Method Development and Application for the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in KwaZulu-Natal, South Africa

By

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A thesis submitted to the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, in fulfilment of the academic requirements for the Master of Science in Analytical and Environmental Chemistry.

School of Chemistry and Physics

June 2018

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Declaration

I, Ayanda Ngubo, herewith affirm that the work reported in this thesis is entirely my original work and other people's work have been fully acknowledged through citation in areas incorporated. The thesis has not been submitted before for any degree or examination purpose to any other high learning institution.

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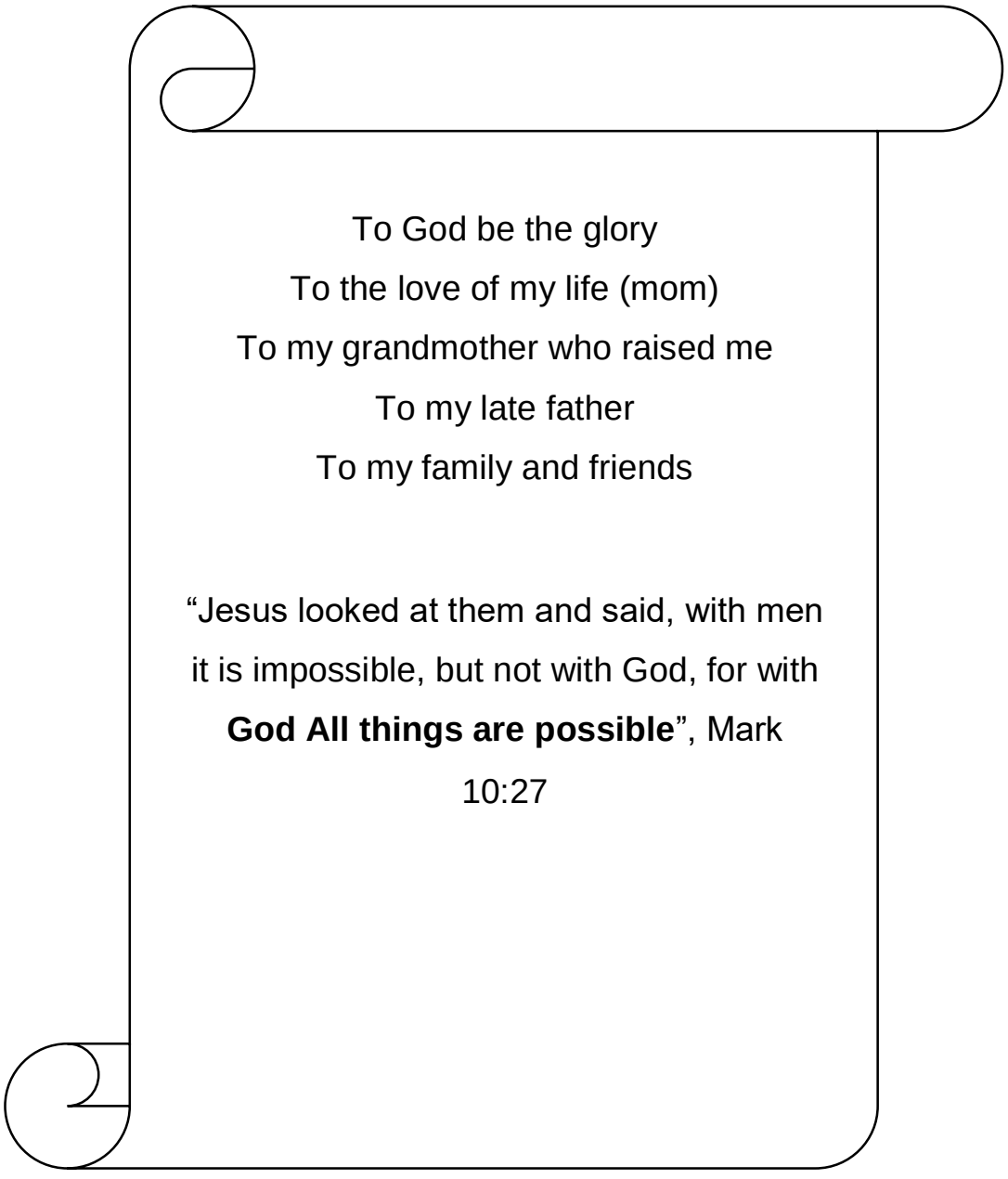
Abstract

This work involved the analysis of PAHs under optimized conditions of gas chromatography-mass spectrometry (GC-MS) method and after extracting by optimised ultrasonic extraction (USE) (for sediment samples) and solid phase extraction (SPE) (for water samples). The accuracy of the entire optimized methods was checked by analysing spiked water and sediment samples at single level representing the natural anticipated concentration levels in the respective matrices. Analytical performance of parameters such as linear range, limit of detection (LOD), limit of quantification (LOQ), extraction efficiency were determined as part of quality assurance. The optimized methods were then applied for the analysis of PAHs in real water and sediment samples. The LOD and LOQ for the optimized SPE method ranged from 0.01- 0.17 µg/L and 0.05-0.5 µg/L. The LOD and LOQ for the optimized USE ranged from 0.008-0.09 and 0.03-0.20 µg/kg.

The percentage recoveries obtained under optimum conditions ranged between 87-121% for SPE and 83-117% for ultrasonic with the relative standard deviation (RSD) of less than 10% for both methods. The concentration levels of PAHs obtained in wastewater, river water and dam water were between 0.186-7.74 µg/L, 2.17-32.94 µg/L and 2.5-3.3 µg/L, respectively. The concentration levels of PAHs obtained in river and dam sediment samples were between 2.8-42.0 µg/kg and 2.8-3.9 µg/kg, respectively.

Dedication



To God be the glory

To the love of my life (mom)

To my grandmother who raised me

To my late father

To my family and friends

"Jesus looked at them and said, with men it is impossible, but not with God, for with **God All things are possible**", Mark 10:27

Acknowledgements

My Sincere gratitude goes to the following people for their contribution:

- ✧ To my heavenly father God, the governor of my life who provided me with strength, courage, wisdom and perseverance.
- ✧ To my mother Princess, for her steadfast love, support and words of wisdom whenever I felt like giving up. A prayerful woman who fears God. I'm forever grateful for the sacrifices she made during my academic period.
- ✧ My grandmother for prayers and teaching me basic principles of life.
- ✧ My uncle Mthandeni, for continuous support and being a role model.
- ✧ I thank my supervisor, Dr P. Mahlambi for her encouragement, support and guidance throughout the duration of this research study.
- ✧ My co-supervisor Prof S. Ojwach for guidance and NRF funding.
- ✧ Ms Z. Ngcingwana for being my lab assistant and a caring mother.
- ✧ Mr Mbhele for assistance with equipment maintenance and troubleshooting.
- ✧ Ms C. Janse van Rensburg for basic lessons on how to operate the GC-MS instrument.
- ✧ Mr B. Gijsbersen for contracting a geographic map for sampling points locations.
- ✧ To the faculty of agriculture, science and engineering for allowing me to conduct this research in their facilities and support.
- ✧ My friends and family for their everlasting love.
- ✧ To everyone not aforementioned whom played a vital part in the compilation of this thesis.

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Abbreviations

°C	Degrees Celsius
Ace	Acenaphthene
Ace(y)	Acenaphthylene
Ant	Anthracene
Ace	Acenaphthene
Ace(y)	Acenaphthylene
Ant	Anthracene
ASE	Accelerated Solvent Extraction
ATSDR	Agency for Toxic Substances and Disease Registry
BaP	Benzo[a]pyrene
BIS	Bishopstowe
C18	Octadecyl
CAM	Campsdrift
COL	College Road
DCM	Dichloromethane
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxyegen
DWA	Department of Water Affairs
ECD	Electron Capture Detection

EPA	Environmental Protection Agency
FID	Flame Ionization Detection
FL	Fluorescence
Flu	Fluorene
GC-MS	Gas Chromatography-Mass Spectrometry
GDP	Gross Domestic Product
H_2O	Water
HLB	Hydrophilic Lipophilic Balance
HMW	High Molecular Weight
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IARC	International Agency for Research on Cancer
IUPAC	International Union of Pure and Applied Chemistry
KZN	KwaZulu-Natal
LLE	Liquid-Liquid Extraction
LMW	Low Molecular Weight
LOD	Limits of Detection
LOQ	Limit of Quantification
MAE	Microwave Assisted Extraction
MeOH	Methanol
MI	Amanzimtoti Influent

MRI	Marrianridge Influent
Nap	Naphthalene
ND	Not Detected
NE	Northern Effluent
NI	Northern Influent
NIP	National Implementation Plan
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polycyclic Biphenyls
PDMS	Polydimethylsiloxane
PID	Photo-Ionization Detector
PMB	Pietermaritzburg
POPs	Persistant Organic Pollutants
Pyr	Pyrene
RDP	Reconstruction Development Programme
RI	Refractive Index
RSD	Relative Standard Deviation
SE	Soxhlet Extraction
SI	Shallcross
SIM	Selected Ion Monitoring
SOP	Standard Operation Procedure
SPE	Solid Phase Extraction

SPME	Solid Phase Microextraction
TDS	Total Dissolved Solids
TIC	Total Ion Chromatogram
TID	Thermionic Ionization Detector
TLC	Thin Layer Chromatography
UHE	Umhlathuzana Effluent
USE	Ultrasonic Extraction
UV	Ultra-Violet
WHO	World's Health Organization
WOH	Woodhouse
WWTP	Wastewater Treatment Plant

Chapter One - Introduction

1.1 **Background review of study**

Modern agricultural and industrial processes (from manufacturing, to mining) generates lots of organic wastes and emissions. Some of the wastes that are generated are harmful to the environment, thus cause health risks and may trigger natural hazards, which become a major concern (Shafy et al., 2016). The released wastes usually contaminate air, water, soil and sediments, thus affecting various ecosystems and natural processes in the environment (Bayowa, 2014). Some of pollutants that are emitted into the environment are resistant to bio-degradation because of their chemical stability. Examples include aromatic compounds, gases, and metal ions and their complexes.

Aromatic compounds form a majority of the group of pollutants termed persistent organic pollutants (POPs). A subgroup of these compounds is polycyclic aromatic hydrocarbons (PAHs). These are mainly generated through incomplete combustion of carbon-containing materials. Due to their persistence and hydrophobic properties, they bio-accumulate in food chains and can be transported between different regions of the globe. Their presence in environmental metrices is influenced by their semi-volatility, poor water solubility and inertness to degradation. They can also be dispersed in the atmosphere through air masses transport to colder climate (MacRae and Anderson, 2009).

In urban settlements, where there is heavy industrial activities as well as generation and disposal of large volumes of domestic wastes, sources of PAHs may include accidental spills of petrochemicals, wastewater, refuse pipe leakage , wood burning and cooking plants (Jones, 1999, Abdel-Shafy and Mansour, 2016). The following schematic diagram summarizes possible sources of PAHs in an urban environment and the fate of such compounds after being transported to their sink destination (Figure 1.1).

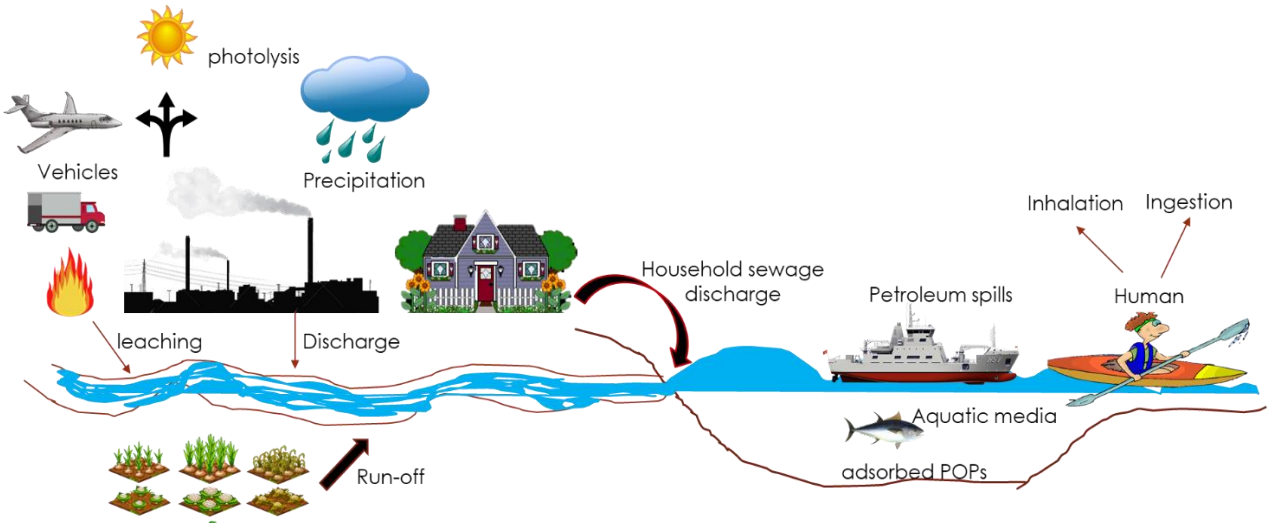

Figure 1. 1 A schematic diagram showing the release of pollutants into the environment (Thamaraiselvan *et al*, 2015).

