# ASSESSMENT OF THE LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SEDIMENTS AND WATER FROM MOKOLO AND BLOOD RIVERS OF THE LIMPOPO PROVINCE, SOUTH AFRICA

**by**

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## DECLARATION

I hereby declare that the dissertation entitled “Assessment of the levels of polycyclic aromatic hydrocarbons (PAHs) in sediments and water from Mokolo and Blood Rivers of the Limpopo Province, South Africa” is my own work. All the sources used or quoted have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

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Signature:

Date:

## DEDICATION

This study is dedicated to my family, my mother Maggie Mogashane and my father Simon Mogashane for the endless support, my brothers, Pholosho and Kgahliso for always motivating me. I also dedicate it to my lovely grannies, siblings, nieces and nephews.

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## ABSTRACT

The presence of polycyclic aromatic hydrocarbons (PAHs) in the environment is of major concern since these compounds are highly persistent, toxic and wide spread pollutants. The aim of this study was to evaluate the levels of PAHs in water and sediment samples collected from Blood and Mokolo Rivers in Limpopo Province, South Africa. Liquid-liquid extraction (LLE) was used for the extraction of PAHs from water, whereas PAHs in sediments were extracted using optimised microwave-assisted extraction (MAE). Furthermore, ultrasonication and a combination of ultrasonication and mechanical agitation were used for the extraction of PAHs from sediments samples. The quantification of sixteen (16) PAHs in water and sediment was carried out using gas chromatography-mass spectrometry (GC-MS) in selected ion monitoring (SIM) mode and by GC-flame ionisation detector (GC-FID).

Concentrations of PAHs in sediments were higher than in water. The highest concentrations of PAHs were obtained in Mokolo River sediments, with the concentration ranging between 0.044 and 51.9 mg/kg. The levels of PAHs recorded in Blood River sediments were lower than those obtained in Mokolo River with concentrations ranging between 0.014 and 3.10 mg/kg. In water samples, higher levels of PAHs were observed in Mokolo River (between 0.0219 and 1.53 µg/L) while lower concentrations were recorded in Blood River (between 0.0121 and 0.433 µg/L). In water and sediment samples from both Rivers, higher molecular weight (HMW) PAH compounds (4-6 rings) were found at greater concentration levels than lower molecular weight (LMW) PAHs (2-3 rings), and this can be attributed to pyrogenic activities in the study areas. The efficiencies and accuracy of the methods for the extraction of PAHs were determined by assessing the recoveries of samples spiked with known amount of standards (for water samples), while a certified reference material (CRM) was used for sediments. Percentage recoveries ranged from 67.6 to 115% for LLE and 83.8 to 125% for MAE for both sample types.

Diagnostic ratio was used for the source identification of PAHs in sediment samples. Several PAHs ratios indicated that both pyrogenic and petrogenic could be the sources of these compounds in both rivers. Toxic equivalency factors (TEFs) and benzo(a)pyrene equivalent (BaPE) were used to quantitatively estimate the PAHs potential human health risk. The assessment of ecotoxicological risk indicated that the

sediment samples collected from Mokolo River are at high toxicity risk while sediments from Blood River are at low sediment toxicity risk.

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| Ace | Acenaphthene |
| Acy | Acenaphthylene |
| ANOVA | Analysis of variance |
| Ant | Anthracene |
| ASE | Accelerated solvent extraction |
| ATSDR | Agency for Toxic Substance and Disease Registery |
| ASTM | American Society for Testing and Materials |
| BaPE | Benzo(a)pyrene equivalent |
| BAnt | Benzo(a)anthracene |
| BaP | Benzo(a)pyrene |
| BbF | Benzo(b)fluoranthene |
| BghiP | Benzo(ghi)perylene |
| BkF | Benzo(k)fluoranthene |
| BR | Blood River |
| Chr | Chrysene |
| CRM | Certified reference material |
| DahAnt | Dibenzo(a,h)antracene |
| DCM | Dichloromethane |
| DEAT | Department of Environmental Affairs and Tourism |
| DNA | Deoxyribonucleic acid |
| EI | Electron ionisation |
| EPA | Environmental Protection Agency |

| | |
|---|---|
| EPAQs | Expert Panel on Air Quality Qtandards |
| ERL | Effect range low |
| ERM | Effect range median |
| ESI | Electrospray ionisation |
| FA-NNC | Factor analysis with nonnegative constraints |
| FID | Flame ionisation detector |
| FLD | Fluorometric detection |
| Fln | Fluoranthene |
| Flu | Fluorene |
| GC | Gas chromatography |
| GC-FID | Gas chromatography-flame ionisation detector |
| GC-MS | Gas chromatography-mass spectrometry |
| HMW | High molecular weight |
| HPLC | High performance liquid chromatography |
| IARC | International Agency for Research on Cancer |
| IDL | Instrument detection limit |
| InP | Indeno(1,23-cd)pyrene |
| IQ | Intelligence quotient |
| LC | Liquid chromatography |
| LLE | Liquid- liquid extraction |
| LMW | Lower molecular weight |
| LWMA | Limpopo water management area |
| LOD | Limit of detection |

| | |
|---|---|
| LOQ | Limit of quantification |
| LRB | Laboratory reagent blank |
| MAC | Maximum allowable concentration |
| MAE | Microwave-assisted extraction |
| MALDI | Matrix-assisted laser desorption ionisation |
| MR | Mokolo River |
| MS | Mass spectrometry |
| MSD | Mass spectrometry detector |
| M/Z | Mass to charge ratio |
| NAD | Nicotinamide adenine dinucleotide |
| NADP | Nicotinamide adenine dinucleotide phosphate |
| Nap | Naphthalene |
| NRF | National Research Foundation |
| PAHs | Polycyclic aromatic hydrocarbons |
| PEL | Probable effect level |
| PFE | Pressurised fluid extraction |
| Phe | Phenanthrene |
| PMF | Positive matrix factorisation |
| Pyr | Pyrene |
| QC | Quality control |
| RSD | Relative standard deviation |
| RT | Retention time |
| SD | Standard deviation |

| | |
|---|---|
| SFE | Supercritical fluid extraction |
| SIM | Selected ion monitoring |
| SPE | Solid phase extraction |
| SPME | Solid phase microextraction |
| SQGs | Sediment quality guidelines |
| TEFs | Toxic equivalency factors |
| TEL | Threshold effect level |
| TIC | Total ion chromatogram |
| TLC | Thin layer chromatography |
| TOF | Time of flight |
| U | Ultrasonication |
| UAM | Combined ultrasonication and mechanical agitation |
| USA | United States of America |
| USEPA | United States Environmental Protection Agency |
| UVD | Ultraviolet detection |
| WHO | World Health Organisation |
| WRC | Water Research Commission |

# CHAPTER 1

# INTRODUCTION

## 1.1 BACKGROUND AND MOTIVATION

### 1.1.1 Background of the study

The pollution of rivers, dams and lakes caused by domestic and industrial wastewater discharges, mining runoff, and other sources threatens our existence and is now a growing threat to water resources in South Africa and other countries (Sibiya, 2012). Pollution in South Africa has affected water quality and impacted public health and the functioning of ecosystems negatively (McCarthy and Humphries, 2013). The main sources of water pollution are believed to be untreated effluents from burning of fossil fuels, municipal, industrial and mining wastewater discharges (Sibiya, 2012; McCarthy and Humphries, 2013).

McCarthy and Humphries (2013) investigated the events surrounding the increasing contamination of Carolina's water supply (Carolina being a town in Mpumalanga, South Africa) with the intentions of identifying a possible cause and to evaluate whether the event has relevance for other dams in the Vaal River catchment system. The analysis of water samples showed that the pollution originated from the Witrandspruit sub catchment where seepage from coal mines had accumulated in a wetland upstream of the dam (McCarthy and Humphries, 2013). Uncontrolled urbanisation is increasing in the country and water experts believe that several cities and town have not yet been able to develop basic utilities for water and environmental services, to keep pace with their rapid growth. This may have lead to the increase in water pollution (Donoghue and Marshall, 2003; Sibiya, 2012). All these industries, wastewater treatment plants and other sources of pollution may contribute to the release and formation of organic compounds such as polycyclic aromatic hydrocarbons (PAHs), which are also considered to be environmental hazards (Countway *et al*., 2003).

The PAHs are organic compounds consisting of two or more fused aromatic rings in a linear or cluster arrangement and do not contain a heteroatom or substituents

(Countway *et al*., 2003; Bayowa, 2014). These compounds are of major environmental concern because they are highly persistent, toxic and wide spread environmental pollutants (Doong and Lin, 2004; Sun *et al*., 2016).

The effects of PAHs are usually known from animal experiments, but because of the similarity of biological systems in different species, it is possible that all mammals including humans can be affected in a similar manner (Escartin and Porte, 1999). Many PAHs are considered to be mutagenic or carcinogenic and believed to cause health problems, including kidney and liver damage (Lotufo and Fleeger, 1991; Nemirovskaya, 2007). Due to their potential toxicity and wide distribution in the natural environment, air, water and sediments, some PAHs are listed as priority monitoring pollutants by the United States Environmental Protection Agency (USEPA). The 16 priority PAHs in USEPA's list are acenaphthene, fluoranthene, benzo(k)fluoranthene, naphthalene, chrysene, benzo(a)pyrene, acenaphthylene, benzo(a)anthracene, dibenzo(a,h)anthracene, anthracene, benzo(b)fluoranthene, benzo(ghi)perylene, phenanthrene, benzo(j)fluoranthene, indeno(1,2,3-c,d)pyrene and pyrene (Kafilzadeh *et al.*, 2011).

Most of the PAHs are among the most powerful carcinogens known to exist. They produce tumors in several organisms through single exposure to microgram quantities (Driscoll *et al*., 2011). Some of PAHs may act at both the site of application and far from the site of absorption and their impacts have been shown in nearly each and every tissue and species tested, regardless of the ways of administration (Zhang *et al*., 2005). The evidence involving PAHs as inducers of cancerous and precancerous lesions is now becoming overwhelming, and this group of substances is likely to be a major contributor to the recent increase in cancer rates. The PAHs were the first substances known to be associated with carcinogenesis (Zhang *et al*., 2005; Sibiya, 2012).

Occupational skin cancer was first reported in London chimney sweeps in 1775 (Gawkrodger, 2004) and also in German coal tar workers in the late 1800's (Diepgen, 2012). Coal tar and pitch were all suspected to be carcinogenic to humans. Studies showed that topical applications of coal tar produced skin tumors in mice and rabbits. Benzo(a)pyrene is one of the PAHs that was identified to be the most carcinogenic compounds in coal tar (Driscoll *et al*., 2011). The carcinogenic activity to humans

caused by tars and shale oils is beyond dispute. In addition to skin cancers, higher occurrence of respiratory tract and upper gastrointestinal tract tumors were mostly associated with occupational exposures to these carcinogens (Bruske-Hohlfeld, 1999; Zhu and Pignatello, 2005).

The PAHs are found almost everywhere in the environment and have been detected in animal and plant tissues (Eisler, 1987; Ciganeck *et al*., 2014), sediments (Mekonnen *et al*., 2015; Sun *et al*., 2016), air (Maliszewska-Kordybach, 1999), soils (Tsibart and Gennadiev, 2013), surface water (Ngabe *et al*., 2000; Nekhavhambe *et al*., 2014), drinking water (World Health Organisation (WHO), 2003a) and groundwater (WHO, 2003b). Humans have probably always been exposed to PAHs due to the natural background level in soils and plants. Avoiding exposure to nanogram quantities of these compounds on a daily basis is now considered to be impossible for all living organisms (Zhu and Pignatello, 2005). Ever since benzo(a)pyrene was identified as a carcinogen at the beginning of this century, the presence of this compound and other PAHs in the environment has received special attention. Many reviews have been published on the toxicological aspects of PAHs in the environment (Zhang *et al*., 2005; Zhu and Pignatello, 2005; Rengarajan *et al*., 2015; Abdel-Shafy and Mansour, 2016; Edokpayi *et al*., 2016).

Generally, PAHs enter water bodies through atmospheric deposition and direct releases of substances through petroleum spills and surface runoff (Abdel-Shafy and Mansour, 2016). Many studies have been conducted recently regarding runoff sources of PAHs (Prabhukumar and Pagilla, 2010; Nekhavhambe *et al*., 2014; Edokpayi *et al*., 2016). Rainfall that runs off parking lots and road surfaces transports PAHs that originated from leaking motor oil, diesel combustion engine, coal gasification, and parking lot sealants in to rivers (Zhang *et al*., 2010; Salih *et al*., 2015). The PAHs usually attach readily to sediment particles, leading to high concentrations in sediments at the bottom of water bodies (Abdel-Shafy and Mansour, 2016).

### 1.1.2 Potential risks associated with polycyclic aromatic hydrocarbons

Considering mutagenicity, carcinogenicity and ubiquity of several PAHs in the environment and atmosphere, the setting of guidelines to limit animal and human exposure is of high priority (Moon *et al*., 2010). Epidemiological research into the

occupational exposure of workers has recognised associations between individual PAHs and human cancer but such compounds serve mainly as markers for exposure to the entire PAH mixture (Delgado-saborit *et al*., 2011). In addition, the only current toxicological data to evaluate the carcinogenic strength of individual PAH is from animals and results are extrapolated to the low quantity to which humans are exposed. This probably makes the assessment of health outcomes and attribution to specific PAH components difficult. Most PAHs are genotoxic carcinogens and thus it is impossible to define an absolutely safe level of exposure (Moon *et al*., 2010).

Dietary intake has been described as a major route for human exposure to PAHs, excluding smoking and occupationally exposed populations. Some of the PAHs in foods can occur as a result of contamination of fruits, vegetables and crops grown in polluted environment (Delgado-saborit *et al*., 2011). These PAHs also accumulate in marine organisms, mainly bivalve mollusks, to the levels greater than their concentrations in the surrounding areas (Net *et al*., 2015). Seafood is also believed to be a major source of proteins and healthy lipids for people. Most of the long-chain omega-3 fatty acids have been reported to have numerous beneficial roles in the human body. Regardless of the health benefits of a seafood diet, an issue of major concern related to continual seafood consumption is the potential risk of exposure to toxic compounds such as PAHs (Delgado-saborit *et al*., 2011).

Some of the subgroups of the population can have higher risks from dietary exposure of PAHs than the normal population. In modern years, a number of epidemiologic studies have described that a major portion of human cancers, such as prostate and lung cancers, are assigned to dietary sources. But, currently little data is present concerning dietary intakes of PAHs and their potential risk from seafood consumption in particular. A dietary intake of PAHs differs greatly between countries and between population groups within countries (Moon *et al*., 2010; Delgado-Saborit *et al*., 2011). Since PAHs are of major concern, maximum allowable limits have been set specifically for those PAHs identified as carcinogenic, toxic and priority pollutants (Oduntan, 2014). Table 1.1 shows some of maximum allowable limits of PAHs in soil and water samples in Spain according to the Agency for Toxic Substance and Disease Registry (ATSDR) and USEPA (Oduntan, 2014).

Table 1.1: Maximum allowable concentrations (MACs) of PAHs in soil and water (Oduntan, 2014)

| | ATSDR | ATSDR | USEPA |
|---|---|---|---|
| PAHs | Soil (mg/kg) | Water (mg/L) | Water (mg/L) |
| Pyrene | 3.0 | 3.0 | |
| Napthalene | 1.0 | 3.0 | |
| Phenanthrene | 3.0 | 3.0 | |
| Benzo[hgi]perylene | 3.0 | 3.0 | |
| Benzo(a) pyrene | 0.3 | 0.005 | |
| Anthracene | 3.0 | 3.0 | |
| Fluoranthene | 3.0 | 3.0 | |
| Acenaphthene | 3.0 | 3.0 | |
| Acenaphthylene | 3.0 | 3.0 | |
| Benzo (a) anthracene | 0.15 | 0.005 | 0.001 |
| Benzo (b) fluoranthene | 0.3 | 0.005 | 0.002 |
| Dibenzo (a) anthracene | 0.3 | 3.0 | 0.004 |
| Fluorene | 3.0 | 0.005 | |
| Indeno[1,2,3-ghi]pyrene | 0.3 | 0.005 | |
| Indene | _ | 0.3 | |
| Chrysene | | | 0.002 |
| Benzo(k)fluoranthene | | | 0.002 |
| Dibenz (a,h) anthracene | | | 0.003 |
| Indenol (1,2,3-c,d)pyrene | | | 0.004 |

### 1.1.3 Significance of the study

The PAHs are often contaminants of major concern due to their chemical and toxicological properties. Composed of numerous aromatic rings, PAHs tend to be highly persistent in the environment, with rather high bioaccumulation toxicity (Expert Panel on Air Quality Standards (EPAQS), 2006). While PAHs may occur naturally in crude oil and ash from forest fires, they are usually found as products of incomplete combustion (EPAQS, 2006). The PAHs are normally found at facilities involved in cooking, and wood preservative (EPAQS, 2006). Researchers have conducted multiple risk measurements at these types of areas (Countway *et al*., 2003; Bayowa, 2014). Due to their accumulation in the food chain, PAHs often drive the eventual risks that are associated with exposures by ingestion of soil and animal products affected by emissions from hazardous environments. In conducting multiple human and ecological risk measures for these facilities, mostly Environmental Protection Agency (EPA) methods are applied, as is, or modified (Countway *et al*., 2003).

The PAHs are believed to be ubiquitous pollutants, which are widely distributed in the environment and with their final destination is usually in soil, sediments and the aquatic environment (Bayowa, 2014). Due to large production activities such as mining, power stations and industrial activities in the current study areas, there are several issues related to pollution and other petroleum associated practices. All these activities may possibly lead to the formation of PAHs as well as other petroleum compounds and pollutants into sediments and waterways. However, petroleum linked activities are not the only source of PAHs released into the environment. Other sources of PAHs may be from pyrogenic activities including municipal, industrial and commercial burning of fuel or hydrocarbons (Bayowa, 2014). Toxic PAHs from contaminated environment may easily enter in the food chain.

Little research work has been done in South Africa on the presence of PAHs in water and sediments (Cele, 2005; Sibiya, 2012; Nekhavhambe *et al*., 2014; Edokpayi *et al*., 2016). Determination and hazard assessment of PAHs in water and sediments in the vicinity of coalmines around Loskop Dam, Mpumalanga have been conducted by Seopela *et al*. (2016). The PAHs in South African sewage sludge samples have been determined by Cele (2005). The PAHs in rivers, surface runoff and sediments in Thohoyandou, Limpopo Province have been investigated by Nekhavhambe *et al*.

(2014). Lephalale area has large production activities such as coal mine and power stations situated near the Mokolo River, which could be the source of PAHs in the river while in the Seshego area, sewage leakage and domestic wastes next to the Blood River may be possible sources of PAHs in the river.

## 1.2 PROBLEM STATEMENT

The PAHs in rivers are thought to cause a human health risk *via* drinking water. They also, however, have a tendency to attach to particles in sediments. Some PAHs are known to be toxic to aquatic animals (Escartin and Porte, 1999). Generally, higher molecular weight PAHs tend to be more stable, persist in the environment longer and are more toxic. Exposure to ultraviolet light can increase toxicity of PAHs and increase toxicity to some aquatic species (Escartin and Porte, 1999). The most significant effect of PAHs toxicity to humans is to cause cancer. Increased incidences of lung and bladder cancers are associated with occupational exposure to PAHs (Nemirovskaya, 2007). Other non-cancer effects are not well understood, though they may include adverse effects on reproduction and on the immune system. The Limpopo Province has a number of rivers possibly receiving PAHs from rainfall runoff from parking lots, power stations, mines and scrap yards (Nekhavhambe *et al*., 2014). For example, in the Seshego area, there is sewage leakage, industrial and domestic wastes next to the Blood River as possible sources of PAHs in the river. In the Lephalale area, there are two power stations and one mine. The Grootegeluk mine produces 18.8 million tonnes (Mt) of final coal products annually. The Medupi power station produces 4 800 MegaWatts (MW) of power from burning of coal in the area (Maswuma *et al*., 2011). An air quality impact assessment has been conducted in Lephalale area due to concerns raised in the region regarding the elevated atmospheric pollutants (Nemirovskaya, 2007; Maswuma *et al*., 2011). The study revealed industries in the area were found to be increasing air pollution through emission of gases into the atmosphere. However, no studies have been conducted in Lephalale and Seshego area to determine the presence and levels of PAHs in the environment. Therefore, monitoring the levels of PAHs in the environment is essential, particularly because of their toxic nature. This study focus on assessing the levels of PAHs in water and

sediment samples collected from Mokolo River situated in Lephalale and Blood River, which is found in Seshego.

## 1.3 AIM AND OBJECTIVES

### 1.3.1 Aim

The aim of this study was to investigate the residual levels of 16 USEPA priority PAHs in water and sediment samples collected from selected sites along Mokolo and Blood Rivers, Limpopo Province.

### 1.3.2 Objectives

The objectives of this study were to:

I. determine the levels of 16 USEPA priority PAHs in the water and sediment samples using gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionisation detector (GC-FID).
II. develop liquid-liquid extraction (LLE) procedure for the determination of PAHs in water samples.
III. optimise a microwave-assisted extraction (MAE) procedure for extracting water insoluble, or slightly water soluble, PAHs from sediments.
IV. develop ultrasonic and mechanical shaking extraction methods for the extraction of PAHs from sediments.
V. validate methods by analysing standard reference materials of sediments.
VI. evaluate the associated health risks by comparing with maximum permissible levels of PAHs in water and sediments.
VII. carry out source identification of PAHs in sediments in the study areas
VIII. evaluate the level of toxicity of PAHs in sediments based on Sediment Quality Guidelines (SQGs), Benzo(a)pyrene equivalent (BaPE) and Toxic equivalency factors (TEFs).

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 INTRODUCTION

In this chapter a literature review of PAHs is presented, focusing mainly on sediments and water. Extraction techniques used in sample preparation are also reviewed. Analytical techniques used for the determination of PAHs in environmental samples are also discussed with more attention given to GC-MS and GC-FID.

## 2.2 CHEMISTRY OF POLYCYCLIC AROMATIC HYDROCARBONS

The PAHs are a group of organic compounds consisting of two or more fused aromatic rings in a linear or cluster arrangement and do not contain a heteroatom (Doong and Lin, 2004; Emoyan *et al*., 2015; Chen *et al*., 2016 ). The PAHs are of major concern since these compounds are highly persistent, toxic (causing cancer) and wide spread environmental pollutants (Doong and Lin, 2004). The PAHs are normally discussed as a group because they are commonly found as mixtures of two or more compounds in the environment (Sibiya, 2012). It must be noted that, while PAHs are generally discussed as a group, the individual compounds are evaluated as separate compound in the risk characterisation. There are more than 100 chemicals in this family of compounds. However, a smaller number are regularly reported at disposal sites (Figure 2.1). The PAHs, which are mostly present at sites but are unreported, can result in the underestimation of potential risks (Andersson *et al*., 2002; Nemirovskaya, 2007).

The PAHs are relatively insolube in water, but they are highly lipophilic. Most of the PAHs with low vapour pressure in air are adsorbed onto particles. However when dissolved in water or adsorbed on particulate matter, they can undergo photodecomposition whenever exposed to ultraviolet light from solar radiation. In addition, PAHs in the atmosphere can react with pollutants such as nitrogen oxides and sulfur dioxide, nitro- and dinitro-PAHs, and sulfonic acids. The PAHs can also be

degraded by some microorganisms in the soil and sediment (Ghosh *et al*., 2000; Kafilzadeh *et al*., 2011).

### 2.2.1 Characteristics of polycyclic aromatic hydrocarbons

The PAHs usually exist as colourless, white or pale yellow solids at a room temperature. The general characteristics of PAHs are high melting and boiling points, low vapour pressure and very low aqueous solubility, which both normally tend to decrease with increasing molecular weight. These are highly lipophilic compounds and therefore, they tend to be very soluble in several organic solvents (Zhang *et al*., 2010).

Both physical and chemical characteristics of PAHs differ with molecular weight. For instance, PAH resistance to reduction, oxidation, and vaporisation increases with increasing molecular weight, however the aqueous solubility of these compounds decreases. In addition, PAHs vary in their behaviour, distribution in the environment, and their impact on biological systems (Kafilzadeh *et al*., 2011; Salih *et al*., 2015). The PAHs can be grouped into two groups according to their physical, chemical, and biological characteristics. Generally, the lower molecular weight PAHs (2 to 3 ring group of PAHs such as naphthalenes, fluorenes, phenanthrenes, and anthracenes) cause acute toxicity to aquatic organisms, while the high molecular weight PAHs, 4 to 7 ring do not. Yet, various members of the high molecular weight PAHs have been known to be carcinogenic (Escartin and Porte, 1999; Zhang *et al*., 2010).

The PAHs are considered to be chemically stable and are also poorly hydrolysed; they are non-polar organic substances. All this could be due to their highly hydrophobic nature and lipophilic characteristics. Several studies have shown that the biochemical persistence of PAHs is influenced by the presence of a dense cloud of pi electrons on both sides of the patterned structure making them highly resistant to nucleophilic attack (Mrozik *et al*., 2003; Salih *et al*., 2015; Gupte *et al*., 2016). Some of the PAHs, such as chrysene, benzo(a)anthracene and benzo(a)fluoranthene, have been found to be vulnerable to oxidation and photo degradation in aqueous environments (Salih *et al*., 2015). This characteristic is determined by the substrates to which they are attached. Other studies conducted on microbial action on PAH found that microbial biodegradation of PAHs is fast for the lower molecular weight compounds such as

naphthalene and phenanthrene, whereas the higher molecular weight fractions such as chrysene and benzo(a)pyrene strongly resist biodegradation by microbes in sediments (Zhang *et al*., 2010; Salih *et al*., 2015). The 16 priority PAHs in USEPA's list are given in Figure 2.1.

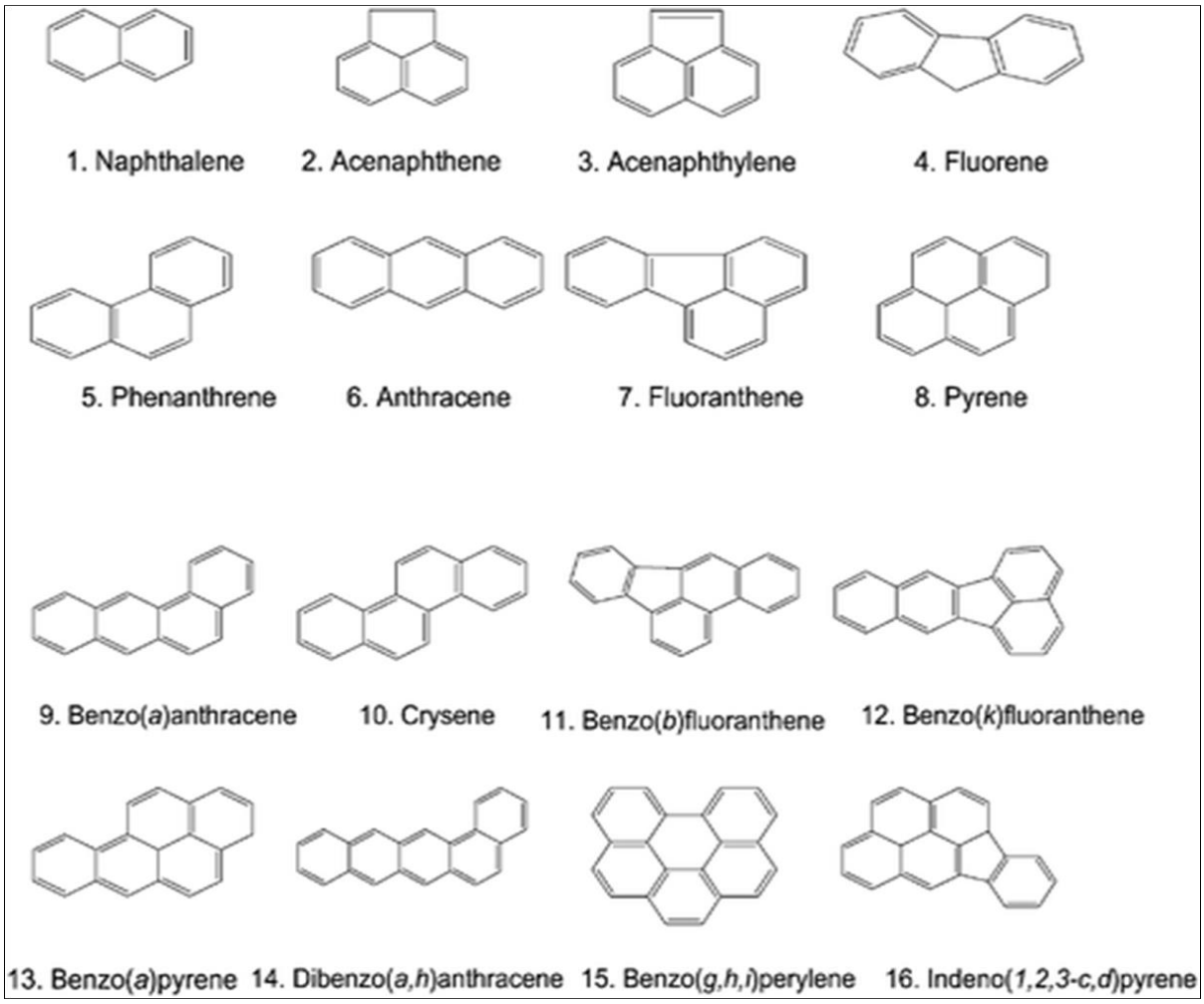



Figure 2.1: The 16 USEPA priority list of PAHs (Yan *et al*., 2004)

### 2.2.2 Formation of polycyclic aromatic hydrocarbons

The PAHs can be formed in several ways in the environment. These include high temperature pyrolysis of organic compounds, low to moderate temperature of sedimentary organic compounds to form fossil fuel, and direct biosynthesis by microbes and plants (Bayowa, 2014; Salih *et al*., 2015). The PAHs are not produced intentionally in the environment, however, they are the by-products of incomplete

organic combustion that come from sources that are increasing because of human activities such as burning or cooking, industrial or vehicular fumes from diesel and petroleum engines. These sources of PAHs are referred to as anthropogenic. The PAHs may also be produced naturally from forest fires and volcanoes. Various PAHs, such as benzo(a)pyrene, were also found to originate from petroleum hydrocarbons in the environment because of accidental or intentional release of petroleum products. The PAHs that are found in the environment in different concentrations are classified into two groups according to their origin, namely pyrogenic and petrogenic (Yunker *et al*., 2002; Salih *et al*., 2015).

## 2.3 SOURCES OF POLYCYCLIC AROMATIC HYDROCARBONS

Hundreds of PAHs are present in environmental mixtures and their sources can therefore, be associated with almost everything in the surrounding environment (Bayowa, 2014). Sources of PAHs in the environment could be divided into natural and anthropogenic sources.

### 2.3.1 Natural sources

Natural sources of PAHs are associated with forest fires and agricultural burning, which contribute the largest concentration of PAHs from a natural source to the environment. The amount of PAHs emitted from these sources varies with the type of organic material burned and type of fire. The PAHs from forest fires tend to sorb to suspended particulates and enter the terrestrial and aquatic environments as atmospheric fallout (Bayowa, 2014).

Another natural source of PAHs occurs in bituminous fossil fuels, such as coal and crude oil deposits, as a result of diagenesis. An example is the low temperature (about 100-150 °C) combustion of organic material over a significant span of time. This process of diagenesis usually favours the formation of alkylated PAHs and the unsubstituted compounds are relatively low in abundance when originating from these sources. Under natural conditions, fossil fuels generally contribute a relatively small amount of PAHs to the environment (Bayowa, 2014). Because most oil deposits are usually trapped beneath layers of rock, the chances are very small of PAHs being

emitted to the surface environment. There are, however, several numbers of petroleum bodies which are capable of contributing PAHs to both atmospheric and aquatic surroundings. These deposits are small and are likely to contribute very little to the overall volume of PAHs in the environment (Jeffrey *et al*., 2007).

Other natural sources of PAHs include volcanic activity and biosynthesis by bacteria and plants. Relative to fires, these sources generally contribute only a small volume of PAHs to the environment. There is still some uncertainty as to whether or not biosynthesis of PAHs in fungi and bacteria is actually occurring or levels of PAHs in these organisms have been acquired from other sources. More experimental techniques and equipment are required to resolve this question (Bayowa, 2014).

### 2.3.2 Anthropogenic sources

Anthropogenic activities have to do with processes, objects, or materials that are derived from human activities, as opposed to those occurring in natural environments without the influence of humans. Anthropogenic sources of PAHs can be further divided into different groups (Samanta *et al*., 2002).

Incomplete combustion of organic materials at high temperature is one of the major anthropogenic sources of PAHs. Studies revealed that there are many other anthropogenic sources of pyrolytic PAHs (Samanta *et al*., 2002; Zhang *et al*., 2010; Kafilzadeh *et al*., 2011; Kafilzadeh, 2015). Any industrial or domestic process in which organic matter is subjected to a high temperature will result in production of PAHs. Recently, treated wood has also been revealed as a minor source of PAHs in water and sediments. Anthropogenic sources can be classified into two categories: sources that can discharge into the atmosphere, and sources that can discharge directly into the body of water. Some of the sources of PAHs, which may discharge directly into aquatic environment include accidental spillage, leakage of PAH-containing fluid, industrial and domestic wastewaters and discharges originating from landfills (Yunker *et al*., 2002; Zhang *et al*., 2010; Bayowa, 2014).

