \times 10^{-6}$ ,  $3.4 \times 10^{-7}$ ,  $2.4 \times 10^{-7}$ ,  $2.2 \times 10^{-7}$  and  $1.1 \times 10^{-4}$  would be associated with concentration of PAHs in soils at these sites. Excess lifetime cancer risk less than 1 in  $10^6$  population exposure ( $1 \times 10^{-6}$ ) is typically considered as negligible under normal exposure and 1 in  $10^4$  ( $1 \times 10^{-4}$ ) under extreme exposure (Peng *et al.*, 2011). The ER from occupational exposure to surface soil based on oral ingestion were all higher than the target risk of  $1 \times 10^{-6}$  for normal exposure but were all within the  $1 \times 10^{-4}$  for extreme exposure for most of the sites except for two sites C and L. However, the resultant risk may have been overestimated, since these calculations were based on exhaustive extraction techniques (for example ultrasonication) which may be different from uptake by the human gut (bioavailability studies).

It is worthy of note that the cancer risks estimated in this study are not consistent with those found in epidemiological studies. For example, Diggs *et al.*, (2011) in their review stated that though laboratory studies pointed towards the likelihood of PAHs causing gastric cancer, epidemiological studies presented contrary evidence. For this reason, whether the high cancer risks estimated from this study was due to the over estimation, the risk should be further estimated based on the PAHs bioavailability or bioaccessibility study. Therefore, the high value of estimated cancer for the exposure group in this study requires further confirmation by using data from bioavailability studies (*In vitro* or *In vivo*) such as FOREShT.

**Table 4.29: Results of MDI (ug/day), Da (µg/[kgday]), Da<sub>BaP<sub>eq</sub></sub> (µg/[kgday]) and ER based PAHs Concentration in Soils from 12 Sites of Different Anthropogenic Activities.**

	K	J	B	D	E	I	H	A	F	G	C	L	<sup>a</sup> Oral MDI food
NAP	0.007	0.006	0.009	0.008	0.007	0.021	0.011	0.438	0.049	0.072	0.045	0.152	7
ACY	0.000	0.000	0.000	0.000	0.002	0.000	0.007	0.005	0.003	<b>0.560</b>	<b>0.170</b>	<b>0.257</b>	0.14
ACP	0.000	0.000	0.000	0.000	0.003	0.000	0.008	0.003	0.002	0.043	0.009	0.008	0.98
FLR	0.000	0.000	0.000	0.001	0.004	0.002	0.003	0.010	0.005	0.124	0.050	0.044	0.59
PHE	0.000	0.001	0.001	0.002	0.006	0.007	0.027	0.064	0.012	<b>5.774</b>	1.204	1.144	1.54
ANT	0.000	0.000	0.000	0.002	0.000	0.004	0.007	0.063	0.002	<b>5.684</b>	<b>0.194</b>	<b>0.264</b>	0.08
FLT	0.000	0.001	0.003	0.002	0.001	0.002	0.021	0.004	0.004	0.173	0.269	<b>0.379</b>	0.35
PYR	0.000	0.001	0.002	0.001	0.002	0.002	0.016	0.016	0.003	<b>3.004</b>	<b>0.717</b>	<b>0.464</b>	0.35
BaA	0.000	0.000	0.001	0.000	0.002	0.000	0.016	0.014	<b>0.168</b>	<b>0.138</b>	<b>0.458</b>	<b>0.473</b>	0.06(0.05)b
CHR	0.000	0.000	0.001	0.004	0.005	0.002	0.024	0.021	<b>1.013</b>	<b>0.752</b>	<b>1.347</b>	<b>0.267</b>	0.11
BbF	0.001	0.001	0.004	0.002	0.002	0.001	0.026	0.001	0.001	0.000	<b>0.539</b>	<b>0.499</b>	0.11
BkF	0.001	0.002	0.005	0.002	0.002	0.002	0.033	0.002	0.002	0.000	<b>0.691</b>	<b>0.684</b>	0.09
BaP	0.000	0.000	0.001	0.001	0.001	0.001	0.010	0.061	0.079	0.000	<b>0.221</b>	0.087	0.11
DaH	0.002	0.002	0.005	0.003	0.005	0.002	0.022	0.014	0.028	<b>0.673</b>	<b>0.673</b>	<b>0.737</b>	0.1
BgP	0.000	0.000	0.001	0.001	0.000	0.000	0.000	0.075	<b>0.058</b>	0.017	0.021	0.016	0.04
IcP	0.001	0.001	0.001	0.002	0.005	0.001	0.044	0.002	0.006	0.000	<b>0.388</b>	<b>0.312</b>	0.06
Sum MDI for PAHs	0.014	0.014	0.034	0.030	0.047	0.046	0.275	0.794	1.436	17.013	6.998	5.787	
D <sub>a</sub>	0.0002	0.0002	0.0005	0.0004	0.0007	0.0007	0.0039	0.0113	0.0205	0.2430	0.1000	0.0827	
Da <sub>BaP<sub>eq</sub></sub> for Sum PAHs	1.1 x10 <sup>-3</sup>	9.7 x10 <sup>-5</sup>	1.6 x10 <sup>-2</sup>	6.7 x10 <sup>-5</sup>	1.0 x10 <sup>-4</sup>	1.9 x10 <sup>-3</sup>	1.1 x10 <sup>-2</sup>	6.3 x10 <sup>-4</sup>	4.6 x10 <sup>-5</sup>	3.4 x10 <sup>-5</sup>	3.1 x10 <sup>-5</sup>	1.5 x10 <sup>-2</sup>	
ER	<b>8.2 x10<sup>-6</sup></b>	<b>7.1 x 10<sup>-7</sup></b>	<b>1.2 x 10<sup>-4</sup></b>	<b>4.9 x 10<sup>-7</sup></b>	<b>7.3 x 10<sup>-7</sup></b>	<b>1.4 x 10<sup>-5</sup></b>	<b>7.9 x 10<sup>-5</sup></b>	<b>4.6 x 10<sup>-6</sup></b>	<b>3.4 x 10<sup>-7</sup></b>	<b>2.4 x 10<sup>-7</sup></b>	<b>2.2 x 10<sup>-7</sup></b>	<b>1.1 x 10<sup>-4</sup></b>	

a---Oral mean daily intake threshold for PAHs in food (oral MDI) (Nathaniel *et al.*, 2009 cited by Lorenzi *et al.*, 2011), b---another Oral MDI (Falco *et al.*, 2003), D<sub>a</sub>-- The annual daily dose exposure also called the average life time daily exposure was also calculated by introducing the EF. Naphthalene: NAP; Acenaphthylene: ACY; Acenaphthene: ACP; Fluorene: FLR; Phenanthrene: PHE; Anthracene: ANT ; Fluoranthene ; FLT ; Pyrene : PYR ; Benz(a)anthracene : BaA ; Chrysene : CHR ; Benzo(b)fluoranthene : BbF ; Benzo(k)fluoranthene : BkF ; Benzo(a)pyrene : BaP ; Indeno(1,2,3-cd)perylene : IcP ; Dibenz(a,h)anthracene : DhA ; Benzo(g,h,i)perylene

#### 4.4.3.4 Chemometric analysis of the concentration of PAHs and RPAHs in soils from different sites.

A strong correlation exist between sum PAHs (Table 4.16) and sum RPAHs (Table 4.17) as shown in Figure 4.17 with a Pearson correlation value of 0.961 (Table 4.30). Using SPSS 16 for windows, the strong positive correlation showed that as the sum PAHs increased the RPAHs also increased the samples analysed. The p value of 0.00 which is less than 0.05 shows that the Pearson correlation value is significant hence, at a p level of 0.05 the null hypothesis that sum PAHs and sum RPAHs are linearly unrelated can be rejected.

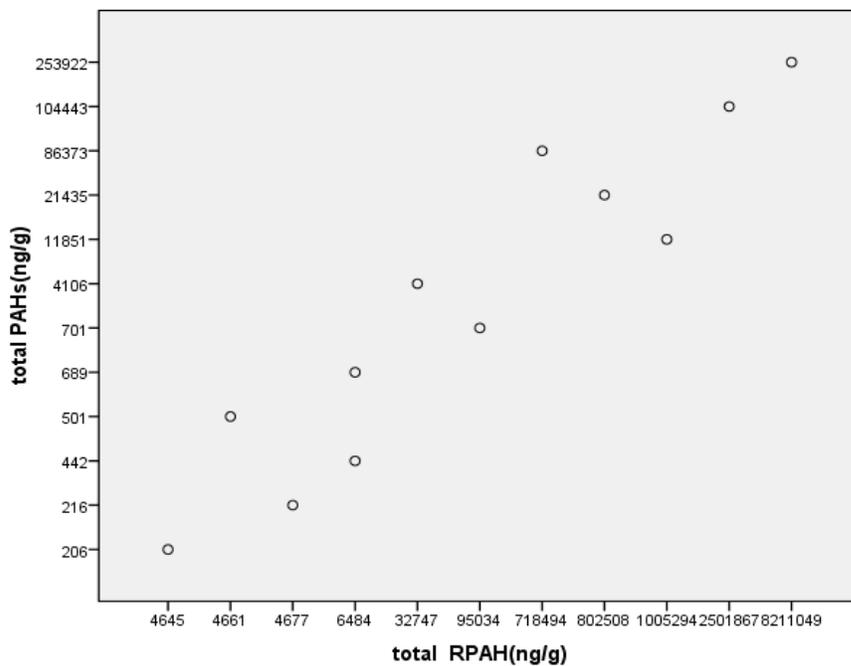


Figure 4.17: Correlation between the Concentration of Sum PAHs and RPAHs in Soil Samples

**Table 4. 30: Correlation Values for Sum PAHs and RPAHs**

		sum PAHs (ng/g)	sum RPAH (ng/g)
sum PAHs (ng/g)	Pearson Correlation	1	0.961
	Sig. (2-tailed)		0.000
	N	12	12
sum RPAH (ng/g)	Pearson Correlation	0.961	1
	Sig. (2-tailed)	0.000	
	N	12	12

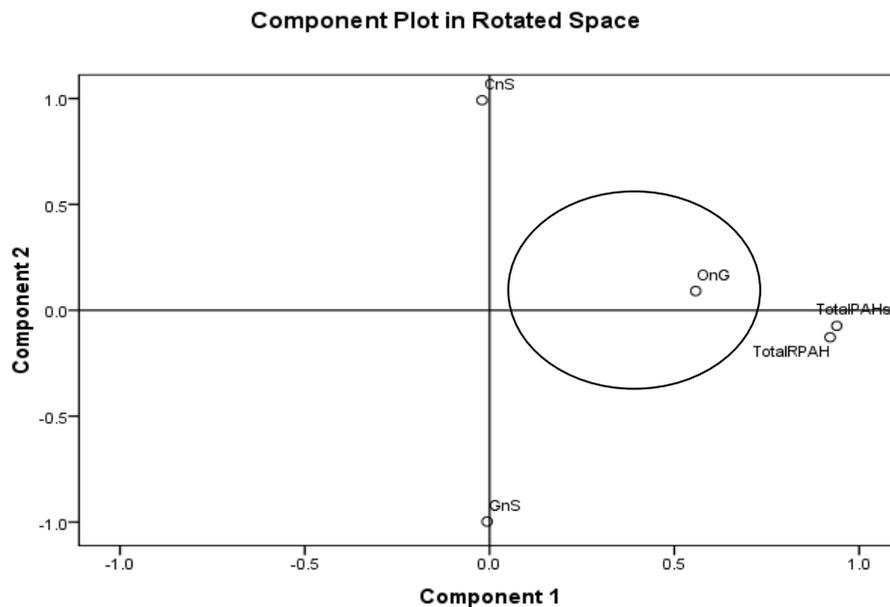
One way ANOVA, for comparing more than two variables together, was determined using Minitab 12.11 for Windows and the results are as shown in Table 4.31. The p value of 0.00 is less than the traditional p value of 0.05 and the *p*-values associated with F is 4.66 with degrees of freedom of 15. This result show that there is a significant difference between the PAHs in different soils and the difference is not due to chance but due to the different activities on the sites.