Although PAHs are not accurately toxic to biota and animals, their accumulation across the food chains, puts predators at high risk from toxicity, since they are difficult to excrete from the body. Exposure to high concentration levels of POPs is very rare, but long-term exposure to low concentration levels may have chronic illness in humans such as immune disorder, reproductive changes, allergies, hypersensitivity, neurotoxicity and cancer (Thamaraiselvan *et al*., 2015).

Owing PAHs low water solubility and high hydrophobic nature, they turn to form colloidal particles with environmental particulates and thus adsorb in water and sediments. They occur as complex mixtures at very low concentrations in most environmental samples (Abdel-Shafy and Mansour, 2016). Some samples especially sediments and liquid effluents are very complex, thus more sensitive analytical methods are required to extract, clean up the extract, separate and detect them. Thus far, the most applicable technique for aqueous samples is the solid-phase extraction (SPE), due to its high extraction efficiency, and high enrichment factor, easy automation, and less consumption of organic solvents (Wilson, 2000). On the other hand, Ultrasonic extraction (USE) technique is a widely used method for extracting soil and sediment samples due to minimal extraction time, simplicity of extraction procedure and apparatus (Banjoo and Nelson, 2005). Gas chromatography-mass spectrometer (GC-MS) is the most suitable instrument for quantitative and qualitative analysis of trace levels of PAHs in environmental samples due to its ability to separate complex organics from volatile samples.

1.2 **Problem statement**

According to the National Implementation Plan (NIP) for persistent organic pollutants, POPs produced by chemical processes from agriculture and industrial sectors are underlined as potent pollutants, that are chemically stable and have a potential to contaminate other locations through long range transportation. Therefore, PAHs evaluation to ensure a sustainable and protected environment for human health is significant as a way of trying to minimize their generation(Fischer *et al.*, 2011).

PAHs fall under a priority group of environmental pollutants which have been reported by the World's Health Organization (WHO) for their persistence and possible toxic effects to both humans and aquatic animals. They are generated mainly by petrogenic and pyrogenic processes. Due to higher chances of generating PAHs in industrial and heavy use of fossil fuels in urban environment as well as the health risk associated with them, it is imperative that the levels of PAHs are investigated and monitored on regular basis (Kielhorn and Boehncke, 2003).

1.3 **Significance of this research**

This study is aimed at measuring the concentration levels of targeted PAHs in water and sediments around KwaZulu-Natal (KZN). An investigation on the presence and levels of the studied compounds in the selected study areas was conducted using optimized extraction method for the identification of the PAHs in water and sediment samples. This data will contribute towards policy making on the allowable concentration levels in KZN.

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Chapter Two – Literature Review

2.1 **Introduction**

Polycyclic aromatic hydrocarbons (PAHs) are among a group of persistent organic pollutants (POPs) that are defined by two or more fused aromatic rings. They occur as complex mixtures and are made up of mainly carbon and hydrogen (Lundestedt, 2003). PAHs have relatively low water solubility constants and have a high lipophilicity. Despite PAHs' low solubility in water, they dissolve in most synthetic organic solvents and adsorbs strongly on organic matters of soils and sediments (Shafy *et al.*, 2016). Some of the PAHs have been listed as priority pollutants by World Health Organization due to persistence to degradation by environmental microbes and their toxic effects to human aquatic animal health. Figure 2.1 shows structures some of the priority PAHs.

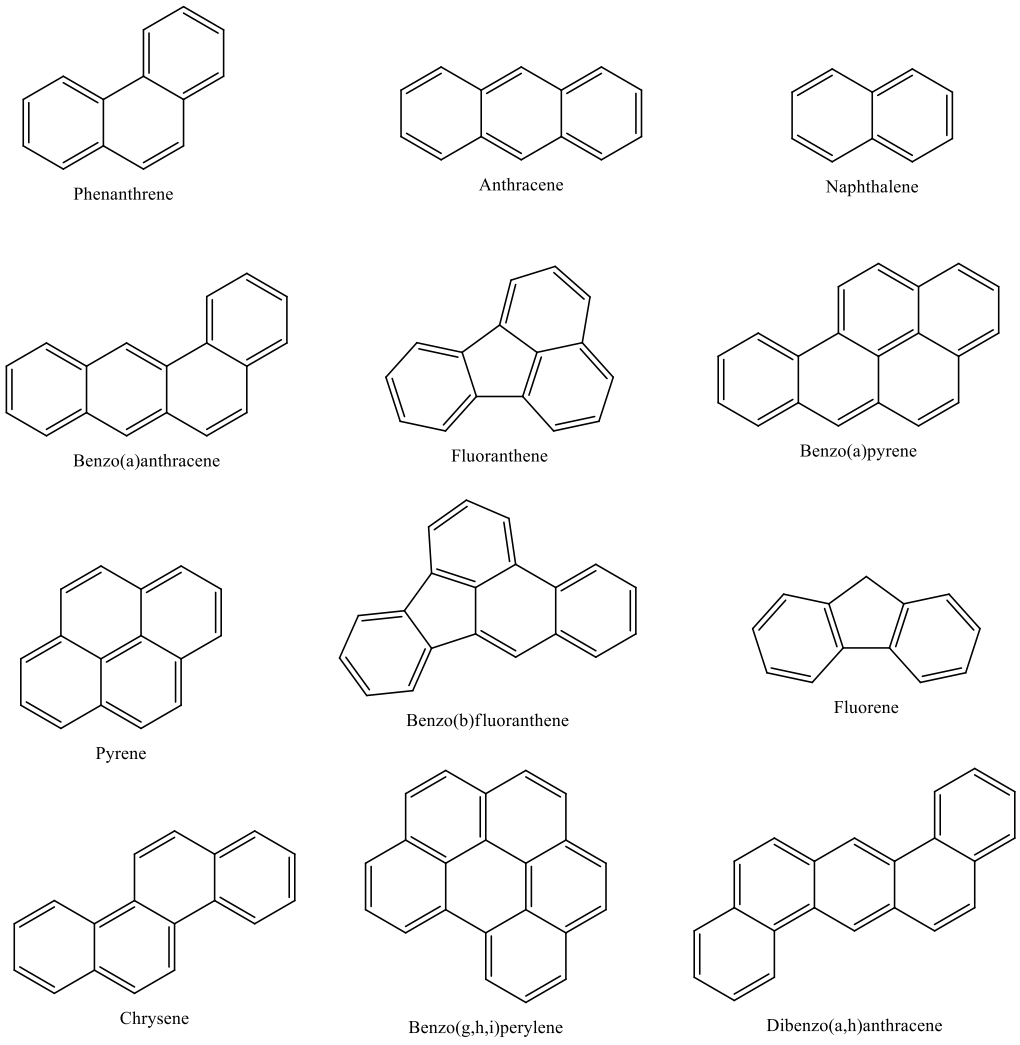

Figure 2. 1 Structures of polycyclic aromatic hydrocarbons.

Most PAHs are colourless, white or pale yellow, and chemically related to each other through aromatic rings. Most are persistent to bio-degradation in the environment due to their chemical stability as a result of delocalization of pi-electrons between sp^2-hybridized carbon atoms in their structures. Resistance to oxidation and reduction increases as the PAH's molecular weight increases, they are hydrophobic and thus more soluble in organic solvents and lipophilic fluids (Nekhavhambe *et al*., 2014).

Their aromaticity and chemical stability confer other properties such as heat resistance, measurable in plane conductivity, resistance to corrosion and a vast range of physiological activities. However, exposure of PAHs to UV-light can induce them to form radicals or reactive species which are toxic to cellular constituents such as proteins, cell membrane and nucleic acids (Fu et al., 2012, IARC, 2010).

PAHs are classified into two groups by the International Union of Pure and Applied Chemistry (IUPAC). Those with two to three fused aromatic rings are called low molecular weight (LMW) PAHs while those with four to six fused aromatic rings are called high molecular weight (HMW) PAHs (Wick *et al*., 2011). LMW-PAHs are more volatile, more susceptible to degradation and they are more soluble in water (Ou *et al*., 2004). On the contrary, HMW-PAHs have low vapour pressures, stable towards bio-degradation, and are nearly insoluble in water. Consequently, only a small amount of PAHs are found dissolved in water while the majority of PAH compounds are sorbed to organic colloids present in environment environmental samples, especially on sediments (Bertilsson and Widenfalk, 2002). Moreover, there is a considerable correlation between their melting point temperatures and molecular weights. Thus, HMW PAHs are less volatile due to their low vapour pressures and are solids at ambient temperatures (Stogiannidis and Laane, 2015). Table 2.1 shows some physical and chemical properties of some commonly occurring PAHs.

Table 2. 1 Properties of the most common PAHs (Satcher, 1995, Gerberding, 2005).

PAH	Number of rings	Formulae	MW (g/mol)	Melting point (˚C)	Boiling point (˚C)	Aqueous solubility mg/L (25˚C)	Log K_{ow}
Naphthalene	2	$C_{10}H_8$	128.19	81	218	31.7	3.29
Acenaphthylene	3	$C_{12}H_8$	152.20	93	279	3.9	4.07
Acenaphthene	3	$C_{12}H_{10}$	178.23	93.4	280	1.9	3.98
Fluorene	3	$C_{13}H_{10}$	166.22	117	295	2.2	4.28
Phenanthrene	3	$C_{14}H_{10}$	178.23	100	340	1.2	4.45
Anthracene	3	$C_{14}H_{10}$	178.23	218	342	0.08	4.45
Pyrene	4	$C_{18}H_{12}$	202.25	156	393	0.08	4.88

*Log K_{ow} = Octanol-water co-efficient

Furthermore, an increase in a molecular weight of PAHs causes an increase in hydrophobicity/lipophilicity and thus a decrease in aqueous solubility and vapour pressure (Alegbeleye, 2015). However, aqueous solubility is affected by temperature and the presence of surfactants that shield PAHs and colloidal organic fractions through micelle formation. An increase in the number of aromatic rings, also increase the boiling point temperature drastically (Luch, 2005). Simply meaning, HMW-PAH can withstand combustion and pyrolysis temperatures, thus resist thermal degradation.