Atmospheric PAH emissions are also divided into two groups: those which originate from non-stationary sources, and those which originate from stationary sources. Stationary sources include coal and gas-fired boilers; coal gasification and catalytic

cracking towers and any other industry that uses wood, petroleum or coal for generating power and heat. These sources contribute large amount of PAHs to the environment and this occurs through the formation of these organic substances during industrial processing or through pyrolysis of the above mentioned fuels for energy generation (Samanta *et al*., 2002; Nguyen *et al*., 2004). These PAHs are combined onto particulates in the air and are then deposited into bodies of water and the surrounding environment (Nguyen *et al*., 2004).

Non-stationary sources of PAHs are associated with automobiles or any other vehicles which use petroleum products as a fuel. Usually temperatures within an internal combustion of engine are usually sufficient to convert a fraction of the fuel or oil into PAHs *via* pyrolysis. These PAHs are then emitted to the atmosphere through exhaust fumes where they sorb onto particulates (Doong and Lin, 2004). Most of the PAHs are then deposited into the environment. Precipitation then washes these PAHs into storm water drainage systems and flushes them into the aquatic environment (Ghosh *et al*., 2001; Samanta *et al*., 2002).

#### *2.3.2.1 Petrogenic sources*

Petrogenic sources are associated with petroleum, including crude oil, fuels and their derivatives. Petroleum is known to be a complex mixture of different organic compounds formed under geological conditions. Petrogenic PAHs are introduced into the aquatic environment through accidental oil spills and municipal and urban runoff. Studies revealed that there has been no observations of common and continuous input of petrogenic PAHs (Guo *et al*., 2007; Jeffrey *et al*., 2007).

Petrogenic PAHs from petroleum sources are found to consist mostly of two to three rings, which are low molecular weight compounds. The higher molecular weight fractions are normally at low concentration less than 100 mg/kg. The products include the same PAHs as in the parent petroleum as well as small amounts that may be produced by catalytic cracking and other refining processes (Nguyen *et al*., 2004). The PAHs found in different refined oils differ, based on the distillation temperature range of the product; for example, the two ringed PAH, naphthalene, is available in gasoline fuels whereas diesel fuels, home heating oils and engine oils can contain four ringed PAHs as well as other different aromatic hydrocarbons (Chen *et al*., 2004).

#### *2.3.2.2 Pyrogenic sources*

Pyrogenic PAHs are associated with incomplete combustion of organic material. Combustion is complete when the application of heat breaks up molecules with the production of carbon dioxide and water. However, when combustion is incomplete, the small organic compounds may condense until new compounds, which may include PAHs, are formed (Chen *et al*., 2004; Bayowa, 2014). Examples of human activities that usually generate PAHs from pyrogenic source are: residential or commercial burning or cooking and industrial or vehicular exhaust from diesel and petroleum engines. The PAHs from pyrogenic sources are normally complex and are mostly dominated by four to five and six rings. The rings generally have their homologous series controlled by the un-alkylated parent compound or sometimes they may contain a homologue with only one or two alkyl substituents (Kafilzadeh *et al*., 2011; Bayowa, 2014).

## 2.4 SOURCE IDENTIFICATION AND APPORTIONMENT

During the identification and apportionment of pollutant sources from the environment, several basic approaches may be used. The two efficient techniques used are Receptor models and Diagnostic ratios (Li and Kamens, 1993; Yunker *et al.*, 2002; Xue *et al*., 2008). The ratios of different PAHs are normally expected to differ depending on the source because of the various conditions in which the PAHs are formed. Receptor models assess contributions from all the major sources according to their observations at sampling sites, and have been mostly employed in source apportionment (Li and Kamens, 1993; Neff *et al*., 2005). Factor analysis with nonnegative constraints (FA-NNC), is known to be one of the advanced receptor models, and has been successfully applied to quantitative identification of organic pollutants in most of the environmental media, including sediments and soils.

Afshar-Mohajer *et al*. (2016) conducted source apportionment of atmospheric PAHs in Palm Beach County, Florida. This study, assessed contributions of major sources of PAHs. Most of the benzene rings were found to be the key parameter in determining the major source. In addition, they found that mobile vehicle sources contributed species with four or less benzene rings, whereas the burning of sugarcane contributed

mainly compounds containing five or more aromatic rings. Results obtained in the same study support more control in the burning of sugarcane and plans to restrict transportation to limit PAH emissions from mobile vehicles (Afshar-Mohajer *et al*., 2016).

Different relationships between PAH compounds have been proved to exist that may indicate information about the procedures that the hydrocarbons have undergone (Li *et al*., 2012). Gas chromatography-mass spectrometry provides data about the relative abundance of organic compounds in a sample, which can then be used to characterise the likely major sources of PAHs in a study area. The aromatic rings that define PAHs may often carry alkylated substituents (Chen *et al*., 2012). The levels of these PAHs can be compared against their unalkylated parent compounds. Several numbers of alkylated PAHs are more common than the parent compounds in petrogenic samples, and less common than the parent compounds in pyrogenic compounds (Kennicutt *et al*., 1994; Mccready *et al*., 2000; Chen *et al*., 2012).

The ratio of high molecular weight (HMW) compounds to low molecular weight compounds (LMW) may indicate the source, since LMW compounds are more common in samples containing petrogenic PAHs and HMW compounds are more common in samples containing pyrogenic PAHs, as most of the HMW molecules are formed at higher temperatures (Li *et al*., 2012; Liu *et al*., 2009). The representative global PAHs profile is usually identified by an abundance of high molecular weight PAHs from high temperature combustion operations (Ma *et al*., 2010). The diagnostic ratio is a most useful tool for the source identification of PAHs in sediment samples (Topal, 2011). Some studies showed that PAHs in sediments with the ratio of fluoranthene to fluoranthene plus pyrene; Fln/(Fln + Pyr) less than 0.4 means petroleum contamination, while Fln/(Fln + Pyr) greater than 0.5 means PAHs are mostly from combustion of grass, wood and coal and $0.4 < Fln/(Fln + Pyr) < 0.5$ from combustion of petroleum. Furthermore, they also showed that the ratio of anthracene to anthracene plus phenanthrene; Ant/ (Ant + Phe) < 0.1 were mostly from petrogenic source, while those with Ant/(Ant + Phe) > 0.1 were mostly from pyrogenic source (Topal, 2011; Nasher *et al*., 2013; Aly Salem *et al*., 2014). Table 2.1 shows the range of diagnostic ratios for PAHs sources (pyrogenic and petrogenic origins of PAHs).

Table 2.1: The range of diagnostic ratios for PAH sources, (Topal, 2011)

| | (LMW PAHs) / (HMW PAHs) | Fln /(Fln+Pyr) | Ant /(Phe+Ant) |
|---|---|---|---|
| **Pyrolytic origin** | <1 | >0.4 | >0.1 |
| **Petrogenic origin** | >1 | <0.4 | <0.1 |

Individual work by Rogers (2002) showed that the ratio of phenanthrene/anthracene (Phe/ Ant) plotted against that of fluorene/pyrene (flu/pyr) can be used to indicate whether PAHs have petrogenic or pyrogenic origins. Stark *et al*. (2003) proved that due to the higher solubility in water of phenanthrene than chrysene, weathering may cause the ratio between them to differ. Samples in which the ratio of phenanthrene to chrysene does not differ have not been subjected to weathering. In addition, Walker *et al.* (2005) also compared this ratio to the ratios of other analytes that have not been assessed in this study, and established this ratio's suitability for differentiation between PAH sources in various locations. The PAHs with petrogenic sources have relatively low fluoranthene/pyrene ratios and high phenanthrene/anthracene ratios while pyrogenic sources generate PAHs with higher fluoranthene/pyrene ratios and lower phenanthrene/anthracene ratios (Li *et al*., 2003; stark *et al*., 2003; Walker *et al*., 2005).

McRae *et al*. (2000) demonstrated that high levels of pyrene, fluoranthene and fluorene and average levels of benzo[b]fluoranthene and indeno[1,2,3-cd]pyrene are associated with the combustion of oil. High levels of pyrene, fluoranthene and phenanthrene are normally associated with incineration (McRae *et al*., 2000). The 16 priority PAHs studied do not generally give enough detailed PAHs distribution data to allow conclusive links between specific PAHs sources and the analysed samples, their main value is in their ability to produce an estimate of the PAHs concentrations (Ravindra *et al*., 2000). Measuring the stable isotope ratios of the PAHs can give far greater details about the major source (Dong and Lee, 2009).

## 2.5 POLYCYCLIC AROMATIC HYDROCARBONS AS ENVIRONMENTAL POLLUTANTS

The PAHs are widely dispersed and settled in the environment as a result of the incomplete combustion of organic matter. Most PAHs are highly toxic, mutagenic and carcinogenic to microorganisms as well as humans. The PAHs are also known to persist in the environment for a long time (Chen *et al*., 2007; Mirsadeghi *et al*., 2011). These compounds are reported to be the most toxic pollutants among the hydrocarbon families (Nemirovskaya, 2007). Therefore, PAHs are considered to be environmental pollutants that can have a harmful effect on animals, microorganism and humans, resulting in the accumulation of toxic substances in the food chain and in some instances, in serious health problems and genetic disorder (Mirsadeghi *et al*., 2011). The PAHs are hardly encountered alone in the environment and most interactions occur with a mixture of PAHs whereby the efficiency of known carcinogenic PAHs can be enhanced (Nemirovskaya, 2007). For example, 1-nitropyrene, a nitrated PAH, is produced during reactions between ketones, the product of burning automobile fuel and airborne nitrogen oxides that take place on the surface of hydrocarbon particles in diesel exhausts (Salih *et al*., 2015).

### 2.5.1 Polycyclic aromatic hydrocarbons in water

Increases in the urban population and industrial development pose major consequences to surrounding water bodies like lakes, rivers and ground water (Song *et al*., 2005; Srogi, 2007a). Understanding the sources, pathways and fate of contaminants in the urban environment is very important for making informed management decisions. Urban areas are referred to as major concentrators and emitters of many of chemicals or substances such as PAHs due to the wide range and intensity of human activities and the characteristics of the built environment (Trapido, 1999; Song *et al*., 2005). Most of the land surface in urban areas is impermeable, covered by buildings and pavement, which prevents rain and snowmelt to soak into the ground; therefore contributing to the increase of runoff. Rainfall in urban areas is converted into urban runoff, which is transported by drainage channels, streams and sewers and ultimately discharged to receiving waters (Srogi, 2007a). Urban runoff discharges may cause physical, chemical, biological and combined impacts on

receiving waters, either of an acute or growing nature and seriously harm water uses in many locations (Tolosa *et al*., 2004; Song *et al*., 2005).

The PAHs enter rivers or surface water mostly through atmospheric fallout, municipal effluents, industrial effluents and oil spillage. Atmospheric fallout may include dry and wet deposition of particles (Srogi, 2007b; Nekhavhambe *et al*., 2014). The PAHs, considered as semi volatile organic compounds, usually exist in the gaseous and the particulate phase in air, and are subject to both vapour and particle washout from the atmosphere mainly during precipitation (Trapido, 1999). Atmospheric deposition is regarded to be an essential input of PAHs to rivers. In addition, it has been estimated that approximately 10–80% of PAHs inputs to the world's oceans are mainly from atmospheric sources (Srogi, 2007a). As a result, urban runoff carries PAHs deposited on surfaces and also mobile-related PAHs from oil drips or spills, tyre particles, and bitumen from road surfaces. A study by Srogi (2007a) demonstrated that higher concentrations of PAHs in urban runoff were found during autumn and winter, because of the high incidence of vehicles in the streets, coupled with the use of heating systems.

The PAHs have low solubility and tend to adsorb to particulate matter, they are mostly found in low concentrations in water bodies (WHO, 2011). Some of the levels of PAHs that have been assessed in water include: marine waters with the levels of non-detected to 11 µg/L and wastewater with levels between <1 and 625 µg/L in European municipalities and North American (WHO, 2011). In South Africa, concentrations of PAHs in surface water around Thohoyandou were measured by Nekhavhambe *et al*. (2014), the level of PAHs ranged between 0.1 and 137 µg/L. The levels of PAHs contamination in water was also determined by Seopela *et al*, (2016) from the Loskop Dam and its tributaries, the concentration in water samples ranged from 1.17 to 14.5 µg/L.

World Health Organization carried out a study in 1997 and reported that the concentration of individual PAHs in surface and coastal waters is 0.05 µg/L and concentration above this point indicates some contamination (WHO, 2003a). Again studies carried out in the United States of America (USA), in four major cities indicated that the total PAHs in drinking water ranged between 4.7 and 600 µg/L and high molecular mass PAHs such as benzo(ghi)perylene, dibenzo(a,h)anthracene and

indeno(1,2,3-cd)pyrene were not detected in the water samples (WHO, 1998; Srogi, 2007b). This could be due to their low solubility in water.

Apart from emission sources, concentrations of PAHs in water also depend on the depth in the sediment core. According to a study carried out by Srogi (2007b), a range of PAHs at different concentrations were found in groundwater samples. Higher concentrations of four-ring compounds were found and the major contributor to this was fluoranthene and pyrene. Naphthalene also dominated in many samples. Total PAH concentrations in groundwater differed widely with depth in the sediment core and several regions of high concentration can be recognised, the highest (742 µg/L) occurred between 50 and 52.5 cm (Srogi, 2007b).

### 2.5.2 Polycyclic aromatic hydrocarbons in sediments

The PAHs tend to accumulate mostly in sediments rather than water (Tripathi *et al*., 2009; Jiao *et al*., 2011; Li *et al*., 2016; Sun *et al*., 2016). Concentrations of PAHs in particular in sediments can range from µg/kg to g/kg depending on the proximity of the area to PAHs source such as industries and municipalities (Kwach and Lalah, 2009; Daso *et al*., 2016; Hu *et al*., 2016). Work by Chen *et al*. (2013) revealed that a measure of the presence of PAHs in soils and sediments could give an indication of the level of this pollutant in the environment. This is influenced mostly by the ability of PAHs to adsorb to dust particles and settle in sediments. Sediment core studies have demonstrated an increase in PAHs concentrations in the past 100-160 years with concentrations peaking in 1950 (Guo *et al*., 2007).

Generally higher molecular weight PAHs, which are hydrophobic compounds and have less solubility in water tend to settle mostly in sediments and can be dissolved in various oily contaminants (Chen *et al*., 2004; Zhao *et al*., 2012). In North America, the total level of PAH in marine sediments ranges from 2.17 to 170 000 ng/g (Wu *et al*., 2012). The concentrations of PAHs in sediments from Nzhelele, Mutshundudi, Mutale, Dzindi and Luvuvhu Rivers, Venda, South Africa ranged from 17.9 to 9 870 µg/kg as reported by Nekhavhambe *et al*. (2014). Seopela *et al.* (2016) carried out a study in Loskop Dam finding the levels of PAHs in sediments ranging between 292 and 2 170 µg/kg.

A study by Rhea *et al*. (2005) from Lakes in Grant Teton National Park, Wyoming revealed that concentrations of PAHs in sediment samples were consistently larger than in water samples, but again varied among sampling events and locations. The study further demonstrated that in August 2001, total detectable PAHs in sediment samples from marina sites in Jackson Lake ranged from 127 to 169 ng/g, while concentrations at non-marina sites ranged from 18 to 311 ng/g. Concentrations of total detectable PAHs in sediment samples collected in Jenny and Taggart Lakes were 19 and 128 ng/g, respectively. Total detectable PAHs in sediment samples from marina sites in Jackson Lake ranged from 8.0 to 19 ng/g in 2002 and from 32 to 47 ng/g in 2003, but then sediment samples collected at non-marina sites in Jackson Lake contained detectable PAH concentrations that ranged from 40 to 471 ng/g in 2002 and from 41 to 274 ng/g in 2003. Total detectable PAHs in sediment samples collected from Jenny Lake in 2002 ranged between 66 and 139 ng/g and were found to be 39 ng/g in 2003. Unlike water samples, a large proportion of the detectable PAH compositions were observed in sediment samples (Rhea *et al*, 2005).

Soclo *et al*. (2000) carried out a study of coastal marine sediments collected from Cotonou (Benin) and Aquitaine (France). The greatest pollution levels were observed for sediments sampled in stations IIB and VIF revealing the harbours of Cotonou (1 410 ng/g) and Verdon (853 ng/g) as being the most contaminated by PAHs among all the studied areas. The surprising part was that, most of the sediment samples collected inside Cotonou harbour (1 205 to 1 411 ng/g) were found to be polluted to a greater extent than those that were sampled in the Bordeaux and Verdon harbours (respectively 491 and 853 ng/g), in spite of many shipping activities registered in the eventual sampling stations (Soclo *et al*., 2000).

The ability of lake sediment cores to store long-term anthropogenic pollution establishes them as natural archives. Work by Warner *et al*. (2016) focused on the influence of smelting and copper shale mining in the Mansfield area of Germany, using mainly the depth profiles of two sediment cores from Lake Suber See. The sediment cores distribute a detailed chronological deposition history of PAHs in the studied area. Further study revealed that both sediment cores are dominated by fluoranthene and PAHs compounds with four aromatic rings (Warner *et al*., 2016).

Hussain *et al*. (2016) investigated effects of different seasons on the residual characteristics and ecological risk of PAHs in sediments from Changdang Lake, China. The study demonstrated that the highest average value of PAHs was 295.28 ng/g in March, 240.91 ng/g in June and 165.81 ng/g in September. Source identification studies based on the analysis of diagnostic ratio suggested that the PAHs in sediments from Changdang Lake were mostly from the mixed combustion source of biomass and petroleum, and the origins of PAHs in different sampling areas have a great deal of temporal and spatial variability during different seasons (Hussain *et al*., 2016).

The bioaccumulation of PAHs from surface sediment into benthic organisms was predicted by Li *et al*. (2016) from a study of Bohai Sea, China. Source contributions to PAHs related toxicity and health risks from the intake of PAHs-contaminated benthic organisms were evaluated based on Positive Matrix Factorisation (PMF) model and Monte Carlo simulation, respectively (Saraga *et al*., 2010). The total concentrations of PAHs ranged between 149.40 and 1211.97 ng/g in sediments of Bohai Sea (BS). Source identification showed that petroleum and vehicular emission, coal combustion and coke ovens constituted 40.0%, 32.2% and 27.8% of PAHs, respectively, but contributed 53.0%, 22.8% and 24.2% of toxicity caused by PAHs in sediment (Li *et al*., 2016).

The quantification of 14 PAHs was done in sediment samples collected from Akaki River, Lake Awassa, and Lake Ziway, Ethiopia by Mekonnen *et al*. (2015). In samples from Akaki River, Lake Awassa, and Lake Ziway, the total content of PAHs evaluated ranged from 0 to 3 070 ng/g, 24.9 to 413 ng/g and 15.0 to 305 ng/g, respectively. In addition, the accuracy of the extraction method employed was determined by extracting and analysing New York/New Jersey waterway sediment standard reference material (SRM 1944). The assessed concentrations of PAHs in SRM 1944 agreed well with the certified values. Source characterisation indicated that the PAHs were mostly from petrogenic origin. Sediments from all sampling areas indicated insignificant levels of toxicity with no risk of adverse biological effects (Mekonnen *et al*., 2015).

### 2.5.3 Polycyclic aromatic hydrocarbons in air

The PAHs are deposited into water, sediment, soil and biological resources through the atmosphere. Wide ranges of atmospheric PAHs concentrations have been measured, with the highest concentrations occurring in urban areas. Levels of PAHs are higher in urban areas due to the heavy traffic and diesel engines used (Mehdinia *et al*., 2015). The PAHs levels are also higher where coal, oil, tires, or agricultural crops are burned. In addition, workplace exposures with higher levels of PAHs in the air may include: coal tar production, coal cooking and smokehouse operations (Mehdinia *et al*., 2015). Atmospheric levels of PAHs are usually higher in winter because of combustion products from heating and reduced thermal- and photo-decomposition (Chen *et al*., 2013). Smoking of cigarettes also increases personal exposure to PAHs daily. In North America, levels of PAHs in the air ranged between 3.7 and 450 ng/m$^3$. Phenanthrene, fluoranthene and pyrene dominate the atmospheric PAHs profile (Mehdinia *et al*., 2015).

The PAHs in the atmosphere are present in the gaseous phase or combined with particulates and tend to condense onto particles at low temperature (Mehdinia *et al*., 2015). At normal temperatures, most atmospheric PAHs are found in the particulate phase. The partitioning of PAHs into gas and particulate phases can also depend on the vapour pressure of the specific PAH (Guo *et al*., 2007). The fate of atmospheric PAHs is influenced by whether the PAHs are in the gaseous or particulate form (Zhang *et al*., 2010).

### 2.5.4 Polycyclic aromatic hydrocarbons in soil

Accumulation of PAHs in soils without direct industrial contamination is considered to be caused by atmospheric deposition after long-range transport. Usually forest fires and airborne pollution deposition are found to be main source of soil PAHs in the environment. The PAHs levels of soil resulting from natural processes are estimated to be in the range of 1 to 10 µg/kg (Wick *et al*., 2011; Han *et al*., 2015). Work done by Wick *et al*. (2011) revealed a total PAH concentration of 0.1 to 55 mg/kg in Welsh soils that resulted from atmospheric deposition with no direct industrial pollution.

Levels of PAHs in soils have increased in the past 100 to 150 years because of rowing industrial activities. Concentrations of PAHs in urban industrial soils can be 10 to 90 times higher than in remote soils (Wick *et al*., 2011). Soils that are found in industrial sites, their PAHs concentrations and type of PAHs differ depending on the type of industry. For example, studies reported total PAH concentrations of 5 863 mg/kg at a creosote production site, 18 704 mg/kg at a wood preserving site, 821 mg/kg at a petrochemical site, and lastly 451 mg/kg in a gas manufacturing plant site (Wick *et al*., 2011). The main pathway of PAH loss in soil is believed to be influenced by degradation through microbial metabolism. Both physical and chemical properties of the particular PAH being degraded can affect this process, including some of the environmental factors such as soil temperature, pH, and oxygen concentration (Wick *et al*., 2011).

### 2.5.5 Polycyclic aromatic hydrocarbons in plants

The PAHs are accumulated in vegetation mainly through atmospheric fallout on and uptake by above ground parts of the plant (Nguyen *et al*., 2004). Concentrations of PAHs in plant tissue in non-industrialised regions usually ranges from 50 to 80 µg/kg (Samanta *et al*., 2002), whilst specific plant tissue concentrations can also depend on plant species, type of PAH, and environmental conditions (Guo *et al*., 2007). Vegetation found in urban areas can have up to 10 times higher PAH levels than rural vegetation and this can be influenced by types of different industrial activities found in urban areas. Work by Samanta *et al*. (2002) found that PAHs can usually be adsorbed into the plant roots, but translocation to the above-ground parts was not likely because plants are not able to transport hydrophobic compounds such as PAHs in xylem.

The exposure to PAHs from food sources is found almost everywhere. The occurrence of PAHs in food is mainly due to either processing techniques or entry into the food chain when either crops or plants are grown in contaminated soil. They can also originate from marine life or fish that live in contaminated water (Huang and Penning, 2014). The risk of cancer has been widely considered as the most essential health concern associated with PAH-contaminated food. A toxicological study revealed that several PAHs can also produce mutagenic, reproductive and neurologic effects (Purcaro, 2015). It is usually hard to effectively reduce the levels of PAHs in food and

their associated health risks because of their ubiquitous nature. Therefore, measures such as the instruction and education of manufacturers and consumers to control PAHs emissions into the environment, and enforcement of safe-limits of PAHs in foods can reduce their intake and health risk (Huang and Penning, 2014; Purcaro, 2015).

### 2.5.6 Polycyclic aromatic hydrocarbons in animals

Researchers have revealed that there are increased incidences of skin, lung, bladder and stomach cancers, also injection-site sarcomas, in animals caused by PAHs compounds (Martinez *et al*., 2004; Wick *et al*., 2011; Leon *et al*., 2014). A study of animals (rodents) showed that some of PAHs can also affect the hematopoietic and immune systems and may have reproductive, neurologic, and developmental effects (Wick *et al*., 2011). Some effects that may be caused are explained below.

## 2.6 POLYCYCLIC AROMATIC HYDROCARBONS IN HUMANS

Humans are usually exposed to PAHs through several ways, namely inhalation of air and re-suspended dust and soil, consumption of water and food, and dermal contact with soil. These sources are relevant to global human exposure. However soil contact generally occurs outdoors. Food and water consumption is usually indoors, inhalation leads to exposure both indoors and outdoors. People spent 60–90% of their time indoors, and hence indoor air can be the most relevant source of PAHs contributing to the inhalation route (Zhang *et al*., 2010).

The PAHs can enter the human body through lungs when air containing these compounds is breathed. Wood, coal and cigarette smoke, and smoke from many different industrial sites may contain PAHs. People living near hazardous waste sites are also likely to be exposed through breathing air containing PAHs. However, it is not really known how rapidly or completely lungs can absorb PAHs (Nguyen *et al*., 2004). Drinking water and swallowing food, soil, or dust particles that contain PAHs are other ways for these chemicals to enter the human body, but absorption is normally slow when PAHs are swallowed. Under normal conditions of environmental exposure, PAHs could enter the human body if the skin comes into contact with soil that contains high levels of PAHs (Chen *et al*., 2013).

The rate at which PAHs can enter the human body by eating, drinking, or through the skin can be affected by the presence of other types of compounds that cause exposure to at the same time the PAHs. The PAHs may enter all the tissues of the human body that contain fat. They tend to be stored mostly in kidneys, liver, and fat. Smaller amounts are stored in adrenal glands and ovaries. The PAHs are changed by tissues in the body into many different substances. Some of these substances can be more or less harmful than the original PAHs. Results from an animal study showed that PAHs do not tend to be stored in the human body for a long time. Most PAHs that can enter the body leave within a few days, primarily in urine (Leon *et al*., 2014).

The PAHs can be harmful to human health under different circumstances. Several types of PAHs, including benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene, which are mostly high molecular weight PAHs, have caused tumors in laboratory animals when inhaled. The tumours also occur when eaten with food or through long skin contact (Martinez *et al*., 2004). Studies using animals have also shown that PAHs can also have harmful effects on body fluids, skin and the body's immune system after both long and short-term exposure. These effects have not yet been reported in people (Martinez *et al*., 2004).

## 2.7 HOW TO REDUCE PAH EXPOSURE

Public awareness of potential PAH exposure through different kinds of activities such as recreational, hobbies and home scenarios may reduce PAH exposure. Usually cigarette smoke contains PAHs and other carcinogenic substances. Exposure to PAHs by smoking cigarettes or passive smoking may increase the risk of exposure to PAHs and PAH-related diseases (ATSDR, 1995). In addition, there are some foods that contain PAHs. Reducing consumption of chargrilled, smoked meats and fish may all reduce exposure to PAHs. The exposure to PAHs might be minimised by always wearing gloves when working with cutting oils, washing immediately after coming in contact with products or contaminated soils containing PAHs, and lastly, by avoiding smoke from campfires. Produce grown in contaminated soil must be washed before consumption and root vegetables should be washed and peeled (ATSDR, 1995).

## 2.8 METABOLISM OF POLYCYCLIC AROMATIC HYDROCARBONS

Exposure to PAHs is never to a single PAH. Thus, understanding what major differences may occur in mixtures of PAHs can give an accurate assessment of their danger. Moreover, understanding the dynamics of complex single metabolism of PAHs and possible effects on the toxicity expression of PAHs is an essential advancement to the exact impact and direct remediation strategies (Chavan and Krishnamurthy, 2012; Leon *et al*., 2014).

The PAHs require a multistep metabolic activation by specific enzymes. The enzyme system that is responsible for PAHs metabolism is the mixed-function oxidase system that requires nicotinamide adenine dinucleotide (NAD) +hydrogen (H) or nicotinamide adenine dinucleotide phosphate (NADP) +hydrogen (H) and molecular oxygen to convert the nonpolar PAHs into polar hydroxy derivatives and arene oxides (figure 2.2). The first reaction is an epoxidation, with benzo(a)pyrene. The product is the corresponding 7, 8-epoxide that, in turn, is subject of epoxide hydrolases to form stereoisomeric dihydrodiols (Moorthy *et al*., 2015). These are converted further to the 7, 8-dihydrodiol-9, 10-epoxide. The terminal oxidase is cytochrome P-450 (CYP1A1). The diol epoxide exist in four stereoisomeric forms of which the key carcinogenic product is benzo(a)pyrene-r-7,t-8-diol-t-9,10-epoxide. The PAH epoxides can then be conjugated with glutathione. This type of conjugation is referred to as a true detoxification reaction and is mediated by glutathione transferase (GSTM1). Epoxides that are not conjugated with glutathione are converted into phenols and diols as mentioned above. These PAH metabolites are sometimes not sufficiently polar to be excreted and are therefore conjugated with sulfuric acids to enable excretion to occur. The hydroxylated derivatives of PAHs may undergo a number of oxidation and hydroxylation reactions. These usually include the conversion of phenols to phenol-epoxides and subsequently to diphenols and triols, diols to tetrols and diol-epoxides, and triols to triol-epoxides (Andersson *et al*., 2002; Moorthy *et al*., 2015). Benzo(a)pyrene as a model of PAHs metabolism given in Figure 2.2 (IARC, 1983).

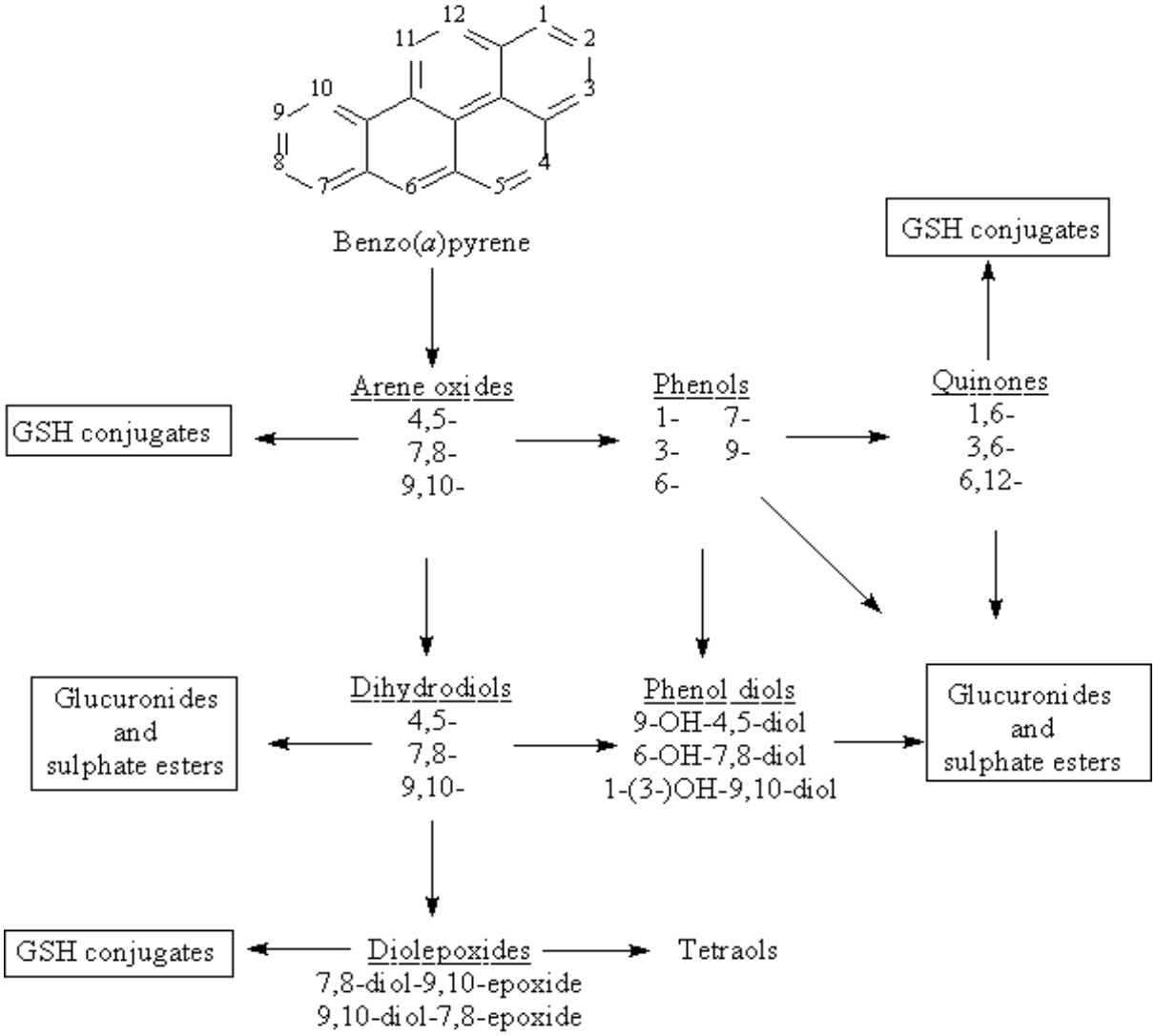



Figure 2.2: Benzo(a)pyrene as a model of PAHs metabolism (IARC, 1983)

## 2.9 ANALYTICAL METHODS FOR DETERMINING PAHs IN ENVIRONMENTAL SAMPLES

### 2.9.1 Sampling

Sampling was an essential part of this study because mistakes that are associated with sampling techniques and the storage of samples, as opposed to the handling of synthetic samples can raise major concerns in the efficiency of detection of PAHs. The method used to sample from a larger population mainly depends on the type of analysis performed during the study. Collection of appropriate water and sediment

samples that meet sampling objectives is important because it can lead to precise and accurate results (EPA, 2001; Zhu *et al*., 2008).