**Table 4.31: One-way Analysis of Variance of the Individual and Sum PAHs**

Analysis of Variance					
Source	DF	SS	MS	F	P
Factor	11	3.992E+09		362897166	4.66
					0.000
Error	180	1.403E+10		77949447	
Sum	191	1.802E+10			
Individual 95% CIs For Mean					
Based on Pooled StDev					
Level	N	Mean	StDev		
K	16	13	26		
J	16	14	21		
B	16	31	36		
D	16	28	27		
E	16	44	31		
I	16	43	75		
H	16	257	177		
A	16	741	1592		
F	16	1340	3733		
G	16	15870	29345		
C	16	6528	6085		
L	16	5398	4552		
Pooled StDev	=	8829	0	7000	

Principal component analysis (PCA) of sum PAHs, sum RPAHs and the physico chemical properties of the soil samples was carried out using SPSS16 for windows. The result revealed that there is a strong relationship among RPAHs, PAHs and oil and grease (OnG) (Figure 4.18, Table 4.33), therefore reflecting common sources for PAHs, RPAHs and OnG. Eigen values were used to determine the number of principal components that should be retained for further study (Table 4. 32). The relationships can be explained by two components (Figure 4.18). The first two principal components had eigen values greater than 1 which explained 82 % of the variance. Component 1 accounts for about 53 % of the variation in the data while Component 2 accounts for 29 % of the variation (Table 4.23). The loadings for

PC1 (Figure 4.18 and Table 4.24) suggests that it is strongly correlated with the sum PAHs, sum RPAHs and OnG in the samples. A negative correlation exists between clay and silt percentage of soil (CnS) and other variables studied here. Hence as clay and silt (CnS) content increase and as gravel and sand (GnS) content of soil decreases, the sum PAHs, sum RPAHs and OnG extractible decreases. The strong correlation found between PAHs and OnG was in agreement with the findings of Oleszczuk and Baran (2005). According to Zuloaga *et al.*, (2000) and Ahangar (2010) the concentration of PAHs extractible from soil are affected by soil size distributions especially the clay content and the nature of organic matter but Means *et al.*, (1980) reported that PAHs in soil is not related to the clay property.



**Figure 4.18: Principal Component Analysis Plot of Sum RPAHs, Sum PAH and the Soil Physico Chemical Properties in Rotated Space (OnG is oil and grease, CnS is clay and silt, GnS is gravel and sand, Total =Sum)**

**Table 4.32: Sum Variance Explained for the Component analysis**

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings
	Sum	% of Variance	Cumulative %		Sum	% of Variance	Cumulative %
1	2.657	53.147	53.147	1	2.657	53.147	53.147
2	1.461	29.220	82.366	2	1.461	29.220	82.366
3	.838	16.762	99.129	3	.838	16.762	99.129
4	.037	.747	99.876	4	.037	.747	99.876
5	.006	.124	100.000	5	.006	.124	100.000

Extraction Method: Principal Component Analysis.

**Table 4.33: Reproduced Correlations**

		GnS	CnS	OnG	SumPAHs	SumRPAH
Reproduced Correlation	GnS	.993	-.992	.029	.275	.325
	CnS	-.992	.993	-.043	-.299	-.348
	OnG	.029	-.043	.297 <sup>a</sup>	.507	.499
	SumPAHs	.275	-.299	.507	.919 <sup>a</sup>	.917
	SumRPAH	.325	-.348	.499	.917	.917 <sup>a</sup>
Residual	GnS		.000	.055	-.017	-.016
	CnS	.000		-.052	.014	.017
	OnG	.055	-.052		-.211	-.212
	SumPAHs	-.017	.014	-.211		.045
	SumRPAH	-.016	.017	-.212	.045	

Extraction Method: Principal Component Analysis.

#### 4.4.5 Bio-Accessibility of PAHs to Human Via an *In Vitro* Model (FORESht).

Assessment of risk associated with amounts of PAHs in soil were based on the Bio-Accessibility study (determined from the Via an *In Vitro* Model (FORESht)).

#### **4.4.5.1 Performance of analytical method for bio-accessibility of PAHs to human via an *In vitro* model (FORESht)**

The performance of the test on spiked portions of the blank extraction solutions from FOREShT is as shown in Figure 4.19 and 4.20. The Barge FOREShT procedure adopted was as in Cave *et al.*, (2010) and Lorenzi *et al.*, (2012). 1ml of FOREShT extract was cleaned up for instrumental analysis. Recovery from 1ml of the FOREShT extract was compared with that of 30ml (the total extract) with the aim of increasing sensitivity. When the total extract was used, poorer recovery values which ranged from 18 % to 72 % for the individual PAHs were observed while better recovery values were obtained when 1ml of FOREShT extract was used in this study as in Figure 4.20. The difference may be due to the break through volume and the influence of high pH of the extract. One important parameter is the breakthrough volume, which determines the maximum volume of water sample which can be introduced into a mass of SPE sorbent (Bielicka-Daszkiewicz and Voelkel, 2009). Breakthrough volume is useful and important to successful SPE performance. The degree of concentration by SPE for a sample volume is determined by the amount of sample that can be extracted without loss of analyte or attaining the breakthrough volume. This volume is a function of the mass of sorbent and hydrophobicity of the solute or analyte and analyte-sorbent interaction. In method development the aim is usually to maximize the ratio of sample volume to sorbent mass (Simpson and Wells, 2000).

Concentration of PAHs from the total FOREShT extract (30 ml) using solvent extraction was also unsuccessful in this study. This was because during recovery of PAHs after the saponification stage of FOREShT using hexane acetone mixture, some solution (water base)

follows the process of taking out the hexane acetone extract. The process of drying the water using  $\text{Na}_2\text{SO}_4$  led to the dissolution of the  $\text{Na}_2\text{SO}_4$  crystals in water such that a slurry was formed which made the removal of concentrated PAHs in hexane impossible. The pH of the aqueous phase from FOREShT extract for the sum extract was high (pH 11) despite the high dilution applied and  $\text{Na}_2\text{SO}_4$  is known to dissolve in alkaline and acidic medium instead of maintaining its hygroscopic nature.

1ml was used as recommended in Cave *et al.*, (2010) and Lorenzi *et al.*, (2012) for further work as earlier mentioned. A comparison of the recovery of PAHs from spiked blank from HLB and C18 SPE cartridge was done and the average result is as seen in Figure 4.20. The generally low recovery of naphthalene may have been due to the volatile nature of naphthalene. The process of extracting and cleaning up of the samples for analysis in this study involved many steps which may have led to the loss of naphthalene hence poor recovery. A similar observation for naphthalene was observed by Lorenzi *et al.*, (2012) where FOREShT was carried out for PAHs. The analytical method was considered appropriate with accuracy (percentage recoveries between 70 % and 130 %) and precision (relative standard deviation < 30 %) was in accordance with USEPA criteria for the quality control and validation of analytical methods (USEPA, 1992) for the other individual PAHs.

In this study, HLB and C18 SPE cartridges were comparable in recovery. There are limited and conflicting reports on this issue of comparison between HLB and C18 SPE cartridges but the findings in this study agree with the findings of Kouzayha *et al.*, (2012) where they studied the recovery of pesticides (organics) using HLB and C18. Good recoveries higher

than 65 % - 68 % for the 67 analyzed pesticides using the C18 and HLB. Cartridges showed similar variation of recovery which is relative standard deviations lower than 9.7 % - 12.3 % were obtained. A look at the individual recovery results for the individual pesticides for C18 and HLB. Cartridges showed similar variation of recovery which is comparable with the results of this study where HLB was better for a particular analyte and C18 for another.

However, in another study by Martín *et al.*, (2011), recoveries of different SPE cartridges for statin drugs (atorvastatin, fluvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin) were compared. C18 cartridge gave better recovery than HLB cartridge. In another comparison study by D'Archivio *et al.*, (2007), HLB and Strata X cartridges provided the best results in the preconcentration of 1L samples, (yielding average recoveries higher than 70 %,) from ground water compared to C18 cartridge. Recoveries of 5 different SPE sorbent types (C18 bonded silica, graphitised carbon black; highly cross-linked polystyrene-divinylbenzene, divinylbenzene-*N*-vinylpyrrolidone (HLB) and surface modified styrene-divinylbenzene were studied in the concentration of 16 pesticides.