2.2 **Formation and production of PAHs**

Incomplete combustion of carbon-containing material leads to the formation of PAHs during industrial processes or other localized combustion related processes (cooking, residential and commercial burning, fumes produced by engines during transportation, burning of waste products, coal tar production, and so forth.) (Satcher *et al.*, 1995). Some PAHs are produced in thermal reactions of appropriate reactants in the presence of

oxygen (examples includes acenaphthylene, acenaphthene, anthracene, chrysene, fluorene, naphthalene, etc.) (Bayowa, 2014): - The equation below shows that in combustion processes, aromatics such as benzene can react with reactive gases such as ethylene to form acenaphthene. This reaction can occur in thermal power generator when coal is combusted to generate heat for heating water (Qi and Zhang, 2004).

$$3C_2H_4 + C_6H_6 + O_2 \xrightarrow[\approx 1173\ K]{\triangle} C_{12}H_{10} + 4H_2O$$

2.3 **Sources of PAHs**

PAHs are found almost everywhere, and their emission sources are both natural and anthropogenic. Natural sources include emission from petroleum products and mined stock piles. Anthropogenic sources involve combustion related processes as well as formation of PAHs during synthetic processes such as destructive distillation, cracking and reforming of synthetic fuels and pyrogenic reactions. The profile of PAHs emitted in coal related production processes differs from PAHs produced from oil refinery processes. Since PAHs are certainly generated and emitted from combustion processes, it is convenient to categorize PAHs as phytogenic (PAHs produced from plants), petrogenic (PAHs associated with combustion and refinement of petroleum products , Pyrogenic for PAHs associated with the combustion of coal (Saber *et al*., 2006)),

2.3.1 **Pyrogenic sources**

Combustion of organic substances under high temperatures and oxygen-depletion (conditions in processes such as pyrolysis, cracking and destruction distillation) are responsible for production and emission of pyrogenic PAHs as waste streams (Saber *et al*., 2006). Pyrogenic PAHs are commonly found in aquatic environment compared to petrogenic PAHs due to a number of pyrogenic sources compared to petrogenic sources. (Guo *et al*., 2007, Wickramasinghe *et al*., 2011). HMW-PAHs such as benzo[*k*]fluoranthene, benzo[*b*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, reach the aquatic environment through direct atmosphere deposition and or via contaminated soils (Budzinski *et al.*, 1997). Environmentalists suggest that LMW-PAHs are often introduced to an aquatic environment by rain washout (Stout et al., 2003).

Between 1990-1995, industrialised countries such as United Kingdom emitted about 117 metric ton of benzo[*a*]pyrene (BaP) from vehicles, natural fires, aluminum production, anode baking, coke production, industrial and domestic combustion processes (Hailwood *et al*., 2001).

In recent decades, there has been a sharp increase on demand for manufacturing of fuel combusted products, manufacturing of gas plant sites, discharge from aluminum smelters and use of free transport systems, and this also increases the amount of mobile PAH emissions, especially in urban areas (Stogiannidis and Laane, 2015). Municipality's surface water runoff and industrial waste discharge to the river contains a mixture of pyrogenic, petrogenic and natural PAH sources (Nawaz *et al.*, 2014).

Unsubstituted PAHs predominates over alkylated PAH homologues (Stout *et al*., 2003). However, when alkylation levels on the environmental matrix increase, the alkylated PAH homologues become more abundant, whereas LMW-PAH becomes less abundant (Boll *et al.*, 2008). An increase in combustion temperature results in a decrease in abundance of alkylated PAH relative to common PAHs (Laflamme and Hites, 1978).

According to Marr *et al.* (1999), naphthalene and other HMW-PAHs dominates Gasoline combustion processes. In areas such as Southern Asia, Canada, and United State of America, where they utilize motorcycles, significant amounts of PAHs are produced (Boll *et al*. 2008), conducted a study in Vietnam where they discovered that the concentrations of PAHs decrease as the distance between the sampling point and the traffic sources increases.

Moreover, biomass combustion at low temperature is one of the sources of emissions of LMW-PAHs (Barra *et al*., 2007), whereas HMW-PAHs are emitted from burning wheat and rice straw (Ravindra *et al*., 2008). Fireplace soot contains significant amounts of LMW-PAHs and HMW-PAHs. Soot with high concentrations of PAHs is most likely to contain LMW-PAHs. Whereas, soot with low concentration levels of PAHs, is dominated by HMW-PAHs (Stark *et al*., 2003). Dominant HMW-PAHs on fireplace soot are benzo[*a*]anthracene, chrysene, benzo[*a*]pyrene, Cyclopenta[*a*]phenanthrene, phenylnaphthalene and benzo[*ghi*]fluoranthene (O'Malley *et al*., 1997). Pinewood resin

combustion produces three ringed alkyl PAHs such as 1,7-dimethylphenanthrene, 1-methyl-7-isopropyl phenanthrene and 1-methylphenanthrene (Yan *et al*., 2005).

Combustion of coal also leads to the production of PAHs in waste gases. PAHs composition in emissions from coal combustion is dependent on temperature and as well as type of coal (Yanker *et al*., 2002). More PAH compounds are produced at lower temperature processes, compared to high temperatures (Chen *et al.*, 2005). PAHs with 2-3 rings namely fluoranthene, anthracene, pyrene, phenanthrene, chrysene and benzo[*k*]fluoranthene dominates in most coal combustion processes (Stout et al., 2001a). Consequently, the production of PAHs in the combustion of coal, coke creosote and other sources during mineral processing results in an increasing concentration of PAHs emissions. Two and three-ringed PAHs (pyrene, naphthalene, acenaphthene, fluorene, fluoranthene and phenanthrene) are found abundant/dominant over four to five-ringed PAHs.

Furthermore, large quantities of PAHs produced from industrial processes. end-up adsorbed in soil and sediments (Stout *et al*., 2003). PAHs can be transported from street dust to the river, dams, and sediments through surface water runoff (Lorenzi *et al*., 2011). Street dust may be contaminated with lubricating oil, automobile exhausts, gasoline, diesel, tyre particles, all of which becomes potential sources of PAHs reaching the water and sediments (Breault *et al*., 2005). A vast range of both LMW-PAHs and HMW-PAHs sourcing is from street dust (i.e. pyrene, phenanthrene, biphenyls, dibenzothiophenes, and benzo[*ghi*]perylene). However, the predominant ones are fluoranthene and pyrene and to the lesser extent, phenanthrene and anthracene (Karlsson and Viklander, 2008).

2.3.2 **Petrogenic sources**

PAHs commonly found in petroleum, crude oil, lubricants, and refinery related products are classified as petrogenic. Thus, accidental spills of petroleum or waste from refining of crude petroleum can contaminate the environmental matrices with such PAHs (Saber *et al*., 2006).

Crude petroleum comprises a mixture of hydrocarbons formed under conditions of high pressures and temperature from fossil animal and plant residues, it can be refined to produce petrol, paraffin, diesel and oil fractions with various proportion of PAH

compounds (Page *et al.*, 1996). Stout *et al.*, (2002) stated that in crude matures there are thermally stable phenanthrene isomers). Petroleum processed products EPA 16 PAHs including their homologues (alkylated naphthalene, fluorene, chrysene series, phenanthrene, and dibenzothiophene) may be produced in crude petroleum processing (Bertilsson and Widenfalk, 2002). The occurrence of these PAHs in significant amounts on sediments may be a good indicator of contamination to petrogenic sources. HMW-PAHs (i.e. Five to six ringed structures: -acenaphthylene, anthracene, and fluoranthene) are often not detected in crude oil and its refined products (Stout *et al.*, 2002). Lubricant fractions are rich in petrogenic PAHs. Low-temperature distillation of crude oil does not produce PAHs. As a result, refined petroleum product does not have any significant concentrations of PAHs, unless the PAH was present in the unprocessed crude oil (Stout *et al.*, 2001b). High-temperature distillation may result in HMW-PAH being produced (i.e. methyl phenanthrene isomers). However, alkylated PAH formed may be removed from diesel processing (Stout and Wang, 2007). Unprocessed diesel comprises of alkylated LMW-PAH and their homologues (Wang *et al.*, 2001).

Illegal dumping of lubricating oils, leakages and spillage can be a significant contamination sources of PAHs in the aquatic environment. Fresh lubricating oils contains low amounts of LMW-PAHs (dibenzothiophenes) compared to used lubricating oils that produce a variety of PAHs such as methyl phenanthrene (Zakaria *et al.*, 2002).

2.4 **Toxicity effects of PAHs**

Plants are susceptible to PAHs toxic effects. Uptake of PAHs through plant roots and their translocation to other parts of the plant can induce growth. Uptake of PAHs by plant roots depends on the concentration of PAHs in the soil, soil type, water solubility, and physical properties of individual PAHs (Zhang *et al.*, 2017). Other plants can convert toxic PAHs to harmless growth hormones (McGuinness and Dowling, 2009).

PAHs have toxicity effects, especially in aquatic animals, and birds. Low concentrations of PAHs have no acute toxic effects on terrestrial invertebrates. However, mammals absorb PAHs during inhalation, dermal conduct, and indigestion thus leads to immunity disorders, tumour development for cancer, reproduction complications, etc. (Shafy *et al.*, 2016).

2.4.1 **Metabolism of PAHs in Mammalians**

According to International Agency for Research on Cancer (IARC), (2010) a significant number of PAHs have a toxicological effect on human health upon long-term exposure to them. Some of these PAHs fall under different groups (Group 1, 2A or 2B) based on their carcinogenic effect(s) on human health. Group 1, 2A or 2B PAHs causes cancer tumour upon inhalation, dermal contact, oral, subcutaneous uptake, intravenous, intratracheal, intraperitoneal or intrabronchial, examples include Benzo[a]pyrene, benzo[k]flouranthene, chrysene, benzo[b]fluoranthene, anthracene, naphthalene. Some of these PAHs are present in unprocessed contaminated foods. This background of contamination of food originates from long range transportation of PAHs from natural and anthropogenic sources to the farming regions that remotes to the sources. Production of other PAHs occurs during high temperatures cooking of plant and animal products, examples includes grilling, frying and roasting (Shafy *et al.*, 2016).

Benzo[a]pyrene (B[a]P) is reported as one of the traditional markers of PAHs that induces cancer. B[a]P becomes activated into electrophilic intermediates during metabolism and thus acts as a carcinogen. Activated B(a)P has a potential to induce lung cancer through three steps (Figure 2.2) (Rengarajan et al., 2015). The first step is the formation of (7R,8S)-epoxy-7,8-dihydrobenzo(a)pyrene(B(a)P-7, 8-oxide catalyzed by cytochrome P450 enzymes. The second step involves catalysis of epoxide hydrolase to form (7R,8S)-epoxy-7,8-dihydrobenzo(a)pyrene (B(a)P-7,8-diol). Cytochrome P450 enzymes produce isomers which act as a carcinogen (BPDE) that bind to DNA to form BPDE-DNA adducts, leading to increasing DNA transcription of defaulted genes (CYP1A1) that induces lung cancer (Rengarajan *et al*., 2015).

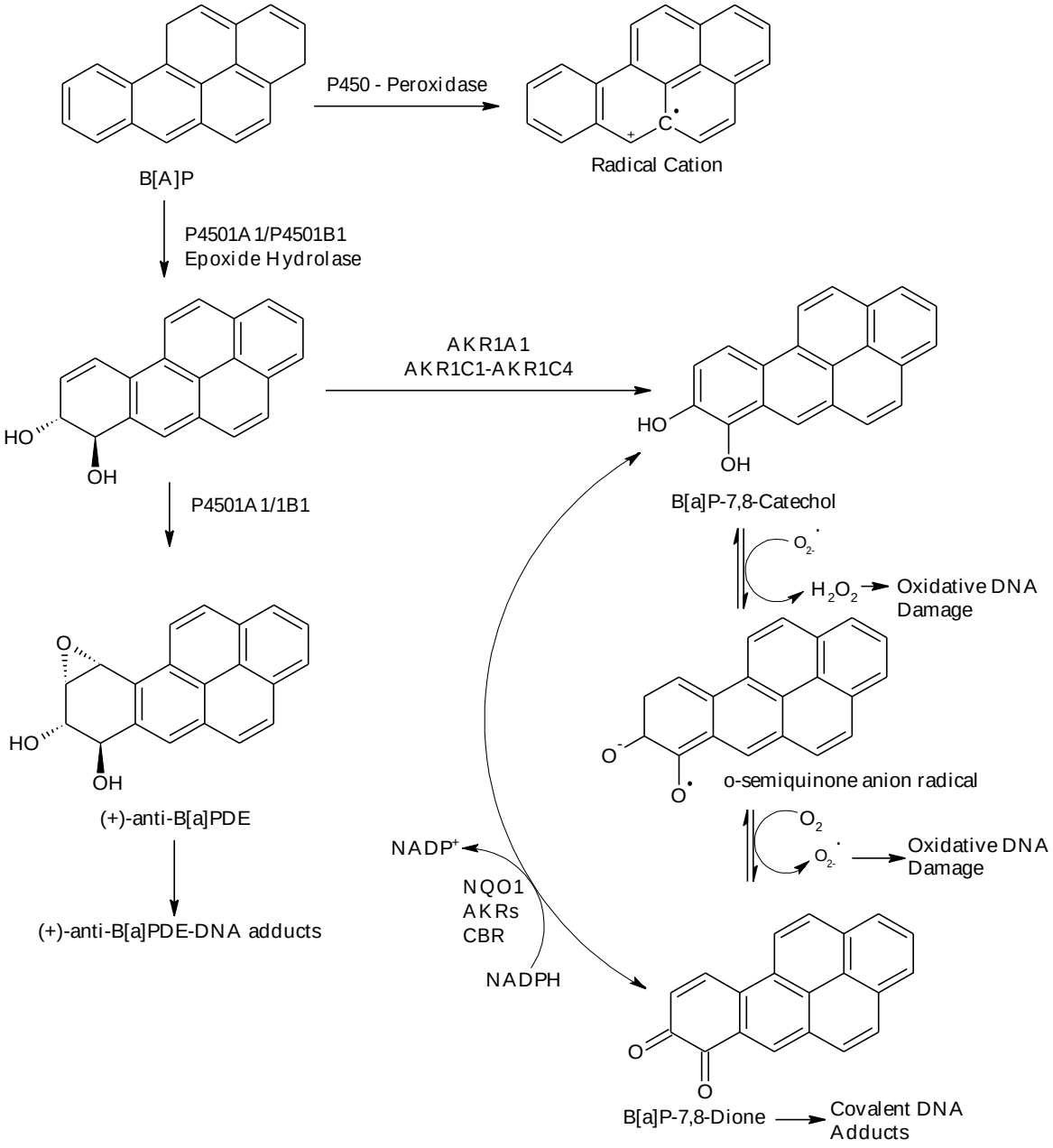

Figure 2. 2 Schematic representation of B[a]P activation for induction of cancer ((Rengarajan et al., 2015).