It is important to understand the sampling objectives when setting up a strategy for sampling water and sediments for PAHs analysis (Chen *et al*., 2007). The survey area should be carefully considered, always making sure it is appropriate to meet the survey objectives (EPA, 2001; Xia *et al*., 2013). The type of sample containers that may be used to store water or sediment samples after collection must be selected corresponding to the type of analysis planned. For instance, plastic and glass bottles of all different sizes corresponding to the materials to be analysed are brought to the site (EPA, 2001). The concentration of gases and some liquids can change if stored for a long time in polyethylene containers because they can pass through material. However, glass has the drawback that it can break easily, but it is suitable for storing organic substances such as PAHs. Usually amber colour glass bottles are most preferred for substances which breakdown under exposure to light such as pesticides and PAHs (EPA, 2001; Leon *et al*., 2014).

### 2.9.2 Extraction and concentration of polycyclic aromatic hydrocarbons from water and sediment samples

Extraction may be explained as taking out of something from something else (Xia *et al*., 2013). All the extraction methods selected in this study influence the accuracy of the results and also determine the total analysis time. In almost all cases, extraction may be used to separate ionic or polar low-molecular-weight substances into an aqueous phase and less polar water-insoluble substances into an immiscible liquid organic phase (Mahgoub, 2016). In addition, compounds can be extracted from solids or liquids using an aqueous or organic solvent. The PAHs are extracted from sediments and water in this study. Many different extraction techniques have been developed and applied for extracting PAHs from sediment and water samples. The extraction techniques to be described here include liquid-liquid extraction, Soxhlet extraction, ultrasonic and mechanical agitation, accelerated solvent extraction, solid-phase extraction and microwave-assisted extraction. Extraction methods and concentration techniques are explained in details below.

#### *2.9.2.1 Liquid-liquid extraction*

Liquid-liquid extraction (LLE) is a separation technique for a wide range of applications in chemical process industries. The most common method of LLE is performed using as separatory funnel (Koch and Shiveler, 2015). It is mostly used for extraction of water samples (Nikolaou *et al*., 2009). This extraction separates components according to their relative solubilities in two immiscible liquids. When the liquids are immiscible; this generally means that they will form two layers when they are together, like oil and water (Yates *et al*., 2013). Some of the compounds are more soluble in the organic layer, which is often an oil while some compounds are more soluble in the aqueous layer (water) (Nikolaou *et al*., 2009; Xia *et al*., 2013).

In a LLE unit, a liquid stream containing the components that must be recovered (solute) is added into an extractor, where it is in contact with a solvent. The two liquids should be immiscible or only slightly miscible (Koch and Shiveler, 2015). These conditions will then allow the solutions to form an emulsion, with one liquid dispersed as droplets in the other. Mass transfer will occur between the dispersed phase and the surrounding liquid which is the continuous phase (Nguyen *et al*., 2004). In order for the two liquids to be eventually separated, they must have different densities. The droplets then accumulate below or above the continuous phase, and this will depend on the liquids relative densities (Leon *et al*., 2014; Chen *et al*., 2016).

The types of solvents used in LLE are chosen to obtain the maximum transfer of the solute from the carrier to the solvent (Tavakoli *et al*., 2008; Chen *et al*., 2016). They must not be completely miscible with the carrier liquid and must have a high affinity for the solute molecules. Generally, an ideal solvent for LLE will normally have the following properties; high boiling point, low viscosity, high resistance to thermal degradation, density difference and high solubility for the solute and low solubility for the carrier liquid (Andersson *et al*., 2002).

Solvents that are usually applied for extracting PAHs from water include benzene, hexane, ethyl acetate, dichloromethane and ether. Dichloromethane has an affinity for non-polar and intermediate polar compounds. Therefore, it is commonly used in applications that require the determination of compounds of varying polarity with high extraction efficiency (Araghi *et al*., 2014; Seopela *et al*., 2016). The standard EPA

Method 610 employs dichloromethane as the extraction solvent for removing PAHs from water by liquid partitioning (Mahgoub, 2016).

Nekhavhambe *et al*. (2014) applied dichloromethane as solvent for extracting PAHs using LLE and obtained individual PAH levels ranging between 0.1 µg/L and 137 µg/L. However, toxicity of dichloromethane is a serious drawback of using the solvent for LLE. Alternative methods that focus on the alteration of the chemical structures of the target compounds by reducing the pH have been developed to avoid using dichloromethane. These techniques not only reduce the large volumes of toxic solvents required, but also the extraction time associated with LLE (Seopela *et al*., 2016).

The use of LLE separates PAHs according to their relative solubilities in different immiscible liquids (Mahgoub, 2016). Kafilzadeh *et al*. (2011) used the following LLE method for extracting PAHs from water using dichloromethane as solvent and found concentration ranging from 0.3 to 65.72 ng/L. In the LLE procedure, the water sample was poured into a separatory funnel and a mixture of 100 mL n-hexane and dichloromethane (1:1 v/v) was added and the flask shaken for 2 min. The water phase was drained and then the organic phase was poured into a glass funnel containing anhydrous sodium sulfate and re-extracted with 50 mL of the same solvent mixture. The extract was concentrated prior to the detection of PAHs (Kafilzadeh *et al*., 2011).

#### *2.9.2.2 Microwave-assisted extraction*

Microwave-assisted extraction (MAE) or simply microwave extraction is a process of using microwave energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent (Nikolaou *et al*., 2009). A modern design of the microwave extraction system contains carousels which can hold approximately sixteen extraction vessels allowing simultaneous multiple extractions. The common advantages of the MAE method are the reductions in solvent usage and time. However, when comparing it to supercritical fluid extraction (SFE), the cost of MAE is moderately small (Lau *et al*., 2010). In addition, this unique heating mechanism provides selective interaction with polar molecules, which significantly enhances the extraction efficiency of organic compounds such as PAHs from sediments (Lau *et al*., 2010). The major disadvantage of this technique is that the solvent needs to be

physically removed from the sample matrix after completion of the extraction of PAHs prior to further analysis. In some cases, samples are pre-treated with activated copper bars to assist the extraction process. It is essential to remove this copper for a clean extract (Shu *et al*., 2000; Lau *et al*., 2010).

Microwave systems for extraction are available in two forms: closed vessel and open vessel systems. A benefit of closed-vessel microwave system is that higher temperatures can be reached due to the increased pressure inside the vessel that raises the boiling point of the solvents used (Sibiya, 2012; Xia *et al*., 2013). There is no loss of volatile substances in a closed system vessel and less solvent is required. There is no need of the repeated addition of solvents and hence the risk of contamination is reduced. The limitations of a closed vessel system include the risk involved in the use of high pressures and the limited amount of sample that can be processed (Nikolaou *et al*., 2009). Open vessels have increased safety because they can be operated at atmospheric pressure and the reagents can be added at any time during the treatment. The major advantage of the open vessel system is the ability to process large samples without the requirement of a cooling process (Nikolaou *et al*., 2009; Araghi *et al*., 2014). The instrument can be purchased at low cost. Limitation of open vessel system is that methods used are usually less precise than the ones used in closed vessel systems. To obtain extraction efficiencies similar to those of closed-vessel systems, the open vessel systems need longer extraction times (Toun *et al*., 2006; Lau *et al*., 2010).

Mekonnen *et al*. (2015) applied MAE, using acetone/n-hexane (1:1, v/v) mixture and this combination of solvents proved to be efficient for the extraction of PAHs from sediments. The measured concentrations of PAHs in SRM 1944 obtained agreed well with the certified values. Seopela *et al*. (2016) also applied MAE using a mixture of acetone/hexane and obtained the total PAH content of sediment extracted ranging from 292-2 170 µg/kg. Photo showing microwave extraction system used in this study is presented in Figure 2.3.


Figure 2.3: The microwave extraction system used in this study

### *2.10.2.3 Mechanical agitation*

Mechanical shaking is a simple and low-cost technique that uses agitation or mixing action to extract the PAHs from sediment samples in a shake-flask placed on a rotary shaker, or it can also be with a magnetic stirrer submersed into the solution directly. Even though it is an easy method with minimal glassware and smaller volumes of extraction solvent, this technique has not been as widely used as Soxhlet and

sonication simply because of the lower extraction efficiency and unsatisfactory quantitative results (Berset *et al*., 1999). However, some studies reported that this method was comparable to the Soxhlet method, the results obtained while using mechanical shaking demonstrated larger variations and less selectivity because of the difficulty in quantifying the PAH extracts (Sun *et al*., 1998; USEPA, 2008). Comparable results were only attainable with long shaking times to extend the contact time with solvent (Lau *et al*., 2010; Oluseyi *et al*., 2011).

#### *2.9.2.4 Ultrasonication*

Ultrasonication is an efficient method when compared to reflux methods for extracting PAHs from soils and sediments. Ultrasonic extraction methods proved to generate comparable or even greater quantities of PAHs than other extraction techniques (Oluseyi *et al*., 2011). However, application of the method gave lower recoveries in some studies (Oluseyi *et al*., 2011; Kumar *et al*., 2014). Agitation may be performed by placing the sample solvent mixture directly into a sonication bath (Lau *et al*., 2010).

Lau *et al*. (2010) stated that sonication was preferable to Soxhlet due to its higher extraction efficiencies and was more economical and easily operated. In addition, Lau *et al*. (2010) noted that similar levels of extraction efficiency to the Soxhlet extraction method can be attained through vigorous sonication. However, the level of extraction efficiency was observed to be highly dependent on the sample matrix and concentration of contaminants in the sample. Contrary to these observations, other studies have indicated that sonication was less efficient than Soxhlet with relatively low recoveries particularly for lower molecular weight PAHs (Sun *et al*., 1998; Berset *et al*., 1999).

The duration of sonication should be carefully monitored to avoid extensive exposure to the irradiation, which may degrade the contaminants in the sample and reduce the extraction rates of PAHs (Oluseyi *et al*., 2011). The decrease in efficiency during excessive sonication is caused by an increase in broken carbonaceous particles and increased contact surface area, which adsorbs the PAHs more readily, causing a reversed adsorption cycle. In addition, further separation techniques such as centrifugation or filtration are usually required after the extraction process (Stephens *et al*., 1994; Lau *et al*., 2010).

#### *2.9.2.5 Soxhlet extraction*

Soxhlet extraction has been widely used as a benchmark technique in the extraction of PAHs from sediment samples. Basically, in the method, the solid sample is placed into a thimble, which is then extracted using an appropriate solvent through the reflux cycle (Oluseyi *et al*., 2011). As soon as 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 extract are siphoned back onto the lower flask where the solvent reboils, and the cycle is repeated until all the sample is completely extracted into the lower flask (Guerin, 1998; Lau *et al*., 2010).

The major drawback of this extraction process is the use of large volumes of solvent, possibly more than 150 mL for the extraction of PAHs from only 10 g of sediment sample. Additionally, this technique is very labour intensive and time consuming, as the solvent has to be refluxed for up to 20 hours to achieve satisfactory extraction efficiencies (Guerin, 1998). Soxhlet extraction has been shown to have relatively poor selectivity for PAHs. Studies showed that the chromatograms of extracts produced through Soxhlet using GC-MS yielded more artefact peaks with branched alkane humps, revealing that compounds such as n-alkanes and humic substances other than PAHs are coextracted using the Soxhlet method (Dean and Xiong, 2000; Kalbe *et al*., 2008). Other minor disadvantage of using the Soxhlet apparatus include the likelihood of sample carryover, the need to fractionise extracts to avoid heavy contamination of the GC injection port, and the difficulty of redissolving dried Soxhlet extracts (Berset *et al*., 1999).

Soxhlet extraction is still one of the preferred methods because of its comparative extraction results despite the nature of sample matrix. Not only does the Soxhlet extraction produce similar results as other methods such as SFE, MAE, accelerated solvent extraction (ASE), and ultrasonic methods, but the results also show small variations with low relative standard deviations (USEPA, 2008; Lau *et al*., 2010). The efficiency of Soxhlet extraction increases with molecular weight, reaching an efficiency range of 85 to 100% for PAHs with more than 4 rings (Lau *et al*., 2010).

#### *2.9.2.6 Accelerated solvent extraction/pressurised fluid extraction*

Accelerated solvent extraction (ASE) or pressurised fluid extraction (PFE) is a new technique, which raises the solvent temperature above its boiling point but maintains it in the liquid phase by elevating the pressure. The high pressure assists in the solubilisation of air bubbles, thereby exposing more of the sample to the extraction solvent while increasing the capacity of the heated solvent to improve solubility. The ASE systems are commercially available for extracting organic compounds such as PAHs from a variety of solid samples. The ASE system consists of several extraction cells on a loading tray proximate to an oven. During extraction, an organic solvent is pumped into the extraction cells preloaded with sediment samples while the temperature and pressure are increased to the desired values (Berset *et al*., 1999; Lau *et al*., 2010).

With the usage of the ASE system, the recovery of PAHs from sediments was reported to be twice that obtained when using Soxhlet extraction (Lau *et al*., 2010). The advantages of ASE includes reduction of solvent consumption and total time required because of the use of high pressures. The extraction method can be fully automated with an online purification column, preventing loss of volatile PAHs, avoiding too long preparation and potential contamination as in the case of mechanical shaking (Kalbe *et al*., 2008; Lau *et al*., 2010).

#### *2.9.2.7 Solid phase extraction*

Solid phase extraction (SPE), is a technique used to clean up a sample that been used for rapid and selective extraction of PAHs from sediment samples. Sediment samples are washed with solvent to leach away unwanted components before the extraction of PAHs with a different solvent into a collection tube (Kootstra *et al*., 1995). When this extraction technique is employed, filtering over an empty SPE column is normally recommended to prevent sediment samples clogging the GC column (Berset *et al*., 1999; Kanchanamayoon and Tatrahun, 2008).

A variation to the SPE of PAHs from sediment is solid phase microextraction (SPME). Lau *et al*. (2010) described the application of the technique for PAH extraction from

sediments. This solvent-free approach uses a small diameter fused-silica fibre coated with the extracting phase and mounted in a syringe-like device for protection and ease of handling. The depth of the injection needle is adjusted for headspace sampling before exposing the fibre, which adsorbs the PAHs from the sediments (Kanchanamayoon and Tatrahun, 2008). The exposed SPME fibre is then transferred directly to the injection port of an analytical instrument such as a GC for analysis. The major benefits of SPME are that it is fast, simple and convenient, which can be done on-site. The configuration of the solid-phase microextractor offers solutions to sampling problems because it can allow extraction of small volume of samples, which can then be analysed without any pre-treatment. The capability of the SPME device to extract small volumes of samples requires extreme precision during manufacturing to achieve homogeneity in the construction of the fibre to provide consistency in extraction outcomes and qualities (Lau *et al*., 2010). One study using SPME revealed that only volatile compounds such as LMW PAHs were detected (Dean and Xiong, 2000; Lau *et al*., 2010). Table 2.2 summarises conventional and modernised extraction techniques.

Table 2.2: Comparison of conventional and modernised extraction techniques (Seopela, 2014; Mahgoub, 2016)

| **Technique** | **Typical sample size and volume** | **Typical solvent volume (mL)** | **Solvent** | **Duration of extraction** | **Advantages** | **Disadvantages** |
|---|---|---|---|---|---|---|
| LLE | 200-900mL | 20-200 | n-hexane and dichloromethane | 3-30min | extracts PAHs both dissolved in the water and adsorbed upon any suspended particles in the sample | Limited selectivity, difficulty of automation and emulsion |
| MAE | 1-10g | 10-40 | Hexane and acetone | 3-30 min | Rapid and multiple extractions, high temperatures, low solvent consumption, no sample or energy loss | Extraction solvent should be active towards microwaves. Sample purification is required. |
| Ultrasonication | 1-30 g | 20-200 | Acetone, acetonitrile, 2-propanol, cyclohexane, methane and dichloromethane | 10-60 min | Multiple extractions, low solvent volumes and high efficiency | Reproducibility must be demonstrated by using replicates. Removal of co-extracted compounds is required |
| SPE | 1-5g | 2-20 | Methanol , cyclohexane | 10-90 min | Fast, low solvent consumption, selectivity, no clean-up or filtration required | Sample losses, from irreversible adsorption on solvent material or loss during elution of contaminants from the extract |
| Soxhlet | 1-30g | 100-600 | dichloromethane | 3-48 h | No filtration required, efficient in extraction | Sample clean-up is needed. Large solvent volumes are used. |
| SPME | 1-5g | Solvent free | Solvent free | 2-4h | Solvent minimization. Fewer steps involve. Minimum sample volume and preparation | little selectivity,<br>Limited capacity of the fiber. Potential contamination of the SPME needle |

## 2.10 TECHNIQUES APPLIED FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS:

A numbers of analytical techniques have been used for the determination of PAHs in environmental samples. The most widely and commonly used are gas chromatography (GC) with either flame ionisation detection (FID) or mass spectrometric detection (MSD), and high-performance liquid chromatography (HPLC) with ultraviolet detection (UVD) or fluorometric detection (FLD). However, there are other techniques that have also been used: thin-layer chromatography (TLC) with UVD or FLD, supercritical fluid chromatography (SFC) with UVD or MSD and liquid chromatography (LC) with MSD (Manoli, 1999). In this review, more attention will be given to GC-MS/FID which proved to be more efficient for the determination of the PAHs. Also, the use of HPLC-UVD for determining PAHs in environmental samples is explained below.

### 2.10.1 Gas chromatography

Gas chromatography is a common type of chromatography used for separating organic compounds such as PAHs. Typical uses of GC involve testing the purity of a particular substance, or separating the different components of a mixture according to their physical properties, such as polarity and boiling point (Araghi *et al*., 2014). The GC is an ideal technique to analyse gas and liquid samples containing many hundreds of different compounds, allowing the analyst to identify both the types of molecular species present and their concentrations (WHO, 1998). In GC, the stationary phase can be a high-boiling liquid and the mobile phase an inert gas such as helium. A GC can also be used to determine how many components are in a mixture. It can also be utilized to separate small amounts of material (Petridis *et al*., 2014).

Generally, in all chromatography, separation occurs when the mixture of the sample is injected into a mobile phase. The mobile phase transfers the sample mixture passed what is referred to as a stationary phase. The stationary phase is usually contained in a tube referred to as a column. Columns can be of glass or stainless steel and have various dimensions (Petridis *et al*., 2014). The mixture of organic compounds (PAHs in this study) in the mobile phase interacts with the stationary phase. Usually each

PAH compound in the mixture interacts at a different rate. As the sample mixture travels through this column, its components go back and forth at different rates between the gas phase and dissolution in the high-boiling liquid, and are thus separated into pure components. Those that can interact the fastest will elute from the column first whereas those that can interact slowest will exit the column last (Petridis *et al*., 2014).

As the instrument is running, the computer generates a graph called chromatogram from the signal (Poster *et al*., 1998; Petridis *et al*., 2014). Each of the peaks in the chromatogram represents the signal created when a PAH compound exits from the GC column into the detector. The x-axis of the chromatogram represent the retention time (RT), and the y-axis the intensity of the signal (Poster *et al*., 1998). Each peak in the chromatogram represents an individual PAH compound that was separated from a sample mixture (USEPA, 1995; Das *et al*., 2008). Figure 2.4 shows Schematic diagram of a GC.

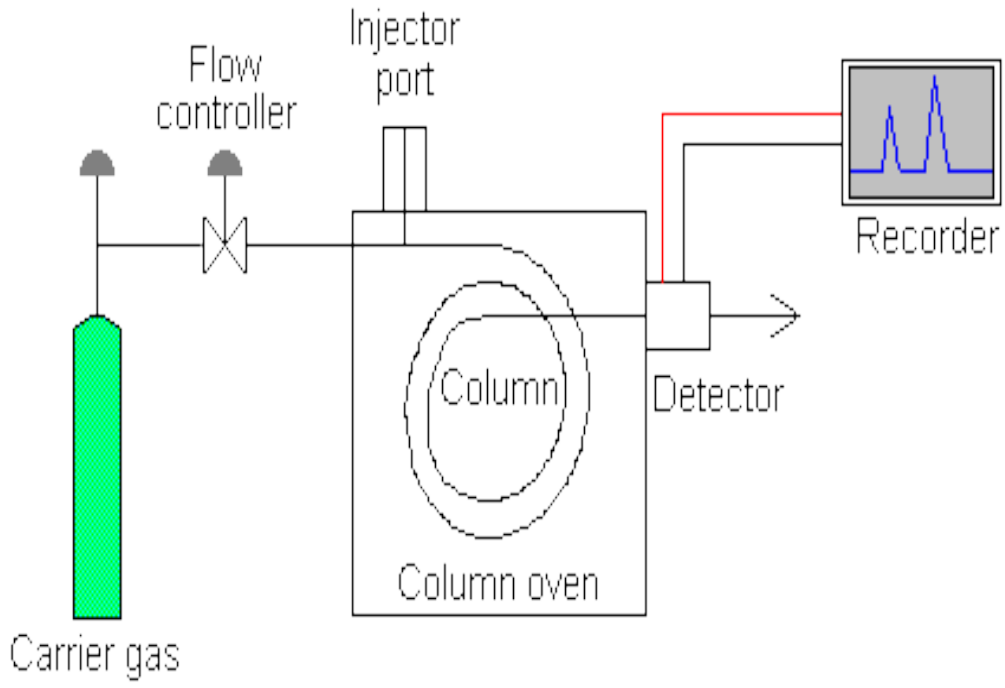



Figure 2.4: Schematic diagram of a gas chromatography (GC) (Bayowa, 2014)

A typical gas chromatograph usually consists of an injection port, a column, carrier gas flow control equipment, heaters and ovens for maintaining the temperatures of the

injection port and column, an integrator chart recorder and a detector as shown in the Figure 2.4 (Bayowa, 2014).

The most critical aspect of GC method development is the selection of an appropriate stationary phase for a specific separation. Methyl and phenyl-substituted polysiloxanes are the most widely used capillary column stationary phase for separation of PAHs in environmental samples (Poster *et al*., 1998). Columns prepared with polysiloxane stationary phases give relatively low background from column bleed, even at high temperatures and with a non-selective detector such as the FID. Columns containing liquid-crystalline stationary phases have shape selectivity aspects that are well suited to the separation of PAH isomers (Poster *et al*., 2006).

The greater selectivity of liquid-crystalline columns has also been used for the separation of methyl-substituted PAHs (USEPA, 2005). Liquid-crystalline columns have some drawbacks, including variations in selectivity, changes in the order of elution of PAHs among different columns, and a limited temperature range. The relatively low temperature limit of early developed liquid-crystalline columns resulted in a limited mass range in the determination of PAHs (Poster *et al*., 2006).

Before using a GC for the separation of PAHs in environmental samples, PAHs in water and sediment samples are normally extracted with solvents, and the extracts are concentrated and cleaned by using SPE procedures to remove potential interfering polar constituents (Simoneit, 1998). Poster *et al*. (2006) evaluated pressurised liquid extraction (PLE) for the determination of PAHs in environmental matrices, and this issue includes a review of PLE in environmental samples. In contemporary analysis of these complex matrices, GC, rather than LC, is usually the preferred technique for separation, identification, and quantification of PAHs, largely because GC normally affords greater selectivity, resolution, and sensitivity (Poster *et al*., 2006).

### 2.10.2 Detectors

There are many detectors which can be used in GC. Different detectors usually have different selectivity. A non-selective detector responds to all compounds except the

carrier gas, while a selective detector responds to a range of compounds with a common physical or chemical property. A specific detector responds to a single chemical compound (Das *et al*., 2008). Detectors can also be divided into concentration dependant and mass flow dependant detectors. The signal from a concentration dependant detector is related to the concentration of solute in the detector. It is therefore essential that the detector be very effective in detecting the result of the analysis and yield a good signal for recording. Currently, there are few detectors used with GC for PAHs quantification. They include flame ionisation detector, fluorescent detector and mass spectrometry. The mass spectrometer is the most preferred detector because it is very sensitive and efficient (Wcislo, 1998; Petridis *et al*., 2014).

#### *2.10.2.1 Mass spectrometric detector*

Mass spectrometry (MS) is a very sensitive technique used to detect, identify and quantify molecules based on their mass to charge ratio (m/z). It is originally developed almost 100 years ago to measure elemental atomic weights and the natural abundance of specific isotopes (Petridis *et al*., 2014). However, the development of methods of macromolecule ionisation, including electrospray ionisation (ESI) and matrix-assisted laser desorption/ionisation (MALDI), enabled the study of protein structure by MS, which allowed most scientists to obtain masses that could be matched to proteins and peptides in databases that can predict the identity of unknown proteins. Technological advances have provided reliable methods to analyse samples in solid, liquid or gas states, whereas sensitivity of current mass spectrometers allows detection of analytes at concentration of $10^{-18}$ moles per litre (Wcislo, 1998; Das *et al*., 2008).

After the separation with GC the charge received by PAHs allows the mass spectrometer to accelerate the ions throughout the remainder of the system. The ions encounter electrical or magnetic fields from mass analysers, which change the paths of individual ions according to their mass to charge ratio. Most used mass analysers include time-of-flight [TOF], quadrupoles and ion traps. Each type has specific characteristics. Mass analysers can be utilised to separate all analytes in a sample for

global analysis, or they can be used like a filter to properly deflect only specific ions towards the detector (Zhi *et al*., 2015).

Mass spectrometers are connected to computers with software that analyses the ion detector data and produces graphs that organise the detected ions based on their individual m/z ratio and relative abundance. Usually these ions can then be processed through databases to predict and identity PAHs based on their m/z ratio (ATSDR, 1995; Takte and Jaiswal, 2011). Mekonnen *et al*. (2015) used GC-MS for the determination of PAHs in sediments and the results were satisfactory since the average recovery of PAHs obtained ranged from 89.0 to 98.2%. The relative standard deviation (RSD) of all samples was also found to be less than 10%.

#### *2.10.2.2 Flame ionisation detector*

The use of flame ionisation detector is also very common for the determination of organic compounds such as PAHs, but it is destructive. It has several components: a stainless steel or alumina body fitted to a flame ignition coil, collector electrode and polarized jet, which is insulated electrically from the body (Figure 2.5). Usually when gas exits from the column, it passes to a small burner where it is combined with hydrogen (fuel gas) and air (oxidant) and combusted (Poster *et al*., 2006). Combustion of PAHs in the flame creates charged particles, which generate small currents between two electrodes (the burner and the collector electrode). The collector electrode collects the generated ionisation current and electrometer converts this to the signal and then amplifying it (Poster *et al*., 2006).

The FID is a useful detector and easy to use for determining organic compounds, including PAHs. It is a mass flow dependent detector; this means that the signal produced is related to the rate at which solutes enter the detector. Gas chromatography with flame ionisation detection (GC-FID) uses support gases such as hydrogen and air. The GC-FID is unaffected by the make-up gas (Bayowa, 2014). The benefits of using a FID in GC include high sensitivity and large linear response range. However, a major drawback is that it destroys the sample. The technique that does not use up the sample during analytical procedures is mass spectrometry. Furthermore, the FID is believed to be useful general detector for the analysis of organic samples (Bayowa, 2014). Analysis of sediment and soil samples from the

Warri and its environs in Nigeria using GC-FID was done by Bayowa (2014). The minimum detection limit obtained for GC-FID used in the analysis was 1 x $10^{-3}$ mg/kg. Kafilzadeh *et al*. (2011) also used a GC-FID for the determination of 16 PAHs in sediment and water and the overall mean recoveries were found to be 96.80% and 91.26% for water and sediment samples, respectively. A schematic representation of a FID used in GC is presented in Figure 2.5.

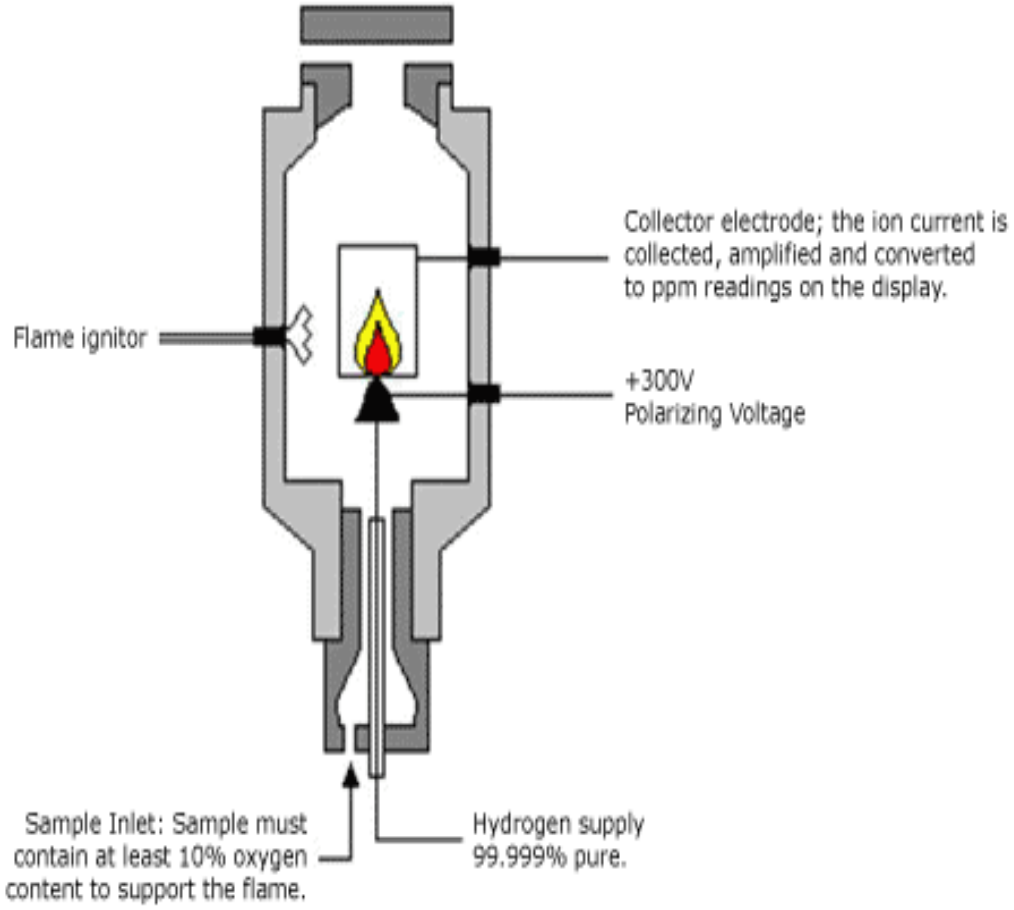



Figure 2.5: Schematic representation of a FID used in GC (Bayowa, 2014)

### 2.10.3 High performance liquid chromatography

High performance liquid chromatography (HPLC) is basically an improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under a high pressures of approximately 400 atmospheres and that makes it much faster (Literathy, 2015). It also allows the use of

very much smaller particle size for the column packing material, which gives a greater surface area for interaction between the stationary phase and the molecules flowing through. This results in improved separation of the PAHs. These methods are highly automated and extremely sensitive (Kumar *et al*., 2014; Literathy, 2015).

The major advantages of the determination of PAH by HPLC are due to the possibility of employing highly sensitive and selective detectors. The molecules of aromatic compounds are known to be capable of absorbing ultraviolet light and certain PAHs exhibit an intense fluorescence. Detectors working in accordance with the above principles have come to be widely used in the analysis of PAH by liquid chromatography (Wegrzyn *et al*., 2006). There are several solvents which exhibit a minimal absorption at the wavelength employed for detection. Detectors used in HPLC for PAHs analysis includes ultraviolet detection (UVD) and fluorometric detection (FLD) (Kumar *et al*., 2014; Literathy, 2015).

### 2.10.4 Supercritical fluid chromatography

Supercritical fluid chromatography (SFC) was initially performed with pure carbon dioxide ($CO_2$) as the mobile phase, but currently SFC is very often carried out in subcritical conditions because $CO_2$ can be modified with an organic modifier or additive in order to increase the solubility of polar compounds; yet carbon dioxide is always the main component of the mobile phase, and its singular properties are advantageous (Bernal *et al*., 2013). These properties are related to the low viscosity and high molecular diffusiveness. In contrast to HPLC, SFC allows the use of higher flow-rates with lower pressure differences through the column, leading to greater efficiency in short analysis times and reduced consumption of organic solvents (Sandra *et al*., 1997).