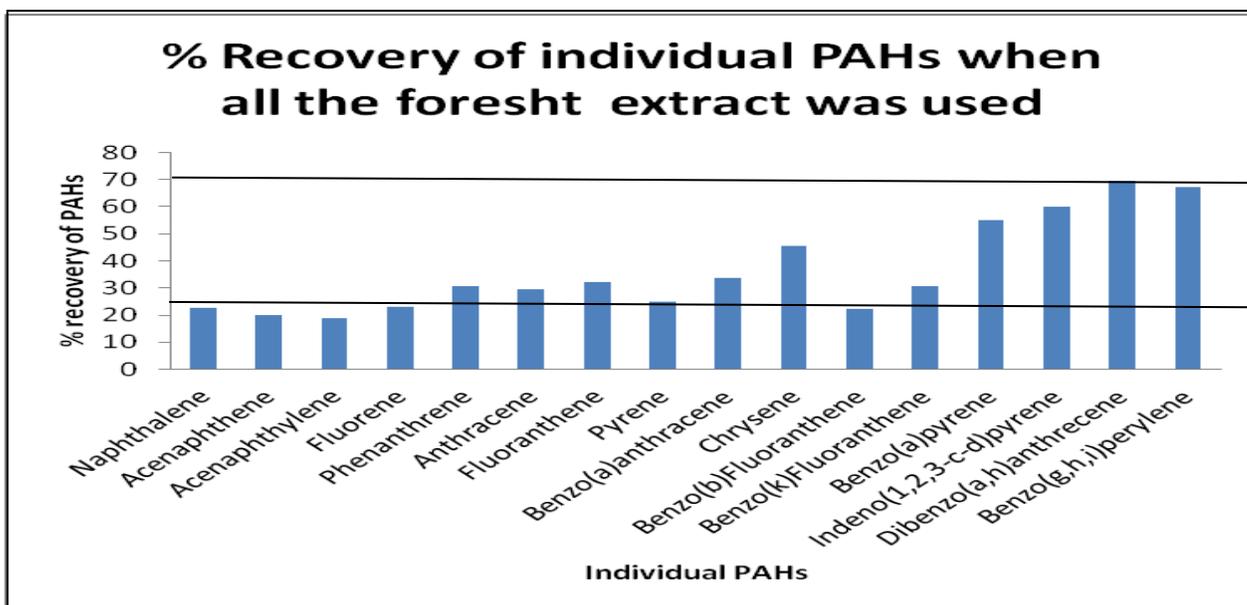


Figure 4.19: Result of FOREShT Performance when all the Extract (30 ml) was Used

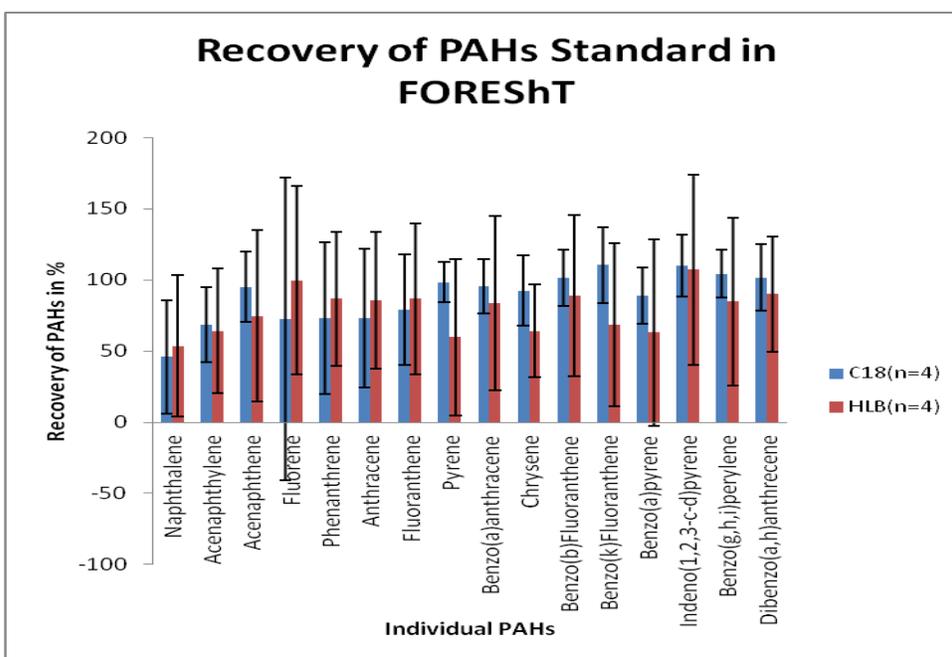


Figure 4.20: Result of FOREShT Performance when 1 ml of FOREShT Extract was used

#### **4.4.5.2: Concentration of bioaccessible PAHs in soils from sites of different anthropogenic activities in Lagos and its Environment**

Soil contamination by chemicals is a widespread problem of concern to industry, employees, communities and regulatory agencies. Exposure to total concentration of chemical (determined by exhaustive rigorous extraction procedures) in soil has been the basis of health risk assessment of soil and has led to overestimation of risk since only a fraction of the total concentration absorbed into the systemic circulation (Turkall *et al.*, 2009, Gomez-Eyles *et al.*, 2010). The most important component in predicting and assessing the risk of hydrophobic organic contaminants (HOCs e.g. PAHs) to living organisms is the bioavailable/bioaccessible fraction in the matrix (Tao *et al.*, 2009).

Heavily contaminated soils which were soils with sum PAHs greater than 1000 ng/g and contaminated soils which were soils with sum PAHs between 600 ng/g to 1000 ng/g (Maliszewska-Kordybach, 2005) were assessed for the bioaccessible PAHs. Oral accessible PAHs to human guts due to involuntary consumption of soil by the workers via an *In vitro* model (FORESHT) for soils was conducted and results obtained are as shown in Tables 4.34 and 4.35. Bioaccessible fraction was expressed as percentage bioaccessible PAH. The result consistently showed naphthalene as the most bioaccessible PAH present for the samples. Sum bioaccessible PAH for the soil in this study ranged between 0.14 % and 41.23 %. Bioavailable/ bioaccessible PAHs from soils in other studies have been found to vary between 10 % and 60 % for soil containing sum PAHs between 10,000 and 300,000 ng/g (Cave *et al.*, 2010), 0.1 to 1.4 % (Van de Wiele *et al.*, 2004), 1 to 3 % in aged crude oil

contaminated soil (Kogel-Knabner *et al.*, 2000) and 0.5 to 2 % in gastro intestinal solubility (Holman *et al.*, 2002). A study by Holman *et al.*, (2002) demonstrated the bioaccessible PAH in three different soil samples ranged from 14 to 40 % using an *in vitro* bioavailability model that simulates gastric digestion and the BaP present in bitumen fragments were largely immobile and typically have low bioavailability/bioaccessibility. This study (Table 4.35 and 4.36) as well as other studies such Holman *et al.*, (2002), Tao *et al.*, (2009) showed that only a small fraction of PAHs in soils is considered to be orally available or accessible to living organisms.

**Table 4.34 : Concentration (ng/g) of Bioaccessible (16 Priority) PAHs Found in Contaminated Soil Samples Collected in the Lagos area, Nigeria.**

Compound Name	A	C	E	F	G	H	I	L	LOD
Compound Name	Dumpsite	Black oil depot	Depot oando	Dumpsite Akoka	Fuel depot coconut	Road side	Trailer Park Ibafo	Mechanic workshop	Compound Name
Naphthalene	299±2.34	92.7±9.15	90.52±34.53	327±24.57	316±132	100±34.53	267±34.52	171±7.96	11.60
Acenaphthylene	ND	ND	ND	ND	ND	ND	ND	ND	0.40
Acenaphthene	ND	ND	ND	ND	ND	ND	ND	ND	8.87
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND	4.43
Phenanthrene	ND	ND	ND	ND	ND	ND	ND	ND	1.20
Anthracene	17.55	29.7±1.23	ND	28.50±9.82	40.13±0.03	28.52±5.40	16.95±34.46	26.24± 1.23	1.20
Fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND	9.00
Pyrene	ND	ND	ND	ND	ND	ND	ND	ND	9.00
Benzo(a)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	3.60
Chrysene	ND	ND	ND	ND	ND	ND	ND	ND	1.63
Benzo(b)Fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND	14.13
Benzo(k)Fluoranthene	ND	ND	ND	ND	ND	ND	ND	ND	12.00
Benzo(a)pyrene	ND	549±0.01	ND	ND	ND	ND	ND	273±1.56	11.43
Ideno(1,2,3-c-d)pyrene	ND	87.61±55.15	ND	ND	ND	ND	ND	ND	20.00
Dibenzo(a,h)anthrecene	194±17.41	ND	ND	ND	ND	ND	ND	100 ±2.88	18.77
Benzo(g,h,i)perylene	ND	ND	ND	ND	ND	ND	ND	ND	5.00
sum PAHs	511±4.32	759±2.03	90.52±18.31	355±0.86	356±6.12	129±12.91	284±0.20	761±18.31	

**Table 4.35: Percentage Bioavailable PAHs to Human Guts Due to Involuntary Consumption of Soil by the Workers via an *In vitro* Model (FORESHt)**

	A	C	E	F	G	H	I	L
<b>Compound Name</b>	<b>Dumpsite</b>	<b>Black oil depot</b>	<b>Depot oando</b>	<b>Dumpsite Akoka</b>	<b>Fuel depot coconut</b>	<b>Road side</b>	<b>Trailer Park Ibafo</b>	<b>Mechanic workshop</b>
<b>Naphthalene</b>	4.59	13.73	86.62	44.57	29.55	62.01	86.76	7.54
<b>Acenaphthylene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Acenaphthene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Fluorene</b>	ND	3.86	ND	ND	ND	ND	ND	0.99
<b>Phenanthrene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Anthracene</b>	1.88	1.03	ND	ND	ND	ND	ND	ND
<b>Fluoranthene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Pyrene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Benzo(a)anthracene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Chrysene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Benzo(b)Fluoranthene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Benzo(k)Fluoranthene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Benzo(a)pyrene</b>	ND	16.66	ND	ND	ND	ND	ND	21.15
<b>Ideno(1,2,3-c-d)pyrene</b>	ND	0.87	ND	ND	ND	ND	ND	ND
<b>Dibenzo(a,h)anthracene</b>	ND	ND	ND	ND	ND	ND	ND	42.82
<b>Benzo(g,h,i)perylene</b>	ND	ND	ND	ND	ND	ND	ND	ND
<b>Sum PAHs</b>	4.32	0.73	12.91	1.66	0.14	3.13	41.23	0.88

#### **4.4.5.3: Risk assessment of PAHs in soils from different anthropogenic sites in Lagos and its Environment based on bio-accessibility of PAHs to human via an *In vitro* model (FORESHt).**

Since bioavailability/bioaccessibility is highly site and source-specific, insufficient data usually makes it difficult to adequately define a value that differs from the default approach of 100%. It is noted that a site-specific assessment of bioavailability/bioaccessibility can be undertaken to carry out risk assessment (Hansen *et al.*, 2007). In this study, site specific bioaccessible PAHs were determined. Based on the concentration of bioaccessible PAHs, re-evaluation of risk associated with PAHs (such as Da, MDI, ER and BaQ<sub>eq</sub>) on sites classified as heavily contaminated and contaminated were calculated and the results are as shown in Table 4.38.

Concentration of the bioaccessible PAHs were used for risk assessment because only the bioaccessible PAHs poses health risk rather than all the PAHs present.