In general, the formation of diol-epoxide derived DNA adducts reacts with covalent DNA adducts and activate the pathway that leads to cancer initiation. Other cancers inducing pathways are from the results of the formation of reactive free radicals from electron oxidation, that reacts with DNA covalent adduct. Naphthalene and its homologues are known to covalently bind to molecules in the liver, lung tissues, kidney and as a resulting enhancing its toxic effects. Formation of Naphthalene-DNA adducts may cause haemolytic anaemia and eye defects (Fu *et al.*, 2012).

2.5 **PAHs in water, soil and sediments**

PAHs deposits into the soil surface through incomplete combustion of organic material and industrial processes and bind to the soil surface, which means their mobility depends on the sorbent particle size and pore throat size of the soils. Also, properties of PAHs also play a vital role in it adsorption and mobility on the soil surface, thus since different PAHs have unique properties to each other, their adsorption and mobility on the soil surface are not the same (EPRI, 2008).

Deposition of PAHs in sediments results from the same processes that regulate deposition to surface soil. Sediments from rural areas are less concentrated with PAHs due to less PAHs sorbet to atmospheric particles settling in streams, lakes, and oceans. On the other hand, there are high concentrations of PAHs in urban areas, due to higher atmospheric deposition resulting from sanitary effluents, roadway runoff, industrial spills, etc., thus ends up being adsorbing on sediments surface (Kafilzadeh, 2015).

2.6 **Health effects**

There is no proper documentation of short-term effects of PAHs, on human health however high concentrations of PAHs in a highly polluted environment increases their toxicity. Some factors that contribute to the impact of PAHs on human health include health condition and age. Mixtures of PAHs can cause skin allergic reactions such as inflammation, nausea, vomiting, diarrhoea and confusion in both animals and humans. PAHs known for skin irritation reaction are benzo(a)pyrene, Anthracene, and naphthalene (Rengarajan *et al.*, 2015).

Chronic health effects may result from long-term exposure to PAH concentrated environments, thus leading to immune system instability, kidney failure, asthma-like symptoms, lungs dis-functioning symptoms, liver failure, etc. (i.e. Naphthalene can cause a breakdown of red blood cells (β-cells and T-cells) if inhaled or ingested in large amounts (Satcher *et al*., 1995).

2.7 **Human exposure to PAHs**

There are three classes of toxicokinetics levels upon exposure to PAHs (absorption, distribution, excretion: - all involving inhalation exposure, oral exposure, dermal

exposure and other routes of exposure). Most PAHs carcinogens enter the human body stream through inhalation of contaminated air, smoking cigarettes, exposure to fireplaces. A common carcinogenic PAH compound found in tobacco is B[a]P, while other PAHs are absorbed in plants from soils surface (Canada, 2016).

A study conducted by Agency for Toxic Substances and Disease Registry (ATSDR) in Environmental Medicine, reported that priority PAHs in ambient air is 0.02-1.2 ng/m^3 in rural areas, while they are 0.15-19.3 ng/m^3 in urban areas. Cigarette smoking and environmental tobacco (containing B[a]P) are other routes of exposure to air. Sullivan and Krieger, (2001) reported that smoking a pack of separated cigarette per day yields 0.4 µg/day of PAHs. Industrial effluents and accidental spills of oil during shipment contaminates water and results in PAH deposition in water. In the United State, untreated water is reported to contain several folds of B[a]P than in drinkable water. The concentration of B[a] P in water is about 0.2 ppb ((ATSDR), 2009).

There is quite a variety of exposure sources of PAHs: - foodstuff, prescription, and non-prescription coal tar products for the treatment of dermatologic disorders, breast milk across the placenta, etc. People working with coal, fire-places, agricultural farms, clinicians are at high risks of exposure potentials (Marston *et al.*, 2001).

2.8 **Effects on aquatic animals**

Studies conducted by Wright *et al.*, (2002), suggested that a global discharge of PAHs is about 80 000 tons per year and those PAHs ends up associating with other environmental matrixes that are carcinogenic and genotoxic. Many studies have reported PAHs toxic effects on fish and bivalve species (Incardona *et al.* 2014, Onozato *et al.* 2016).
Another study by Ikenaka *et al.*, (2012), was conducted to examine the impact toxicity of benzo[a]pyrene in aquatic species (*Ceriodaphnia reticulate* and *Daphnia Magna).* The levels of B[a]P were found to be 4.3 µg/L in *Ceriodaphnia reticulate* species and 4.7 µg/L in *Daphnia Magna* species. Their results findings signified that short-term exposure to low concentrations of B[a]P might impact the abundance of zooplankton family group. However, their study also suggested further investigations for specific toxic impacts in specific species, physiological and molecular analysis (Ikenaka *et al.*, 2012).

2.9 **Allowable concentration levels of PAHs in soil and water.**

The Agency for Toxic Substances and Disease Registry (ATSDR) case studies in Environmental Medicine Toxicity of Polycyclic Aromatic Hydrocarbons (PAHs) has set up standards and regulations for PAH exposure in the environment. Table 1 shows the allowable concentrations of commonly investigated PAHs in water and soil.

Table 2. 2 Allowable levels of PAHs in water and soil ((ATSDR), 2009).

PAH	MAC (soil), ppm	MAC (water), ppm
Naphthalene	1.0	3.0
Acenaphthylene	3.0	3.0
Acenaphthene	3.0	3.0
Fluorene	3.0	3.0
Phenanthrene	3.0	3.0
Anthracene	3.0	3.0
Pyrene	3.0	3.0
Benzo[hgi]perylene	3.0	3.0
Benzo[a]pyrene	0.3	0.005
Benzo[a]anthracene	0.15	0.005
Benzo[k]fluoranthene	0.3	0.005
Benzo[b]fluoranthene	0.3	0.005
Dibenzo[a]anthracene	0.3	0.005
Indeno[1,2,3-ghi] pyerene	0.3	0.005
Indene	-	3.0

2.10 **Analysis of PAHs in environmental matrices**

2.10.1 **Sampling strategy**

Sampling process involves selecting a portion of material small enough to represent a part of the environment sampled. A sampling plan is the most vital and critical part of any analytical work, because improper sampling could result in great relative errors and absolute errors (Madrid and Zayas, 2007). In general, errors due to instrumentation are

very low, however when investigating trace analytes in sub-μg/L levels, possible errors due to improper sampling and sample prepartion are very high (Batley, 1999). When sampling, a standard operation procedure (SOP) should be written and aligned to a sampling purpose. SOP should incorporate the aspects of when, where and how the samples are collected, preparation of sampling site instruments, sample record-keeping, sample containers and storage, and sample pre-treatment procedure (Stoeppler, 1997). Storage and handling of sample is of paramount importance, because improper starage and handling could result in the following: - decomposition of analyte by means of UV irradiation (specially organic compounds), temperature, microbial activity, loss of analyte due to volatilization and chemical reactions with the container. In most analytical sampling protocols, brown-glass bottles are used for protecting the samples from external agents, and samples are stored at low temperatures (4˚C), whereas sediments and biota have to be frozen (Madrid and Zayas, 2007).

A well validated and established method for measuring levels of chemical pollutants is a three steps monitoring mode: - bottle sampling, extraction and instrument analysis. Using this method, surface water is normally collected at about 0.5 meters from the surface of water. Other non-conventional sampling methods are the use of water samplers and peristatic pumps, but the methods have major disavantages such as their cost and mantainance. All field samples require a chain of custody (COC) where all the parameters that may influence the results obtained are recorded (i.e. location, site observations, sample physico-chemical parameters, date and time of collection) (Pitard and Gys, 1993).

2.10.2 **Extraction techniques**

There are various analytical extraction techniques that are used for extraction of PAHs. These include soxhlet extraction, mechanical agitation, accelerated solvent extraction (ASE), sonication, microwave-assisted extraction (MAE), solid phase extraction (SPE), Sub-critical fluid extraction and many more. However, all these techniques offer different advantages over one another, depending on the nature of the sample and the nature of the analysis (Khan *et al.*, 2005).

2.10.2.1 **Solid sample extraction**

2.10.2.1.1 **Soxhlet extraction (SE)**

This technique is used to extract non-to semi-volatile organic analytes on a solid matrix using a solvent that continually evaporates from a still-pot, whereby the extraction thimble in the condenser apparatus are connected to the sample (Castro and Ayuso *et al*, 2000). Extraction occurs via evaporation and condensation of solvent which is collected into an extraction chamber with side chamber that triggers its draining back into the still-pot when it is full using appropriate solvents (Sporring *et al*, 2005). Drawbacks of this technique are that it requires large volumes of solvents for extraction and it is time-consuming since reflux might take more than 24 hours. The Soxhlet condenser requires continuous running of water through the condenser for cooling. More significantly, soxhlet extraction has a poor selectivity of PAHs compared to other organic analytes (Dean and Xiong, 2000).

In a study by Nikolic *et al*., (2017) homogenized soil samples spiked with a mixture of 16 PAHs were extracted using 300 ml volume of dichloromethane for 8 hours. The sample was pre-concentrated to 5 mL by rotary evaporation, followed by separation and cleaning by column chromatography technique. The extracts were analysed on GC-MS and the recoveries of PAHs were in a range 75-111% for all PAHs investigated. However, this technique uses large volumes of environmentally unfriendly solvents, takes long to extract (Nikolic et al., 2017).

2.10.2.2 **Accelerated solvent extraction (ASE)**

ASE method is an efficient method for extracting PAHs from solid samples. The method is the fast, safe, and adaptable to automation. Extracting PAHs solid samples can be accomplished with small amounts of solvents. ASE uses elevated temperatures and pressures to increase the extraction efficiency of analyte from its matrixes. Due to increased solubility and decreased viscosity extraction media and analyte, enhanced diffusion rate of the analyte into the solvent is achievable. ASE results in high recoveries of PAHs compared to traditional methods and is relatively more accurate, with an improved relative standard deviation (Lau et al., 2010). However, this technique is costly to run due to the high temperatures and pressures that are required.

Accelerated solvent extraction (ASE) procedure coupled to gas chromatography-mass spectrometry was employed in the study by Belo *et al*., (2017) whereby fermented and dried cocoa beans were spiked with known concentrations if targeted PAHs analytes and the recoveries ranged between 2.16-107.02%. The method development indicated some of the PAHs were not efficiently extracted, therefore, it is vital that the ASE method may be further optimized before the method can be adopted (Belo *et al*., 2017).