SFC is usually used to determine low concentrations of organic compounds such as PAHs and high molecular weight molecules. It is normally used in pharmaceutical drug analysis, as well as in the analysis of petroleum, polymers and propellants (Sandra *et al*., 1997). The SFC is similar to GC and LC, but uses liquid carbon dioxide as the mobile phase so the flow path is highly pressurised (Bernal *et al*., 2013). In addition, due to the properties of carbon dioxide, low viscosity, high eluting power, separations

obtained by SFC for the determination of PAHs are generally faster than by liquid solvents (Lesellier, 1999).

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 INTRODUCTION

This chapter gives detailed description of the study areas, the purity of reagents, standards, apparatus and instrumentation. It also includes the approach followed in this research such as sampling, sample preservation, sample preparation and sample analysis.

## 3.2 DESCRIPTION OF THE STUDY AREAS

### 3.2.1 Mokolo River

Lephalale is one of the areas considered to be rich in minerals in Limpopo Province. This area has two power stations and one coal mine. These industries use water from Mokolo River, which is situated in the Lephalale area. The River covers an area of approximately 8 387 km² (Maswuma *et al*., 2011; Limpopo water management area (LWMA), 2012). The Mokolo River and its tributaries rise in the western part of the Waterberg district, and flows through a flat area until it enters the Mokolo Dam. From this point, the river flows through a flat area until it converges with the Limpopo River. Construction of the Mokolo Dam commenced in 1970 and completed in 1980, mainly to supply water to the Eskom Matimba Power Station, Grootegeluk Coal Mine, Lephalale Municipality, agricultural activities for irrigation and recently newly built Medupi power station (Pienaar, 2009). Approximately 87% of water from Mokolo River is used for agricultural purposes while 13% for mining, power generations and industries (Maswuma *et al*., 2011). Irrigation is currently the largest water user in the area. The Lephalale area contributes significantly towards agricultural activities in Limpopo Province (Pienaar, 2009). There is also a significant amount of irrigation from groundwater. Lephalale area is one of the rural areas where some people still depend on water from the rivers for domestic activities (Department of environmental affairs and tourism (DEAT), 2006; LWMA, 2011).

Eskom Matimba as well as, Medupi power stations and Grootgeluk coal mine, which occur in close proximity to the Mokolo River, are likely to pollute the Lephalale area. These industries could possibly be the major sources of PAHs in the Mokolo River. There are other activities in the area, including sand mining that takes place in the lower sections of the Mokolo River before the river enters the Mokolo dam. The map showing ten sampling sites and a photograph showing condition of sampling at site 4,

which is next to sand mining and agricultural activities in Mokolo River, are presented in Figures 3.1 and 3.2, respectively.

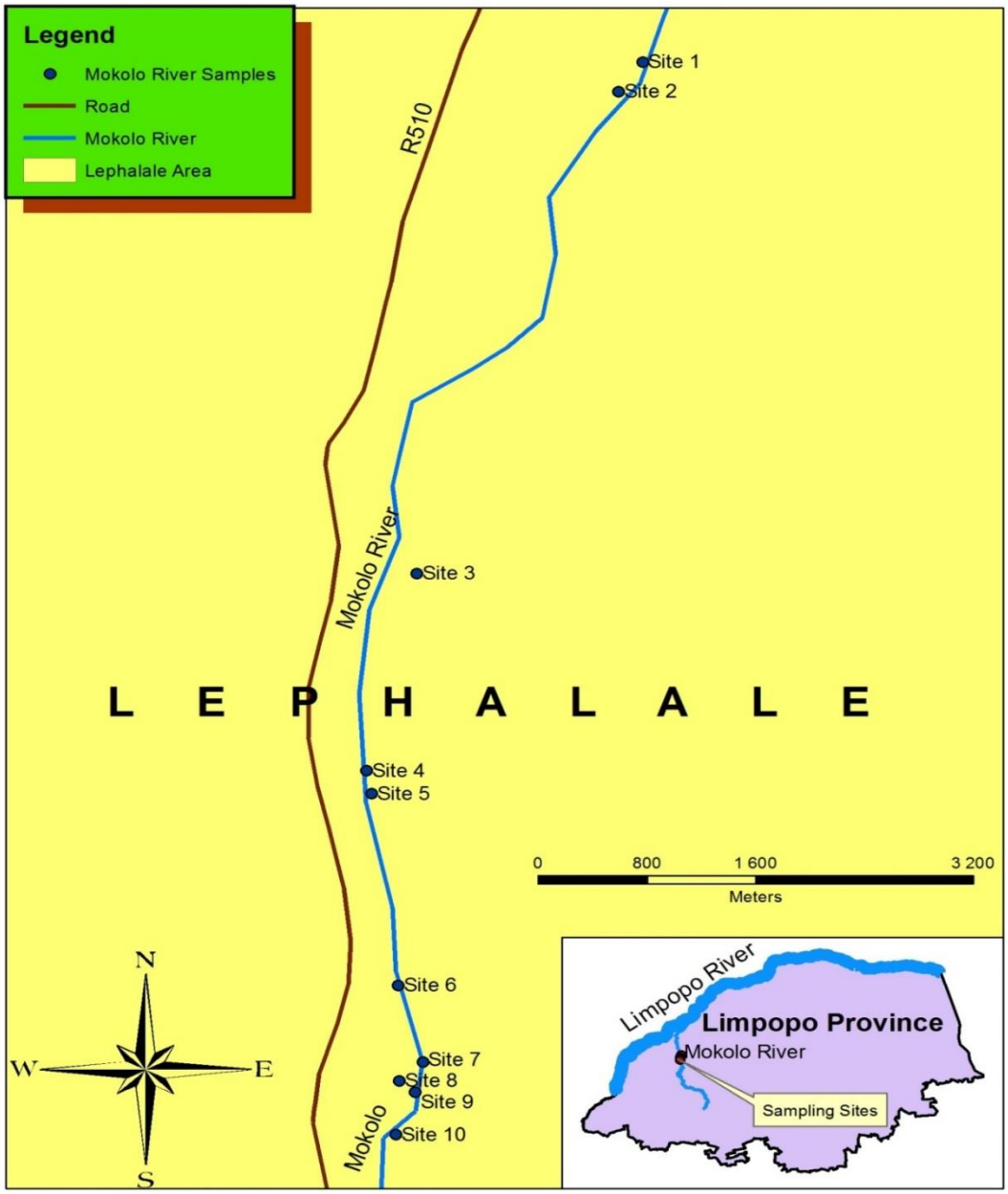



Figure 3.1: Map showing ten sampling sites in Mokolo River

Figure 3.2: Sampling site at Mokolo River

### 3.2.2 Blood River

Blood River, also known as Mulaudzi River, is found in Seshego area, Limpopo Province. Blood River is a tributary of the Sand River. This river meets the Sand River from the west, just north of Polokwane, and flows to the small Seshego Dam. The Sand River flows by the western edge of Polokwane and continues until it reaches the Limpopo River (van Vuuren, 2006). A photograph showing the dumping site near Blood River in Seshego area is presented in Figure 3.3. These domestic wastes could be a possible source of contaminants in the river and are found next to sampling points 4 and 5.

Figure 3.3: A dumping site near Blood River in Seshego area

Water from Blood River is mainly used for agricultural and domestic activities. Animals from the area drink the same water. Seshego is one of the areas around Polokwane that, is highly polluted. Possible sources of pollution could be leaking of sewage observed during sampling, domestic and industrial wastes and other activities taking place in the area and around Polokwane. Thus, all these wastes and activities could possibly be sources of PAHs in the river. Blood River also has activities taking place in the upper site of the river where people from around the area and outside Polokwane are mining sand. Sand mining in this area could have a negative impact on the river system. Therefore, it is essential to assess the levels of PAHs in water and sediment samples collected from Blood River. The map showing sampling sites and conditions

of sampling sites 6 and 7 in Blood River are presented in Figures 3.4 and 3.5. There is sewage leaking opposite sampling sites 7 and 8 that flows directly into the river and this might be a possible source of contamination (Figure 3.5). The sampling sites 6 and 7 could also be acting as a drain, receiving waste water from residential areas found in close proximity to the river.


Figure 3.4: Map showing ten (10) sampling sites in Blood River

Figure 3.5: The conditions of the Blood River in Seshego area

The sampling point below (Figure 3.6) is found next to the bridge in Blood River. This portion has water most of the year. This sampling point was also next to a dumping site, few residential houses and agricultural activities. The condition of sampling point 4 is shown in Figure 3.6.

Figure 3.6: Sampling site 4 at Blood River

## 3.3 SAMPLE COLLECTION, HANDLING, PRESERVATION AND STORAGE

Water and sediment samples were collected from 10 sampling sites along Mokolo River (Figure 3.1) and 10 sampling sites in Blood River (Figure 3.4). Water samples were collected in pre-cleaned brown glass bottles at 40 cm below water level from all the different sampling sites in this study. To avoid contamination, these brown glass bottles were thoroughly washed, and rinsed with acetone and dichloromethane (DCM). Brown glass bottles were rinsed twice with the sample at the site before the water samples were collected. Samples were then transported to the laboratory on crushed ice in a cooler box and stored at 4°C until extraction and analysis were carried out.

Sediment samples were taken at a depth of about 15 cm from the surface of the sediment at the same site and time as water sampling took place. Samples were air dried for 5-6 days, ground using an agate mortar and pestle and passed through 250 µm sieve and stored until extraction and analysis.

## 3.4 REAGENTS, APPARATUS AND INSTRUMENTATION

All the solutions were prepared using high purity solvents. Acetone, dichloromethane, methane, hexane, nitric acid, hydrochloric acid and diethyl ether used in this research were all obtained from Sigma-Aldrich (Chemie GmbH, Germany). Boiling chips added to the liquids so that the solution can boil easily were purchased from Sigma-Aldrich (Chemie GmbH, USA). Anhydrous sodium sulfate, $Na_2SO_4$ (Sigma-Aldrich Chemie GmbH, Germany) was used for drying samples. Activated copper was prepared by mixing copper powder (Alfa Aesar GmbH & Co KG, Germany) with a 35% hydrochloric acid. Once activated, the powder was rinsed three times with de-ionised water, followed by methanol, and lastly with diethyl ether. After washing, the activated copper was dried under vacuum. Ultra-pure water was generated by easy ultra-pure water system (Shangai Carrex Analytic Instrument Co., Ltd, China), and was used as a blank for PAHs from water samples and to rinse glassware. Hydrophilic polypropylene membrane filter (0.45 µm) and filter papers (70 mm) were purchased from Sigma-Aldrich (Chemie GmbH, Germany) and Schleicher and Schuell, (Germany), respectively. A Buchi-evaporator R-200, equipped with a heating bath and vacuum pump v-700 (Labotec SA, RSA) was used to concentrate the extracts. A Reacti-Vap™ Evaporating Unit used to control a gentle stream of nitrogen, was purchased from Pierce (Illinois). A Vortex (Lasec SA, RSA) was used to mix vials containing samples and an automatic pipette (Glison Inc., USA) was used to measure volume of liquids. The samples were also filtered with a 0.45 µm PVDF syringe filter (Merck Millipore, USA).

An EPA 610-N PAH kit, containing the 16 US EPA priority PAHs, was purchased from Supelco (Bellfonte, USA). The following reference standards: naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr), benzo(a)anthracene (BAnt), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF),

benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InP), dibenzo(a,h)anthracene (DahAnt), and benzo(g,h,i)perylene (BghiP) were supplied by Supelco (Bellfonte, USA). Certified reference material (CRM-104) of sediments (Sigma-Aldrich Chemie GmbH, USA) was used to validate the method. The efficiency of extraction method selected for water samples was evaluated by spiking de-ionised water with known amounts of a standard mixture consisting of PAHs. Standards were prepared from 1000 mg/L stock solutions. The following analyte concentrations were prepared to monitor the extraction efficiency used for water samples, 100 µg/L of LMW PAHs (Nap, Acy, Ace, Flu, Phe and Ant), 5.00 µg/L of BkF and 10.0 µg/L of each of the remaining PAHs. Suitable volumes of internal standards, Ace-D10, Chr-D12 and Per-D12 (Sigma-Aldrich Chemie GmbH, USA) were added to each mixture. De-ionised water was used as the laboratory reagent blank (LRB). The LRB and standards were used to evaluate the performance of the LLE method. A 1.00 mL aliquot of the solution containing different concentrations of analytes was added to 600 mL deionised water and extracted using the extraction method chosen for water samples. The standard deviations (SD) and relative standard deviation (RSD) were determined for all sets of results to monitor the precision of the results.

## 3.5 SAMPLE PREPARATION

### 3.5.1 Microwave extraction of PAHs in sediment samples

A CEM MARS Microwave system (CEM, USA) was used for extraction of PAHs in sediment samples. Homogenised sediment sample (5.00 g) was weighed into extraction vessels. The extraction was done with a mixture of 30 mL 1:1 (v/v) acetone: hexane for 30 min at temperature of 110 °C, pressure of 800 psi and power of 1600 W. After the vessels had cool to room temperature, the extracts were filtered with 70 mm filter paper, collected in 250 mL round bottom flasks and evaporated to approximately 2 mL in a Buchi-evaporator, equipped with a heating bath and vacuum pump at a temperature of 40°C. Activated copper was added to each sample for desulfurisation, followed by the addition of anhydrous sodium sulfate to dry the extract and then filtered through 0.45 µm PVDF syringe filter. The extract was evaporated to dryness under a gentle stream of nitrogen. The internal standards, Ace-D10, Chr-D12 and Per-D12, were added to the extract followed by 1 mL of dichloromethane and

transferred to 1.5 mL brown vial. All the samples were mixed thoroughly using a vortex and analysed by GC-MS and GC-FID.

### 3.5.2 Liquid-liquid extraction of PAHs in water samples

Liquid-liquid extraction was used for the extraction of PAHs from water samples. Water samples were passed through 70 mm filter paper. This was followed by further filtration by passing them through a 0.45 µm hydrophilic polypropylene membrane filter. In a separation funnel, 50 mL of dichloromethane was added to 600 mL of the water sample and shaken gently for about 2 min before the two phase separated. The organic phase was drained from the separation funnel, and collected and combined in a 500 mL Erlenmeyer flask. The procedure was performed three times using 50, 30 and 20 mL of dichloromethane. The three combined extracts were dried with anhydrous sodium sulfate. After drying, the sample extract was collected in a 250 mL round bottom flask containing boiling chips. The extract was concentrated to a volume of approximately 3 mL using the Buchi-evaporator, as above, at a temperature of 40°C. The extract was dried with anhydrous sodium sulfate. The extract was evaporated to dryness under a gentle stream of nitrogen. Suitable volumes of internal standards, Ace-D10, Chr-D12 and Per-D12, were added to all the extracts. All samples were diluted appropriately and made up to final volume of 1.00 mL, with DCM by using an automatic pipette (Model P1000). A Vortex-Genie 2, (Model G560E) was used to thoroughly mix the contents in a 1.5 mL amber sample tube before analysis by GC-MS and GC-FID.

### 3.5.3 Ultrasonication procedure

Ultrasonication technique was used for extraction of PAHs from sediments. During the process, 1.00 g of sediment was accurately weighed into a 25mL amber bottle. A 20 mL portion of 1:1 (v/v) acetone: hexane was added to the bottle. The bottles were sealed with screw cap closure lined with PTFE-faced silicon rubber washer and shaken vigorously to suspend the contents. The bottles were sonicated in an ultrasonic bath for 60 min at ±50 °C. The extraction solutions were then centrifuged for 10 min at 2000 rpm. The volume of the extracts was reduced to approximately 2 mL using a rotary evaporator. Activated copper was added to the sample to desulfurise the

solution. The extracts were dried with anhydrous sodium sulfate and filtered through a 0.45 µm PVDF syringe filter. The solution was evaporated to dryness under a gentle stream of nitrogen. Suitable volumes of internal standards, Ace-D10, Chr-D12 and Per-D12 were added to all the extracts and reconstituted to 1 mL with DCM by using an automatic pipette and mixed thoroughly using a vortex before analysis by GC-FID.

### 3.5.4 Combination of mechanical shaking and ultrasonication procedure

Mechanical shaking and ultrasonication were used for extraction of PAHs from sediment samples. A 1.00 g of sediment sample was accurately weighed into 25 mL amber bottle. A 20 mL portion of 1:1 (v/v) acetone: hexane mixture was added to the bottle. The bottles were sealed with screw cap closure lined with PTFE-faced silicon rubber washer. A platform shaker was used to shake the contents for 25 min at 2000 rpm. The bottles were sonicated in an ultrasonic bath for 60min at ±50 °C. The extraction solutions were then centrifuged for 10 min at 2000 rpm. The volume of the extracts was reduced to approximately 2 mL using a rotary evaporator. Activated copper was added to the sample to desulfurise the solution. The extracts were dried with anhydrous sodium sulfate and filtered through a 0.45 µm PVDF syringe filter. The solution was evaporated to dryness under a gentle stream of nitrogen. Suitable volumes of internal standards, Ace-D10, Chr-D12 and Per-D12 were added to all the extracts and reconstituted to 1 mL with DCM by using an automatic pipette and mixed thoroughly using a vortex before analysis by GC-FID.

## 3.6 CONSTRUCTION OF CALIBRATION CURVES

The calibration curves were done using the commercially available PAHs standards. The standards were prepared as directed by the manufacturer. Stock solutions (1000 mg/L) of these PAHs standards were individually prepared by accurately weighing 0.010 g of each pure PAH standard, dissolving it and diluting to 10.0 mL in a volumetric flask with DCM. After the optimisation of gas chromatographic conditions, calibration was achieved by both external and internal standardisation. For external standard calibration, different concentrations of each of the analytes of interest were prepared from 10 mg/L intermediate solutions. Calibration curves were plotted using peak areas as a function of concentration for each of the PAH standards. Internal standard

calibration curves were also constructed for comparison. Quantification of PAHs was carried out by GC-MS/FID.

## 3.7 DETERMINATION OF LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

To determine the limit of detection (LOD) and limit of quantification (LOQ) of the entire analytical procedure, reagent blanks were prepared following the same procedures as the samples. As mentioned, stock solutions of 1000 mg/L of the 16 PAHs and three internal standards, were prepared from pure standards using DCM for GC-FID. The GC was set at the optimum temperature and 1.00 µL of the prepared reagent blanks was injected and the GC run as usual. Regression analysis data were used to calculate the LOD and LOQ for each PAH. Standard deviations were also calculated from the regression analysis data of the reagents blanks and concentrations for each of the PAH standard measured. The LOD is three times the standard deviation while LOQ is ten times standard deviation of the average of all individually prepared reagent blank solutions.

## 3.8 DETERMINATION OF PAHS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

Analysis of water and sediment samples was done using GC-MS. Both the identification and quantitative determination of PAHs in sediments and water were performed on an Agilent GC 7890A series (Agilent Technologies, USA), which was coupled to a Triple Axis (Model 5975C) quadrupole MS. The MS was equipped with an electron impact (EI) ionisation source. In this case separation occurs as the vapour constituent partition between the gas and liquid phases. The sample was automatically detected as it comes out from the column by a mass spectroscopic detector. The operational conditions used for GC-MS analysis are presented in Table 3.1.

Table 3.1: Operating conditions used for the determination of PAHs in sediment and water samples using GC-MS

| **Agilent GC 7890A with 5975C MSD** | |
|---|---|
| Inlet temperature | 310°C |
| Pressure | 15 psi |
| Mode | Pulsed splitless |
| Injection pulse pressure | 40 psi until 2 min |
| Purge flow to split vent | 30 mL/min at 1min |
| Column | D65-MS |
| Agilent column 122 | 5532UI: 30mχ250 µm χ 0.25µm |
| Injection volume | 1 µL |
| Carrier gas | Helium |
| He flow | 1.33 mL/min |
| Ionisation energy | 70 eV |

## 3.9 DETERMINATION OF PAHs BY GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR

Analysis of samples was also carried out by GC-FID (Agilent Technologies, USA). Important instrumental parameters were optimised before starting with analysis. The important parameters for GC-FID include; inlet temperature, carrier gas (He) flow rates and oven temperature program parameters such as initial oven temperature, ramp rate and initial time. The detector parameters were fuel flow, utility flow, and make-up gas flow rate and flame ionisation detector (FID) temperature. Operational conditions used for GC-FID are given in Tables 3.2 and 3.3.

Table 3.2: Operating conditions used for determination and quantification of PAHs using GC-FID

| **Agilent 7820A GC with FID** | |
|---|---|
| Column type | HP-5 |
| Length | 30 m |
| Internal diameter | 320 µm |
| Film thickness | 0.25 µm |
| Injection volume | Splitless 1 µL |
| Carrier gas | Helium |
| He flow | 6.5 mL/min |
| He flow (make-up gas) | 25 mL/min |
| $H_2$ flow | 30 mL/min |
| Air | 400 mL/min |
| Make-up gas | Hydrogen |
| Average velocity | 79.473 cm/sec |
| FID temperature | 300°C |
| Injector temperature | 280°C |
| Purge flow to split vent | 40 mL/min at 0.5 min |

Table 3.3: Oven temperature program for GC-FID

| | Rate<br>°C/min | Value<br>°C | Hold time<br>Min |
|---|---|---|---|
| Initial | | 75 | 0.5 |
| Ramp 1 | 10 | 200 | 5 |
| Ramp 2 | 10 | 280 | 10 |

## 3.10 QUALITY CONTROL/ASSURANCE

The extraction method for sediments was validated using certified reference material of sediments containing PAHs. Assessment of the accuracy of the results and analysis

of PAHs determined in sediments samples was verified using CRM-104. The efficiency of the extraction method chosen for water samples was evaluated by spiking de-ionised water with known amounts of standard mixture consisting of PAHs.

## 3.11 STATISTICAL ANALYSIS

The data was analysed using one-way analysis of variance (ANOVA) to investigate statistical significance of differences in the mean concentration of the 16 PAHs determined in sediment and water samples from Mokolo and Blood Rivers. A probability level of $P = 0.05$ was considered statistically significant. The SPSS Statistics software was used for statistical analysis.

# CHAPTER 4

# RESULTS AND DISCUSSION

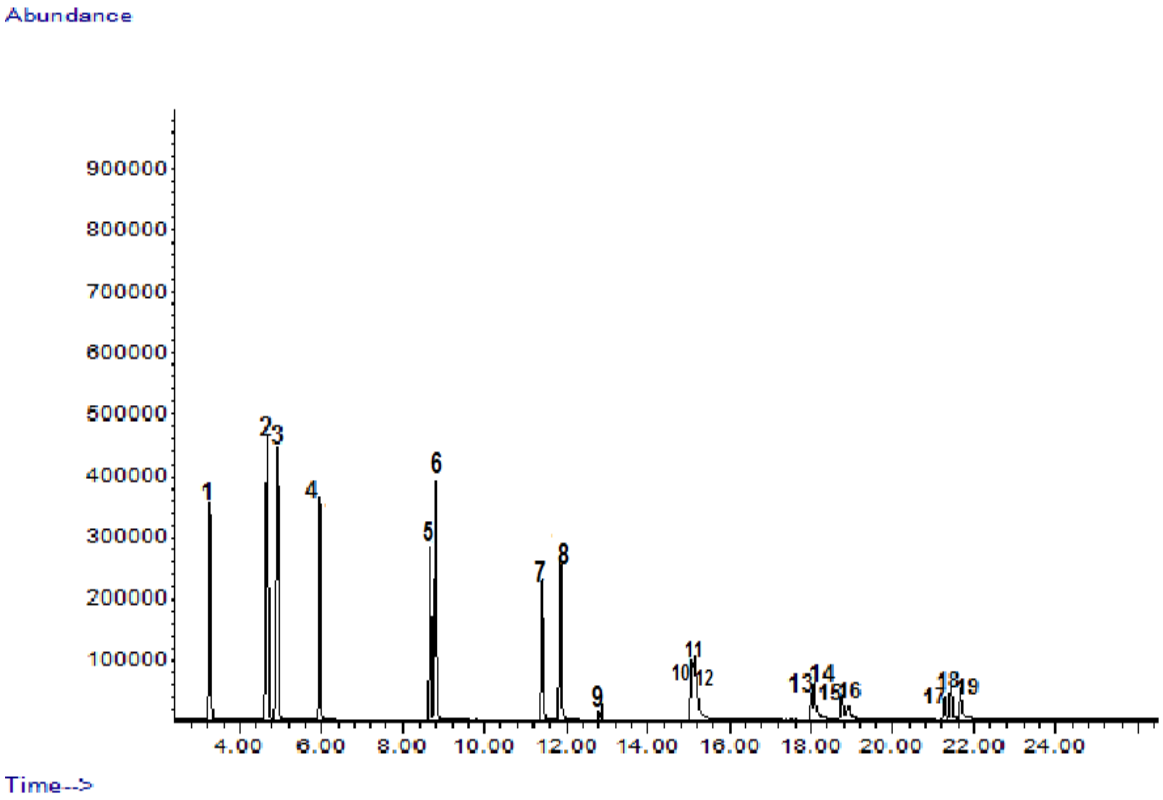
## 4.1 INTRODUCTION

This section summarises and discusses the concentrations of PAHs in water and sediment samples from Mokolo and Blood Rivers obtained using GC-MS and GC-FID. The results including mean concentration and standard deviation of all samples are given in tables and graphs. It also includes regression parameters, calibration curves, LODs and LOQs. Evaluation of accuracy and precision of the methods employed for quantification of PAHs are also given. Risk assessment and source identification are also presented in this section.

## 4.2 DETERMINATION OF THE PAHs BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

### 4.2.1 Identification of PAHs using gas chromatography-mass spectrometry

The identification and quantification of PAHs in sediment samples was carried out by GC-MS. In this study, the identification of PAHs was carried out first by analysing standards containing the USEPA priority PAHs and they were separated with a flat baseline. Figure 4.1 is a chromatogram indicating that PAHs and internal standards, were completely separated. The identification of these compounds were confirmed by their retention times, molecular mass and abundance of confirmation ions from the PAHs standards. The chromatograms of a mixed standard containing the USEPA priority PAHs and internal standards obtained in this study were comparable to those obtained by Seopela *et al*. (2016). Benzo(a)anthracene and Chrysene eluted from the column at the same time as one of the internal standard (Chr-D12) but they were identified by their ions. The target and qualifier ions for each PAH together with their retention times for selected ion monitoring (SIM) of GC-MS are given in Table 4.1.




PAH 1 to 17: naphthalene, acenaphthyene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and internal standards (3,11 and 14: acenaphthene-D10, chrysene-D12 and perylene-D12).

Figure 4.1: A total ion chromatogram of a mixed standard containing the USEPA priority PAHs and internal standards, obtained by GC-MS

Table 4.1: Ions selected for each PAH for selected ion monitoring (SIM) of PAHs by GC-MS (target ion in bold)

| PAHs compounds | Ions selected for each PAH m/z | Retention time (RT), min |
|---|---|---|
| Naphthalene (Nap) | **128,** 127, 129, 102 | 3.26 |
| Acenaphthylene (Acy) | **152,** 151, 76.1, 150 | 4.67 |
| Acenaphthene-D10 (Ace-D10) | **162,** 164, 160, 163 | 4.93 |
| Acenaphthene (Ace) | **153,** 154, 152, 76.1 | 5.96 |
| Fluorene (Flu) | **166,** 165, 167, 82.4 | 8.65 |
| Phenanthrene (Phe) | **178,** 176, 76.1, 179 | 8.78 |
| Anthracene (Ant) | **179,** 178, 176, 89.1 | 11.4 |
| Fluoranthene (Fln) | **202,** 203, 200, 201 | 11.8 |
| Pyrene (Pyr) | **203,** 202, 200, 201 | 13.6 |
| Benzo(a)anthracene (BAnt) | **228,** 226, 114, 229 | 15.1 |
| Chrysene-D12 (Chr-D12) | **240,** 236, 241, 120 | 15.1 |
| Chrysene (Chr) | **228,** 226, 229, 114 | 15.2 |
| Benzo(b)fluoranthene (BbF) | **252,** 253, 250, 126 | 17.9 |
| Benzo(k)fluoranthene (BkF) | **253,** 251, 250, 126 | 18.1 |
| Benzo(a)pyrene (BaP) | **254,** 126, 250, 253 | 18.8 |
| Perylene-D12 (Per-D12) | **264,** 266, 265, 132 | 18.9 |
| Indeno(1,2,3-cd)pyrene (InP) | **276,** 277, 274, 138 | 21.3 |
| Dibenzo(a,h)anthracene (DahAnt) | **278,** 279, 276, 139 | 21.4 |
| Benzo(ghi)perylene (BghiP) | **279,** 138, 277, 274 | 21.7 |

### 4.2.2 Calibration curve

Calibration standards were prepared using different concentrations for each PAH through dilution of stock solutions with DCM. The prepared standard solutions for each PAH compound were analysed using GC-MS and the peak areas quantified. The calibration curves were constructed using peak area *versus* concentration. The calibration curves for the 16 US EPA priority PAHs constructed showed good linearity since all the $R^2$ values were ≥0.995. The lowest regression value obtained was for Flu with value of 0.9954 and Phe had the highest value of 0.9996. Both quantitative and qualitative data of all the 16 priority PAHs were achieved through GC-MS in SIM mode. The calibration curves for 16 PAHs were prepared using external standardisation. An example of external calibration curve obtained is presented in Figure 4.2. The correlation coefficients and calibration equations for all 16 PAHs are presented in Table 4.2.

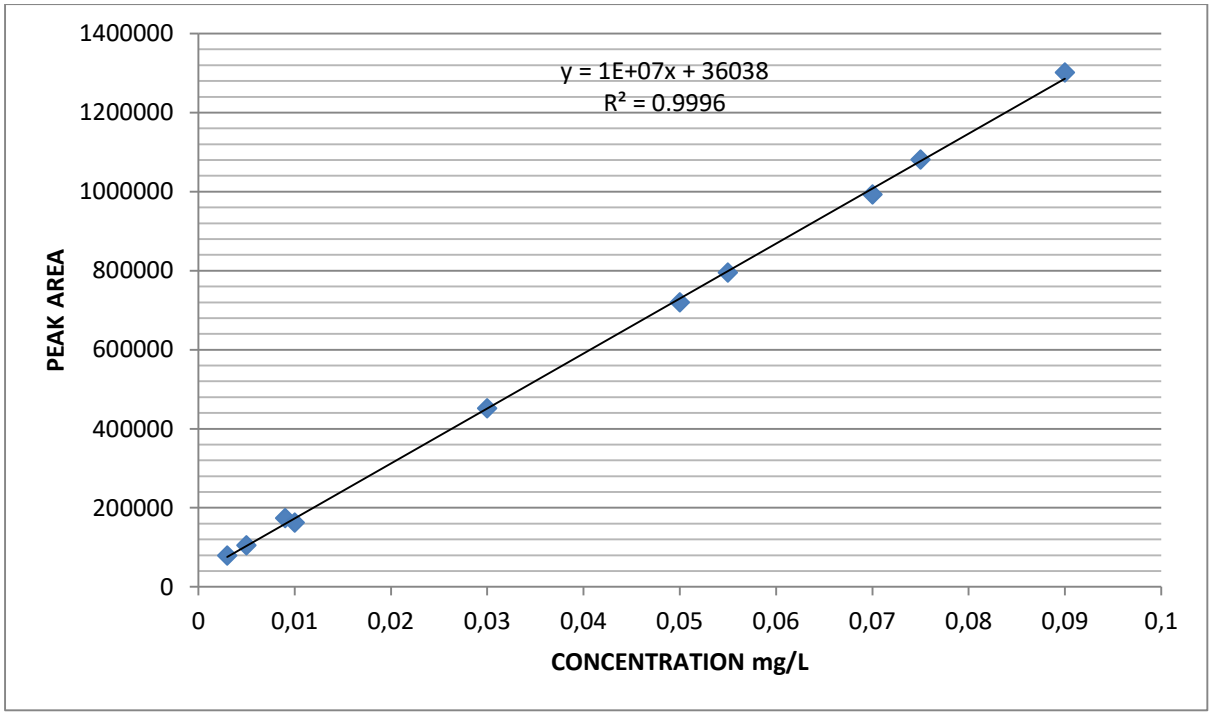



Figure 4.2: Calibration curve obtained for phenanthrene by GC-MS

Table 4.2: Correlation coefficient and calibration equation obtained for all the analytes by GC-MS/SIM

| Compound | Correlation coefficient, $R^2$ | Calibration equation | LOD (ng/g) | LOQ (ng/g) |
|---|---|---|---|---|
| Naphthalene (Nap) | 0.9969 | Y=2E+07x +7102,2 | 0.0224 | 0.0746 |
| Acenaphthylene(Acy) | 0.9981 | Y=2E+07x+38086 | 0.0167 | 0.0557 |
| Acenaphthene (Ace) | 0.9973 | Y=1E+07x+27210 | 0.00471 | 0.0157 |
| Fluorene (Flu) | 0.9954 | Y=1E+07x+25702 | 0.0471 | 0.157 |
| Phenanthrene (Phe) | 0.9996 | Y=1E+07x+36038 | 0.00858 | 0.0286 |
| Anthracene (Ant) | 0.9979 | Y=8E+06x+9755,5 | 0.0341 | 0.115 |
| Fluoranthene (Fln) | 0.9979 | Y=1E+07x+24387 | 0.0165 | 0.0549 |
| Pyrene (Pyr) | 0.9965 | Y=2E+07x+15882 | 0.0115 | 0.0385 |
| Benzo(a)anthracene (BAnt) | 0.9993 | Y=1E+07x +50899 | 0.556 | 1.85 |
| Chrysene (Chr) | 0.9983 | Y=2E+07x +11982 | 0.0793 | 0.264 |
| Benzo(b)fluoranthene (BbF) | 0.9992 | Y=1E+07x +14781 | 0.0457 | 0.107 |
| Benzo(k)fluoranthene (BkF) | 0.9991 | Y=9E+06x +77,485 | 0.00459 | 0.0152 |
| Benzo(a)pyrene (BaP) | 0.9992 | Y=7E+06x +5327,1 | 0.943 | 3.14 |
| Indeno(1,2,3-cd)pyrene (InP) | 0.9982 | Y=4E+06x +108,27 | 0.00659 | 0.0219 |
| Dibenzo(a,h)anthracene (DahAnt) | 0.9989 | Y=6E+06x +3973,1 | 0.00895 | 0.0298 |
| Benzo(ghi)perylene (BghiP) | 0.9984 | Y=6E+06x +10064 | 0.675 | 2.25 |

### 4.2.3 Determination of limits of detection and quantification

The LODs were calculated to assess the sensitivity of the instrument. The LOD and LOQ were obtained by analysing the blank samples. The LODs and LOQs for each PAH were then calculated from the standard deviation of the concentration of the blank samples. The LODs were obtained taking the usual definition, namely the analyte concentration that gives a signal greater than three times the standard deviation of background noise. The LODs values obtained ranged between 0.00459 and 0.943 ng/g while LOQs ranged from 0.0152 to 3.14 ng/g. Mekonnen *et al*. (2015) reported LODs and LOQs values ranging from 1.28 to 3.92 ng/g and 4.27 to 13.1 ng/g for PAHs respectively, in sediment samples using GC-MS. These values are higher than the LODs and LOQs obtained in this study for sediment samples. The LODs and LOQs values for each PAH are given in Table 4.2.