##### *1) BaP equivalence dose.*

Sum BaP<sub>eq</sub> in this study had values between 0.09 ng/g (road side soil and Premium motor spirit / kerosene depot) and 637 ng/g (Depot and loading point for used for black oil). Sum BaP<sub>eq</sub> dose order for samples studied was E, H, I < F, G < A < L < C (Table 4.36). BaP<sub>eq</sub> dose of 48 ng/g and 892 ng/g were determined for rural soil in India (Agarwal, 2009), 1009 ng/g for traffic soil from Delhi India, 650 ng/g for surface soils of Agra, India (Masih and Taneja, 2006) and 124 ng/g for soil from Tarragona, Spain (Nadal *et al.*, 2004). The values in this study for sites of similar activities were lower than findings of previous studies. This may have been as a result of the approach employed in this study. Previous studies employed the traditional risk

assessment approach of employing the concentration of all the PAHs found present in the matrices ( usually derived from exhaustive techniques) while the BaP<sub>eq</sub> in this study were base on the bioaccessible PAHs concentration derived from the FOREShT method.

**Table 4.36: BaP<sub>eq</sub> dose (ng/g) and Sum BaP<sub>eq</sub> dose (ng/g) for the PAHs in Soils Classified as Contaminated Soils Collected in Lagos, Nigeria.**

58 ± 44	A	C	E	F	G	H	I	L
Compound Name	Dumpsite	Black oil depot	Depot oando	Dumpsite Akoka	Fuel depot coconut	Road side	Trailer Park Ibafo	Mechanic workshop
<b>Naphthalene</b>	0.30	0.09	0.09	0.33	0.32	0.10	0.27	0.17
<b>Acenaphthylene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Acenaphthene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Fluorene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Phenanthrene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Anthracene</b>	0.18	0.30	0.00	0.29	0.40	0.29	0.17	0.26
<b>Fluoranthene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Pyrene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Benzo(a)anthracene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Chrysene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Benzo(b)Fluoranthene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Benzo(k)Fluoranthene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Benzo(a)pyrene</b>	0.00	549.26	0.00	0.00	0.00	0.00	0.00	273.93
<b>Ideno(1,2,3-c-d)pyrene</b>	0.00	87.61	0.00	0.00	0.00	0.00	0.00	0.00
<b>Dibenzo(a,h)anthracene</b>	1.94	0.00	0.00	0.00	0.00	0.00	0.00	1.01
<b>Benzo(g,h,i)perylene</b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>Sum BaP<sub>eq</sub> dose</b>	2.41	637.27	0.09	0.61	0.72	0.39	0.44	275.37

2) *The mean dietary intake (MDI) of PAHs based on bioaccessibility study.*

Lorenzi *et al.*, (2011) in their study estimated risk by comparing the MDI for soil with oral mean daily intake threshold for PAHs in food (oral MDI). In this study, a comparison of MDI for the composite soils (based on the concentration of bioaccessible PAH) with oral MDI for PAHs in food was carried out and the result showed that all the PAHs in composite samples studied were less than the oral MDI for PAHs in food (Table 4.37). This indicates no risk associated with activities on these sites based on the MDI risk assessment approach.

3) *Cancer Estimate (ER) of PAHs Based on Bioaccessibility study.*

The ER results given in Table 4.37 are based on the bioaccessible PAHs. In this study, the estimated sum annual daily intake of PAHs is associated with ER values of  $2.5 \times 10^{-9}$ ,  $6.5 \times 10^{-7}$ ,  $5.5 \times 10^{-10}$ ,  $2.7 \times 10^{-9}$ ,  $6.5 \times 10^{-10}$ ,  $9.5 \times 10^{-10}$ ,  $2.0 \times 10^{-9}$  and  $4.1 \times 10^{-7}$  for an adult (body weight of 70 kg), with exposures of 40 work years (if a person works for 25–65 years of age) working on sites where composite soil samples A, C, E, F, G, H, I, and L respectively were sampled. The overall estimated theoretical cancer risk from occupational exposure to surface soils based on bioaccessible oral ingestion were all lower than both the target risk of  $1 \times 10^{-6}$  for normal exposure and the  $1 \times 10^{-4}$  for extreme exposure all the sites. The sum ER combining the child and adult exposure periods of 30 years for adult and 6 years for a child was  $2.3 \times 10^{-5}$  based on sum PAHs (ODH, 2011).  $8.2 \times 10^{-6}$ ,  $7.1 \times 10^{-7}$ ,  $1.2 \times 10^{-4}$ ,  $4.9 \times 10^{-7}$ ,  $7.3 \times 10^{-7}$ ,  $1.4 \times 10^{-5}$ ,  $7.9 \times 10^{-5}$ ,  $4.6 \times 10^{-6}$ ,  $3.4 \times 10^{-7}$ ,  $2.4 \times 10^{-7}$ ,  $2.2 \times 10^{-7}$  and  $1.1 \times 10^{-4}$  were the ERs values based on the sum concentrations of 16 priority PAHs (from exhaustive

extraction) for adults (body weight of 70 kg) working on sites where the composite soil samples were taken. However using the bioaccessible PAHs which are the PAHs that cause the actual risk, the risk associated with sites in this study were lower and less than the normal exposure limit of  $1 \times 10^{-6}$ . However, it should be noted here that using these values provides only a theoretical estimate of risk. Since the actual risk of cancer is unknown and could be as low as zero.

PAHs are lipophilic and as a result they bioaccumulate in plants and other living organisms reaching levels that cause toxicological effects (Manoli *et al.*, 2004). Hence caution should still be exercised to reduce exposures on sites with high levels of PAHs.

**Table 4.37: MDI ( $\mu\text{g}/\text{day}$ ), Da ( $\mu\text{g}/[\text{kgday}]$ ), Da<sub>BaP<sub>eq</sub></sub> ( $\mu\text{g}/[\text{kgday}]$ ) and ER Based Bioaccessibility data for Contaminated and Highly Contaminated Soil.**

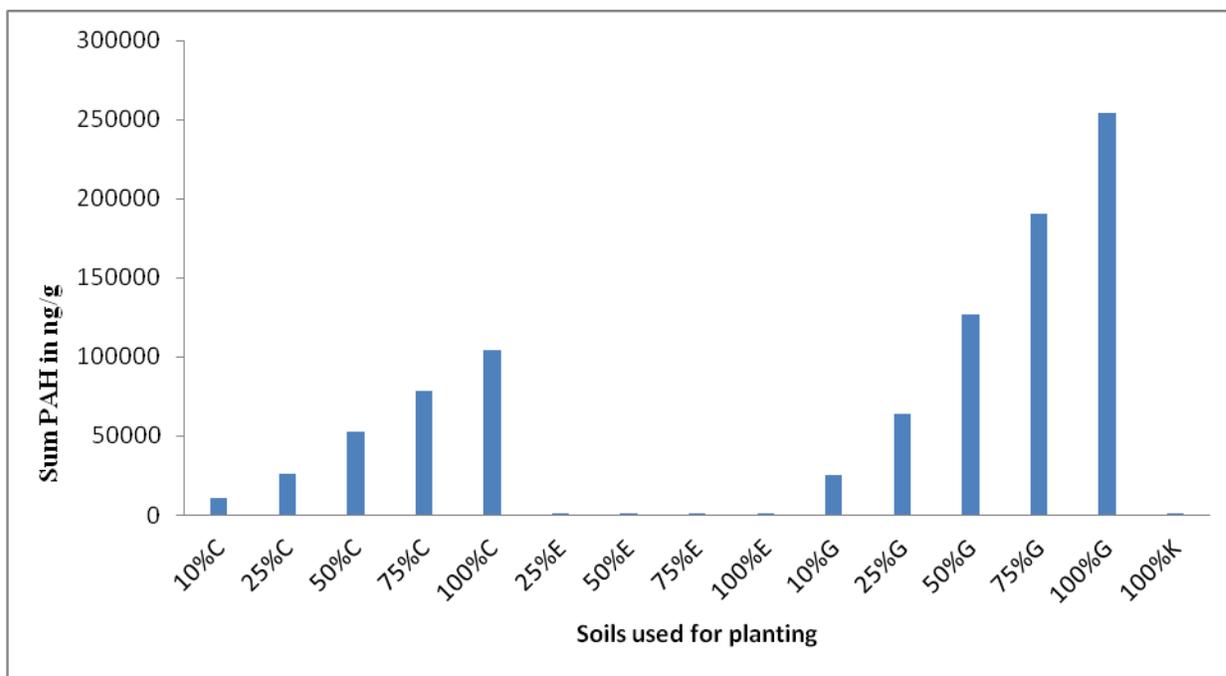
MDI	A	C	E	F	G	H	I	L	<sup>a</sup> Oral MDI food
NAP	0.03	0.01	0.01	0.03	0.03	0.01	0.03	0.02	7
ACY	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14
ACP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.98
FLR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.59
PHE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.54
ANT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
FLT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35
PYR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35
BaA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
CHR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
BbF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11
BkF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
BaP	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.03	0.11
DaH	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.1
BgP	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.04
IcP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
Sum MDI of Sum bioavailable PAHs	0.05	0.08	0.01	0.04	0.04	0.01	0.03	0.06	
Da of Sum bioavailable PAHs	0.0004891	0.0007270	0.0000875	0.00034	0.000341	0.000123	0.000272	0.000548	
Da <sub>BaP<sub>eq</sub></sub> Sum bioavailable PAHs	3.388 $\times 10^{-07}$	8.83 $\times 10^{-05}$	7.5 $\times 10^{-08}$	3.67 $\times 10^{-07}$	8.96 $\times 10^{-08}$	1.3 $\times 10^{-07}$	2.71 $\times 10^{-07}$	5.59 $\times 10^{-05}$	
ER based on Bioavailable PAHs	2.473 $\times 10^{-09}$	6.45 $\times 10^{-07}$	5.48 $\times 10^{-10}$	2.68 $\times 10^{-09}$	6.54 $\times 10^{-10}$	9.47 $\times 10^{-10}$	1.98 $\times 10^{-09}$	4.08 $\times 10^{-07}$	

Naphthalene: NAP; Acenaphthylene: ACY; Acenaphthene: ACP; Fluorene: FLR; Phenanthrene: PHE; Anthracene: ANT ; Fluoranthene ; FLT ; Pyrene : PYR ; Benz(a)anthracene : BaA ; Chrysene : CHR ; Benzo(b)fluoranthene : BbF ; Benzo(k)fluoranthene : BkF ; Benzo(a)pyrene : BaP ; Indeno(1,2,3-cd)perylene : IcP ; Dibenz(a,h)anthracene : DhA ; Benzo(g,h,i)perylene : DhA

## **4.5 POLYCYCLIC AROMATIC HYDROCARBON UPTAKE BY SOME LOCAL EDIBLE PLANTS IN SOUTH WEST NIGERIA FROM POTTED SOILS (BIOAVAILABLE PAHS IN PLANTS)**

### **4.5.1 Concentration of PAHs in Plants and Soil**

PAH concentrations of the composite soil samples were analysed and the results are as earlier seen in Table 4.26. Four composite soils were used for the bioavailability study based on the sum PAH concentrations. Soils used were heavily contaminated soils (soil with sum PAHs concentration >1000 ng/g) and significantly contaminated soils (soils with sum PAHs concentration between 600 and 1000 ng/g) (Maliszewska-Kordybach, 2005, Okedeyi *et al.*, 2012). The forest soil was used as the control soil. Sum concentrations of the 16 priority USEPA PAHs in the contaminated soils were 104,443, 701 and 253,922 ng/g (Table 4.26) respectively for soils C, E and G respectively while for the control (K) 207 ng/g was determined. To study bioaccessibility of PAHs from soil to plants (bioavailable PAHs in plant), soils C, E and G were mixed in different ratios with soil K to generate soils of varying PAH concentrations. To these soils were fertilizer added and bagged ready for planting. The PAH concentrations of the resulting soil mixtures were determined and the results are as shown in Figure 4.21.