2.10.2.3 **Microwave-Assisted Extraction (MAE)**

MAE is another useful for the extraction of analytes from a solid. A sample-solvent mixture is subjected to microwave radiation from a magnetron generator. This is used to rapidly heat the polar solvent which in turn heat-up the sample matrices to release analyte into the solution. MAE is a preferred method compared to conventional heating due to rapid heating that enables fast reaction analysis (Mahat *et al*., 2014). Advantages of MAE are a reduction of solvent volumes, shorter real-time analysis, and better control of reaction conditions (i.e. duration, pressure, and temperature). Its unique mechanism enables enhanced selective interaction of polar molecules with the solvent thus increases extraction efficiency. However, like any other method, MAE has drawbacks such as excessive heating ma decompose thermally sensitive analytes (Flotron *et al*., 2003).

Results obtained in a case study by Mahat *et al*., (2014) on soils and sediments showed average recoveries ranging from 75-105%. The relative standard deviation for concentrations was ranging between 2.6-8.2%, showing excellent reproducibility of results. Though the results are reasonably good, maintenance of MAE is costly; thus, alternative technique may be used instead of MAE.

2.10.2.4 **Ultrasonic extraction (USE)**

Ultrasonic extraction uses intense ultrasound radiation as an energy source at high frequencies (16 kHz) to extract organic analytes from a permeable solid matrix through pressure waves. Ultrasonication forms vacuum energy regions through compressions and rarefactions of solvent molecules that allows disruption and pulverization of the solid matrix (Agency, 1996). Ultrasound may be introduced into the sample solvent mixture by the ultrasonic probe or through submerging a glass container (conical flask with stopper) into an ultrasonic bath.

The direct use of the ultrasonic probe in soils or sediments matrix samples may results in erosion of the probe tip, thus leading to an imprecise supply of ultrasonic energy and poor extraction efficiency. Furthermore, sufficient ultrasonic energy is achievable when fine-tuning the ultrasonicator to the appropriate power, and functional disrupter horn (Webster, 2006).

Ultrasonic bath extraction technique is an advantageous extraction technique because it requires less time for the extraction. However, the instrument should run using specific operating conditions to allow maximum extraction. Also, maximum extraction is attainable by increasing the number of extraction cycles. Each cycle is repeated using the same amount of fresh solvent and the solvent extracts combined at the end. Excess solvent extracts is removed from the extract after separation from sedimented soil matrices via vacuum filtration or through centrifugation (Khan *et al*., 2005).

In a study by Banjoo and Nelson, (2005) on the improved ultrasonication procedure for the determining PAHs in sediments, average recoveries ranging from 76-96% were obtained when wet homogenized sediments were extracted using a mixture of n-hexane-acetone (1:1, v/v) for an hour.

2.10.3 **Liquid sample extraction**

2.10.3.1 **Liquid-liquid extraction (LLE)**

In this technique, a sample analyte is partitioned between two immiscible liquids. Extraction takes place between an aqueous phase and the organic phase. In principle, more polar hydrophilic compounds interact better with the aqueous phase, while non-polar hydrophobic compounds have better interactions with the organic solvents (Dean, 2009). In theory, distribution of analyte into the two solvents is dependent on the equilibrium distribution coefficient and distribution ratio (Rezaei and Nedjate, 2003). Equilibrium distribution coefficient is achieved by shaking the mixture in a separating funnel, allowing the non-polar/polar extract (X) to absorb into the organic phase/aqueous phase (Sato and Sato, 1992).

$$X(aq) \longleftrightarrow X(org)$$

Multiple batch extractions ensure maximum extraction efficiency using the same small batch of solvent volumes. The batch extracts are then combined and pre-concentrated for direct analysis by either high-performance liquid chromatography (HPLC) or gas chromatography (GC). Chemical parameter variations such as the pH may affect the extraction efficiency when a weak acid or weak base is used as an aqueous phase. Thus the distribution ratio is used to correct discrepancies (Stauffer *et al*., 2008). A major practical drawback to this technique is emulsion formation when an aqueous phase containing surfactants or fatty acids is employed. Though the LLE technique offers some good advantages, such as cost-effectiveness of the apparatus, an emulsion may result in poor selectivity and efficiency of the extraction technique (Cantwell and Losier, 2002).

A study by Farajzadeh et al., (2016) on the coupling of homogeneous LLE and dispersive liquid-liquid microextraction for the extraction and preconcentration of PAHs from water samples and analysis by GC was conducted. The LODs and LOQs of the targeted PAHs were in a range of 0.08-0.20 µg/L and 0.29-0.66 µg/L. The linear range was 0.66-4000 µg/L for acenaphthene, 0.57-1600 µg/L for phenanthrene, 0.40 – 1290 µg/L for anthracene, and 0.29-135 µg/L for pyrene. Precision ranged from 4-6% RSD values for 50 µg/L spiked water sample. Extraction efficiency for targeted PAHs ranged from 77-94%.

2.10.3.2 **Solid-phase extraction (SPE)**

SPE uses a solid chromatographic packing material (stationary phases; silica) to separate components of the samples based on their adsorption to the stationary phase and interactions with the mobile or eluting phase. Furthermore, gravitational force acting on the cartridge increases hydrostatic pressure in the column as the mobile phase flows through the column. Hydrostatic pressure facilitates desorption equilibrium and fractioning of compounds, despite their solubility in the mobile phase (Andrade et al., 2016).

This technique is often used for selective removal of matrixes/interferences, and for a clean-up crude extract. SPE offers a variety of benefits compared to other analytical approaches. Some of these benefits include clean-up of interferences in complex sample matrix, thus purifying it before a chromatographic separation, reducing analyte ion

suppression, fractionating of crude extracts and analysing them as separate collected fractions and to trace concentrations of low concentrated analytes (Poole, 2002).

SPE functions the same chromatographic principles as HPLC or UPLC. By manipulating chromatographic conditions such as changing the choice of sorbent (stationary phase) and mobile phase (solvent), SPE can be used for sample fractionation, trace concentration of analyte, matrix effects reduction (Ronald, 2008). Depending on the choice of the stationary phase and mobile phase combination, SPE can separate samples based on polarity, electrical charge and molecular size. Non-polar compounds (e.g. hydrocarbons) have a high affinity for binding to non-polar sorbent (e.g. cyanopropylsilyl-[CN], n-octylsilyl-[C_{18}], and n-octadecylsilyl-[$C_{18,}$ ODS]) or partitioning on a non-polar mobile phase. SPE interferences can potentially suppress the analyte's signal strength on the detector. Thus removal of interferences from a sample matrices may result in a significant reduction of signal strength of the interferences (Zwir-Ferenc and Biziuk*, 2006).

Depending on the polarity of the analyte and its interferences, one can use the following modes; Normal-phase SPE, Reversed-phase SPE, Ion-exchange and affinity SPE. Normal-phase SPE involves packing of the SPE cartridge with a polar stationary phase (silica) while using a lesser non-polar mobile phase (i.e. hexane). Reversed-phase SPE is merely the opposite of the Normal-phase SPE (Arsenault, 2012).

Reversed-phase SPE involves four steps: - column conditioning and equilibration, sample loading, washing, and elution of analyte. The purpose of conditioning the sorbent column is to increase the active surface area of the chemically bonded non-polar sorbent (C_2, and C_{18}), thus activating binding sites for the analytes to be retained. To escape de-wetting phenomena on SPE, the sorbent should be prevented from drying-out. After conditioning the sorbent (C_8 or C_{18}), the sample is loaded, and then a small volume of a selective organic solvent is used for washing to remove impurities (example includes water soluble interferences when water is used as a washing solvent). After washing, the analytes are eluted from the cartridge and collected as a single or multiple fractions of target analytes from the solid state (Camel, 2003).

SPE has a range of industrial and academic applications. Applications such as determination of PAHs in seafood, wastewater and river water samples; melamine and cyanuric in infant formula, multi-Residue determination of veterinary drugs in milk, ingestions, endocrine-disrupting compounds in river water, organochlorine pesticides and PCBs in Soil (Arsenault, 2012). Depending on the nature of SPE eluent, SPE coupling to gas chromatography (GC) or liquid chromatography for sample signal detection on qualitative and quantitative analysis is advantageous (Arsenault, 2012).

Wang *et al*., (2016) used of solid-phase extraction coupled with gas chromatography and mass spectrometry for the determination of chlorinated polycyclic aromatic hydrocarbons in water. An Oasis HLB stationary phase was used as a stationary phase for the SPE. The recoveries ranged from 82.5 to 102.6% with a relative standard deviation below 9.2%. Their results suggested that indeed SPE coupled to GC-MS can be used to reliably quantify PAHs in water samples under the specified conditions.

2.10.3.3 **Solid phase microextraction (SPME)**

In SPME, a coated-silica fibre (stationary phase) is used as a sorbent to adsorb organic compounds. Thus preconcentration of the organic compound takes place in the sorbent (Xu *et al*., 2013). After preconcentration, the adsorbed compounds are then transferred into a separating instrument, which then allows quantitation of the analyte. A stationary phase with greater affinity for the analyte is essential. Thus, it is imperative to do investigations on the analyte properties and the sorbent properties. A Commonly reported stationary phase for non-polar phase analysis on the SPME is polydimethylsiloxane (PDMS). PDMS stationary phase is advantageous due to it physical diameter and high stability on elevated temperatures (Pozo-Bayón *et al*., 2001). PDMS may also be coupled to GC or HPLC. However, the coupling to GC is more common (Lappas and Lappas, 2016).

A study by Ke *et al.*, (2014) on the determination of nine PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, and chrysene) in leather products using headspace SPME coupled to GC-MS showed good linearity of the extraction method ranging from 0.1-20 µg/L. The recoveries obtained were very good and ranged from 81.7-124.9 %.

2.11 **Detection techniques**

Principles of chromatography are based on the separation of sample components due to their differences in the partitioning coefficient between two phases as well as their structural properties and compositions. In general, chromatographic technique contains a stationary phase (column: - tube packed with porous particles or bonded phases on a micro-solid support) and a mobile phase (liquid/gas that transports sample components). Separation solely depends on the affinity of sample components to interact with the stationary phase, strong interactions between analyte molecules with stationary phase leads to slow separation. Various separation techniques can be employed for sample components separation (i.e. Thin-Layer Chromatography (TLC), Gas Chromatography, liquid chromatography and Paper Chromatography) (Astefanei *et al*., 2017).

2.11.1 **High-performance liquid chromatography (HPLC)**

HPLC is used to quantify and separate non-volatile samples in a solution. Practically, HPLC is based on chromatographic theory fundamentals (i.e. retention time, resolution, and sensitivity) and is equipped with a mobile phase reservoir, pump, sample injection, detector, and data display connected in series (Figure 2.3).

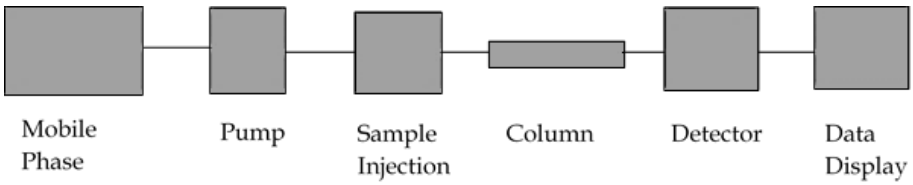

Figure 2. 3 Schematic representation of High-Performance Liquid Chromatography (Moreno-Arribas and Polo, 2003).

During separation, there are two elution techniques for pumping the mobile phase: - In the Isocratic method, the composition of mobile phase remains does not change throughout sample separation process, while in gradient method, mobile phase changes composition throughout the process (Schoenmakers *et al*., 1999).

2.11.1.1 **HPLC detectors**

Main types of detectors in HPLC are Ultraviolet (UV-Vis), fluorescence (FL), refractive index (RI), diode array, electrochemical and conductivity detector. Each detector has its advantage over the other, depending on the composition of the sample (Arti *et al*., 2011). UV-Vis detector works prior excitation of atomic species by photons in a VUV spectral range. Coupling of the UV-Vis detector to chromatographic instruments enables the detector functioning for both qualitative and quantitative analysis. UV-Vis detector is mass sensitive, meaning that the response is directly proportional to the concentration of analyte over time. Therefore, alteration of the time probe in the flow cell may improve the signal of the analyte recorded (Schug *et al*., 2014).