### 4.2.4 Quantification of PAHs in sediments by gas chromatography-mass spectrometry

The 16 USEPA priority PAHs were quantified based on the calibration curves obtained by analysing different concentrations of standards using GC-MS. The concentrations of PAHs in sediment samples collected from Mokolo and Blood Rivers are presented in Tables 4.3 and 4.4, respectively.

Table 4.3: Concentrations of PAHs in sediment samples collected from Mokolo River in ng/g, dry weight

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | 0.793±0.015 | <0.0167 | <0.00471 | <0.0471 | 1.09±0.11 | 2.26±0.16 | <0.0165 | <0.0115 |
| S2 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | <0.0341 | <0.0165 | <0.0115 |
| S3 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | <0.0341 | <0.0165 | <0.0115 |
| S4 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 2.93±0.6 | 4.51±0.2 | 0.390±0.092 | <0.0115 |
| S5 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 1.03±0.08 | 1.91±0.09 | <0.0165 | <0.0115 |
| S6 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 0.577±0.66 | 0.313±0.071 | <0.0165 | <0.0115 |
| S7 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 2.26±0.3 | 3.67±0.7 | 0.315±0.020 | 0.474±0.016 |
| S8 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | <0.0341 | <0.0165 | <0.0115 |
| S9 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | <0.0341 | <0.0165 | <0.0115 |
| S10 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 1.16±0.09 | 2.02±0.013 | <0.0165 | <0.0115 |

Table 4.3 continued

| Site | BAnt | Chr | BbF | BkF | BaP | InP | Dah-Ant | BghiP |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S2 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S3 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S4 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S5 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S6 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S7 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S8 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S9 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S10 | <0.556 | <0.0793 | <0.0457 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |

Table 4.4: Concentrations of PAHs in sediments collected from Blood River in ng/g, dry weight

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | <0.0341 | 0.302±0.032 | 0.020±0.0011 |
| S2 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | <0.0341 | <0.0165 | <0.0115 |
| S3 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 1.78±0.004 | 3.09±0.005 | 0.10±0.001 | <0.0115 |
| S4 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | <0.0341 | <0.0165 | <0.0115 |
| S5 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 1.05±0.003 | 1.16±0.003 | <0.0165 | 0.511±0.001 |
| S6 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | 0.433±0.01 | <0.0165 | 0.46±0.0004 |
| S7 | 0.3±0.0003 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | 0.433±0.001 | <0.0165 | 0.46±0.0004 |
| S8 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | <0.00858 | 1.18±0.002 | <0.0165 | <0.0115 |
| S9 | <0.0224 | <0.0167 | <0.00471 | 0.403±0.0002 | 0.607±0.001 | 1.57±0.002 | <0.0165 | 0.3±0.0003 |
| S10 | <0.0224 | <0.0167 | <0.00471 | <0.0471 | 1.74±0.003 | 2.53±0.001 | <0.0165 | <0.0115 |

Table 4.4 continued

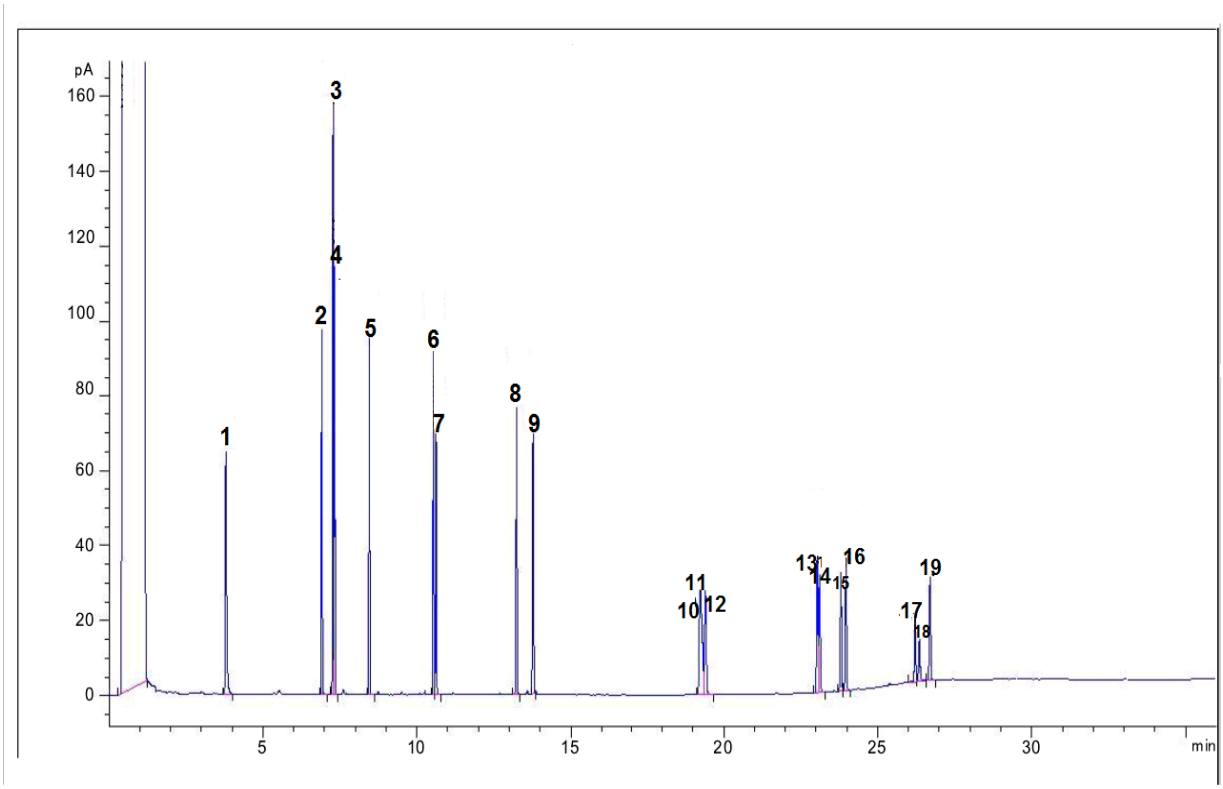
| Site | BAnt | Chr | BbF | BkF | BaP | InP | Dah-Ant | BghiP |
|---|---|---|---|---|---|---|---|---|
| S1 | 74.7±0.082 | 36±0.041 | 0.81±0.001 | <0.00459 | <0.943 | <0.00659 | <0.00895 | 3.04±0.003 |
| S2 | 130±0.021 | 62.7±0.010 | 1.06±0.0002 | <0.00459 | 2.92±0.003 | 0.86±0.002 | <0.00895 | 1.57±0.002 |
| S3 | 99.1±0.010 | 47.3±0.003 | 3.12±0.001 | 3.60±0.005 | 1.21±0.02 | <0.00659 | <0.00895 | 2.85±0.003 |
| S4 | 94.7±0.039 | 45.8±0.018 | 0.75±0.001 | <0.00459 | 2.70±0.008 | 5.11±0.013 | <0.00895 | 3.57±0.010 |
| S5 | 122±0.012 | 59.1±0.048 | 0.86±0.0002 | <0.00459 | 4.57±0.017 | <0.00659 | <0.00895 | <0.675 |
| S6 | 57.2±0.046 | 27.5±0.022 | 0.48±0.001 | <0.00459 | <0.943 | <0.00659 | <0.00895 | 17.4±0.011 |
| S7 | 57.2±0.046 | 27.5±0.022 | 0.48±0.001 | <0.00459 | <0.943 | 11.3±0.018 | 4.80±0.010 | 11.9±0.015 |
| S8 | 87±0.044 | 44.7±0.012 | 0.474±0.011 | <0.00459 | <0.943 | <0.00659 | <0.00895 | <0.675 |
| S9 | 139±0.258 | 67.8±0.092 | 2.11±0.002 | 2.85±0.003 | 3.47±0.001 | 20.1±0.002 | 2.10±0.002 | 21.3±0.017 |
| S10 | <0.556 | <0.0793 | 2.75±0.006 | 2.66±0.008 | <0.943 | 6.27±0.012 | 0.784±0.001 | 5.20±0.010 |

The concentrations of PAHs in sediment samples of Mokolo and Blood Rivers are presented in Tables 4.3 and 4.4, respectively. In Mokolo River, the PAHs concentrations ranged from <LOD to 4.51 ng/g. The lowest and highest concentration obtained was for anthracene in sample 6 and sample 4, respectively. In Blood River, PAH concentrations ranged between <LOD and 139 ng/g. The lowest concentration was obtained in sample 1 for Pyr while highest concentration was observed in sample 9 for BAnt. Higher concentrations were measured in Blood River sediments while concentrations obtained in Mokolo River were very low. In Mokolo River all HMW PAHs were not detected, while in Blood River most of the LMW compounds were not detected. The concentrations vary at different sampling sites in both rivers. Furthermore, the concentrations of all the PAHs decreases slightly from sampling site 1 to 10, this might be caused by different sources of PAH found around the rivers. However, the analysis using the GC-MS could not be continued due to problems encountered with the MS detector. The problem was with the filament of the Agilent MSD that generates electrons used to ionise the samples. Because of this problem (broken filaments) good chromatograms that show complete separation could not be obtained and also it was difficult to identify the PAHs of interest. Therefore, no further analysis was done by GC-MS. The GC-FID, which is also the preferred technique for qualitative and quantitative analysis of PAHs in environmental samples was used.

## 4.3 DETERMINATION OF THE PAHs BY GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR

### 4.3.1 Identification of polycyclic aromatic hydrocarbons by gas chromatography-flame ionisation detector

The sediment and water samples were analysed by GC-FID. The 16 US EPA priority PAHs determined include, naphthalene (Nap), acenaphthene (Ace), acenaphthylene (Acy), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr), benzo(a)anthracene (BAnt), chrysene (Chr), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), indeno(1,2,3-cd)pyrene (InP), dibenzo(a,h)anthracene (DahAnt), and benzo(g,h,i)perylene (BghiP). Several researchers used GC-FID successfully for qualitative and quantitative analysis of US EPA priority PAHs (Tahir *et al*., 2008; Kafilzadeh *et al*., 2011; Topal, 2011; Bayowa, 2014; Nekhavhambe *et al*., 2014). The GC-FID technique used in the present study was assessed by analysing a 1 mg/L mixture of the 16 priority PAH standards and three internal standards, resulting in the chromatogram given in Figure 4.3. All 16 PAHs were successfully separated with the exception of BAnt and Chr that co-eluted with one of the internal standards (Chr-D12). However, BAnt, Chr and Chr-D12 were identified by their retention times since they were different, namely, 19.2, 19.4 and 19.3 min, respectively. The successful separation of PAHs confirmed the GC-FID as the technique of choice for this study. Figure 4.3 shows chromatogram indicating that all 16 PAHs and 3 internal standards were completely separated in scan mode.




PAH 1 to 19: Nap, Acy, Ace, Flu, Phe, Ant, Fln, Pyr, BAnt, Chr, BbF, BkF, BghiP, InP, DahAnt, BaP and internal standards (3, 11 and 16: acenaphthene-D10, chrysene-D12 and perylene-D12

Figure 4.3: Chromatogram of a mixed standard containing the 16 USEPA priority PAHs and internal standards, obtained by GC-FID

### 4.3.2 Construction of calibration curves

Calibration standards were prepared by using different concentrations for each compound of interest through dilution of stock solutions with DCM. The prepared standard solutions for each PAH compound were analysed by GC-FID. Calibration curves for the 16 USEPA priority PAHs were constructed by plotting the response ratios, As/Ais *versus* the concentration ratios, Cs/Cis, where As and Ais are the peak areas of the analyte and internal standard and Cs and Cis their concentrations, in mg/L, respectively (Seopela *et al*., 2016). Internal standardisation was proven to be more effective technique than external standardisation (De Oliveira *et al*., 2009; Usher *et al*., 2015). The internal standard technique is mostly used to improve the precisions of results where volume errors are difficult to predict and control. Examples of types

of errors that are reduced by the use of an internal standard are those caused by evaporation of solvents, injection errors, and complex sample preparation involving transfers, extractions, and dilutions (Usher *et al*., 2015). Thus, internal standard calibration was applied in this study. A representative calibration curve obtained by internal standardisation is given in Figure 4.4.

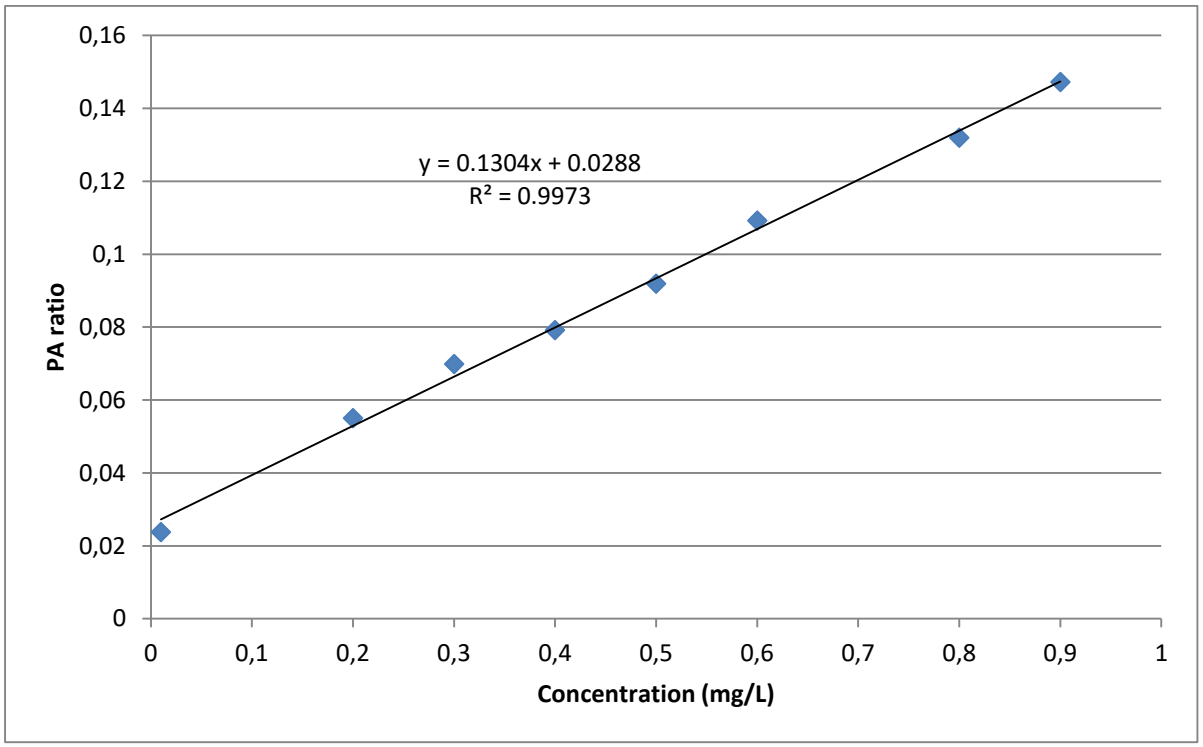



Figure 4.4: An example of an internal standard calibration curve prepared for anthracene constructed from GC-FID results

Linear calibration curves were obtained for all standard concentration ranges that were determined. The correlation coefficients for the linear regression obtained ranged from 0.9367 to 0.9973. The lowest regression value obtained was for DahAnt, Fln, Pyr and BaP with values lower than 0.99. The calibration curves were used to determine concentrations of each PAH compound found in water and sediment samples.

### 4.3.3 Limit of detection and limit of quantification

The LOD is normally explained as the lowest quantity or concentration of a component that may be consistently detected with a given analytical technique. The LOD represents the level below which we cannot be sure whether or not the analyte is

actually present. It follows from this that no analytical technique can ever conclusively prove that a particular chemical substance is not present in a sample; only that it cannot be detected. The LOD was measured for each PAH (Tables 4.5 and 4.6). The LODs were determined by analysing 16 USEPA priority PAHs standards, over the range of 1 to 6 000 µg/L and then calculating the standard deviation from the measured concentrations of the standards. The LODs in the present study for water analysis procedure ranged between 0.0112 and 0.0401 µg/L while the LOQs ranged between 0.0372 and 0.119 µg/L.

For sediments analysis procedure, the LODs ranged from 0.00214 to 0.0214 mg/kg while the LOQs ranged between 0.0223 and 0.0801 mg/kg as obtained for MAE. The LODs ranged from 0.00605 to 0.359 mg/kg and 0.0121 to 0.322 mg/kg as obtained for combination of ultrasonication and mechanical shaking and ultrasonication, respectively. However, the LOQs ranged from 0.0202 to 1.21 for combination of ultrasonication and mechanical shaking and between 0.0403 and 1.07 mg/kg for ultrasonication. Furthermore, the lowest LODs and LOQs are observed for MAE as compared to other two extraction techniques. Any PAH concentration in sediment or water samples with values less than the LOD were recorded as below LOD. The LODs and LOQs obtained in the current study are comparable to those reported by Seopela *et al*. (2016). Chemical formulae of PAHs, number of rings, retention times, regression parameters, LODs and LOQs obtained from analysis of standards by GC-FID for methods applied for analysis of sediment and water samples are given in Tables 4.5 and 4.6, respectively.

Table 4.5: Regression parameters, limit of detection and quantification (mg/kg) applied to sediments obtained from analysis of standards by GC-FID

| PAHs | Chemical formula | No. of rings | Retention time (Min) | $R^2$ | LOD (MAE) | LOQ (MAE) | LOD (UAM) | LOQ (UAM) | LOD (U) | LOQ (U) |
|---|---|---|---|---|---|---|---|---|---|---|
| Nap | $C_{10}H_8$ | 2 | 3.759 | 0.996 | 0.0124 | 0.0412 | 0.0927 | 0.309 | 0.185 | 0.618 |
| Acy | $C_{12}H_8$ | 3 | 6.911 | 0.9946 | 0.0214 | 0.0715 | 0.0536 | 0.179 | 0.322 | 1.07 |
| Ace | $C_{12}H_{10}$ | 3 | 7.327 | 0.9956 | 0.0140 | 0.0467 | 0.351 | 1.17 | 0.0701 | 0.234 |
| Flu | $C_{13}H_{10}$ | 3 | 8.447 | 0.9965 | 0.0110 | 0.0368 | 0.0276 | 0.0921 | 0.0552 | 0.184 |
| Phe | $C_{14}H_{10}$ | 3 | 10.537 | 0.9945 | 0.0110 | 0.0385 | 0.0288 | 0.0961 | 0.0578 | 0.193 |
| Ant | $C_{14}H_{10}$ | 3 | 10.640 | 0.9973 | 0.0103 | 0.0342 | 0.0257 | 0.0855 | 0.0513 | 0.171 |
| Fln | $C_{16}H_{10}$ | 4 | 13.249 | 0.9786 | 0.0117 | 0.0392 | 0.0293 | 0.0979 | 0.0587 | 0.198 |
| Pyr | $C_{16}H_{10}$ | 4 | 13.782 | 0.9715 | 0.0117 | 0.0391 | 0.0293 | 0.0976 | 0.0585 | 0.195 |
| BAnt | $C_{18}H_{12}$ | 4 | 19.240 | 0.9953 | 0.00999 | 0.0333 | 0.248 | 0.826 | 0.0499 | 0.167 |
| Chr | $C_{18}H_{12}$ | 4 | 19.411 | 0.9956 | 0.0144 | 0.0479 | 0.359 | 1.21 | 0.0719 | 0.239 |
| BbF | $C_{20}H_{12}$ | 5 | 23.044 | 0.9973 | 0.00743 | 0.0248 | 0.0186 | 0.0619 | 0.0371 | 0.124 |
| BkF | $C_{20}H_{12}$ | 5 | 23.108 | 0.9953 | 0.00743 | 0.0239 | 0.0167 | 0.0557 | 0.0356 | 0.119 |
| BghiP | $C_{22}H_{12}$ | 6 | 23.806 | 0.9952 | 0.00668 | 0.0223 | 0.0171 | 0.0571 | 0.0334 | 0.111 |
| InP | $C_{22}H_{12}$ | 6 | 26.225 | 0.9951 | 0.0195 | 0.0649 | 0.0487 | 0.162 | 0.0974 | 0.325 |
| DahAnt | $C_{22}H_{14}$ | 5 | 26.349 | 0.9367 | 0.00241 | 0.0801 | 0.00605 | 0.0202 | 0.0121 | 0.0403 |
| BaP | $C_{20}H_{12}$ | 5 | 26.711 | 0.9807 | 0.0210 | 0.0702 | 0.0525 | 0.175 | 0.105 | 0.350 |

MAE=Microwave-assisted extraction, U=Ultrasonication, UAM=Combination of ultrasonication and mechanical shaking

Table 4.6: Regression parameters, limit of detection and quantification applied to water obtained from analysis of standards by GC-FID

| PAHs | Chemical formula | No. of rings | Retention time Min | $R^2$ | LOD (µg/L) | LOQ (µg/L) |
|---|---|---|---|---|---|---|
| Nap | $C_{10}H_8$ | 2 | 3.759 | 0.996 | 0.0206 | 0.0688 |
| Acy | $C_{12}H_8$ | 3 | 6.911 | 0.9946 | 0.0358 | 0.119 |
| Ace | $C_{12}H_{10}$ | 3 | 7.327 | 0.9956 | 0.0234 | 0.0780 |
| Flu | $C_{13}H_{10}$ | 3 | 8.447 | 0.9965 | 0.0185 | 0.0615 |
| Phe | $C_{14}H_{10}$ | 3 | 10.537 | 0.9945 | 0.0193 | 0.0644 |
| Ant | $C_{14}H_{10}$ | 3 | 10.640 | 0.9973 | 0.0171 | 0.0571 |
| Fln | $C_{16}H_{10}$ | 4 | 13.249 | 0.9786 | 0.0196 | 0.0653 |
| Pyr | $C_{16}H_{10}$ | 4 | 13.782 | 0.9715 | 0.0196 | 0.0652 |
| BAnt | $C_{18}H_{12}$ | 4 | 19.240 | 0.9953 | 0.0166 | 0.0556 |
| Chr | $C_{18}H_{12}$ | 4 | 19.411 | 0.9956 | 0.0240 | 0.0801 |
| BbF | $C_{20}H_{12}$ | 5 | 23.044 | 0.9973 | 0.0124 | 0.0414 |
| BkF | $C_{20}H_{12}$ | 5 | 23.108 | 0.9953 | 0.0124 | 0.0397 |
| BghiP | $C_{22}H_{12}$ | 6 | 23.806 | 0.9952 | 0.0112 | 0.0372 |
| InP | $C_{22}H_{12}$ | 6 | 26.225 | 0.9951 | 0.0325 | 0.108 |
| DahAnt | $C_{22}H_{14}$ | 5 | 26.349 | 0.9367 | 0.0401 | 0.133 |
| BaP | $C_{20}H_{12}$ | 5 | 26.711 | 0.9807 | 0.0351 | 0.117 |

## 4.4 EVALUATION OF ACCURACY AND PRECISION OF THE METHODS EMPLOYED FOR QUANTIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS

The extraction efficiency of each method was validated before its application to entire sediment and water samples. The MAE method applied in the current study was validated by analysing a certified reference material (CRM-104 of sediments) containing mixture of PAHs. The LRB was used with known amount of standards for determining recoveries for water samples, as discussed in Section 3.4. The

concentrations and percentage recoveries of each PAH in CRM-104 obtained using GC-FID are presented in Table 4.7 while percentage recoveries for validating the LLE method are given in Table 4.8.

### 4.4.1 Validation of MAE, ultrasonication and combination of ultrasonication and mechanical shaking techniques for determination of PAHs in sediments

Various methods of extraction of PAHs from sediment samples and determination of PAH have been proposed and several studies have been conducted to compare the different extraction techniques (Kumar *et al*., 2014; Lau *et al*., 2010; Oluseyi *et al*., 2011; Seopela *et al*., 2016). The MAE, ultrasonication and combination of ultrasonication and mechanical shaking techniques were compared by evaluating the percentage recoveries of 16 PAHs from CRM-104 as determined by GC-FID. The recoveries obtained for these PAHs are given in Table 4.7. The percentage recoveries obtained from ultrasonication ranged from 32.4 to 98.5% with the % RSD values ranging between 0.279 and 8.91%. Benzo(b)fluoranthene showed lowest percentage recovery of 32.4% while the highest percentage recovery of 98.5% was obtained for chrysene. The percentage recoveries obtained from a combination of ultrasonication and mechanical shaking ranged from 23.1 to 86.5% with the % RSD values ranging between 0.261 and 8.7%. Dibenzo(a,h)antracene demonstrated the highest recovery (86.5%) while only 23.1% was recovered for acenaphthene. However, results from this study showed that extraction performed using ultrasonic bath and a combination of ultrasonication and mechanical agitation gave lower recoveries and was less efficient. The low extraction efficiencies might be caused by losses of PAHs that occur during concentrating of extracts with the rotary evaporator before analysis. The percentage recoveries obtained from MAE ranged from 83.8 to 125% with % RSD values ranging between 0.317 and 7.53%. Phenanthrene had the lowest percent recovery of 83.8% while the highest percentage recovery of 125% was obtained for Indeno(1,2,3-cd)pyrene. The percentage recovery for LMW PAHs (Nap-Ant) ranged from 83.8 to 117%, whereas the percentage recovery for HMW PAHs (Fln-BghiP) ranged from 92.7 to 125% and this shows that higher percentage recoveries were obtained for HMW compounds. The LMW compounds might have been lost during the evaporation step

(Seopela *et al*., 2016). The precision obtained for the ultrasonication, combined ultrasonication and mechanical shaking and MAE techniques was acceptable, since the % RSD values obtained for these three methods ranged from 0.261 to 8.91(Table 4.7).

The recoveries obtained in the present study indicated that MAE is more efficient than ultrasonication and combination of ultrasonication and mechanical shaking since the percentage recoveries for all the PAHs are above 80 % for the MAE method. Mekonnen *et al*. (2015) and Seopela *et al*. (2016) all reported average percentage recoveries of more than 80% for selected PAHs obtained with MAE, which is comparable to the percent recoveries obtained in the current study. As can be seen in Table 4.7, higher recoveries and better precision were obtained by MAE using GC-FID analysis. From these results it can be concluded that MAE is a suitable technique for extraction of PAHs from sediment samples. Therefore, all the sediment samples collected from Mokolo and Blood Rivers were extracted by MAE. Certified concentrations and percentage recoveries of PAHs in CRM-104 for method validation obtained by GC-FID are given in Table 4.7.

Table 4.7: Certified and measured concentrations and percentage recoveries of PAHs in CRM-104 for method validation obtained by GC-FID after MAE, ultrasonication (U) and a combination of ultrasonication and mechanical shaking (UAM)

| PAH | measured value (µg/Kg) (MAE) | certified value (µg/Kg) | %Recovery (MAE) | %RSD (MAE) | Measured value (µg/Kg) (U) | %Recovery (U) | %RSD (U) | Measured value(µg/Kg) (UAM) | %Recovery (UAM) | %RSD (UAM) |
|---|---|---|---|---|---|---|---|---|---|---|
| Nap | 398±2.84 | 414 | 96.1 | 0.715 | 239±8.5 | 57.7 | 3.56 | 189±1.45 | 45.7 | 0.767 |
| Acy | 599±1.9 | 511 | 117 | 0.745 | 412±1.15 | 80.6 | 0.279 | 365±0.954 | 71.4 | 0.261 |
| Ace | 604±4.5 | 528 | 114 | 0.317 | 187±4.5 | 35.4 | 2.41 | 122±2.9 | 23.1 | 2.39 |
| Flu | 369±14.1 | 392 | 94.3 | 3.82 | 269±3.41 | 68.6 | 1.28 | 338±5.5 | 86.2 | 1.62 |
| Phe | 397±21.6 | 474 | 83.8 | 5.44 | 421±9.8 | 89 | 2.32 | 325±12.5 | 68.6 | 3.85 |
| Ant | 237±1.63 | 282 | 84.3 | 0.688 | 255±7.1 | 91 | 2.78 | 238±19.8 | 84.4 | 8.3 |
| Fln | 394±28.5 | 456 | 86.4 | 7.23 | 285±20.5 | 63 | 7.2 | 216±8.9 | 47.4 | 4.12 |
| Pyr | 331±16.1 | 302 | 109 | 4.86 | 286±15.6 | 95 | 5.46 | 247±1.45 | 81.8 | 0.587 |
| BAnt | 364±3.2 | 412 | 88.4 | 0.879 | 189±7.1 | 45.8 | 3.8 | 322±14.9 | 78.2 | 4.63 |
| Chr | 203±12.4 | 201 | 101 | 6.12 | 198±17.7 | 98.5 | 8.91 | 128±1.35 | 63.7 | 1.05 |
| BbF | 70.6±0.9 | 58.6 | 120 | 1.27 | 19±0.15 | 32.4 | 0.78 | 28.4±2.47 | 48.4 | 8.7 |
| BkF | 299±2.6 | 323 | 92.7 | 0.869 | 323±5.6 | 82 | 2.1 | 271±0.98 | 83.9 | 0.361 |
| BghiP | 298.8±9 | 305 | 97.9 | 3.01 | 256±2.3 | 84 | 0.898 | 189±5.75 | 61.9 | 3.04 |
| InP | 338±35.5 | 270 | 125 | 10.5 | 131±1.91 | 48.5 | 1.45 | 224±16.7 | 82.9 | 7.45 |
| DahAnt | 164±3.3 | 164 | 100 | 2.01 | 139±4.9 | 84.7 | 3.53 | 142±4.3 | 86.5 | 3.03 |
| BaP | 215±36.2 | 180 | 119 | 7.53 | 145±6.2 | 81.1 | 4.28 | 154±6.2 | 85.6 | 4.03 |

### 4.4.2 Validation of LLE method for determination of PAHs in water

The LLE method has been successfully applied by some researchers for extraction of PAHs from water samples (Kafilzadeh *et al*., 2011; Nekhavhambe *et al*., 2014; Kafilzadeh, 2015; Edokpayi *et al*., 2016; Seopela *et al*., 2016). The efficiency of the LLE method for extraction of PAHs from water was determined by assessing the percentage recoveries of LRB spiked with a known amount of standards. Higher percentage recoveries were obtained for all the compounds (above 80%) with the exception of Nap and DahAnt, which were 67.6 and 75.2%, respectively. The low extraction efficiencies of Nap and DahAnt might be caused by losses of PAHs that occur during concentrating of extracts with the rotary evaporator before analysis (Nekhavhambe *et al*., 2014; Seopela *et al*., 2016). The perecentage recoveries for all 16 PAHs ranged from 67.6 to 115% with the % RSD values ranging between 0.0534 and 3.91%, indicating good precision. The recoveries obtained in this study are comparable to the percentage recoveries reported by Nekhavhambe *et al*. (2014) and Seopela *et al*. (2016), however higher recoveries and better precision were obtained in the current study. The higher recoveries and better precision obtained by LLE method compared to solid-phase extraction conducted by Seopela *et al*. (2016) proved LLE as a suitable method for extraction of PAHs from water samples. Therefore, all the water samples collected from Mokolo and Blood Rivers were extracted by LLE. The percentage recoveries and % RSD of each PAH in water samples are presented in Table 4.8.