**Figure 4.21: Sum Concentration of the 16 priority PAHs Found in Soils used for Planting.**

Eight different commonly consumed vegetables [*Corchorus olitorius* (Ewedu), *Celosia argentea* (Soko), *Amaranthus cruentus* L (Grain amaranthus/ Tete), *Basella alba* (Amunu tutu/White Spinash), *Lactuca sativa* (Lettuce), *Allium ascalonicum* (Spring Onions/Alubasa elewe), *Talinum triangulare* (Water leaf), *Telfairia occidentale* (Ugwu)] in Nigeria were planted on prepared soils and their mixes. Some soils were not able to grow plants planted on them probably because of the oil content of the soils and the contamination level. Table 4.38 shows a summary of the soils on which specific plants grew. All the plants in this study grew on the control soil (K). *Telfairia occidentale* (Ugwu) consistently grew on all the soils and their mixes. This suggests *Telfairia occidentale* (Ugwu) has some special property which enabled it

grow even in 100 % heavily contaminated soils with high oil contents (4.92 % to 1.41 %). *Lactuca sativa* (Lettuce) had least rate of growth followed by *Celosia argentea* (Soko). *Lactuca sativa* (Lettuce) did not grow on the heavily contaminated soil and only grew on the 25 % contaminated soil (25 % C: 75 % K mix). This suggests that *Celosia argentea* (Soko) and *Lactuca sativa* (Lettuce) may be used as bio indicators for polluted soils.

**Table 4.38: A summary of the soils on which specific plants grew**

Plants Soil	10 % C	25 % C	50 % C	75 % C	100 % C	25 % E	50 % E	75 % E	100 % E	10 % G	25 % G	50 % G	75 % G	100 % G	100 % K
Soko															
Tete															
Spinach															
Ugwu															
Waterleaf															
spring onions															
Lettuce															
Ewedu															

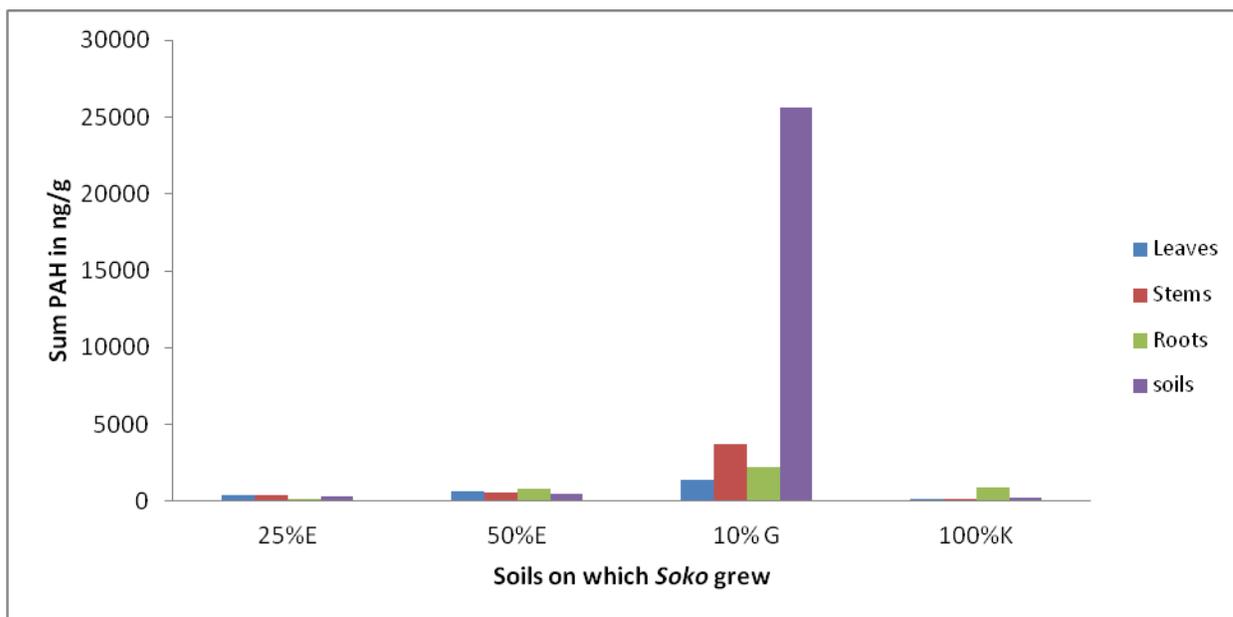
 Represents soils where plants grew  Represents soils where plants did not grow

The 16 priority USEPA PAHs were analysed in the different parts of the plants. Concentrations of the individual 16 priority USEPA PAHs were summed. Figures 4.22 to 4.30 shows the sum PAHs concentration in different plant parts studied. Amount of PAHs in plants grown on soils from contaminated sites were compared with plants grown on the control and results revealed increased concentration of PAHs in plants from contaminated sites. There was a positive correlation with PAHs concentrations in the plants to the amounts in the soil. Soils with higher concentration of PAHs had plants with higher concentration of PAHs. *Talinum triangulare* (water leaf) even though it could not grow on all soils in this study, had a higher uptake of PAHs (668 -7490 ng/g) than other plants tested. *Telfairia occidentale* (Ugwu) grew on all the

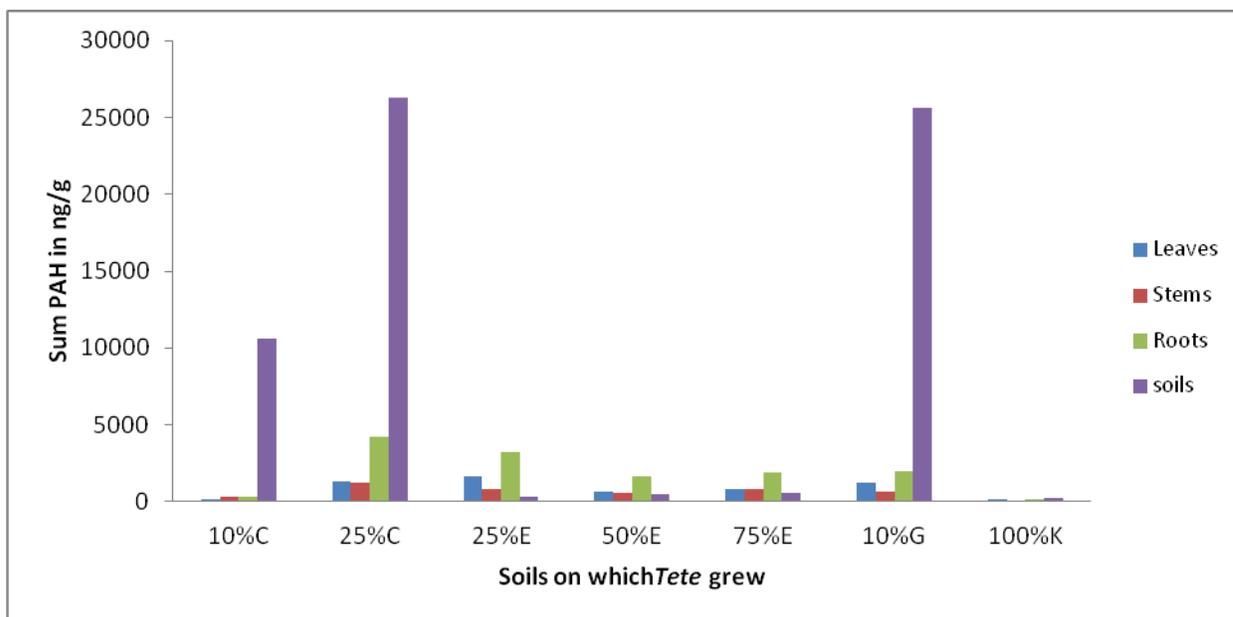
experimental soil in this study but did not have the highest concentration of PAH. This suggests *Telfairia occidentale* (Ugwu) has some features that sustained it in soils where other plants could not grow. This ability of *Telfairia occidentale* (Ugwu) makes it a potential crop for phyto remediation of PAH contaminated sites. The levels of PAHs in the roots were in most plants higher than the stem and the levels in most stems were greater than the levels in leaves. This finding was similar to the findings of other researcher who worked on plant uptake of PAHs (Vácha *et al.*, 2010, Zitka *et al.*, 2012).