Figure 2.4, shows fluorescence detector manufactured for HPLC, equipped with a miniature tungsten-halogen lamp in series with the flow cell. Two silica lenses connected in series to the excitation filters direct energy to the flow cell. A spherical lens collects emissions from the flow cell and minimizes background radiation. Further removal of spectral interferences occurs in the emission filter; thus the photomultiplier only detects emitted radiation of the analyte of interest (Johnson *et al*., 1977).

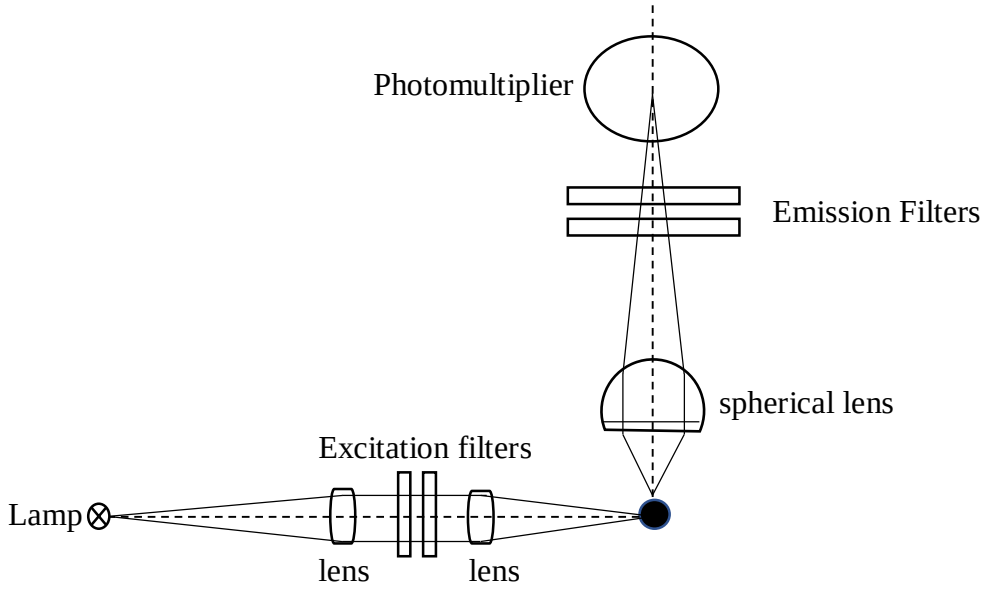

Figure 2. 4 Optical diagram of the fluorometer ((Johnson et al., 1977).

RI detector fine-tuning enables control of the four refractive index monitoring methods which are the angle of deviation, reflection method, critical angle method, and the Christiansen method (Scott, 1986). Figure 2.5 shows a schematic diagram of the water's refractometer. The refractometer measures the deflection of the light beam when the light passes through the sample and reference liquids producing a refractive index. In theory, an incandescent lamp supplies light beam that passes through the optical mask slits, which are responsible for the confinement of the beam within the sample cell. Lenses connected in series with the mask then focus the beam between the sample and reference liquids into the mirror. Lights beam then get reflected by the mirror back into the sample and reference liquids, through the lenses and into the photocell. Finally, generation of a signal by the change in location of the beam signal in the photocell which then gets amplified and recorded into the computer (Scott, 1977).

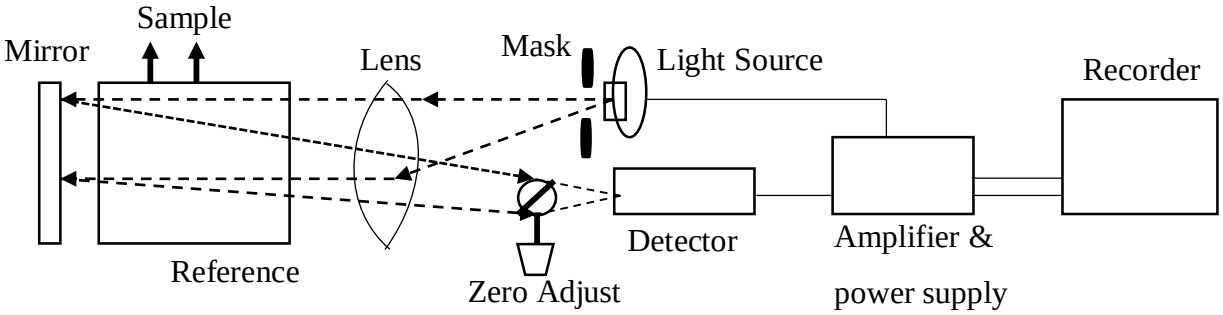

Figure 2. 5 A schematic representation of the waters refractometer detector (Scott, 1977).

Diode array detector (DAD) records a compound's signals at different wavelengths simultaneously at repetition rates, then average them to give a single chromatographic peak on a spectrum (Scheer, 1995). Critical parameters of the DAD are the slit width, bandwidth and the reference wavelength range. Enhancing the bandwidth of the detector results in the increase of sensitivity; thus, reducing the background noise. However, selectivity and the spectral resolution can be lost due to the increase of the bandwidth. Resolution can be corrected by using a narrower slit width, while sensitivity can be enhanced by using a more wide slit width (Gras *et al*., 2017).

Electrochemical detection can be conducted using five different measurement methods which are potentiometric, conductometric, coulometric, voltammetric, and amperometric (Ivaska, 2008). In these methods, concentration-dependent parameters such as resistance, current and voltage are measured, while other parameters are kept constant to obtain a signal of interest. Voltammetry measurements are conducted by passing electrical current that acts as a function of applied potential in a cell and is directly proportional to the sample concentration (Businova *et al*., 2012). The amperometric measurement method is a subgroup of voltammetry, where a constant potential is supplied in a cell (Guy and Walker, 2016). Coulometry involves the integration of current/potential over a given period. According to Faraday's law, the electron reaction is directly proportional to the charge produced during consumption of the electrolyte's number of moles and the amount of an electrolyte used. Potentiometric methods give maximum selectivity compared to volumetric and coulometric measurements because all the compounds are recorded in different wavelengths in potentiometric measurements (Ševčík, 1976).

A case study was conducted by Zhang *et al.* (2016) on the simultaneous determination of 16 PAHs in reclaimed water using SPE followed by ultra-performance convergence chromatography with photodiode array detection. The results showed that the developed method can separate all 16 PAHs within 4 minutes and with a reasonable limit of detection ranging from 0.4-4 µg/L. This makes it an appropriate method for analysing bulk water samples for risk assessment of PAHs.

2.11.2 **Gas Chromatography (GC)**

GC separation is based on the difference in analyte volatility, whereby the samples must evaporate within a GC temperature range. Due to the high resolving power of GC it is the widely used separation techniques for successful determination of PAH concentrations from environmental matrices. Separation of analytes occurs at GC stationary phase consist of a thin immobilized film on a support coated inside the column that partitions the analyte when a mobile phase (carrier gas: - helium or an unreactive gas such a nitrogen) is passed through it (Poster *et al*., 2006).

The analyte is partitioned into the stationary phase and swept by carrier gas and thus elution from the column occurs at different time intervals (retention time) depending on the nature of interactions between the analyte and stationary phase. However, factors

such as chromatographic conditions: column length and diameter, separation temperature mode, stationary liquid phase, and film thickness, play a significant role on the retention time (Poster *et al.*, 2006). Injection conditions (speed, sample size, liner size, and temperature), solvent type and volume, solvent properties and temperature programming all have an impact on the retention time. GC is equipped with a retention gap working in conjugate with capillary columns to remove non-volatile compounds, thus limiting column contaminants and enhancing the peak shape by re-concentrating the analyte (Barron, 2014).

Capillary column stationary phase selection for specific PAHs is very important to avoid PAH separation problems. The most widely used stationary phase for separation of vaporized analytes are methyl and phenyl substituted polysiloxane). Partitioning occurs mainly on the stationary phase, where the mobile phase (gases) simply sweeps the gases in and out of the stationary phase, thus leading to an establishment of gaseous-liquid equilibria across the column. Temperature programming is critical in achieving full resolution of nearly co-eluting analytes. The flow rate affects separation efficiency (N, H) via the van Demeter relationship.

GC-MS separate compounds based on their relative interactions with a stationary phase swept by an inert carrier gas. The evaporated gas phase molecules establish an equilibrium upon interactions with the stationary phase (Hoffman and stroobant, 2007). The equilibrium is controlled by the temperature (high temperatures favor equilibrium shift towards mobile phase, while low temperatures favors stationary phase) (Parris, 1976). The temperature can be held isotherm for a maximum resolution of peaks on the resulting TIC spectrum (Raad *et al.*, 2016). It is therefore important that the analysis temperature and the rates at which they are ramped are chosen properly. Gas molecules are ionized by a beam of electrons, thus producing positive ions that are analyzed by a quadrupole mass filter. The positive ions are then discriminated based on specified mass-to-charge ratio, *m/*z (Rubakhin and Sweedler, 2010). The mass spectrum gives information about the retention time and the electron ionization spectrum.

A sample is introduced into the GC-MS instrument through injection port manually or using an autosampler (Figure 2.6). The sample then enters a programmable oven space equipped with a column packed with either coated silica particles or hallow capillary

column coated onto the inner wall. As sample components then get volatilized, ionized and fragmented as they exit the column into a detector (Hussain and Maqbool, 2014).

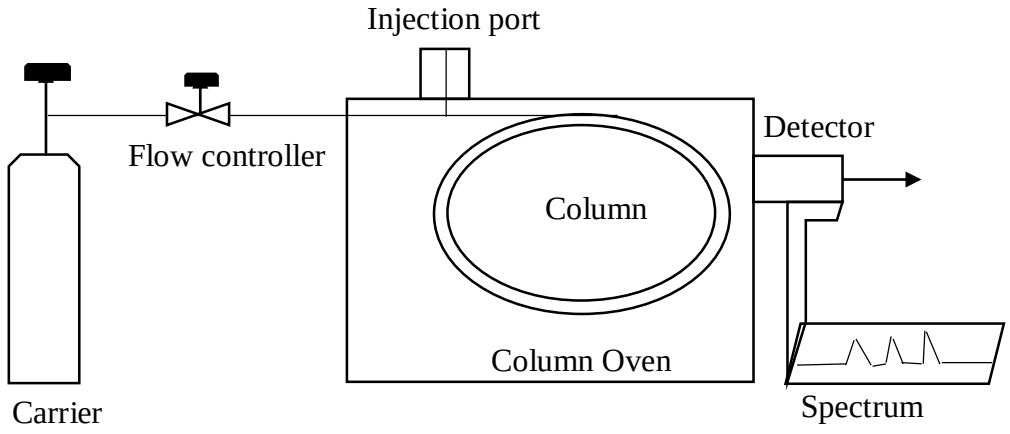

Figure 2. 6 A schematic representation of Gas chromatography.

A column that leads to low background noise even at elevated temperatures is preferred and advantageous when equipped with selective or non-selective detectors. Advantages of gas chromatography over liquid chromatography are the fact that GC is rapid and inexpensive. However, GC has some few drawbacks when wanting to analyze samples with low volatility (Poster *et al*., 2006).

2.11.2.1 **Gas Chromatography detectors**

Commonly used GC detectors are flame ionization detectors (FID), electron capture detector (ECD), photo-ionization detector (PID), mass spectrometer (MS) and thermionic ionization detector (TID). Their response to analyte can be specific or others non-specific. Some detectors respond to concentration changes (examples include UV absorption detector, fluorescence detector, photoionization detector, electron capture detector, and thermal conductivity detector) while others respond to the mass of the analyte (examples include FID, nitrogen phosphorus detector, mass spectrometer, and atomic emission detector). An ideal detector for the use in GC should have enhanced sensitivity, a good linear dynamic range, independence on mobile phase characteristics, response index or linearity, detector response, detector time constant, sensitive to temperature and pressure, sensitive to mobile phase flow rate, etc. FID gives satisfactory performance compared to other detectors thus far (Scott, 2003). GC-MS allows for greater sensitivity and selectivity for the analysis of PAHs (Arrebola *et al.*, 2005).