Table 4.8: Percentage recoveries of PAHs in water samples for method validation obtained by GC-FID after LLE

| PAH | Spiked level (µg/L) | Amount measured (µg/L) | %Recovery | %RSD |
|---|---|---|---|---|
| Nap | 100 | 67.7±0.99 | 67.6 | 1.46 |
| Acy | 100 | 106±0.27 | 106 | 0.254 |
| Ace | 100 | 104±0.35 | 104 | 0.338 |
| Flu | 100 | 108±0.171 | 108 | 0.158 |
| Phe | 100 | 89.3±0.489 | 89.3 | 0.548 |
| Ant | 100 | 109±0.0583 | 109 | 0.0534 |
| Fln | 10 | 10.2±0.0484 | 102 | 0.475 |
| Pyr | 10 | 11.5±0.0197 | 115 | 0.171 |
| BAnt | 10 | 11±0.0438 | 110 | 0.398 |
| Chr | 10 | 10.9±0.0695 | 109 | 0.638 |
| BbF | 10 | 10.7±0.0912 | 107 | 0.852 |
| BkF | 5 | 5.35±0.117 | 107 | 2.18 |
| BghiP | 10 | 10.8±0.0757 | 108 | 0.701 |
| InP | 10 | 11±0.292 | 110 | 0.265 |
| DahAnt | 10 | 7.52±0.294 | 75.2 | 3.91 |
| BaP | 10 | 11.1±0.14 | 111 | 1.26 |

## 4.5 QUANTIFICATION OF PAHs IN WATER SAMPLES

### 4.5.1 Concentrations of PAHs in water samples collected from Blood River

The results of analysis of the water samples from Blood River are presented in Table 4.9. The LLE method was used for the determination of the 16 US EPA PAHs from water samples. The results including mean and standard deviation of individual PAHs were obtained in milligram per litre (mg/L) and they were converted to microgram per litre (µg/L). The levels of PAHs in water samples from Blood River in all sampling sites ranged between 0.0121 and 0.433 µg/L. Most of LMW compounds with fewer rings (Nap, Ace, Acy, Flu, Phe and Ant) were not detected while most of HMW PAHs were found. Benzo(a)anthracene had the highest concentration in most of the sampling sites compared to all the PAHs. Benzo(a)pyrene was found below LOD in all sampling sites while DahAnt was also not detected except for site one. Chrysene, BkF and InP were also below the LOD in most sampling sites as can be seen in Table 4.9. Levels of the PAHs in water samples collected from Blood River are given in Table 4.9.

Table 4.9: Concentrations (µg/L) of the PAHs in water samples collected from Blood River

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | 0.179±0.0211 | 0.135±0.0111 | 0.169±0.0206 |
| S2 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.119±0.00749 |
| S3 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.127±0.0150 |
| S4 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.289±0.0216 |
| S5 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.346±0.0431 |
| S6 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.137±0.0175 |
| S7 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.159±0.0110 |
| S8 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.232±0.00649 |
| S9 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.192±0.00413 |
| S10 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.179±0.0118 |

Table 4.9 continued…..

| Site | BAnt | Chr | BbF | BkF | BghiP | InP | DahAnt | BaP |
|---|---|---|---|---|---|---|---|---|
| S1 | 0.392±0..0142 | <0.0240 | 0.174±0.0143 | <0.0124 | <0.0112 | 0.0729±0.00230 | 0.0850±0.00296 | <0.0351 |
| S2 | 0.433±0.0212 | <0.0240 | <0.0124 | <0.0124 | 0.0478±0.00178 | <0.0325 | <0.0401 | <0.0351 |
| S3 | 0.406±0.0199 | <0.0240 | 0.0386±0.00216 | 0.0146±0.00139 | 0.0121±0.00110 | 0.0587±0.00354 | <0.0401 | <0.0351 |
| S4 | <0.0166 | <0.0240 | <0.0124 | 0.0197±0.00139 | 0.0156±0.00174 | <0.0325 | <0.0401 | <0.0351 |
| S5 | 0.166±0.00108 | <0.0240 | 0.0330±0.00211 | <0.0124 | 0.0433±0.00173 | <0.0325 | <0.0401 | <0.0351 |
| S6 | 0.209±0.0133 | <0.0240 | 0.0256±0.00112 | <0.0124 | 0.0247±0.00217 | <0.0325 | <0.0401 | <0.0351 |
| S7 | 0.151±0.00622 | <0.0240 | 0.0192±0.00181 | <0.0124 | 0.0263±0.00123 | <0.0325 | <0.0401 | <0.0351 |
| S8 | <0.0166 | 0.0266±0.00250 | 0.0455±0.00277 | <0.0124 | 0.0570±0.00612 | <0.0325 | <0.0401 | <0.0351 |
| S9 | <0.0166 | <0.0240 | 0.0438±0.00120 | <0.0124 | 0.0209±0.00110 | <0.0325 | <0.0401 | <0.0351 |
| S10 | 0.239±0.0144 | 0.0315±0.00291 | <0.0124 | <0.0124 | <0.0112 | <0.0325 | <0.0401 | <0.0351 |

### 4.5.2 Concentrations of PAHs in water samples collected from Mokolo River

Levels of PAHs in the water samples from Mokolo River ranged between 0.0219 and 1.53 µg/L. Most of LMW compounds with fewer rings (Nap, Ace, Acy, Flu, Phe and Ant) were not detected while HMW PAHs were found at most of the sampling sites. Benzo(a)anthracene, InP and DahAnt were present in higher concentrations when compared to other PAHs; however their concentration values vary at different sampling sites. Although HMW PAHs are present in higher concentration, the majority of them, including BkF, BghiP, InP, DahAnt and BaP, were below the LOD at most of the sampling sites. Levels of the PAHs in water samples collected from Mokolo River are presented in Table 4.10.

Table 4.10: Concentrations (µg/L) of the PAHs in water samples collected from Mokolo River

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.0421±0.000514 |
| S2 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | <0.0196 |
| S3 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | <0.0196 |
| S4 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | 0.0326±0.00135 | 0.0989±0.000295 |
| S5 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | 0.172±0.00584 | 0.260±0.00184 | 0.166±0.0195 |
| S6 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | <0.0196 |
| S7 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | <0.0196 |
| S8 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | 0.0718±0.00178 | 0.308±0.0133 |
| S9 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | 0.0969±0.00507 | 0.327±0.0228 |
| S10 | <0.0206 | <0.0358 | <0.0234 | <0.0185 | <0.0193 | <0.0171 | <0.0196 | 0.309±0.0108 |

Table 4.10 continued…..

| Site | BAnt | Chr | BbF | BkF | BghiP | InP | DahAnt | BaP |
|---|---|---|---|---|---|---|---|---|
| S1 | 0.783±0.00744 | 0.0922±0.00844 | 0.0362±0.00118 | 0.0481±0.00183 | <0.0112 | 1.16±0.0459 | 1.53±0.0522 | 0.384±0.00146 |
| S2 | 0.721±0.0212 | 0.0317±0.00225 | <0.0124 | <0.0124 | 0.0332±0.00118 | <0.0325 | <0.0401 | <0.0351 |
| S3 | 0.295±0.0144 | 0.0899±0.00219 | <0.0124 | <0.0124 | <0.0112 | <0.0325 | <0.0401 | <0.0351 |
| S4 | 0.632±0.0210 | 0.119±0.0106 | 0.0219±0.00172 | <0.0124 | <0.0112 | <0.0325 | <0.0401 | <0.0351 |
| S5 | <0.0166 | 0.138±0.00248 | 0.487±0.00173 | 0.786±0.00324 | 0.586±0.0199 | <0.0325 | <0.0401 | 0.474±0.00152 |
| S6 | 0.385±0.00225 | 0.0719±0.00112 | <0.0124 | <0.0124 | <0.0112 | <0.0325 | <0.0401 | <0.0351 |
| S7 | 0.504±0.0139 | 0.0877±0.00857 | <0.0124 | <0.0124 | <0.0112 | <0.0325 | <0.0401 | <0.0351 |
| S8 | 0.630±0.00412 | <0.0240 | 0.0338±0.000768 | <0.0124 | <0.0112 | 0.0576±0.00104 | <0.0401 | <0.0351 |
| S9 | 0.104±0.00255 | <0.0240 | <0.0124 | <0.0124 | <0.0112 | 0.0723±0.00245 | <0.0401 | <0.0351 |
| S10 | 0.347±0.00621 | 0.0454±0.00283 | <0.0124 | <0.0124 | <0.0112 | <0.0325 | <0.0401 | <0.0351 |

## 4.6 COMPARISONS OF PAHs CONCENTRATION IN MOKOLO AND BLOOD RIVER WATER SAMPLES

Generally, the concentrations of PAHs were higher in the sediment than in water samples in both Blood and Mokolo Rivers. This may be due to the hydrophobic nature of PAHs because they tend to adsorb on the surface of sediment samples since they are not soluble in water (Edokpayi *et al*., 2016; Seopela *et al*., 2016). In addition, HMW PAHs (≥ 4 aromatic rings) are less water-soluble, less volatile and more lipophilic than LMW PAHs (≤3 aromatic rings) (Edokpayi *et al*., 2016). Comparisons and distribution of the PAHs of each water sample collected from the MR and BR, as obtained following LLE, are presented in Figures 4.5 and 4.6.

Naphthalene, Acy, Ace, Flu, and Phe were not detected in either river while Ant was only detected once in sample 1 and sample 5 of Blood and Mokolo Rivers, respectively. The concentrations of PAHs detected in Mokolo River are higher than the levels in Blood River. Dibenzo(a,h)anthracene, InP, BAnt, BkF, BbF, BaP and BghiP contributed the greatest part to the total concentration of PAHs found in the MR. Dibenzo(a,h)anthracene had the highest concentration in MR while the highest concentration in the BR was observed for BAnt (Figure 4.10). A Study by Nekhavhambe *et al*. (2014) reported levels of PAHs in river water samples collected in the Vhembe District of South Africa. The PAH levels ranged between 0.1 and 137 µg/L for river water samples. Their results revealed higher total PAH concentrations in water compared to this study. This might be due to different possible sources found in the study areas as they will not contribute the same amount of PAHs to the rivers. Therefore, the low concentrations of PAHs found in water samples of Mokolo and Blood Rivers in this study might be caused by several factors, including seasonal variation in human activities releasing PAHs and the dilution of PAHs due to rain (Seopela *et al*., 2016). The concentrations of PAHs in water samples from Mokolo and Blood Rivers were subjected to one-way analysis of variance (ANOVA) to determine the significance of the observed difference at 95% confidence level. There were no significant differences observed since the total concentration revealed insignificant difference ($P$>0.05) in Mokolo and Blood Rivers.

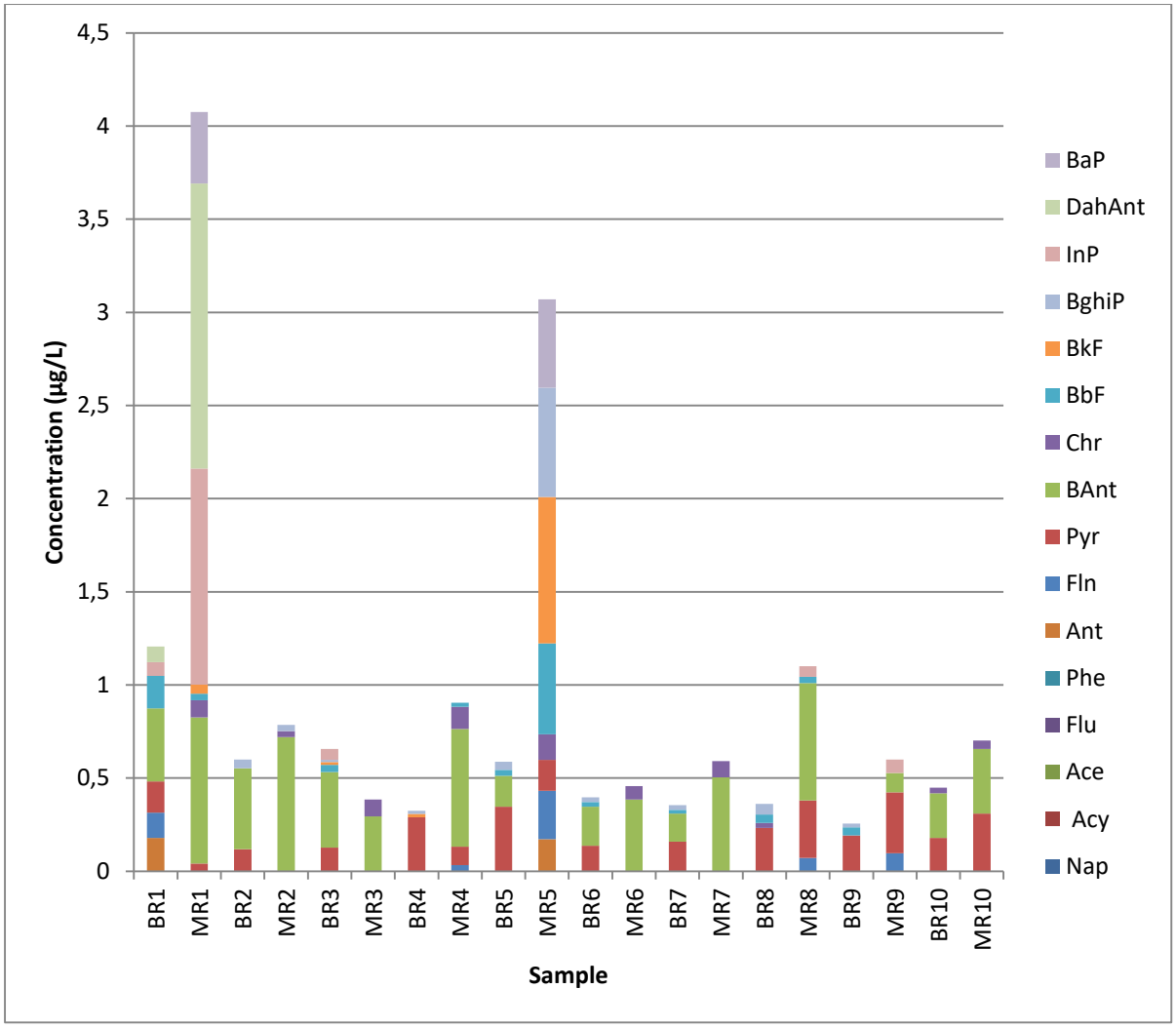



Figure 4.5: Comparisons of the concentration of each water samples collected from MR and BR as obtained following LLE

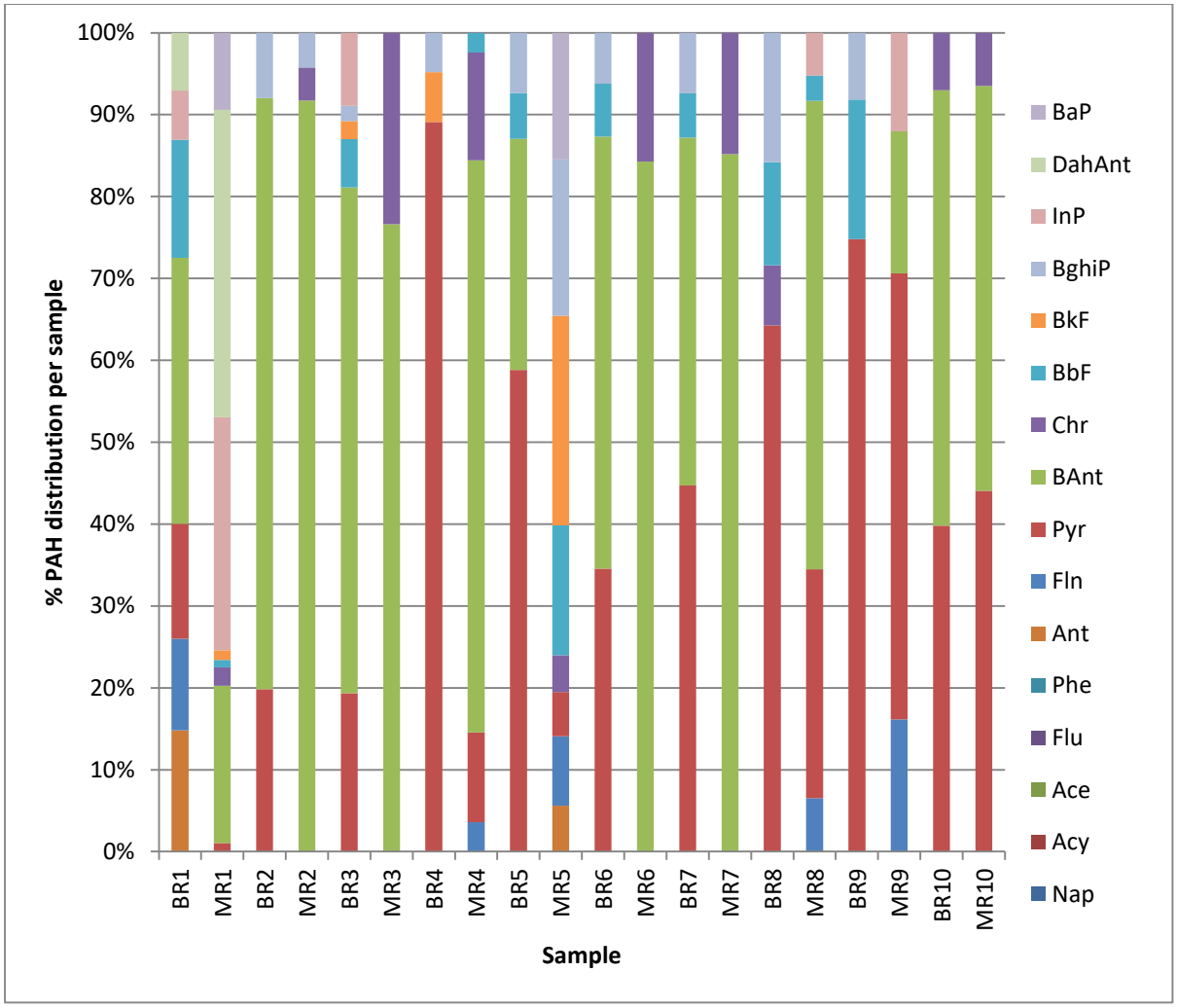
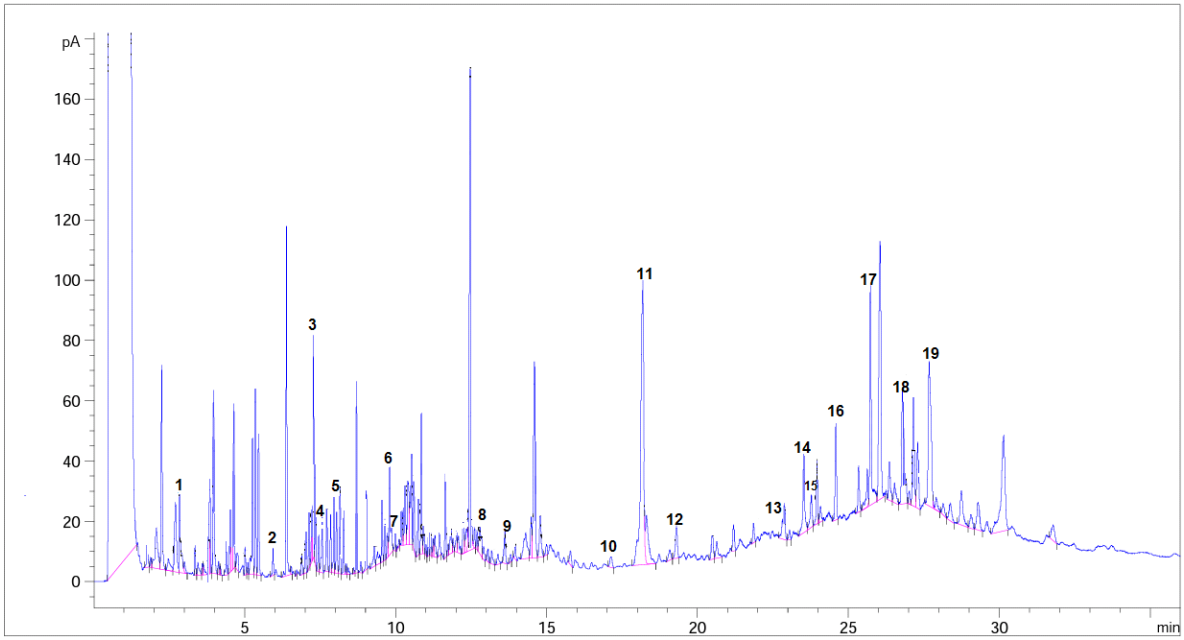



Figure 4.6: Distribution of the 16 PAHs in water samples from MR and BR as obtained following LLE

## 4.7 QUANTIFICATION OF PAHs IN SEDIMENTS AFTER MAE METHOD

The results of analysis of the sediment samples from Blood and Mokolo Rivers are presented in Tables 4.11 and 4.12, respectively. After optimisation, MAE was used for the determination of the 16 US EPA PAHs from sediment samples. The results including mean and standard deviation of individual PAHs were obtained in milligram per litre (mg/L) and converted to milligram per kilogram (mg/kg). Figure 4.7 shows a chromatogram indicating the presence of the PAHs in the extract of sediment sample 1 collected from Blood River. The complexity of the sediment samples is also observed, clearly illustrated by the presence of peaks other than compounds of interest in the chromatogram (Figure 4.7).




PAH 1 to 19: Nap, Acy, Ace, Flu, Phe, Ant, Fln, Pyr, BAnt, Chr, BbF, BkF, BghiP, InP, DahAnt, BaP and internal standards (3, 11 and 16: acenaphthene-D10, chrysene-D12 and perylene-D12)

Figure 4.7: A chromatogram (PAH 1-19) indicating the presence of the PAHs in the sediment sample 1 collected from Blood River

Table 4.11: Concentrations of the PAHs in sediments collected from Blood River in mg/kg

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | 0.061±0.005 | <0.0214 | 0.130±0.010 | 0.230±0.007 | 0.257±0.014 | 0.300±0.004 | 0.064±0.002 | <0.0117 |
| S2 | 0.016±0.001 | <0.0214 | 0.133±0.008 | 0.283±0.011 | 0.422±0.001 | 0.499±0.010 | 0.078±0.003 | 0.182±0.004 |
| S3 | <0.0124 | <0.0214 | 0.189±0.001 | 0.210±0.004 | 0.344±0.030 | 0.485±0.013 | <0.0117 | 0.158±0.010 |
| S4 | 0.087±0.003 | <0.0214 | 0.112±0.006 | 0.191±0.019 | 0.136±0.003 | 0.386±0.010 | <0.0117 | 0.228±0.017 |
| S5 | 0.014±0.001 | <0.0214 | 0.172±0.022 | <0.0110 | <0.0110 | 0.547±0.025 | 0.035±0.002 | 0.222±0.015 |
| S6 | <0.0124 | <0.0214 | 0.073±0.005 | 0.030±0.001 | <0.0110 | 0.356±0.005 | <0.0117 | 0.270±0.004 |
| S7 | 0.027±0.001 | <0.0214 | 0.095±0.009 | 0.016±0.001 | 0.106±0.001 | 0.453±0.035 | 0.099±0.008 | 0.208±0.010 |
| S8 | 0.059±0.003 | <0.0214 | 0.093±0.009 | <0.0110 | <0.0110 | 0.303±0.011 | 0.079±0.006 | <0.0117 |
| S9 | 0.078±0.003 | 0.025±0.001 | <0.0140 | 0.213±0.014 | 0.439±0.041 | 0.951±0.058 | 0.071±0.001 | <0.0117 |
| S10 | 0.072±0.001 | <0.0214 | 0.286±0.001 | 0.135±0.003 | 0.433±0.004 | 1.38±0.022 | 0.288±0.004 | 0.233±0.001 |

Table 4.11 continued…..

| Site | BAnt | Chr | BbF | BkF | BghiP | InP | DahAnt | BaP |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.00999 | <0.0144 | <0.00743 | 0.590±0.008 | <0.00668 | 0.447±0.036 | 0.700±0.001 | 0.184±0.001 |
| S2 | 0.237±0.022 | <0.0144 | 0.145±0.008 | 0.681±0.032 | 0.136±0.005 | <0.0195 | <0.00241 | 0.194±0.006 |
| S3 | 0.222±0.014 | 0.186±0.016 | 0.144±0.007 | 0.719±0.048 | 0.270±0.020 | <0.0195 | <0.00241 | 0.270±0.001 |
| S4 | 0.129±0.003 | <0.0144 | 0.080±0.001 | <0.00743 | 0.178±0.004 | <0.0195 | 0.681±0.034 | 0.121±0.002 |
| S5 | 0.214±0.004 | 0.176±0.006 | 0.116±0.003 | 0.130±0.013 | <0.00668 | 0.122±0.009 | <0.00241 | <0.0210 |
| S6 | 0.118±0.004 | 0.124±0.007 | 0.073±0.007 | <0.00743 | 0.247±0.037 | 0.128±0.005 | 2.13±0.034 | 1.74±0.108 |
| S7 | 0.196±0.012 | 0.143±0.001 | <0.00743 | <0.00743 | <0.00668 | 0.287±0.011 | 1.14±0.063 | <0.0210 |
| S8 | 0.229±0.020 | 0.121±0.001 | <0.00743 | 0.243±0.014 | <0.00668 | 0.104±0.008 | 0.639±0.027 | <0.0210 |
| S9 | <0.00999 | <0.0144 | <0.00743 | 1.69±0.083 | <0.00668 | 0.398±0.019 | <0.00241 | <0.0210 |
| S10 | 0.260±0.020 | <0.0144 | 0.273±0.001 | 3.10±0.220 | 1.54±0.112 | 0.558±0.002 | 2.07±0.072 | 0.761±0.053 |

The levels of PAHs in the sediment samples collected from Blood River ranged from 0.014 to 3.10 mg/kg. All of the 16 PAHs quantified in sediment samples from Blood River were found to be above LOD in most samples. Of the six LMW compounds (Nap-Ant) the lowest concentration was obtained for naphthalene (0.014 mg/kg) while the highest concentration was observed for Anthracene (1.38 mg/kg) at sampling site 10. From the HMW PAHs (Fln-BghiP), the highest concentration was observed for BkF while Fln had the lowest concentration. Acenaphthylene was below the LOD value of 0.0214 in all sediment samples except for sampling site 9. Chrysene was also below the LOD in sampling sites 1,2,4,9 and 10. The LMW compounds (two to three ringed PAHs) such as naphthalene and acenaphthylene had lower concentrations, while HMW compounds ($\geq$ 4 ringed PAHs) were present in higher concentrations. Since the HMW compounds are more lipophilic than LMW compounds, concentrations of HMW PAHs in sediment samples are expected to be higher than those obtained for LMW compounds (Kafilzadeh, 2015). Dibenzo(a,h)anthracene and BkF were present at higher concentrations than any of the other 16 PAHs in all sampling sites. However, their concentration values vary at different sampling sites. Furthermore, the concentrations of all the PAHs increased slightly from sampling site 1 to 10, this might be caused by different sources of PAH found around the river.

Table 4.12: Concentrations (mg/kg) of the PAHs in sediments collected from Mokolo River

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.0124 | <0.0214 | <0.0140 | <0.0110 | 3.64±0.055 | 4.83±0.107 | 1.30±0.129 | 0.535±0.034 |
| S2 | <0.0124 | <0.0214 | 48.1±1.14 | 7.52±0.540 | <0.0110 | 10.0±0.93 | 18.6±1.45 | 28.2±1.90 |
| S3 | <0.0124 | <0.0214 | <0.0140 | 3.47±0.195 | 11.2±0.927 | 17.2±0.683 | 13.2±0.075 | 22.2±1.66 |
| S4 | <0.0124 | <0.0214 | 2.02±0.082 | 12.2±0.986 | 25.7±1.84 | <0.0103 | 21.1±1.31 | <0.0117 |
| S5 | 0.769±0.016 | 0.044±0.003 | 4.71±0.455 | 1.05±0.077 | <0.0110 | 11.8±1.02 | 3.17±0.258 | 2.15±0.069 |
| S6 | 0.233±0.018 | 0.616±0.004 | 5.04±0.495 | <0.0110 | 6.30±0.302 | 10.4±1.01 | 2.52±0.096 | 21.7±1.26 |
| S7 | 0.197±0.018 | 0.786±0.060 | 4.53±0.037 | 1.33±0.170 | 5.98±0.089 | 13.5±0.158 | 9.00±0.450 | 3.70±0.038 |
| S8 | 0.906±0.012 | 0.813±0.042 | 0.213±0.001 | <0.0110 | 0.816±0.010 | <0.0103 | <0.0117 | <0.0117 |
| S9 | <0.0124 | 1.07±0.101 | 0.284±0.006 | <0.0110 | <0.0110 | 1.27±0.035 | <0.0117 | 1.09±0.054 |
| S10 | <0.0124 | <0.0214 | 0.795±0.074 | 0.992±0.047 | 1.90±0.094 | 2.19±0.108 | 0.219±0.016 | <0.0117 |

Table 4.12 continued…..

| Site | BAnt | Chr | BbF | BkF | BghiP | InP | DahAnt | BaP |
|---|---|---|---|---|---|---|---|---|
| S1 | 1.69±0.132 | <0.0144 | 2.53±0.188 | <0.00743 | 3.95±0.302 | 4.03±0.347 | 8.35±0.174 | 6.53±0.490 |
| S2 | 28.0±1.20 | <0.0144 | <0.00743 | <0.00743 | 46.4±2.13 | 4.69±0.158 | 21.7±1.50 | 3.82±0.366 |
| S3 | <0.00999 | 10.2±0.696 | 20.5±1.32 | 8.13±0.706 | 24.3±0.207 | 2.91±0.133 | <0.00241 | 4.99±0.407 |
| S4 | <0.00999 | <0.0144 | <0.00743 | <0.00743 | 51.9±5.02 | <0.0195 | <0.00241 | <0.0210 |
| S5 | <0.00999 | <0.0144 | <0.00743 | <0.00743 | <0.00668 | 1.39±0.015 | <0.00241 | 3.31±0.303 |
| S6 | 20.1±0.297 | <0.0144 | 5.30±0.615 | 42.0±4.08 | 5.33±0.106 | 0.078±0.002 | 0.726±0.013 | <0.0210 |
| S7 | 18.3±0.359 | <0.0144 | 20.7±1.00 | <0.00743 | 8.01±0.286 | 0.183±0.012 | 3.77±0.181 | 3.86±0.213 |
| S8 | 1.13±0.014 | <0.0144 | <0.00743 | 4.57±0.380 | <0.00668 | <0.0195 | 0.198±0.018 | 1.74±0.096 |
| S9 | 0.868±0.018 | <0.0144 | 0.664±0.033 | 6.33±0.319 | 0.596±0.019 | <0.0195 | <0.00241 | <0.0210 |
| S10 | 0.922±0.068 | <0.0144 | 0.329±0.024 | 5.51±0.306 | <0.00668 | 3.78±0.030 | <0.00241 | 0.98±0.081 |

The concentrations of PAHs in the sediment samples collected from Mokolo River ranged from 0.044 to 51.9 mg/kg. Acenaphthylene and Nap showed lower concentrations and were found below LOD at most of sampling sites compared to other LMW PAHs. Benzo(ghi)perylene and pyrene showed higher mean concentration as compared to all other PAHs. Chrysene was not detected in all ten sampling sites except for sampling site 3 which contained concentration of 10.2 mg/kg and the reason for this is unclear at this stage. All 16 PAHs were detected in almost all ten sampling sites except for chrysene. Generally, LMW compounds demonstrated lower concentration than HMW PAHs which showed higher concentration values and their values vary at different sampling sites.

## 4.8 COMPARISONS OF PAHs CONCENTRATION IN MOKOLO AND BLOOD RIVER SEDIMENTS

Comparisons and distribution of PAH concentrations of each sediment sample collected from Mokolo River (MR) and Blood River (BR), as obtained following MAE, are presented in Figures 4.8 and 4.9, respectively. Sediment samples in MR had higher PAH concentrations as compared to BR sediments as can be seen in Figure 4.8. Sample 2 in MR contained higher total PAH concentrations of about 220 mg/kg than all samples from MR and BR. Sampling sites 2,3,4,6 and 7 in MR contained higher concentration than all other sites in MR and BR. Benzo(g,h,i)perylene and Ace contributed the greatest portion to the total PAH concentration in MR with values of 48.1 and 51.9 mg/kg at samples 2 and 4, respectively. Most of the HMW compounds ($\geq$ 3 ringed PAHs) also contributed significantly to the total concentration in MR, these compounds being BkF, Pyr, BAnt, BaP, Fln, BbF, Ant and Phe. Concentration distributions of PAHs in MR sediment shown in Figures 4.8 and 4.9 demonstrate that the sediment PAHs content is higher in MR from sample 1 to 7 and gradually decreases in the direction toward sampling sites 8 to 10. This indicates that the sources of PAHs in MR sediment samples might be likely due to industries in the area. As can be seen in Figure 4.8, BR had lower concentrations of PAHs in sediment than in MR as some of compounds in BR were below LOD or at very low concentrations. Edokpayi *et al*. (2016) determined PAHs in sediment samples in Vhembe District of South Africa and found concentration ranging between 27.10 and 55.93 mg/kg, which

are comparable to the concentration found in this study in MR (0.044 to 51.9 mg/kg). The concentrations of PAHs found in Blood River are lower than those in MR and the PAH concentrations reported by Edokpayi *et al*. (2016).