Vácha *et al.*, (2010) studied the influence of soil load with polycyclic aromatic hydrocarbons (PAHs) on their contents in selected plants (*Raphanus sativus* var and *Radicula duo* variety and carrot (*Daucus carota*), 3 varieties of Nantes and with parsley (*Petroselinum crispum*)). The roots of tested plants were loaded with PAHs especially the lower molecular weight PAHs compared with the higher molecular weight PAHs in soils which were found to be transferred into the root. In another study by Zitka *et al.*, (2012) the uptake of fluoroanthrene by plants was highest in roots exposed to contaminated soils. They attributed the high concentrations of fluoranthrene in root to the lipophilicity of PAH. Fluoranthrene (like other PAHs) because of its lipophilic property accumulates in the cellular compartments such as plasma membrane and membrane-based organelles known to contain lipids. When plants grow on PAH contaminated soils, initially PAHs adsorbs to plant cell walls, and they then gradually diffuse into subcellular fractions of tissues. Hence transpiration and the lipid content of root cell fractions are the main drivers of the subcellular partition of PAHs in roots and determine the accumulation of lipophilic compounds. The diffusion rate is related to the concentration gradient (Kang *et al.*,

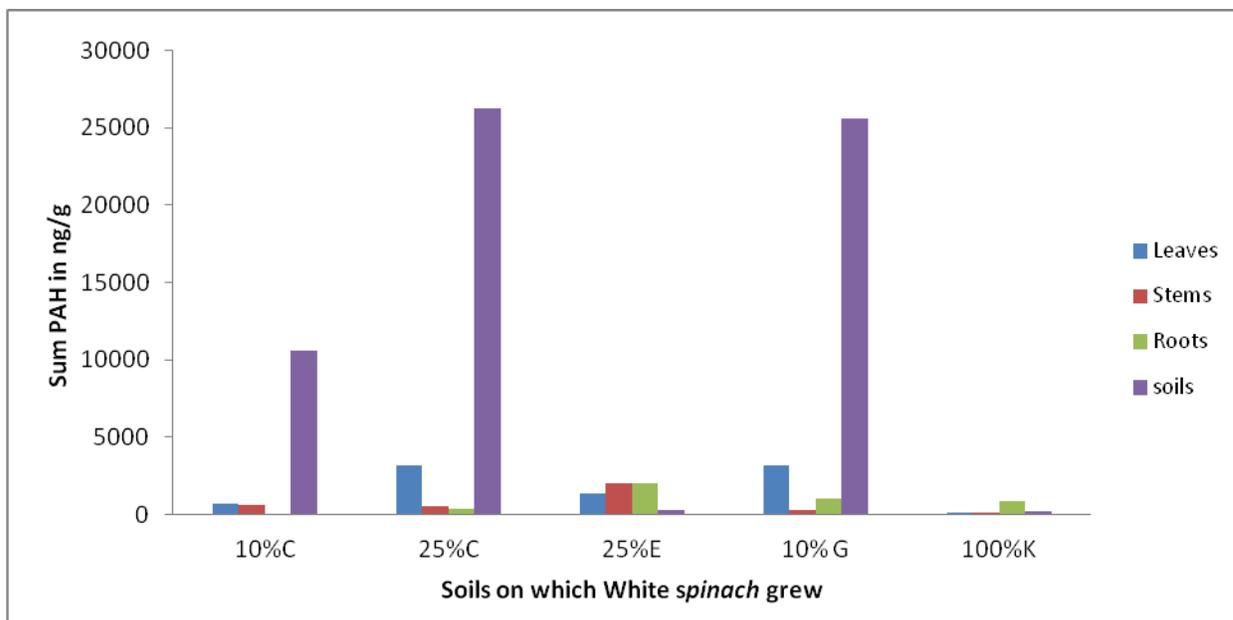
2010) from soil to plant. Immobilisation and accumulation of PAHs in the roots compared to the leaves and stem may be as a result of possible chemical modification of PAHs due to subsequent interactions with numerous hydroxyl groups of polysaccharides of cell walls as cellulose, hemicelluloses and pectin substances (Kang *et al.*, 2010). Un-Nisa and Rashid (2015) in their study found that the Vetiver grass (*Vetiveria zizanioides*) effectively removes PAHs from soil and that the level of uptake is significantly higher in root and shoot on contaminated soils compared with the levels in the control. *Vetiveria zizanioides* was recommended as a potentially, promising plant species, for the removal of PAHs from soils.



**Figure 4.22: Sum PAH in the different parts of *Celosia argentea* (Soko) Plants and in Soils on Which They Grew**



**Figure 4.23: Sum PAH in the Different parts of *Amaranthus cruentus L* (Grain Amaranthus/ Tete) Plants and in Soils on which they Grew**



**Figure 4.24. Sum PAH in the Different parts of *Basella alba* (Amunu tutu/White Spinash) Plant and in Soils on Which They Grew**

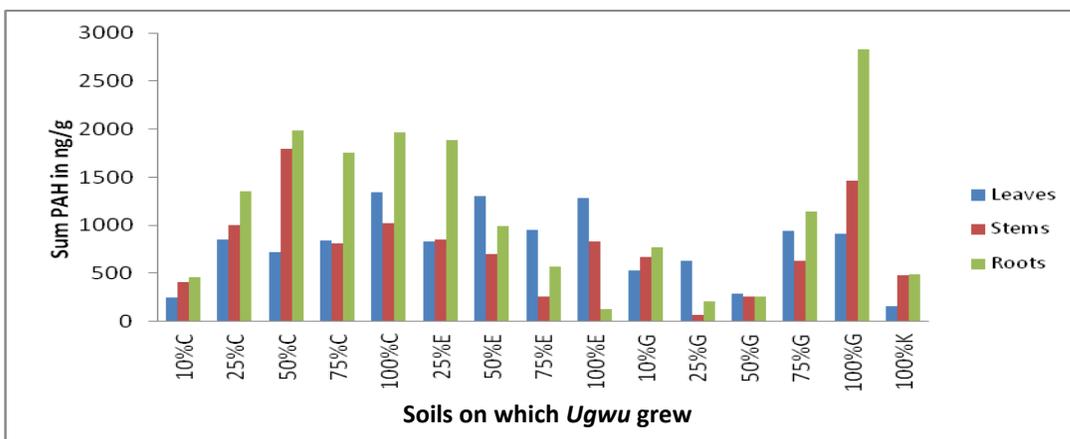


Figure 4.25 Sum PAH in the Different parts *Telfairia occidentalis* (Ugwu) Plant and in Soils on which they Grew

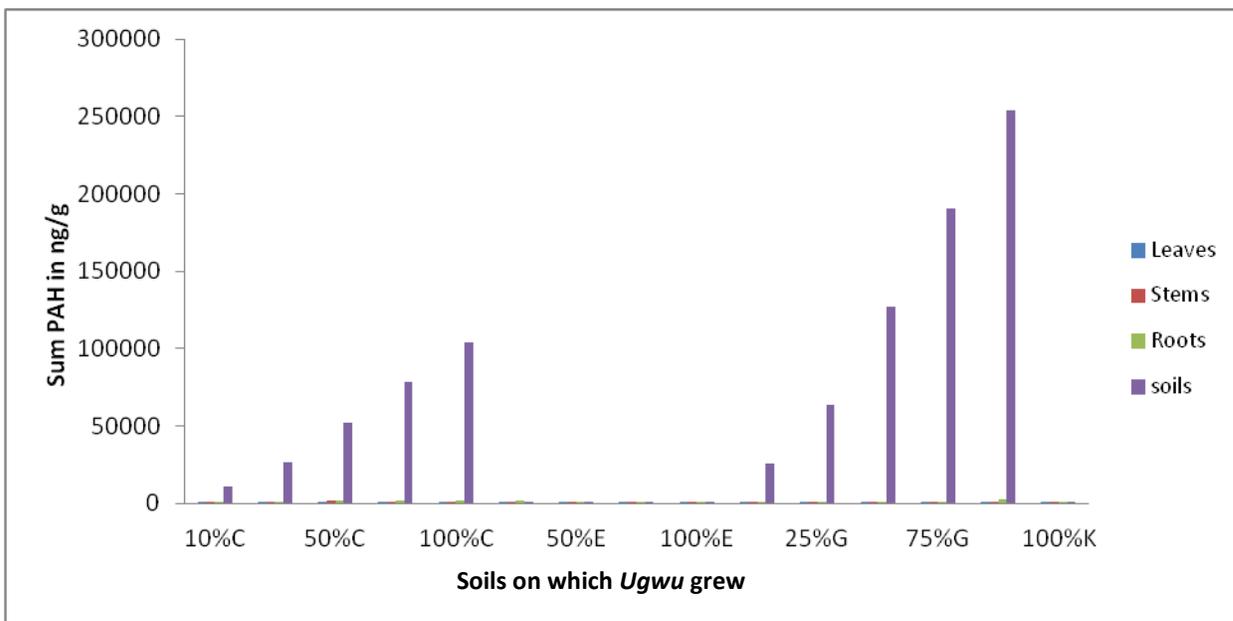
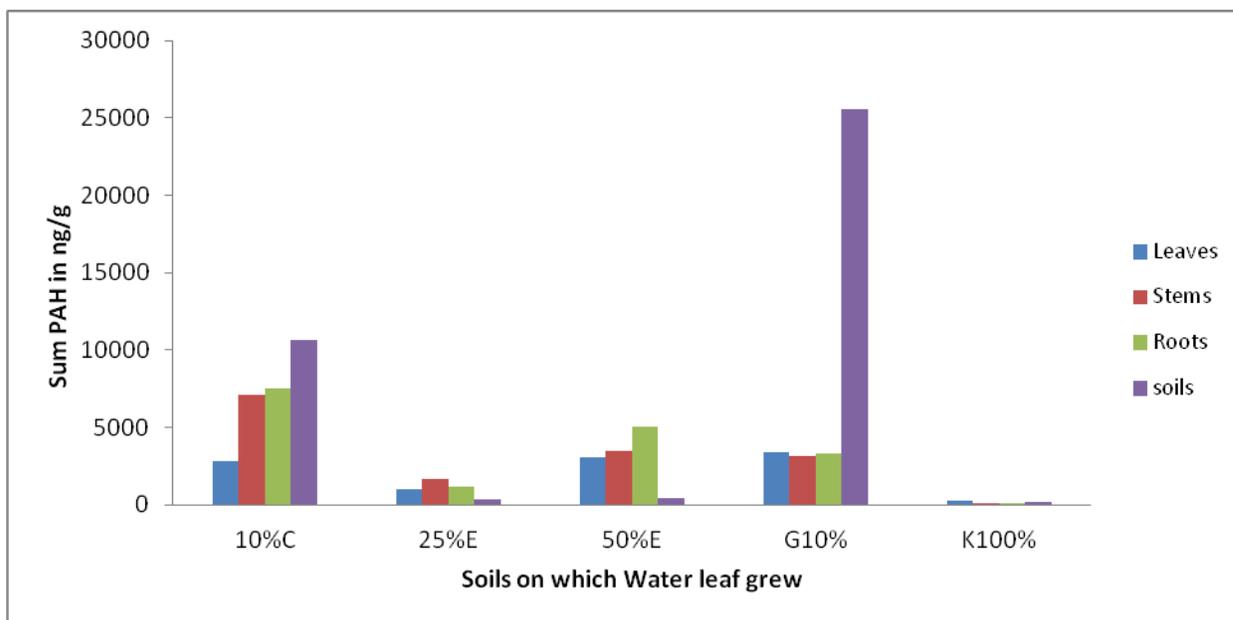
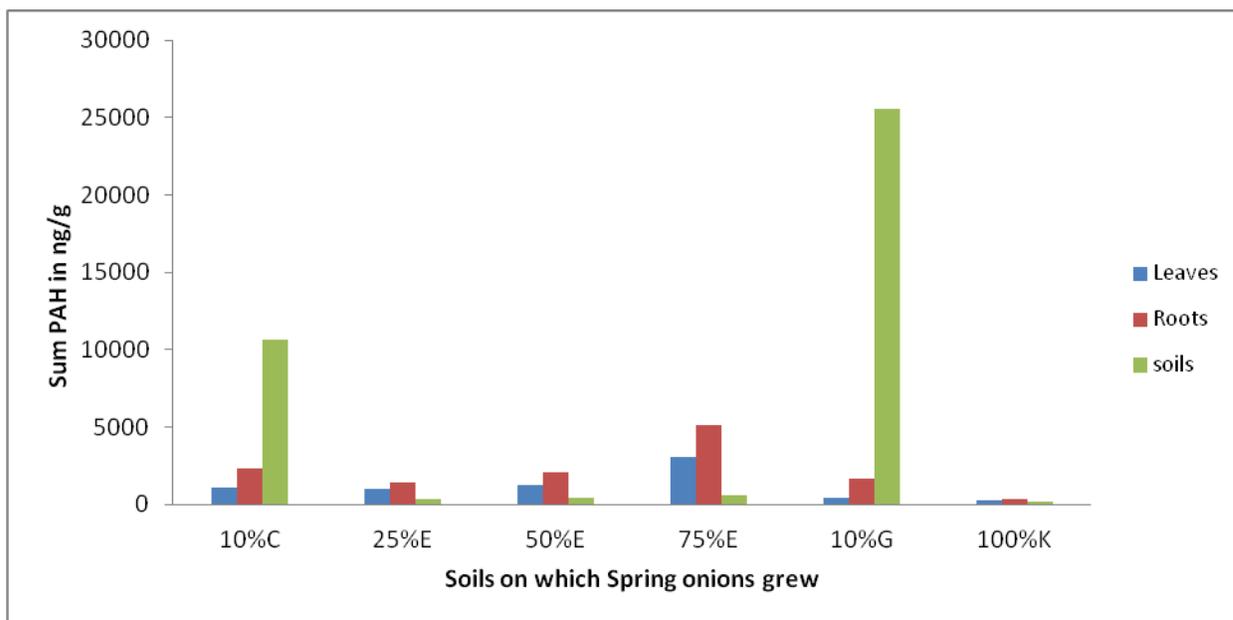