Flame ionization detector (FID) is incorporated with a hypodermic needle, and two electrodes, responsible for measuring of electrical residence between electrodes. Linearity and sensitivity of this detector are dependent on the flame temperature introduced (Stirling and Ho, 1960). When volatile gaseous analytes are passed through an FID detector, they are thermally decomposed and ionized in the organic hydrogen-air flame and the created ions and electrons are attracted into the ion collector electrodes by the polarizing voltage, which then produce a current corresponding to the amount of sample burned. The electrometer then measures the current produced and converts it into digital form, thus giving a signal on the recorder (Sudhakar *et al*., 2016). FID is sensitive to most compounds, besides O_2, H_2S, NO, CO, H_2O, N_2O, SO_2, CS_2, and NH_3 where the detector has a limited sensitivity or no sensitivity (Putorti, 1997).

Electron capture detector (ECD) measures the electrical conductivity of analyte when exposed to radioactive radiation and functions to create negatively charged ions species from a reaction of electronegatively charged species with thermal electrons from the radioactive source. Ionization of carrier gas by beta particles from a radioactive source results in a production of low energy thermal electrons. These electrons then produce current, which are then measured (Hinshaw *et al*., 2010). ECD has maximum selectivity and sensitivity for chlorinated, fluorinated, and brominated electronegative compounds (Lasa *et al*., 1994).

Thermal ionization detector (TID) is sensitive to both organic and inorganic compounds with different thermal conductivity and heat capacity from the carrier gas. Advantages of this detector include good thermal stability and does not suffer from isotropic effects(Stirling and Ho, 1960).

A mass spectrometer can be coupled to a gas chromatograph as a detector. The separated analytes from GC are ionized to produce molecular and fragmented ions to give ions. Those ions are further discriminated according to their mass-to-charge ratios in a mass analyzer and based on their relative abundance (Hoffmann, 2007). The ions are converted into an electric signal that is measured by the computer to produce data. To avoid collision of gaseous molecules that may lead to a formation of extraneous matrix ions. The MS source, analyzer and detector operates under high vacuum (low pressure).

However, other possible collisions are monitored by considering the kinetic theory of gases (Trimpin, 2016). There is a variety of ionization sources that can be used to produce MS ions (examples include electron ionization, chemical ionization, and field ionization). However, depending on the type of analysis, ionization sources are normally chosen by considering the physico-chemical parameters of the analyte and the internal energy transfer during the ionization process.

A study by Yan *et al.,* (2018) on the determination of 18 PAHs in surface water using simplified liquid-liquid micro-extraction and pseudo-MRM GC/MS/MS was conducted. The use of a third quadrupole mass filter in the developed method enabled enhanced selectivity and sensitivity of the instrument to PAHs. The LODs were within 0.002 µg/L for all PAH compounds, while the LOQs were recorded as within 0.01 µg/L for all PAH compounds. The use of helium gas with collision energy in this study enabled noise reduction improving signal-to-noise, thus resulting on good chromatographic separation of all PAHs.

A study conducted by Banjoo *et al*., (2005) on the determination of PAHs in West Indies sediments showed very good recoveries ranging from 76-120%, and the LOD and LOQ calculated to be 1-2 µg/kg and 3-6 µg/kg. These results suggested that GC-MS method that was used for the analysis is very sensitive and selective to low levels of PAHs.

Furthermore, a study by Young *et al*., (2011) showed good recoveries (90-106%) using SPE and GC-MS for oyster samples spiked with 16 priority PAHs (Naphthalene, acenaphthene, Acenaphthylene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo[b]flouranthene, Benzo[k]flouranthene, Benzo[a]pyrene, Indeno(1,2,3-cd) pyrene, Dibenz(a,h)anthracene, Benzo[ghi]pertlene.

2.12 **References**

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Chapter Three – Research Aim & Objective

3.1 **Aim**

The aim of this study is based on the research hypothesis mentioned below, thus is formulated to answer the hypothesis.

- To optimized solid phase extraction (SPE) and ultrasonication extraction (USE) methods for the extraction of PAHs in water and sediment analysis by GC-MS.

3.2 **Objectives**

The aim of this work will be achieved by the following objectives:

- To optimize SPE method extraction conditions using spiked distilled water samples.
- To apply the optimized SPE method for the extraction of PAHs in the river, wastewater and dam water samples in KwaZulu-Natal.
- To optimize an ultrasonication method extraction conditions using spiked sediment samples.
- To apply the optimized methods for the extraction of PAHs in sediment samples.
- To separate, identify and quantify PAHs in river water, wastewater, and sediment samples using GC-MS.
- To investigate the seasonal effect on the concentration levels of PAHs in two different seasons in wastewater.

3.3 **Research hypothesis**

- There are high concentration levels of PAHs in river, wastewater, dam water and river sediments in Kwa-Zulu Natal because of anthropogenic activities occurring around the chosen cites of study.
- Optimizing conditions for extraction and detection methods can improve existing analytical techniques for determination of PAHs in water and sediments.

3.4 **Research questions**

- What are the optimum SPE and USE conditions for extracting the selected PAHs in water and sediments, respectively?
- What are conditions that can be optimised to obtain high recoveries of all PAHs of interest?
- Are the seven targeted PAH compounds present in river water, wastewater and sediments samples collected in KwaZulu-Natal?
- If they are present, what are the concentration levels of individual PAH and are they within the acceptable limits?
- Are the concentration levels comparable in river, waste, dam and sediment samples?
- Is there any seasonal effect on the concentration levels of PAHs in river, waste, dam and sediment samples?

3.5 **Justification of study**

According to the National Implementation Plan (NIP) for persistent organic pollutants (POPs such as PAHs, pesticides, PCBs, etc.) produced through chemicals processes on our daily basis by agriculture and industrial sectors are persistence in the environment and can be transported globally. Thus, studies towards creating a sustainable and protective environment for human health is necessary (Fischer *et al*., 2011). PAHs fall under a priority group of environmental pollutants reviewed in many research articles and by the World's Health Organization (WHO) for their persistence to degradation and bioaccumulation and hence potential toxic effects on both humans and aquatic animals (Ranjan *et al*., 2017, Shafy *et al*., 2016). Their source of origin can be petrogenic or pyrogenic. Therefore, the levels of PAHs must be investigated in areas where there are thermally powered and fuel processing industries such as petroleum processing, steel processing, automobile exhausts, wastewater treatment plants, industrial discharge, petroleum spills, domestic, stationary, etc. This research work is aimed at investigating if targeted PAHs are not at the levels that can pose threat to biota animals and humans health.The prevalence of PAHs in pristine environments particularly in water is present at very low concentration levels in the environment due to their low solubility in water. Therefore, development of reliable methods that can be able to extract, concentrate them in the solution and detect them at trace level is of importance. The aim of this work was therefore to optimise and apply GC-MS with SPE and USE method for qualitative and quantitative GC-MS analysis of PAHs in river, wastewater, and dam water as well as river and dam sediments.

Chapter Four – Research Methodology

4.1 **Introduction**

This section of the thesis provides a description of the study area and the analytical experimental procedures which were followed. In addition, sampling and analysis techniques adopted in this study for the reliable measurements of PAHs in water and sediment samples will be presented. The measurements involved a three-steps procedure: sampling, extraction by solid-phase extraction (for liquid samples) and ultrasonic extraction (for soil samples), and then separation and detection by performed by GC-MS.

4.2 **Sampling plan**

Samples of wastewater, river water and sediments were taken from Durban wastewater treatment plants, Pietermaritzburg river and Howick dams. These sites were considered a representative sample for assuming PAHs occurrence from potential urban-related activities in KwaZulu-Natal (KZN) Province. Water samples were collected from twelve sampling sites around KZN in spring and autumn seasons, while sediment samples were collected with foil in Cedera agricultural farm and along Msunduzi river in spring season. Sediment samples were left to dry for a week in a secured air extracting fume-hood. They were ground to a homogenized powder before extraction the optimized ultrasonication procedure.

Five sampling sites along Msunduzi river were chosen as to assess the systematic variations of PAHs along Msunduzi river Pietermaritzburg. Four surface samples (influent & effluent) were collected from four water treatment plants around the Durban area: at Umhlathuzana, Umbilo, Manzimtoti, and Northern treatment works. Pre-cleaned amber glass bottles with screwed Teflon caps were used to collect water samples, this is important in minimizing relative errors due to volatilization of analyte and loss of analyte due to sample exposure to UV irradiation light. Samples were then transported to the laboratory in ice covered boxes and immediately stored in the fridge at 4 °C. Physical parameters such as conductivity, temperature, dissolved oxygen, salinity, total dissolved solids and pH were measured at each sampling site (Table 3.1-3.3). Samples were collected in May and August 2017. These were analysed within a week from the date of sampling.

4.3 **Standards and reagents**

Dichloromethane (99.9%) and *n*-hexane (97%) were purchased from Sigma-Aldrich Laborchemikalien (Steinheim, Germany). Methanol (99.9%) was from Merck KGaA (Darmstadt, Germany). Naphthalene (99%), acenaphthene (99%), fluorene (98%), were purchased from Sigma-Aldrich (Steinheim, Germany). Anthracene (98.8%) was purchased from Fluka (Steinheim, Germany) and phenanthrene (99%) and pyrene (99%) were from Merk (St Louis, MO, USA). All reagents were used without further purification.

4.4 **Equipment**

Eumax ultrasonic cleaner bath purchased from LABOTEC (Elma, Singen, Germany) was used for extraction of PAHs in solid samples. Supelco SPE Vac-Elut unit, wsa purchased Sigma-Adrich (Germany) was used to extract PAHs in liquid samples. Oasis hydrophilic-lipophilic balance (HLB) cartridge (60 mg, 3 ml), obtained from Waters (Ireland) was first optimized for the controlled elution of PAHs from the SPE before applying to field samples. Edward 8 vacuum pump was used to dry the SPE cartridges. Holdoph-Basis Hei-VAP Value rotor evaporator purchased from Holdoph (Germany) was used to pre-concentrate the samples before GC-MS analysis. Quantitative analysis of PAHs was conducted using Gas Chromatography (GC) coupled to a Mass Spectrometry (MS) QP-2010 series (Shimadzu, Japan). A capillary column InertCap 5MS/Sil 30 m (I.D. = 0.25 mm, film thickness = 0.25 µm, Japan) was used for separation of PAHs.

4.5 **Preparation of standard solutions**

100 mg/L of PAHs stock solution was prepared by accurately weighing 10 mg of each targeted PAH (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene and pyrene) and transferred into a 100 mL volumetric flask. The volume was made to the mark using *n*-hexane ensuring that all PAHs are completely dissolved. Sequential standards (0.2 to 1.0 mg/L) were prepared from the stock solution and analyzed using a GC-MS instrument. Quantification of PAHs was performed through external calibration method, where the peak areas of the prepared standards were plotted against known concentrations of PAHs to construct a calibration curve. Calibration curves obtained are shown in Figure 5.2.

4.6 **GC-MS instrumental conditions**

Quantitative analysis of PAHs was conducted using Gas Chromatography (GC) coupled to a Mass Spectrometry (MS) QP-2010 series. A capillary column InertCap 5MS/Sil was used for analyte separation with 99.99% helium as a carrier gas at constant flow rate at 1.32 mL/min. Injection temperature was set to 260 °C. The injections were done in a splitless mode and the injection volume was 3 µl. The oven temperature was held at 40 °C for 1 min, then programmed within the range 40-100 °C at a rate of 15°C/min, after which the second ramp was at 10 °C/min applied up to 210 °C, the temperature was held for 2 min. At 210 °C before a third ramp at a rate of 5 °C/min was applied up to 310 °C, at which it held for 8 min. Separated PAHs were ionized using a 70eV beam and transferred into a single quadrupole and two-dimensional, multi-electron detector. MS was running under selected ion monitoring (SIM) mode set to detect characteristic ions of the targeted PAHs. This leave out non-specific ions from interferences from the sample matrices and thus enhancing sensitivity. Analyte quantification was done by setting specific mass-charge ration *(m/z*), naphthalene 128, Acenaphthylene 152, Acenaphthene 154, Fluorene 166, Phenanthrene and anthracene 178, and pyrene 202.

4.7 **Description of the study area – KwaZulu-Natal Province**

KwaZulu-Natal (KZN) is the largest province in South Africa second most populated province (11.1 million people) (Francis Scott and Magagula, 2017). Majority of the land in KZN is regarded as rural, where a large percentage of people lives. Majority of the rural folk lives in poverty and rely on subsistent farming and do not have access to piped water. However, there is data indicating that only 15.3% of the households were dependent on their agricultural products as a source of income, while some were employed by industries in the city (Pauw *et al*., 2005).