Looking at the results from this study, it can be deduced that the lower and higher levels of PAHs in the sediment samples from both Blood and Mokolo Rivers are most likely due to the different activities going on around them. For example in MR, there are large production activities such as coal mine and power stations situated near the river, which could be sources of PAHs. All these could be responsible for the high PAHs levels of 0.044 to 51.9 mg/kg in sediment samples in MR. The lower concentrations for sediment samples of 0.014 to 3.10 mg/kg in the BR would also indicate that there are few activities taking place around or within the study area. There is sewage leakage and domestic wastes next to the BR as possible sources of PAHs in the river. Exact sources of PAHs in sediments samples from both rivers were found when conducting source apportionment of PAHs. The concentrations of PAHs in sediment samples from two rivers (BR and MR) were subjected to one-way analysis of variance to determine the significance of the observed difference at 95% confidence level. The null hypothesis was rejected since the total concentration revealed significant difference ($P$<0.05) in Mokolo and Blood Rivers.

As discussed already in Section 1.1.2, maximum allowable concentration (MAC) have been set specifically for those PAHs prescribed as carcinogenic, toxic and priority pollutant (Oduntan, 2014). Some of MAC of PAHs in soil and water samples in Spain according to the ATSDR and USEPA (Oduntan, 2014) are presented in Table 1.1. As can be seen in Tables 4.11 and 4.12, in sediment samples, all the PAHs were above the MAC in Mokolo River except naphthalene and acenaphthylene, which were less than the MAC. However, in Blood River, Ant, BAnt, InP, DahAnt, and Bap were all above MAC. These results indicate a potential risk to humans. All the PAHs measured in both study areas were below the MAC in all of the river water samples. Comparisons and distribution of PAHs concentrations of sediment samples collected from Mokolo and Blood Rivers are presented in Figures 4.8 and 4.9, respectively.

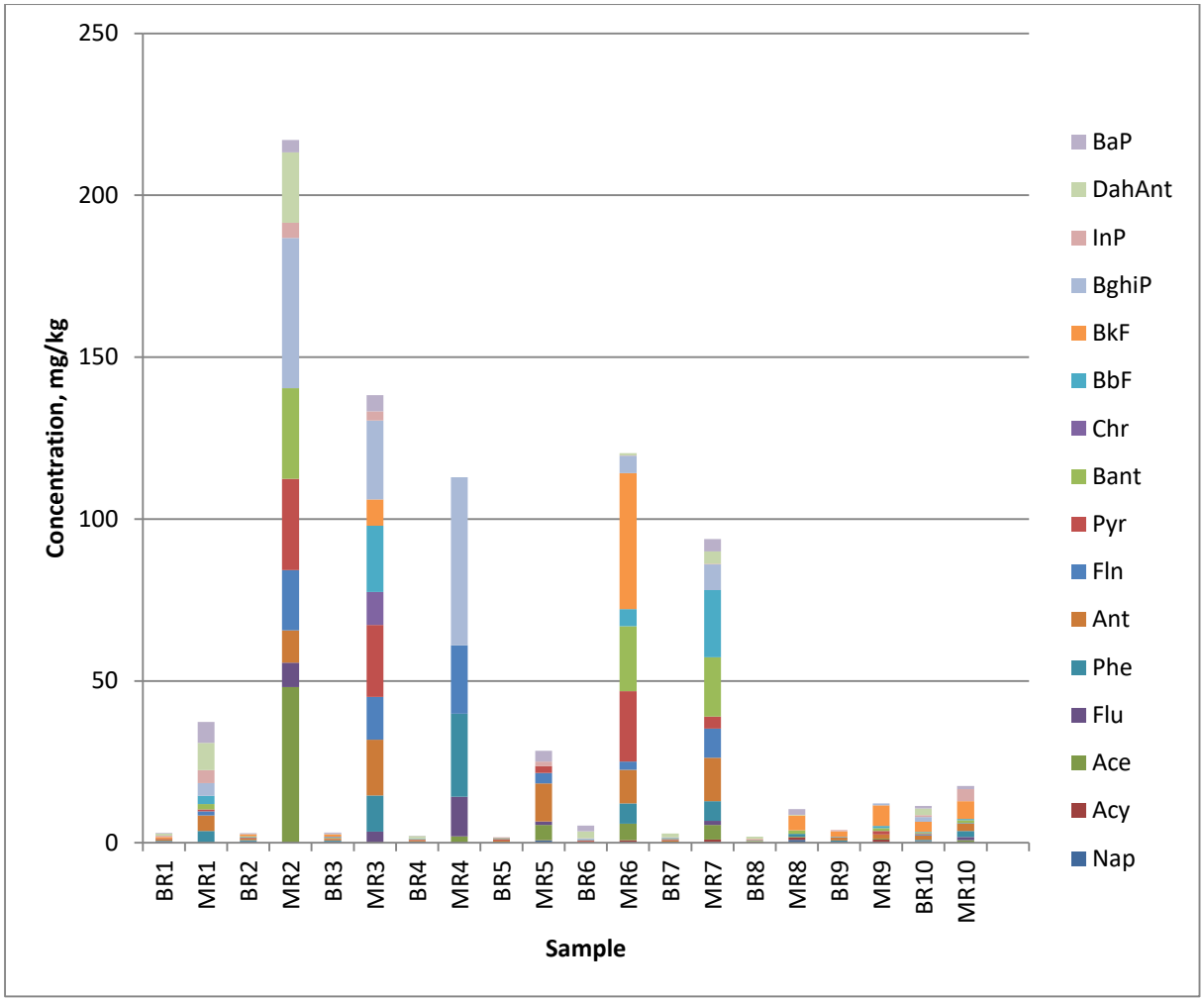



Figure 4.8: Comparisons of the concentration of PAHs in each sediment samples collected from MR and BR as obtained following MAE method

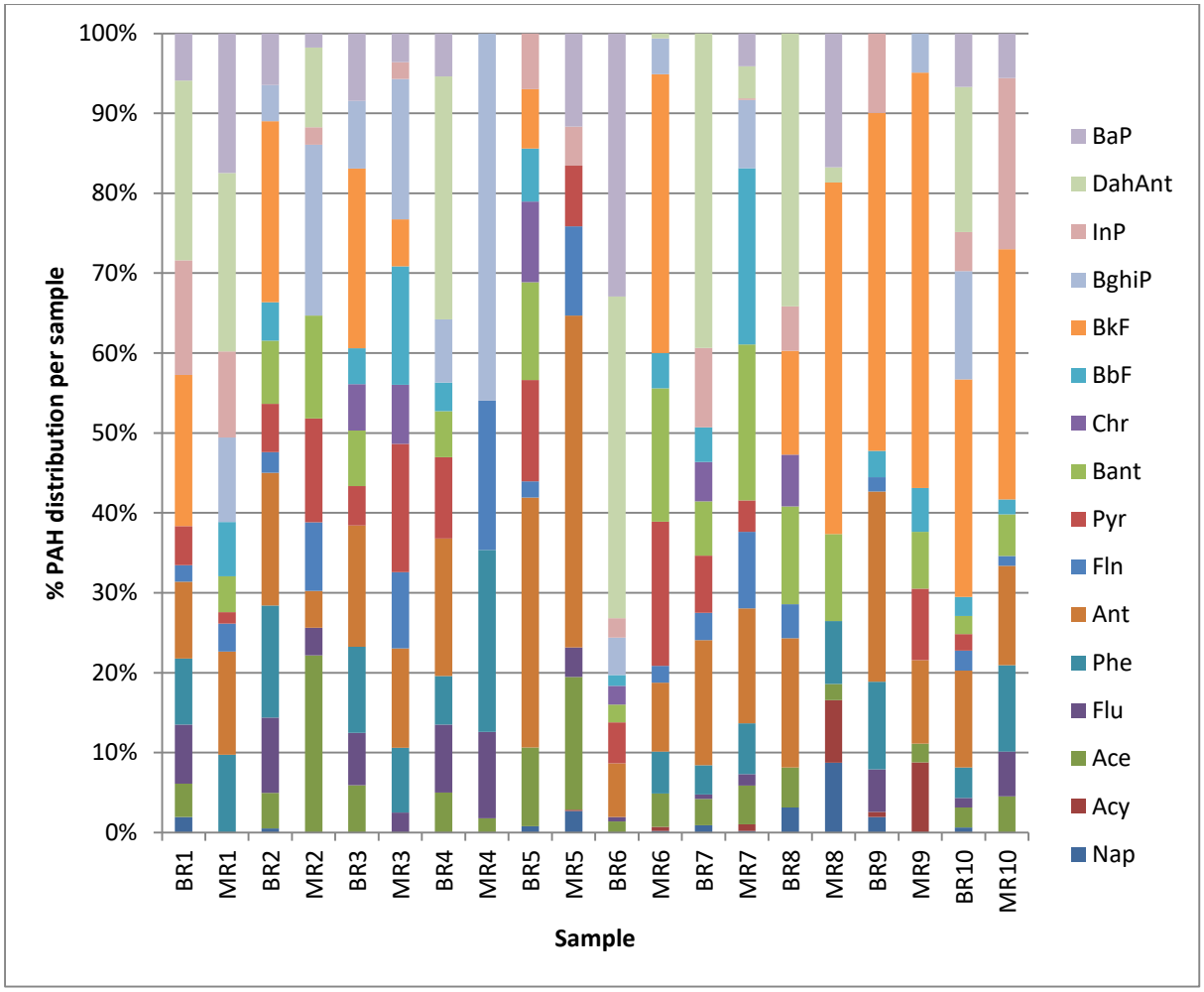



Figure 4.9: Distribution of the 16 PAHs in sediment samples from MR and BR as obtained following MAE method

## 4.9 DETERMINATION OF THE 16 US EPA PRIORITY PAHs IN SEDIMENT SAMPLES FROM BLOOD RIVER BY ULTRASONICATION AND COMBINATION OF ULTRASONICATION AND MECHANICAL SHAKING

### 4.9.1 Determination of PAHs in Blood River sediments by combination of ultrasonication and mechanical shaking

The concentration of PAHs in sediment samples collected in Blood River ranged from 0.045 to 35.1 mg/kg as obtained following extraction by a combination of ultrasonication and mechanical shaking. Chrysene was not detected in all sites while naphthalene was detected in samples collected from sites 9 and 10. Most of the LMW compounds (Nap, Ace, Acy, Flu, Phe and Ant) were detected at lower concentrations, while the HMW PAHs detected showed higher concentrations in most of the sampling sites. Anthracene had the lowest concentration of 0.045 mg/kg followed by Ace with 0.057 mg/kg while DahAnt had the highest concentration of 35.1 mg/kg. Acenaphthene, Fln, BkF and BaP were detected in samples from almost all sites except those from sites 6, 3, 1 and 7, respectively. Concentrations of PAHs obtained by the combination of ultrasonication and mechanical shaking in Blood River sediments are given in Table 4.13.

Table 4.13: Concentrations (mg/kg) of the PAHs extracted by combination of ultrasonication and mechanical shaking, Blood River sediments

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.0927 | <0.0536 | 0.889±0.0301 | 0.080±0.0039 | <0.0288 | 0.045±0.0029 | 0.058±0.0055 | 1.78±0.367 |
| S2 | <0.0927 | <0.0536 | <0.351 | 0.174±0.006 | 0.070±0.003 | <0.0257 | 0.232±0.0089 | 0.425±0.045 |
| S3 | <0.0927 | <0.0536 | 0.654±0.0121 | 0.213±0.010 | <0.0288 | <0.0257 | <0.0293 | <0.0293 |
| S6 | <0.0927 | 0.125±0.022 | <0.351 | <0.0276 | 1.05±0.0217 | 0.309±0.080 | 0.505±0.103 | <0.0293 |
| S7 | <0.0927 | <0.0536 | <0.351 | <0.0276 | <0.0288 | <0.0257 | 0.258±0.022 | 0.075±0.002 |
| S9 | 0.203±0.002 | <0.0536 | 0.363±0.065 | 0.255±0.054 | 0.701±0.085 | 0.502±0.163 | 0.320±0.020 | 0.097±0.016 |
| S10 | 0.300±0.006 | 0.271±0.037 | <0.351 | 0.258±0.044 | 0.647±0.069 | 0.325±0.048 | 0.263±0.002 | 0.400±0.059 |

Table 4.13 continued……

| Site | BAnt | Chr | BbF | BkF | BghiP | InP | DahAnt | BaP |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.248 | <0.359 | 4.11±0.137 | 0.548±0.0779 | <0.0171 | <0.0487 | 27.7±3.85 | 12.8±0.998 |
| S2 | <0.248 | <0.359 | 0.353±0.010 | 5.82±0.966 | <0.0171 | 1.71±0.255 | 35.1±3.45 | 17.3±0.889 |
| S3 | <0.248 | <0.359 | 0.935±0.003 | 0.247±0.0338 | <0.0171 | <0.0487 | <0.00605 | 18.8±1.38 |
| S6 | <0.248 | <0.359 | <0.0186 | 1.16±0.23 | <0.0171 | 0.299±0.057 | <0.00605 | <0.0525 |
| S7 | <0.248 | <0.359 | 1.05±0.105 | <0.0167 | 0.513±0.093 | 9.64±1.68 | 1.85±0.325 | 2.43±0.315 |
| S9 | 0.253±0.040 | <0.359 | <0.0186 | 0.575±0.036 | 0.297±0.052 | 5.08±0.817 | 0.870±0.127 | 2.72±0.479 |
| S10 | <0.248 | <0.359 | 0.345±0.006 | 1.014±0.106 | 0.407±0.017 | 4.14±0.466 | <0.00605 | 1.80±0.224 |

### 4.9.2 Determination of PAHs in Blood River sediments by Ultrasonication method

Generally the LMW compounds were detected in lower concentration than the HMW PAHs (≥ 4 aromatic rings) after extraction of PAHs from Blood River sediment samples by ultrasonication. Chrysene was found below the LOD in all sampling sites. Naphthalene, Acy and Ant were detected in sample collected from site 7. Furthermore, the levels of PAHs ranged from 0.081 to 8.59 mg/kg and DahAnt had the highest concentration, while Pyr the lowest. Concentrations of PAHs obtained by ultrasonication extraction method from Blood River sediments are presented in Table 4.14.

Table 4.14: Concentrations (mg/kg) of the PAHs extracted by ultrasonication, Blood River sediments

| Site | Nap | Acy | Ace | Flu | Phe | Ant | Fln | Pyr |
|---|---|---|---|---|---|---|---|---|
| S1 | <0.185 | <0.322 | <0.0701 | <0.0552 | <0.0578 | <0.0513 | <0.0587 | 0.892±0.0300 |
| S3 | <0.185 | <0.322 | 0.198±0.0107 | <0.0552 | 0.130±0.023 | <0.0513 | 0.085±0.0086 | <0.0585 |
| S5 | <0.185 | <0.322 | 0.349±0.042 | 0.084±0.017 | <0.0578 | <0.0513 | <0.0587 | 0.081±0.0058 |
| S6 | <0.185 | <0.322 | 0.195±0.013 | <0.0552 | <0.0578 | <0.0513 | 0.332±0.002 | 1.49±0.235 |
| S7 | 0.192±0.0026 | 0.69±0.0017 | 0.292±0.0100 | 0.325±0.017 | 0.946±0.0308 | 0.340±0.044 | 0.335±0.002 | 0.538±0.0235 |
| S9 | <0.185 | <0.322 | 0.432±0.0145 | <0.0552 | <0.0578 | <0.0513 | <0.0587 | <0.0585 |
| S10 | <0.185 | <0.322 | 0.139±0.021 | 0.206±0.003 | <0.0578 | <0.0513 | <0.0587 | 0.425±0.0235 |

Table 4.14 continued…..

| Site | BAnt | Chr | BbF | BkF | BghiP | InP | DahAnt | BaP |
|---|---|---|---|---|---|---|---|---|
| S1 | 0.551±0.040 | <0.0719 | 0.202±0.0105 | <0.0356 | <0.0334 | 0.954±0.106 | 0.253±0.0468 | <0.105 |
| S3 | 0.543±0.011 | <0.0719 | <0.0371 | 0.056±0.0038 | <0.0334 | <0.0974 | 4.86±0.154 | <0.105 |
| S5 | 0.559±0.006 | <0.0719 | 0.614±0.017 | <0.0356 | <0.0334 | 4.62±0.101 | <0.0121 | 0.883±0.029 |
| S6 | 0.575±0.046 | <0.0719 | 0.663±0.067 | 1.45±0.0102 | 0.795±0.076 | <0.0974 | <0.0121 | 10.1±0.005 |
| S7 | 0.760±0.116 | <0.0719 | <0.0371 | 3.80±0.116 | 1.50±0.257 | 1.38±0.094 | 3.53±0.201 | <0.105 |
| S9 | 0.436±0.046 | <0.0719 | <0.0371 | 1.75±0.359 | 1.98±0.005 | 1.61±0.205 | 8.59±0.049 | 1.19±0.095 |
| S10 | 0.508±0.005 | <0.0719 | 0.832±0.101 | 2.69±0.414 | <0.0334 | 2.44±0.377 | 8.38±0.295 | 0.703±0.110 |

## 4.10 COMPARISON OF THREE EXTRACTION METHODS

To compare the three extraction techniques used in the current study, six sediment samples from Blood River were selected and the PAHs extracted and measured using GC-FID with the same column and instrumental conditions. Three extraction techniques (MAE, ultrasonication and combination of ultrasonication and mechanical shaking) were optimised for the quantification of PAHs in Blood River sediment samples. According to these results (Figures 4.10 and 4.11), in terms of concentration, ultrasonication and combination of ultrasonication and mechanical shaking yielded higher concentrations of PAHs than the measured concentrations after MAE. The PAH concentrations of Blood River sediment samples by extraction with ultrasonic bath and combination of ultrasonication and mechanical shaking ranged from 0.081 to 8.59 mg/kg and between 0.045 and 35.1 mg/kg, respectively (Tables 4.13 and 4.14), while for MAE the PAH concentrations ranged between 0.014 and 3.10 mg/kg (Table 4.10). Higher values for the concentrations of compounds, including DahAnt, BaP and InP with more rings (≥5 fused aromatic rings), were found when PAHs were extracted from sediments by combined ultrasonic bath and mechanical shaking, while LMW compounds had lower concentrations. Furthermore, the concentrations of the LMW compounds were also lower when ultrasonic bath and MAE were used to extract the PAHs from sediment samples. From the results obtained using the three extraction techniques it was observed that higher concentrations were obtained for HMW compounds than LMW compounds. This is in agreement with the findings of Bayowa (2014), who reasoned that HMW PAHs, which are hydrophobic compounds and are less soluble in water tend to settle mostly in sediments.

According to the results of the present study obtained by using three different extraction techniques, in terms of precision and extraction efficiency, the MAE was the preferred procedure for the extraction of PAHs from sediment samples. Generally, the precision obtained for ultrasonication and combined ultrasonication and mechanical shaking was poor, since the % RSD values of both methods ranged between 0.0477 and 20.5% and 0.314 to 20.9%, respectively. The MAE had the smallest % RSD compared to the other two techniques with the value ranging between 0.055 and 9.98% for all PAHs from Mokolo and Blood Rivers sediment samples. Furthermore,

the results from MAE were evaluated using the certified reference material (CRM) for efficiency of the extraction method and the results were satisfactory obtaining average recoveries ranging from 83.8 to 125% (Table 4.7). However, some studies indicated that extraction performed using an ultrasonic bath gave lower recoveries and was less efficient (Lau *et al*., 2010; Oluseyi *et al*., 2011). As already indicated in Section 2.9, sonication and combination of ultrasonication and mechanical agitation require more time because further separation techniques such as centrifugation or filtration are usually applied after the extraction process (Lau *et al*., 2010). Seopela *et al*. (2016) applied combined ultrasonication and mechanical shaker for the extraction of PAHs from sediment samples. However, the precision of the results was poor with the % RSD ranging between 1.01 and 26.8%, and the time spent for combined ultrasonication and mechanical shaker was 1h30 min while MAE took 30 min. The reproducibility and extraction efficiency obtained using MAE were higher. For these reasons, namely the higher recoveries and better precision obtained using MAE; MAE was selected as the most suitable method for extraction of PAHs from sediment samples. Thus, all the sediment samples collected from Mokolo and Blood Rivers were extracted by MAE. Mekonnen *et al*. (2015) and Seopela *et al*. (2016) successfully applied MAE for the extraction of PAHs from sediment samples and obtained higher recoveries than with other methods. The ANOVA was used to determine the statistical significance of results obtained by MAE, ultrasonication and the combined ultrasonication and mechanical shaking methods. The null hypothesis was rejected since there was significant difference ($P$<0.05) between the results from these three methods. Comparisons and distribution of the concentration of sediment samples collected in BR as obtained following MAE, ultrasonication and the combined ultrasonication and mechanical shaking are presented in Figures 4.10 and 4.11.

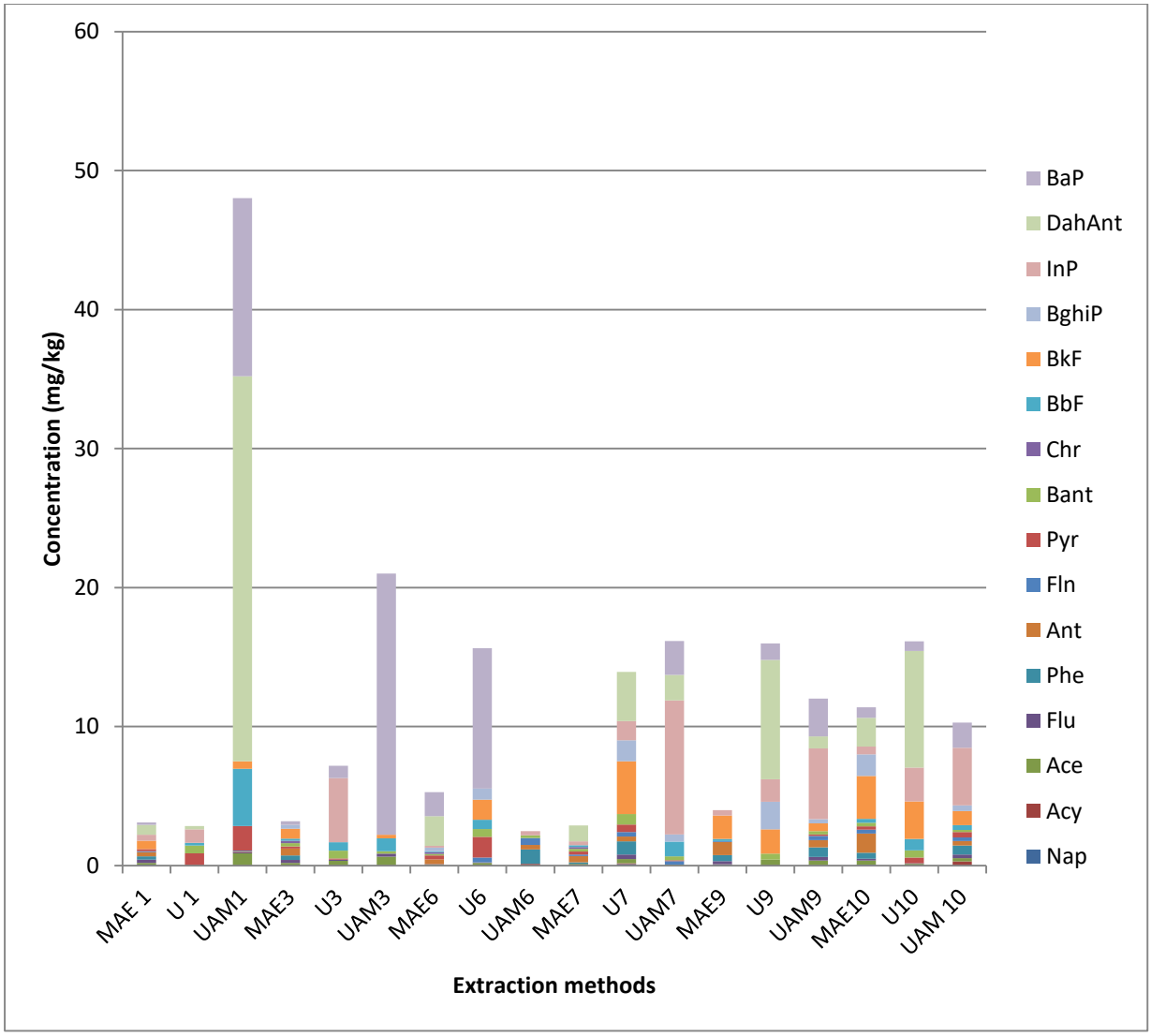



Figure 4.10: Comparisons of the concentration of sediment samples collected from BR as obtained following MAE, U and combination of UAM

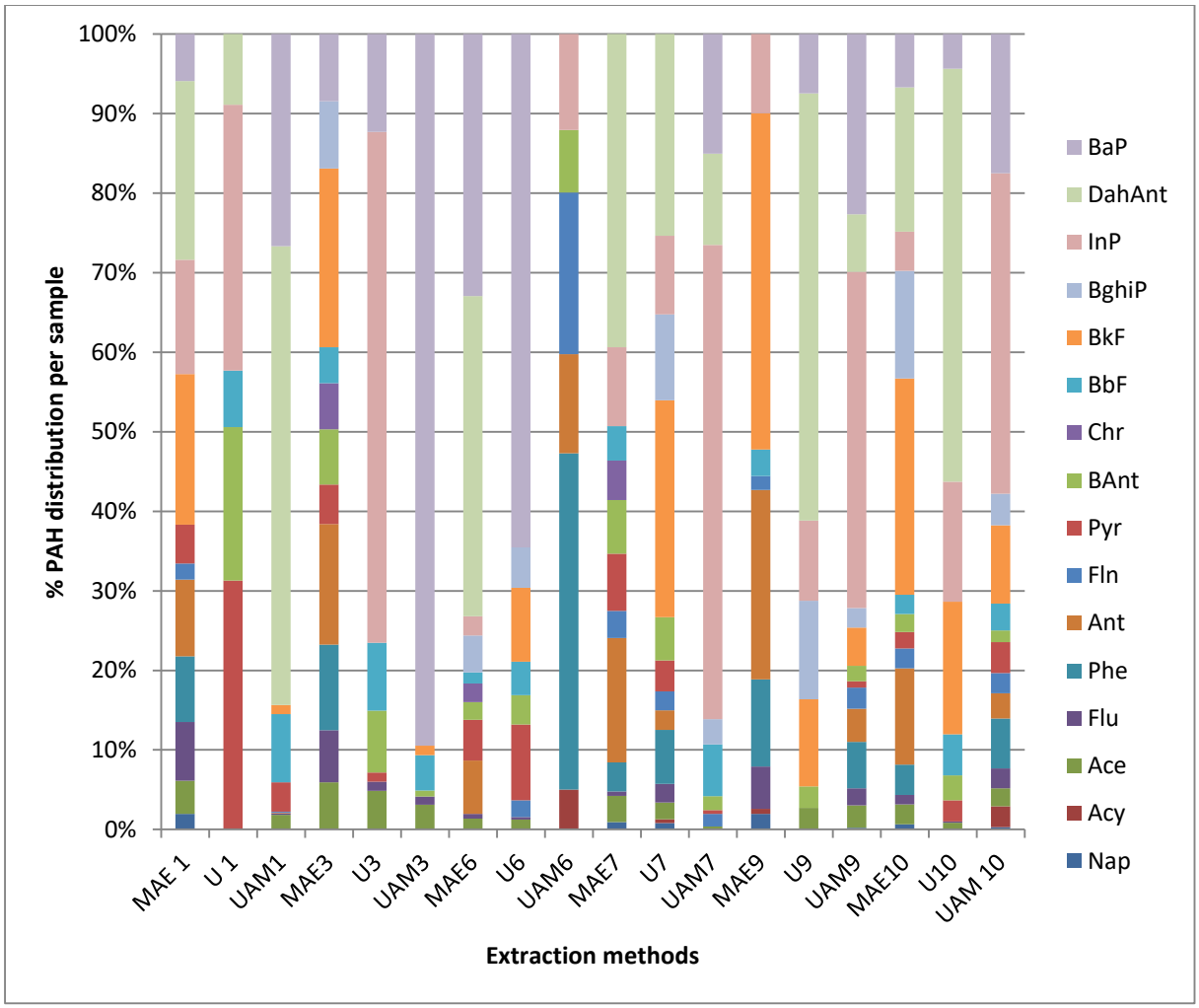



Figure 4.11: Distribution of the 16 PAHs in sediment samples collected from BR as obtained following MAE, U and UAM

## 4.11 COMPARISON OF CONCENTRATIONS OF PAHs IN WATER AND SEDIMENTS REPORTED FROM DIFFERENT LOCATIONS IN THE WORLD

The levels of PAHs obtained in water and sediments from Mokolo and Blood Rivers were compared with the other measured concentrations of PAHs found by different researchers from around the world and South Africa. The total concentrations of PAHs in water samples collected from Mokolo and Blood Rivers were lower than those reported from South Africa and other countries. Lower total concentrations of the 16 PAHs were reported for water samples from around the world (Table 4.15), and the concentrations are comparable with those observed in the present study. The total concentrations of PAHs determined in sediment samples in the present study were found to be higher than those reported by other researchers from South Africa and other countries (Table 4.15). However, higher concentrations of PAHs in sediments have been reported in other studies from around the world, highlighting the seriousness of PAHs pollution in the environment (Kumar *et al*., 2014). The total concentrations of PAH in water and sediments reported from different locations in the world are compared in Table 4.15.

Table 4.15: Total concentrations of PAHs in water and sediment samples reported from different locations in the world

| **Locationn** | **Number of PAHs** | **PAHs concentrations range in water, µg/L** | **PAHs concentrations range in sediment, mg/kg** | **Reference** |
|---|---|---|---|---|
| Thohoyandou, Limpopo Province, South Africa | 6 | 0.1-137 | 0.0179-9.87 | Nekhavhambe *et al*., 2014 |
| Vhembe District, South Africa | 16 | 13174-26382 | 27.10-55.93 | Edokpayi *et al*., 2016 |
| Loskop Dam, South Africa | 16 | 1.17-14.5 | 0.292-2.17 | Seopela *et al*., 2016 |
| Dalian Bay, China | 24 | 0.015-1.16 | 0.064-2.1 | Liu *et al*., 2013 |
| Qiantang River, China | 15 | 0.0703-1.84 | 0.0913-0.614 | Chen *et al*., 2007 |
| East Arjun Nagar Delhi, India | 16 | 5.87-35.3 | 0.921-18.8 | Kumar *et al*., 2014 |
| Yellow River, China | 15 | 0.179-0.369 | 0.031-0.133 | Li *et al*., 2006 |
| Blood River, South Africa | 16 | 0.0121-0.433 | 0.014-3.10 | This study |
| Mokolo River, South Africa | 16 | 0.0219-1.53 | 0.044-51.9 | This study |

## 4.12 IDENTIFICATION OF PAHs SOURCE IN SEDIMENTS

As previously discussed in Section 2.4, the diagnostic ratio is most useful tool for the source identification of PAHs in sediment samples (Topal, 2011). The ratio of HMW to LMW compounds may indicate a PAH's source, since LMW compounds are more common in samples containing petrogenic PAHs and HMW compounds are more common in samples containing pyrogenic PAHs, as most of the HMW molecules are formed at higher temperatures (Liu *et al*., 2009; Li *et al*., 2012). The ratio of 2 to 3 rings and 4 to 6 rings are used in this study to differentiate between the two PAHs sources (pyrogenic and petrogenic) (Aly Salem *et al*., 2014). Several molecular ratios, including Nap/Fln, Phe/Ant, Fln/Pyr, Chr/BAnt, and Pyr/BaP have been used in some studies for interpreting PAH compositions and identifying possible sources (Li *et al*., 2006; Jiao *et al*., 2011; Aly Salem *et al*., 2014; Seopela *et al*., 2016). The ratio Phe/Ant has been developed to study the petrogenic or pyrogenic sources of PAHs in sediments (Li *et al*., 2006).

The concentration ratios of PAHs with the same molecular weight such as Flu/ (Flu + Pyr) and Ant/ (Ant + Phe) were used in the current study to identify the possible PAH origins in sediments from Blood and Mokolo Rivers. The PAHs in sediments with the ratio Ant/ (Ant + Phe) < 0.1 indicate petroleum contamination, while Ant/ (Ant + Phe) > 0.1 suggests pyrogenic source (Li *et al*., 2006; Nasher *et al*., 2013). In the present study, the values of the Ant/Ant+Phe ratios were between 0.539 and 1.00 with the mean value of 0.766 in Blood River sediments, which indicates that the PAHs are from a pyrogenic source. A possible contribution source of PAH in the Blood River could be due to combustion of organic matter.