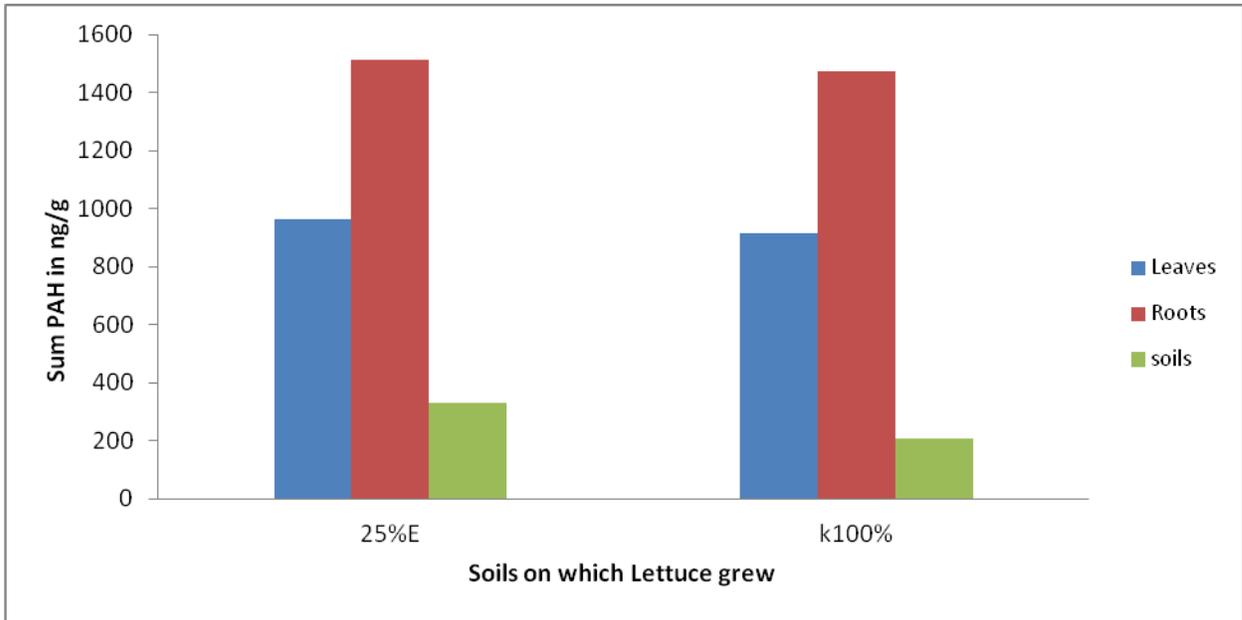
Figure 4.26: Sum PAH in the soils and Different Parts of *Telfairia occidentalis* (Ugwu) Plants and in Soils on Which they Grew



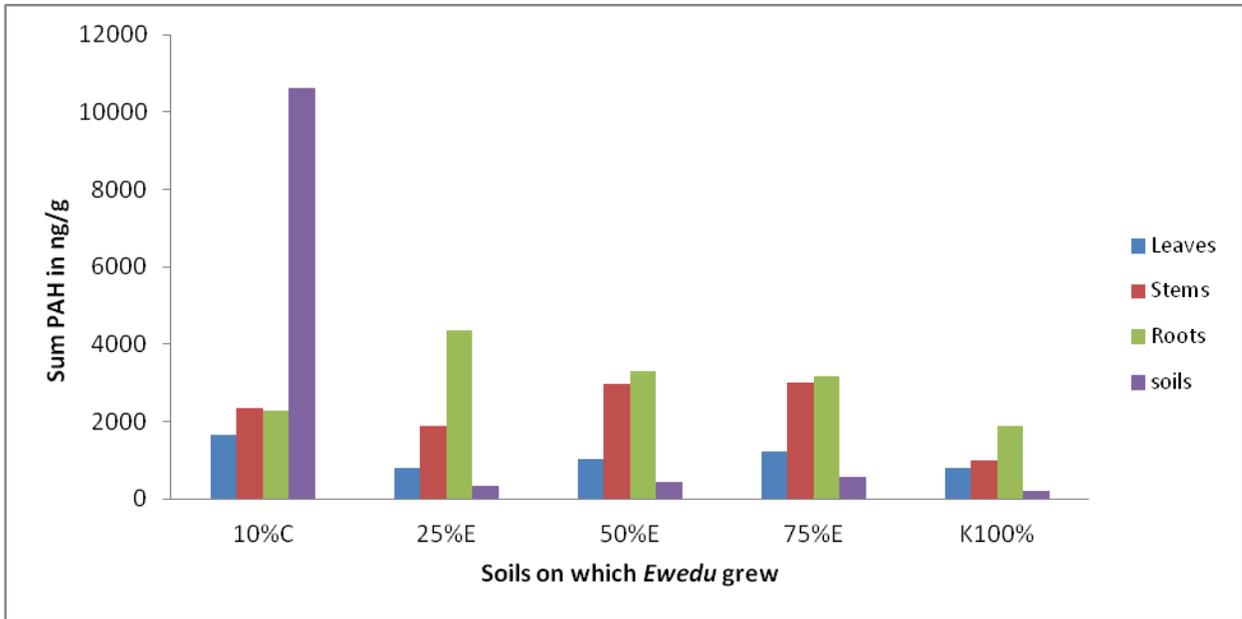
**Figure 4.27: Sum PAH in the Different Parts of *Talinum triangulare* (Water leaf ) plants and in Soils on which they Grew**



**Figure 4.28: Sum PAH in the Different Parts of *Allium ascalonicum* (Spring onions) plants and in Soils on which they Grew**



**Figure 4.29: Sum PAH in the Different parts of *Lactuca sativa* (Lettuce) plants and in Soils on Which They Grew**



**Figure 4.30: Sum PAH in the Different parts of *Corchorus olitorius* (Ewedu) plants and in Soils on Which They Grew**

## 4.7 SUMMARY OF FINDINGS

The study found that the various smoking methods (sawdust, charcoal and fire wood) contributed varying amount of PAHs to the fishes. Combustion temperature during the generation of smoke was particularly critical to PAHs formation and smoking generally increased the concentration of PAH in the fishes. At high temperatures, less smoke was produced and at lower temperatures more smoke was produced. The Sum PAH concentration of the oven dried fishes was the least. Most of the individual PAHs were not detected in the oven dried fish samples. This may be due to the smokeless nature of oven process. Smoked *Arius heude loti* had highest level of PAHs followed by smoked *Cynoglossus senegalensis* and then smoked *Hake sp.* of the smoked fishes. This may be related to the oil content of the fishes. *Arius heude loti* had the highest oil content (4.8 mg/g) of the three fishes investigated, followed by *Cynoglossus senegalensis* (3.4 mg/g) and *Hake Sp* (2.3 mg/g). The oil content of the fish and the temperature of the smoking process were found to affect the level of PAHs.

MDI and comparisons of the PAH levels with guideline values were used for the risk assessment of fishes. MDI values for smoked fishes were calculated based on an IR of 25 g/day for adult. *Arius heude loti* (cat fish, *Aro*) gave highest MDI values followed by *Cynoglossus senegalensis* (*Abo*) and then *Hake sp* (*Panla*) this may be related to the oil content in the fishes. This suggests that risk increases from less oily smoked fishes to more oily fishes and from oven to sawdust smoking method. The 5.0 µg/kg BaP maximum level for smoked meat and fish has been established by the European Commission (Regulation (EC) No 208/2005)

(Lorenzo *et al.*, 2011). All the smoked fishes in this study were lower than the 5.0 µg/kg BaP limit set except for oven dried *Cynoglossus senegalensis* (Sole) (5.6 µg/kg).

The proximate analysis of the nutrients in the samples of raw and roasted corn (*Zea mays*), yam (*Dioscorea Sagittifolia*) ripe and unripe plantain (*Musa paradisiaca*) showed that all the samples have significantly different nutritional compositions. There are indications that all the food samples are good sources of nutrients, however, the results show that both the raw and the roasted food samples contain certain levels of PAHs, with the roasted food samples having higher levels of sum PAHs as a result of the food processing. Except for roasted corn, BaP was not detected in the raw and roasted food samples; therefore, it can be assumed that both raw and roasted foods do not present a health risk for humans based on comparison with limit. The EU has established a maximum permissible level for BaP of 5 µg/kg for smoked meat and smoked fish products and a maximum limit for BaP present in foodstuffs (as a result of the use of smoking-flavour agents) at 0.03 µg/kg. From the result of roasting of the food samples, it was obvious that the concentration of sum PAHs increased and for this reason an alternative process should be introduced. Considering the carcinogenic potential of the PAHs, the reduction of these contaminants in the diet is highly desirable and special attention must be given to the intake of roasted foods since considerable amount of PAHs can be ingested in a single meal.