The Flu/Pyr and Flu/ (Flu + Pyr) ratios are also used as indicators for assessing the attribution of PAH pollution in sediments (Aly Salem *et al*., 2014). Furthermore, if Flu/ (Flu + Pyr) >0.5, the pyrogenic source from the combustion of grass, wood, or coal is suggested. However, if the ratio of Flu/ (Flu +Pyr) is between 0.4 and 0.5, PAHs are mostly from the combustion of petroleum; if the ratio <0.4, petroleum contamination is suggested (Aly Salem *et al*., 2014). As can be seen in Table 4.16, the ratio Flu/ (Flu +Pyr) is between 0.4 and 0.5, revealing that petroleum combustion could be the source of PAHs in Blood River sediments. Moreover, a BAnt / BAnt+Chr ratio of <0.2 normally

implies a petrogenic origin, 0.2 to 0.35 indicates a mixed petrogenic and pyrogenic origin, and >0.35 indicates pyrogenic origin (Nasher *et al*., 2013). Values of this ratio from Blood River sediments ranged from 0.448 to 1.00 with the mean value of 0.581, which indicates pyrogenic origin. It could be concluded that PAHs in sediment samples collected in Blood River originated from petroleum and combustion of organic matter.

As shown in Table 4.17, the values of the Ant/ Ant+Phe ratio ranged from 0.535 to 1.00 with the mean value of 0.603 in Mokolo River sediments, which indicates that the PAHs are from a pyrogenic source while the ratio of Flu/ (Flu +Pyr) is <0.4 indicating petrogenic origin. Additionally, the BAnt / BAnt+Chr ratio was >0.35, which indicates pyrogenic origin (Table 4.17). These results easily indicate that PAHs in Mokolo River sediments originate from both pyrogenic and petrogenic origin. The diagnostic PAH ratios of sediments from Blood and Mokolo Rivers are given in Tables 4.16 and 4.17, respectively.

Table 4.16: Diagnostic PAH ratios in the sediment samples collected from Blood River

| | Phe / Ant | Ant / Phe+Ant | Flu / Pyr | Flu / Flu+Pyr | BAnt / BAnt+Chr |
|---|---|---|---|---|---|
| Site 1 | 0.857 | 0.539 | 0 | 1.00 | 0 |
| Site 2 | 0.847 | 0.542 | 1.56 | 0.609 | 1.00 |
| Site 3 | 0.709 | 0.585 | 1.32 | 0.571 | 0.544 |
| Site 4 | 0.352 | 0.739 | 0.838 | 0.456 | 1.00 |
| Site 5 | 0 | 1.00 | 0 | 0 | 0.549 |
| Site 6 | 0 | 1.00 | 0.111 | 0.1 | 0.488 |
| Site 7 | 0.234 | 0.810 | 0.0769 | 0.0714 | 0.578 |
| Site 8 | 0 | 1.00 | 0 | 0 | 0.654 |
| Site 9 | 0.462 | 0.684 | 0 | 1.00 | 0 |
| Site 10 | 0.314 | 0.761 | 0.579 | 0.367 | 1.00 |
| Mean | 0.378 | 0.766 | 0.448 | 0.417 | 0.581 |

Table 4.17: Diagnostic PAH ratios in the sediment samples collected from Mokolo River

| | Phe / Ant | Ant / Phe+Ant | Flu / Pyr | Flu / Flu+Pyr | BAnt / BAnt+Chr |
|---|---|---|---|---|---|
| Site 1 | 0.754 | 0.570 | 0 | 0 | 1.00 |
| Site 2 | 0 | 1.00 | 0.267 | 0.211 | 1.00 |
| Site 3 | 0.651 | 0.607 | 0.156 | 0.135 | 0 |
| Site 4 | 0 | 0 | 0 | 1.00 | 0 |
| Site 5 | 0 | 1.00 | 0.488 | 0.328 | 1.00 |
| Site 6 | 0.606 | 0.623 | 0 | 0 | 1.00 |
| Site 7 | 0.443 | 0.693 | 0.359 | 0.264 | 1.00 |
| Site 8 | 0 | 0 | 0 | 0 | 1.00 |
| Site 9 | 0 | 1.00 | 0 | 0 | 1.00 |
| Site 10 | 0.868 | 0.535 | 0 | 1.00 | 1.00 |
| Mean | 0.332 | 0.603 | 0.127 | 0.294 | 0.7 |

## 4.13 POTENTIAL ECOSYSTEM RISK ASSESSMENT

The PAHs in sediments may be themselves toxic to aquatic life or may be a source of chemicals that bioaccumulate in the food chain (Wenning and Ingersoll, 2002). The main aim of a sediment test was to investigate whether chemicals are detrimental to or are bioaccumulated by benthic organisms. The tests may be used to assess the interactive toxic impact of complex chemical mixtures in sediments. Furthermore, prior knowledge of specific pathways of interactions among sediments and test organisms is not necessary when conducting the tests (Wenning and Ingersoll, 2002). Sediment

chemistry data alone do not provide a sufficient basis for assessing the threat posed by sediment associated contaminants to aquatic organisms. Interpretive tools are also needed to determine if sediment associated contaminants are available at concentrations that could potentially harm the designated uses of the aquatic environment (Mekonnen *et al*., 2015). A number of methods have been developed for determining the toxicity of chemicals in sediments (American Society for Testing and Material (ASTM), 2014). Sediment assessment methods for directly measuring of the biological effects on organisms and animals often include the use of sediment quality guidelines (SQGs) (Swartz *et al*., 1995; Wenning and Ingersoll, 2002; Long *et al*., 2006). The SQGs are defined as the measured chemical concentrations intended to be protective of biological resources, or predictive of harmful effects to those resources (Wenning and Ingersoll, 2002). Furthermore, the SQGs assess sediment contamination and also assist practitioners in sediment evaluation and management to formulate risk management decisions (Long *et al*., 2006). The SQGs also provide a scientifically defensible basis to assess the potential impacts of sediment-associated contaminants on aquatic organisms (Mekonnen *et al*., 2015). These SQGs have been developed for many potentially toxic chemicals (i.e., trace elements, chlorinated organic, and PAHs) (MacDonald *et al*., 2000; Mekonnen *et al*., 2015; Zaghden *et al*., 2017).

The SQGs for evaluating sediment quality relative to the potential for harmful effects on sediment-dwelling organisms have been derived in previous national studies using various statistical approaches, including the EqP approach (MacDonald *et al*., 1996), screening-level concentration approach (Swartz, 1999), effects range–low (ERL) (Field *et al*., 1999) and effects range–median (ERM) approaches (Long *et al*., 2005), threshold-effects level (TEL) (Swartz *et al*., 1995) and probable-effects level (PEL) approaches (MacDonald *et al*., 1996). Mekonnen *et al*. (2015) used the two sets of SQGs, ERL/ERM, and TEL/PEL to assess the toxicity of PAHs on the aquatic organisms in the sediment of Akaki River, Lake Awassa and Lake Ziway in Ethiopia. In addition to this, Cardellicchio *et al*. (2007) also used two sets of SQGs, ERL/ERM and TEL/PEL for assessing sediment quality from Mar Piccolo in Taranto, Italy. Therefore, the potential toxicity of PAHs in the sediments on the aquatic organisms in Mokolo and Blood Rivers was evaluated according to the SQGs, based on the ERL

and ERM target values. In the current study, the comparisons of chemical concentrations of PAHs with SQGs are presented in Table 4.18.

The ERL and ERM values define three concentration ranges for a chemical, including those that were rarely (below the ERL), at concentration below ERL biological effect rarely occur, at concentration ≥ERL and ≤ERM biological effect occasionally occur and at concentration >ERM, negative biological effect regularly occur and sediment samples were predicted to be toxic (Cardellicchio *et al*., 2007; Nasher *et al*., 2013; Zaghden *et al*., 2017). The concentration ranges of the individual PAH are illustrated in Table 4.18 for sediments samples from Mokolo and Blood Rivers. The concentrations less than the ERL values were observed in some sampling sites while others have concentrations higher than the ERL. The measured concentrations of PAHs in all sediment samples from Blood River were below the ERL except for Ace, Flu and BkF which recorded average values greater than the ERL but still less than the ERM, indicating that PAHs in Blood River sediment samples have no adverse biological effects except for Ace, Flu and BkF. These may occasionally cause negative toxic effects but not acute effects. However, all the individual PAHs (except naphthalene) in sediment samples from Mokolo River were above ERM, which indicates that negative biological effect may frequently occur in this study area and sediment samples were predicted to be toxic. Only naphthalene in this area recorded an average value higher than the ERL but still less than the ERM, which indicates that biological effect may occasionally occur (Table 4.18).

Table 4.18: PAH levels in sediments from Mokolo and Blood Rivers, South Africa, compared with sediment quality guideline values (Cardellicchio *et al*., 2007; Aly Salem *et al*., 2014; Mekonnen *et al*., 2015)

| **Compounds** | **SQG values (ng/g dm)** | | **This study PAH concentration ng/g (Blood River)** | | | **This study PAH concentration ng/g (Mokolo River)** | | |
|---|---|---|---|---|---|---|---|---|
| | ERL | ERM | MEAN | MIN | MAX | MEAN | MIN | MAX |
| **Naphthalene** | 160 | 2100 | 41.4 | 0 | 87 | 211 | 0 | 906 |
| **Acenaphthylene** | 44 | 640 | 2.5 | 0 | 25 | 333 | 0 | 1070 |
| **Acenaphthene** | 16 | 500 | 128 | 0 | 286 | 6570 | 0 | 48100 |
| **Fluorene** | 19 | 540 | 131 | 0 | 283 | 2670 | 0 | 12200 |
| **Phenanthrene** | 240 | 1500 | 214 | 0 | 439 | 5550 | 0 | 25700 |
| **Anthracene** | 853 | 1100 | 566 | 300 | 1380 | 7100 | 0 | 17200 |
| **Fluoranthene** | 600 | 5100 | 71.4 | 0 | 288 | 7110 | 0 | 21100 |
| **Pyrene** | 665 | 2600 | 150 | 0 | 270 | 7960 | 0 | 28200 |
| **Benzo[a]anthracene** | 261 | 1600 | 161 | 0 | 260 | 7100 | 0 | 28000 |
| **Chrysene** | 384 | 2800 | 75 | 0 | 186 | 1020 | 0 | 10200 |
| **Benzo[b]fluoranthene** | 320 | 1880 | 83.1 | 0 | 273 | 5000 | 0 | 20700 |
| **Benzo[k]fluoranthene** | 280 | 1620 | 715 | 0 | 130 | 6650 | 0 | 42000 |
| **Benzo[a]pyrene** | 430 | 1600 | 237 | 0 | 1540 | 14100 | 0 | 51900 |
| **Indeno[1,2,3-cd]anthracene** | NA | NA | 204 | 0 | 558 | 1710 | 0 | 4690 |
| **Dibenzo(a,h)anthracene** | 63.4 | 260 | 736 | 0 | 2070 | 3470 | 0 | 21700 |
| **Benzo[ghi]perylene** | 430 | 1600 | 327 | 0 | 1740 | 2520 | 0 | 6530 |

## 4.14 RISK ASSESSMENT

There are several PAHs that are known to have carcinogenic effects and are hence of concern (Aly Salem *et al*., 2014). The BaP equivalent (BaPE) was used to quantitatively estimate the PAHs potential human health risk. The toxicity assessment of Mokolo and Blood Rivers sediments was conducted according to the concentration of some known potentially carcinogenic PAHs, including BAnt, BbF, BkF, BaP, DahAnt, and InP (Nasher *et al*., 2013). The BaPE was determined using the following equation (Aly Salem *et al*., 2014):

BaPE= BAnt * 0:06 + BbF * 0:07 + BKf * 0:07 + BaP + DahAnt * 0:06 + InP * 0:08.

The PAHs risk assessment in various sediments has been reported around the world (Wang *et al*., 2009; Nasher *et al*., 2013; Aly Salem *et al*., 2014). However, there is no available information on the PAHs quantitative risk assessment. According to the Canadian soil quality guidelines, soils containing values <0.1 mg/kg BaPE are considered uncontaminated, soils containing values ranging between 0.1 and 1.0 mg/kg BaP are considered slightly contaminated and soils containing 1 to 10 mg/kg BaPE are considered to be significantly contaminated (Yang *et al*., 2014). The BaPE values in the current study for all sediment samples from Blood River ranged from 0.039 to 1.89 mg/kg with the highest value obtained in site 6. The results calculated showed that the sediment samples are slightly contaminated and there is no risk in this study area. Mokolo River recorded highest values of BaPE ranging between 0 to 7.63 mg/kg, which indicates that PAHs from this study area have relatively higher toxicity as compared to Blood River. Aly Salem *et al*. (2014) carried out risk assessment of sediment samples of the Red Sea, Egypt and obtained BaPE values ranging between 0 and 45.3 ng/g. However, higher levels of BaPE values were recorded in the current study compared to those reported from Egypt (Aly Salem *et al*., 2014). The BaPE values calculated in this study are presented in Table 4.19.

Table 4.19: Calculated concentration values of BaPE and TEQs in sediments from Mokolo and Blood Rivers in mg/kg

| | BaPE Blood River (mg/kg) | TEQs Blood River Mg/kg | BaPE Mokolo River (mg/kg) | TEQs Mokolo River Mg/kg |
|---|---|---|---|---|
| Site 1 | 0.303 | 0.935 | 7.63 | 15.7 |
| Site 2 | 0.266 | 0.239 | 7.18 | 28.7 |
| Site 3 | 0.312 | 0.314 | 7.23 | 7.42 |
| Site 4 | 0.175 | 0.823 | 0 | 0 |
| Site 5 | 0.039 | 0.0467 | 3.42 | 3.45 |
| Site 6 | 1.89 | 3.90 | 4.57 | 3.69 |
| Site 7 | 0.103 | 1.19 | 6.65 | 11.5 |
| Site 8 | 0.077 | 0.675 | 2.14 | 2.11 |
| Site 9 | 0.150 | 0.0567 | 0.542 | 0.217 |
| Site 10 | 1.18 | 2.97 | 1.75 | 1.54 |

## 4.15 TOXIC EQUIVALENT FACTOR

Toxic equivalency factors (TEFs) of seven known carcinogenic PAHs (BAnt, BaP, BbF, BkF, Chr, DahAnt and InP), were used to quantitatively assess the potential toxicological significance to human health (Aly Salem *et al*., 2014). Out of the seven carcinogenic PAHs, BaP is the only one having enough toxicological data for derivation of a carcinogenic factor, and the carcinogenicity of other PAHs was assessed relatively to BaP (Aly Salem *et al*., 2014). The potential toxicity of PAHs in sediment samples was assessed by calculating the total toxic BaP equivalent (TEQ carc) for all known carcinogenic PAHs using the following equation (Nasher *et al*., 2013; Aly Salem *et al*., 2014):

Total TEQ carc = $\Sigma Ci\times$ TEF$i$ carc,

where $Ci$ is the concentration of individual carcinogenic PAH (ng/g d.w.) and TEF$i$ carc (toxic equivalency factors) is the toxic factor of carcinogenic PAHs relative to BaP. The US EPA established the TEFs for each carcinogenic PAH: 0.1 for BAnt, 0.001 for Chr, 0.1 for BbF, 0.01 for BkF, 1 for BaP, 0.1 for InP, and 1 for DahAnt (Nasher *et al*., 2013; Aly Salem *et al*., 2014). Aly Salem *et al*. (2014) reported the total TEQs values of sediments collected from Red Sea, Egypt, the values ranging between 0 and 72.27 ng/g, with the average value of 2.94 ng/g. In the present study, the TEQs values ranged from 0.0467 to 3.90 mg/kg in Blood River sediments, while Mokolo River sediments recorded higher values of TEQs ranging between 0 and 28.7 mg/kg (Table 4.19). In comparison with studies reported in other countries, TEQs values were higher in the sediments collected in the current study than those of other areas reported in the literature, such as sediment samples from Red Sea in Egypt (Aly Salem *et al*., 2014) and Langkawi Island in Malaysia (Nasher *et al*., 2013). These results indicate a negative potential risk to human health. The TEQs values calculated in this study are presented in Table 4.19.

# CHAPTER 5

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 CONCLUSIONS

The 16 US EPA priority PAHs were detected in all sediment samples collected from Mokolo and Blood Rivers while only 11 were detected in water samples from the same rivers. Optimised methods, LLE and MAE, were applied for the extraction of the 16 US EPA priority PAHs from water and sediment samples, both methods were more efficient than others investigated. The efficiency of LLE for extraction of PAHs from water samples was determined by assessing the percentage recoveries of LRB spiked with PAHs standard (Table 4.8). Higher percentage recoveries (above 80%) were recorded for all of the PAHs extracted by LLE. The MAE method was successfully validated by using a suitable CRM, obtaining quantitative percentage recoveries (above 80%). The MAE was preferred for the extraction of PAHs from sediment samples due to higher extraction efficiency and better precision than ultrasonication and combined ultrasonication and mechanical shaker which demonstrated poor precision. The concentrations in the sediments are commonly higher than that of water, and that also indicated that PAH levels are unacceptable in Mokolo River. This study also showed that sediment samples were more contaminated than the water samples. The highest concentrations of PAHs were obtained in Mokolo River sediments, with the concentrations ranging from 0.044 to 51.9 mg/kg (Table 4.12). These high values are clearly due to industries found in close proximity to the river. The PAHs recorded in Blood River sediments were lower than those obtained in the Mokolo River with concentrations ranging between 0.014 and 3.10 mg/kg (Table 4.11). In water samples, higher PAHs concentrations were obtained in Mokolo River (0.0219 to 1.53 µg/L) compared to the levels in Blood River (0.0121 to 0.433 µg/L). In water and sediment samples from both Rivers, HMW PAH compounds (4-6 rings) were found in higher concentrations than LMW PAHs (2-3 rings).

The PAHs levels in sediments obtained in the present study are considerably higher than some values reported in other studies around South Africa, which may be related to climatic conditions while concentrations of PAHs in water samples collected from both rivers were lower than those reported for various river water. Most PAHs levels

detected in both study areas were below the MAC in all of the river water samples. In sediment samples from both rivers, most of the PAHs were above the MAC, indicating a potential health risk to human beings.

Diagnostic ratio was used for the identification of the source of the PAHs in the sediment samples. Several PAH ratios indicated that both pyrogenic and petrogenic could be sources of those compounds in both rivers. This source analysis showed that the PAHs in both rivers might have different origins. Although there are fewer industries around Blood River, there are industrial, municipal and domestic wastes as well as, sewage leaking directly into the rivers around the study area and this could be possible source of contaminants in the river. Higher PAHs concentrations in sediment samples in Mokolo River might be due to industrial wastes, wild fires, commercial and agricultural activities by residents and traders, which are not appropriately monitored by the relevant authorities.

The assessment of ecotoxicological risk indicated that the sediment samples collected from Mokolo River are at high toxicity risk while sediments from Blood River are at low sediment toxicity risk according to the SQGs for PAHs (Table 4.18). The values obtained should not exceed the estimated values (SQGs) to avoid adverse biological effects. Blood River had most mean concentrations less than ERL while few were between the ERL and ERM. Mokolo River had most mean concentration above ERM. According to the results of this study, occasionally and frequently adverse biological effects, including cancer, reproductive and physiological disorders, may occur in fish, birds and mammals. The BaPE and TEQs values were higher in the sediments samples collected from Mokolo River in this study than those reported in other studies around the world (Table 4.19). This BaPE and TEQs values indicates that PAHs from Mokolo River showed relatively higher toxicity while results from Blood River revealed lower toxicity.

## 5.2 RECOMMENDATIONS

The results obtained in the present study areas have revealed PAH levels that are above SQGs. Thus, it is essential that monitoring programme be implemented to reduce the effects of these PAHs on the environment. This should be well implemented by relevant authorities. Consistent monitoring has to be conducted to assess the long term impact of PAHs. This would assist in ensuring their concentrations remain constant for a long time since they are known to be persistent in the environment. Industries should also be made to understand and appreciate their environmental responsibilities. Moreover, the public in general should be educated about the environment and be well informed on PAHs, their source, health impact and how to minimise their release into the environment. Several studies on seasonal variation of PAHs in water and sediment samples should also be done in order to have an idea of this effect. More studies should be conducted on fish samples proving that levels and presence of PAHs or any other pollutants in fish may have a direct bearing on human health.

## 5.3 CONFERENCE PRESENTATION

Mogashane, T.M., Ambushe, A.A., Mujuru, M. and McCrindle, R.I. Assessment of the levels of polycyclic aromatic hydrocarbons in sediments and water, South African Chemical Institute (SACI) Conference, 29th of November 2015 to the 4th of December 2015, at Southern Sun, Elangeni Hotel in Durban. *Poster presentation*

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## Appendix

Figures showing chromatograms indicating the presence of the PAHs in the sediment and water samples collected from Mokolo and Blood Rivers analysed with GC-FID.

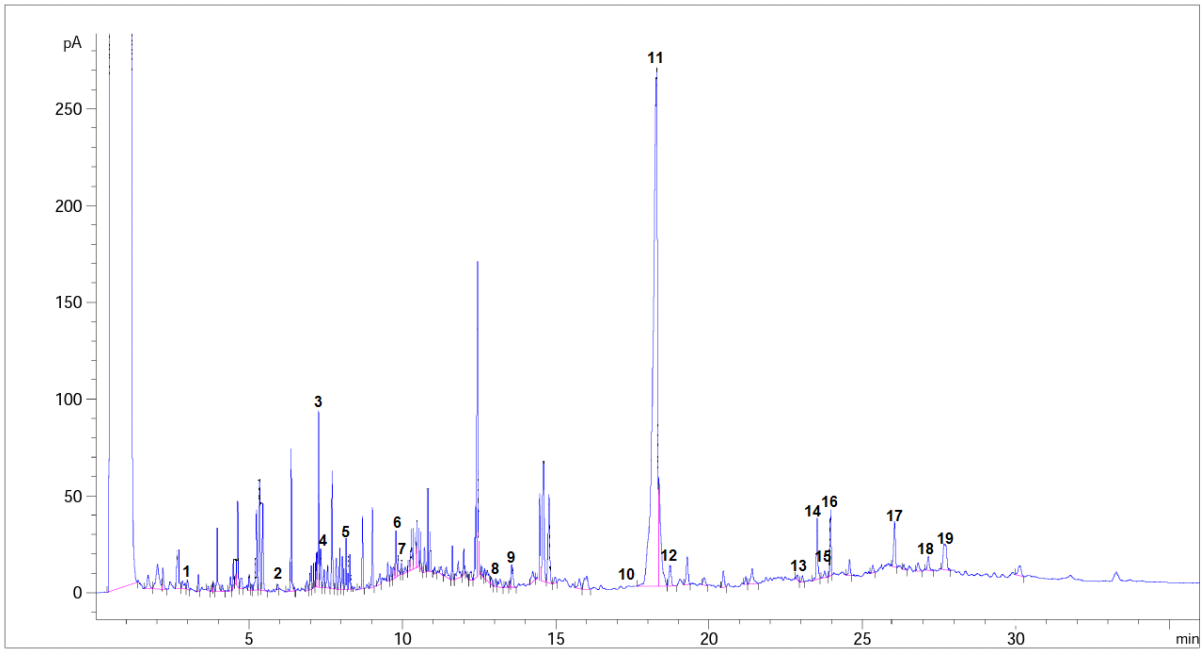

Figure A1: Chromatogram indicating the presence of the PAHs in the extract of sample 10, sediments sample collected from Mokolo River

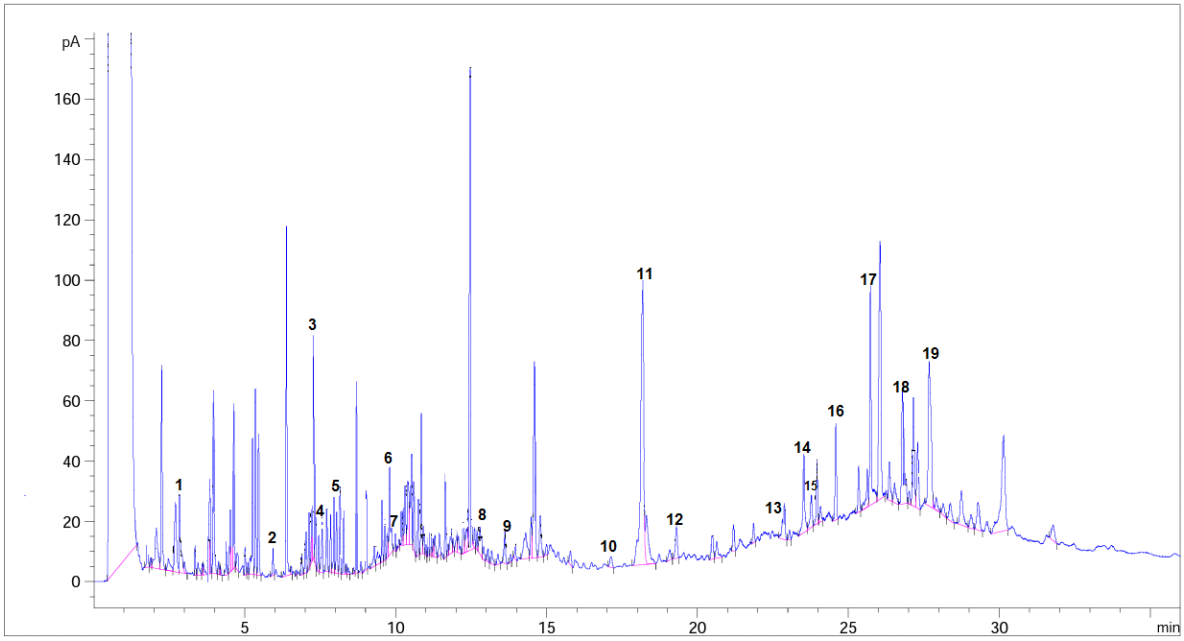


Figure A2: Chromatogram indicating the presence of the PAHs in the extract of sample 1, sediments sample collected from Blood River

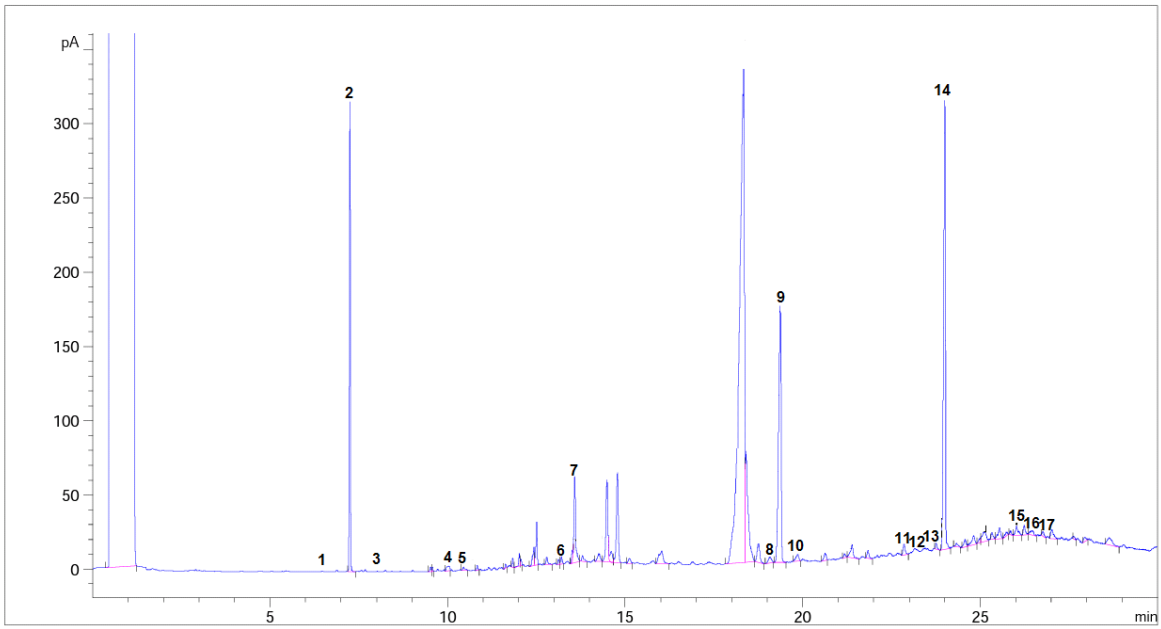


Figure A3: Chromatogram indicating the presence of the PAHs in the extract of sample 9, water sample collected from Mokolo River

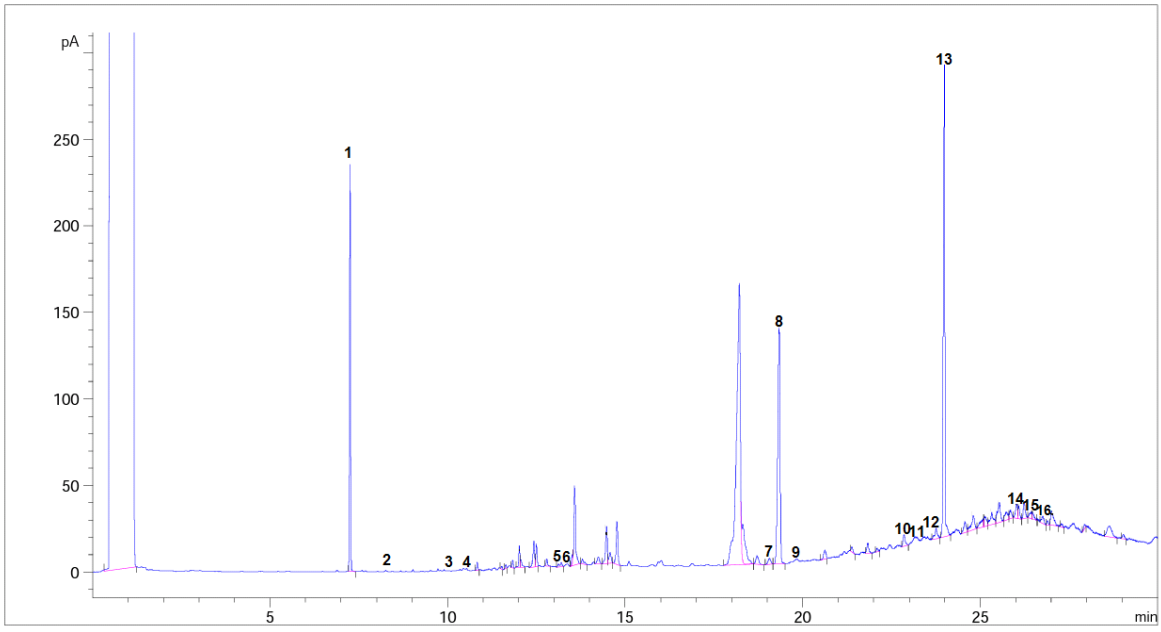


Figure A4: Chromatogram indicating the presence of the PAHs in the extract of sample 8, water sample collected from Blood River

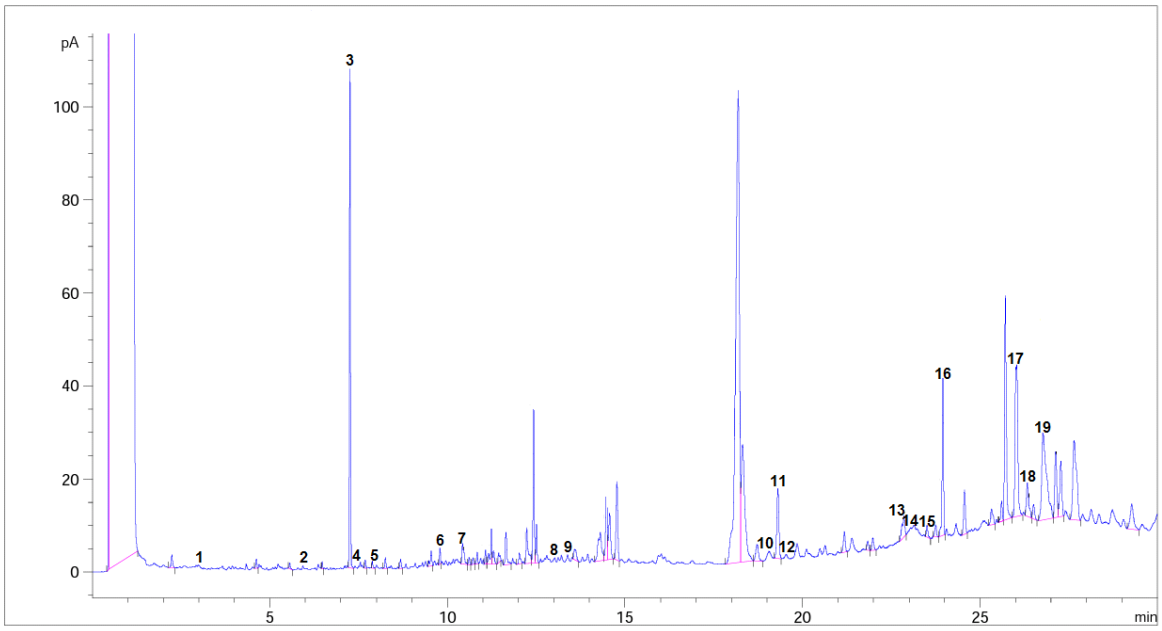


Figure A5: Chromatogram indicating the presence of the PAHs in the extract of sediment sample 1 as obtained following ultrasonication extraction method