Certified reference materials (CRM) are used to assist with the method development, validation of accurate methods of analysis, ensuring traceable measurement results at a working level and within uncertainty range. A SIM method using the GC-MS was developed and validated with CRM172-100g. To improve on extraction method, a certified reference material was extracted with seven different HPLC grade solvents. Solvents usually employed in extraction studies of PAHs such as acetone, hexane, ethyl acetate, acetone: hexane (Luo *et al.*, 2012), dichloromethane, acetonitrile and propan-2-ol were compared for their extractability of PAHs. The polarity of the selected solvents, range from polar organic solvent (acetonitrile, acetone) to mildly polar organic solvents (propan-2-ol, dichloromethane) to non polar organic solvents. The solvent miscibility with water ranged between miscible (acetone, propan- 2-ol and acetonitrile) and immiscible (ethyl acetate, n-hexane, dichloromethane). Extracts were cleaned up and analysed on the GC-MS (HP6890N/5973MSD) by the optimised SIM method for the 16 USEPA priority PAHs. N-hexane: acetone (1:1) consistently gave (better) results between the prediction interval. In this study, n-hexane: acetone (1:1) was therefore the preferred extraction solvent.

The USEPA 16 priority PAHs were analysed for and their concentrations quantified in samples from sites of different anthropogenic activities in the Lagos area by GC-MS. Concentration of PAHs for these sites were used to access the associated health risk to humans who work on the sites. Soils from sites A (a dump site), C (a black oil dump site), F ( a dump site in Akoka), G (petroleum depot Coconut Island), H ( Road side in lagos) and L (Mechanic workshop in Onike) were classified as heavily contaminated sites in this study hence require remediation.

However, only C, G and L exceeded the 'New Dutch List' intervention concentration of 40000 ng/g. Based on the Dutch list only sites C, G and L require remediation. Sum BaP<sub>eq</sub> at different sampling sites in this study had values which ranged between 0.0 mg/g (K,) to 16.7 mg/g (C, lubricating oil depot soil). Soils classified as heavily contaminated (A, C, F, G, H and L) still had a higher total BaP<sub>eq</sub> compared with other soils. MDI for soil samples was calculated and compared with that of food. Some of the individual PAHs in sample A, C, F, G, H and L exceeded the recommended MDI value for food, indicating some risk associated with activities on these sites based on this ingestion estimate. The overall cancer risk from exposure to surface soils based on oral ingestion is not above health guidelines of 1 in 10,000 for extreme exposure except for composite soil samples C and L which exceed the value for extreme exposure. However, it should be noted here that the resultant risk could be overestimated, since these calculations were based on exhaustive extraction techniques (ultrasonication), which may be different from uptake by the human guts shown by the bioavailability/ bioaccessibility studies.

Revaluation of risk associated with workers on the contaminated and heavily contaminated sites were carried out using the FOREShT Method (an *in vitro* gastrointestinal model). Bioaccessible PAHs were measured. The concentration of bioaccessible PAHs was subsequently used for risk assessment. Soils from sites A, C, E, F, G, H, I and L were classified as heavily contaminated and contaminated based on their sum PAHs, showed that only a percentage of PAHs in soils is considered to be bioaccessible to living organisms (humans) (0.73 to 41.23 %). MDI for contaminated and heavily contaminated soil samples was calculated and compared with that of food. The MDI results showed that no risk was associated

with bioaccessible PAH. Indicating no risk associated with activities on these sites based on this ingestion estimate. The overall cancer risk from exposure to surface soil based on oral ingestion is not above health guidelines ER for all the sites were below the  $1 \times 10^{-6}$  and  $1 \times 10^{-4}$  values for normal exposure and extreme exposure respectively. This showed that though PAHs were present the amount of bioaccessible PAH based on oral risk assessment will not cause cancer. However, it should be noted here that using these values provides only a theoretical estimate of risk. Since the actual risk of cancer is unknown and could be as low as zero.

PAHs are lipophics and as a result; bio accumulates in plants and other living organisms reaching levels that can cause toxicological effects (Manoli *et al.*, 2004). Hence caution should still be exercised to reduce exposures on sites with high levels of PAHs. Three soils (G, C, E) and a control (k) were used to grow 8 different edible plants. Many of the plants subsequently died. As a result, soils were diluted with control matrix to give 75, 50, 25 and 10 % contaminated soils to enable growth. The concentrations of PAHs in these new soils were determined. Concentrations of PAHs in the plants were also determined. Generally the concentrations in the roots were higher than in other plants parts. The levels of PAH in the stems, were higher than in the leaves. Since the leaves are what is actually consumed, in the edibles plants, studied less risk is therefore associated with consumption of the leaves. *Ugwu* grew consistently on all the soils. The uptake of PAHs by plants was proportional to the concentration of PAHs in the different soils. *Tainum triangulare* (water leaf) though could not grow on the soils and their mixes, but it took up more PAHs than other plants tested. The level of PAHs in plants grown on contaminated sites compared to the control site revealed increased

concentrations of PAHs in the plants. There was a positive correlation with PAHs concentrations in the plants when compared to the levels in the soil. The summary of findings in this study is as tabulated in Table 4.39.

**Table 4.39: Summary of findings from this study**

S/N	Objective	Findings
1	To Validate/optimize analytical method for quantification of PAHs in complex matrices.	An optimised single ion monitoring method was generated for analyses of the sixteen priority PAHs, 25 alkylated PAHs and 6 deuterated PAHs was generated
2	To quantitate and access the risk of the PAHs in smoked fishes and roasted foods (yam, corn and plantain).	<p>a. PAH in roasted foods and smoked fishes were quantified and varying amount of PAHs were observed.</p> <p>b. Certain risks were associated with the concentration of PAHs in the food and fish samples especially due to their ability to bio accumulate in the body.</p>
3	To quantify of the sum and bioassessible PAHs in soils from sites of different anthropogenic activities using exhaustive and <i>in vitro</i> techniques respectively.	<p>a. Varying amounts of PAHs were found in the sites of different anthropogenic sites.</p> <p>b. There were relationships between the activities and PAH levels on the sites.</p> <p>a. Not all the PAHs present in the sites are Bio accessible.</p>
4.	To utilize other risk assessment approachess to access human exposure to PAHs from sites of different anthropogenic activities.	<p>a. Using the different risk assessment approaches varying amount of risk were associated with the different sites of anthropogenic activities.</p> <p>b. Base on the Dutch limits at the current level, some site require remediation however base on bioaccessible PAHs determined from the FOREShT study, the risk are below the limits for MDI and ER</p>
5	To determine the level of uptake of PAHs from soil by tropical edible plants	a. Tropical edible plants can take up PAHs from soils but with varying capacities and care should be exercised with where we source our vegetables for consumption from.

#### 4.8 CONCLUSION

Smoking and roasting were found to generally increase the PAHs levels in fishes and foods. The various smoking methods contributed PAHs in varying degrees. The Sawdust smoked fishes consistently had the highest level of PAHs of all the smoked fishes followed by the fire wood and then the charcoal. The oil content of the fish and the temperature of the smoking process were found to affect the PAHs level. The results reveal that the the concentration of PAHs in smoked fishes and roasted food samples possess certain risks especially due to their ability to bio accumulate in the body. An optimised single ion monitoring method for the analyses of 25 alkylated PAHs 6 deuterated PAHs and the 16 priority PAHs has been generated. Of the Twelve composite soils from sites of different activities analysed for PAHs, sites A, C, F, G, H and L were heavily contaminated sites. However, only C, G and L exceeded the 'New Dutch List' intervention concentration of 40.0 mg/g. The soils classified as heavily contaminated (A, C, F, G, H and L) still had a higher total BaP<sub>eq</sub> compared with other soils. MDI of some individual PAHs in sample A, C, F, G, H and L exceeded the recommended MDI value for food while for other six samples had their values within limit. These risk assessment indicates some risk based on this ingestion estimate. The overall cancer risk from exposure to surface soil based on oral ingestion is not above health guidelines of 1 in 10,000 except for composite soil samples C and L. Since these calculations were based on exhaustive extraction techniques (ultrasonication), which may be different from uptake by the human gut (bioaccessibility/ bioavailability studies) a site-specific assessment based bioaccessibility of PAHs in the soils was carried out. The FOREShT *in vitro* gastrointestinal model was used to ascertain their bioaccessible PAHs. The concentration of bioaccessible PAHs was used

subsequently for risk assessments. The results indicate that though soils from sites A, C, F, G, H and L were classified as heavily contaminated (<1000 ng/g) and E,I, as contaminated (1000 to 600 ng/g) soils based on their total concentration of PAHs, only a percentage (0.7- 41.2%) was bio-accessible for uptake by humans. Based on the bioaccessible fraction, the MDI results showed that no risk was associated with the bio-accessible fraction of PAHs, the overall cancer risk from exposure to surface soil based on oral ingestion was not above the approved health guidelines of 1 in 10,000 for extreme exposures.

Tropical edible plants were found to take up PAHs from soils but with varying capacities and care should be exercised with where we source our vegetables for consumption from. Generally the concentrations in the roots were higher than in other plants parts. The levels of PAH in the stems, were higher than in the leaves.

#### **4.7 CONTRIBUTION TO KNOWLEDGE**

The research has been able to add the following contributions to the body of existing knowledge:

- I. A development of an improved/ optimised GC-MS method using the SIM mode for quantification of PAHs and alkylated PAHs in complex matrices.
  
- II. Quantification and risk assessment of PAHs in smoked Nigerian fishes and roasted staple foods (yam, plantain and corn) for the first time.
  
- III. For the first time, a comprehensive risk assessment of PAHs in soils in Nigeria, using the estimated cancer risk (ER), annual daily dose exposure (Da), mean daily intake (MDI) and bioaccessible PAHs risk assessment methods.
  
- IV. Quantification for the first time of the uptake pattern of PAHs by local tropical edible plants.

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## APPENDICES

### Appendix One: *Publications from this research*

- 1) SILVA, B. O., ADETUNDE, O. T., OLUSEYI, T. O., OLAYINKA, K. O. & ALO, B. 2011. Effects of the methods of smoking on the levels of polycyclic aromatic hydrocarbons (PAHs) in some locally consumed fishes in Nigeria. *African Journal of Food Science*, **5**, 384-391.
  
- 2) ADETUNDE, O. T., OLUSEYI, T. O., OYEYIOLA, A. O., SILVA, B. O., OLAYINKA, K. O. & ALO, B. I. 2012. Effects of roasting on the proximate composition and levels of polycyclic aromatic hydrocarbons in some roasted nigerian delicacies. *Journal of Emerging Trends in Engineering and Applied Sciences*, **3**, 857- 862.
  
- 3) ADETUNDE, O. T., MILLS, G. A., OLAYINKA, K. O. & ALO, B. I. 2014. Assessment of occupational exposure to polycyclic aromatic hydrocarbons via involuntary ingestion of soil from contaminated soils in Lagos, Nigeria. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, **49**, 1661–1671.