KZN has ten district municipalities which are uMgungundlovu, uThukela, eThekwini, King Cetshwayo, Metropolitan, uMzinyathi, Amajuba, Zululand, uMkhanyakude and iLembe. uMgungundlovu (Pietermaritzburg) and eThekwini (Durban) districts municipalities are major contributors to the overall gross domestic product (GDP) of the province, through manufacturing, electricity generation, construction, finance, mining, transport, commercial agriculture and retail sectors (Francis Scott and Magagula, 2017). Some of these activities are directly or indirectly contributors to the generation and release of harmful pollutants such as PAHs into the environment. These pollutants productivity causes health risk to the natural

ecosystems as well as human health. Therefore, studies in uMgungundlovu Municipality: - Pietermaritzburg and eThekwini Municipality- were considered for the study in order to understand environmental pollution due to PAHs in KwaZulu-Natal.

Sampling site locations

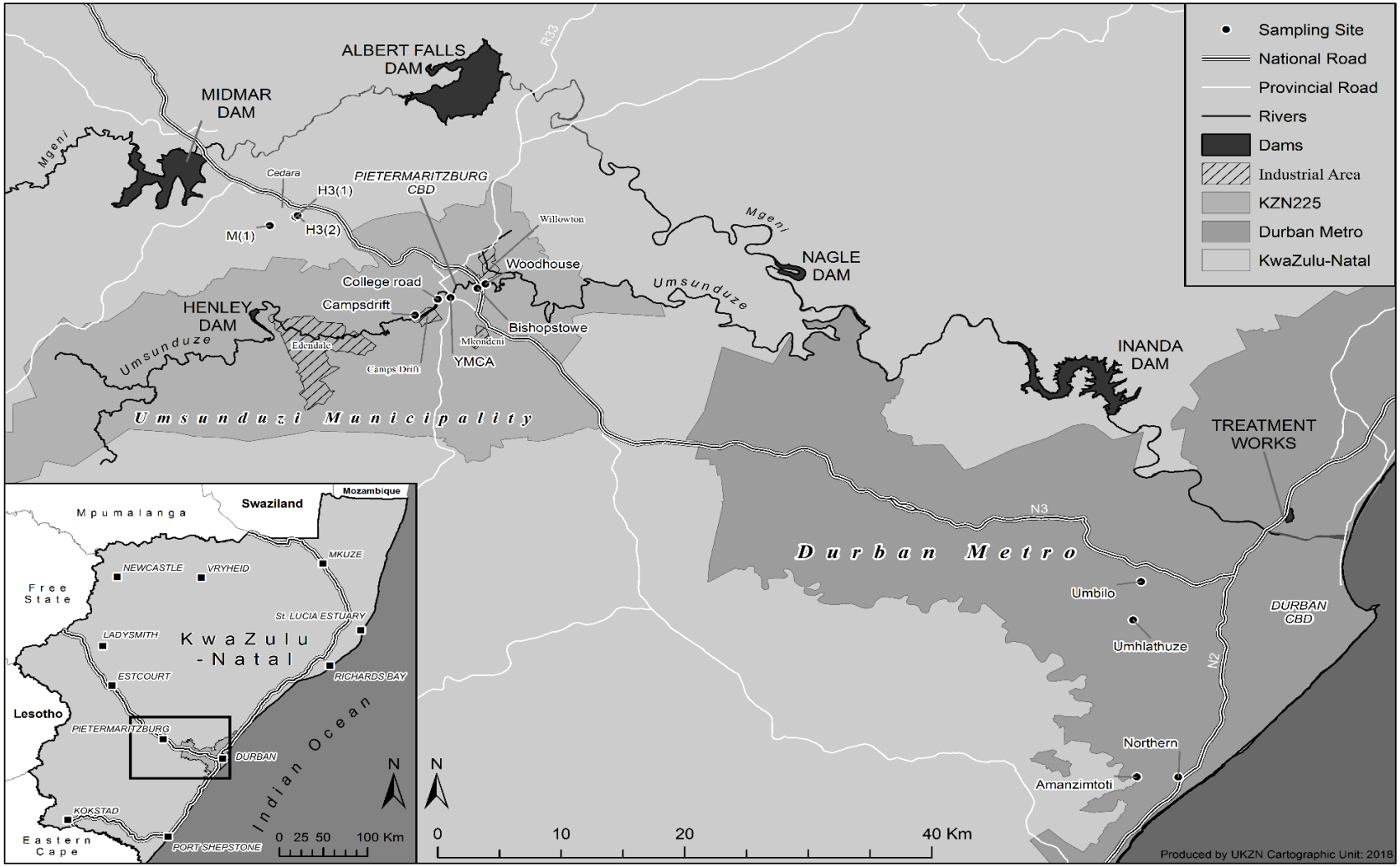

Plate 4. 1 Graphic map constructed by Gijsbersen B. showing the location of twelve sampling points. Along Msunduzi river: - Camps Drift (CD), College Road (CR), Woodhouse (WH), and Bishpstowe (BS); Cedara: - M (1), H (3), and (H (2), and Durban: -Umbilo, Umhlathuzana, Northern treatment works and Amanzimtoti.

4.7.1 **First study area: - Pietermaritzburg**

Pietermaritzburg is a provincial Legislature and capital City of KwaZulu-Natal province. The city has major industries (Steel processing industries (SOMTA), Aluminum Semi-Fabricator (HULAMIN)), as well as cloth and foot wear manufacturing industries, food industries in Eastwood. Most of these industries are located near Msunduzi river. This river supplies also some communities and animals with water. Due to the proximity of the industries to the river, they contribute to the pollution of the river; some occasional bursting of sewerage pipes and illegal dumping of waste have been pointed as other worrisome contributors to the sharp decline of the Msunduzi river's health state (Clacey, 2016). Moreover, Pietermaritzburg Msunduzi river hosts international competitions such as the International Canoe Federation (ICF) Marathon, where canoeists utilize the river course for their recreational sports, thus may expose participants to POPs (Oliver and Hill, 2006).

The increase in traffic volumes in the city has seen a rise in pollutants related to exhaust fumes. Deposited emissions of pollutants such as PAHs will be washed into the river by rain water runoff. In addition, the city is experiencing high volumes of illegal dumping of waste near the river because there is only one main dumping site located (Darvil waste disposal) in New England road. This put water resources at risk to contamination by toxic compounds (Mphambukeli *et al*., 2017).

Campsdrift area is about 750 m from the central business district (CBD) of the city of Pietermaritzburg and is dominated by industries which could contributes significantly to pollution of water and sediments because of their possible discharge into the river (Plate 4.2). Other contributors of PAHs could be waste from informal settlements and residential compounds. play part in the contamination of uMsunduzi river in Camps Drift area (Plate 4.2). These settlements are usually highly populated resulting in an extensive sewerage discharge into the river, thus affecting the quality of water. Moreover, illegal dumping of waste from Willowton industrial area also contribute to contamination (Affairs, 2018).

Plate 4. 2 Camps Drift sampling point, coordinates: S 29˚37'40.5" E 30˚21'43.6".

College road sampling point is about 750 m from Camps drift. Additional contamination of the river occurs at this point due to illegal dumping of waste and automobile exhaust systems fallout or deposition that may be some of the contributors of PAHs into the water (Plate 4.3). It was therefore imperative that the presence of POPs at this sampling point be investigated, due to exposure possibility to human's using the river for fishing and recreational activities (Oliver and Hill, 2006).

Plate 4. 3 College road sampling point, coordinates: *S 29˚36'53.6" E 30˚22'43.2".*

Woodhouse sampling point is at the Wensleydale area and it is also within the 1 km radial zone from the CBD. It is approximately 500 m from an informal human settlement of the Manor area, residential and manufacturing industries in Eastwood and Sobantu areas. Due to improper waste disposal from residences that takes place near the river which could contain POPs (Plate 4.4), it is possible that PAHs may be present in this area.

Plate 4. 4 Woodhouse sampling point, coordinates: S 29˚36'53.6" E 30˚22'43.2".

Bishopstowe is near the medium residential suburb of Lincon Meade about 5 km from the city centre. It is also located close to Darville wastewater treatment plant, one of the city's major waste water treatment plant. Darville handles waste from residential area. Treated water (disinfected by chlorination) from Darville treatment plants is discharged into Msunduzi river at this area (Plate 4.5), therefore allowing additional Msunduzi river contamination by POPs from household, industries, and hospitals, since the Darville treatment plants are not designed to get rid of PAHs. Also, crop production farms located near the river are potential sources of POPs found in this area, because automobile exhaust systems that produce PAHs are used during planting and harvesting.

Plate 4. 5 Treated water from Darville treatment plant mixing with uMsunduzi river. Coordinates: S 29˚36'21.5" E 30˚24'27.0".

4.7.2 **Second study area: - Durban**

One of the objectives of the eThekwini Municipality strategic plan is to create a sustainable environment that promotes beneficiary of Durban's ecosystem. Durban is the largest city in the province of KwaZulu-Natal (South Africa) run by eThekwini Municipality. It is also a prominent tourist destination of KwaZulu-Natal. Due to industrial and domestic activities, both marine ecosystem gets affected by the amount of sewage disposal (EThekwini, 2016).

Furthermore, economic transformation and developments in the eThekwini municipality results in an increase demand of certified water supply meeting the standards set by the Department of Water Affairs (DWA) (EThekwini, 2016). It is very important that recycling of water is utilized. Waste from central and urban regions of eThekwini municipality are directed into wastewater treatment plants through sewage pipes and thus several persistence organic pollutants end-up in wastewater influent. Moreover, most rural and informal settlements lack reliable sewage infrastructure and wastes from temporary ablution can get washed off to sewage line during storm water runoff, thus affecting the quality of water accessible to aquatic species and humans (EThekwini, 2016).

The eThekwini Municipality manage their solid waste through landfills disposal, and therefore chemical decomposition on landfill sites can cause contamination of air and water via leaching and rain water runoff. In this study area, the concentration levels of PAHs at wastewater treatment plants was determined before (influent) and after (effluent) treatment. The wastewater treatment plants (WWTPs) were chosen based on their accessibility and approval of appointments made. The samples were collected in four waste water treatment plants around Durban which includes Manzimtoti, Umbilo, Umhlathuzana and Northern treatment works (Plates 4.6-4.9).

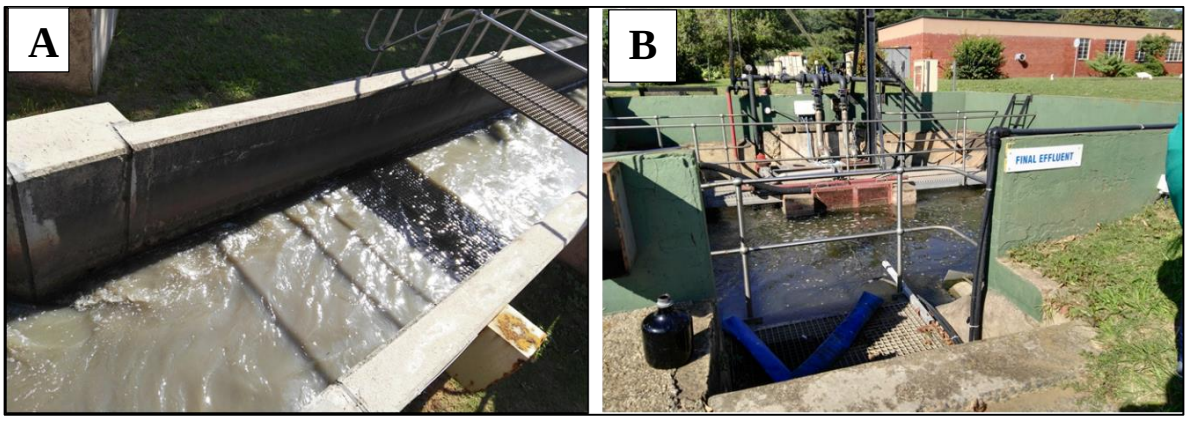

Plate 4. 6 Durban wastewater treatment plant, A): - Influent, B): - Effluent. Coordinates: - Manzimtoti S 30˚00'29,7" E 30˚54'54,9".

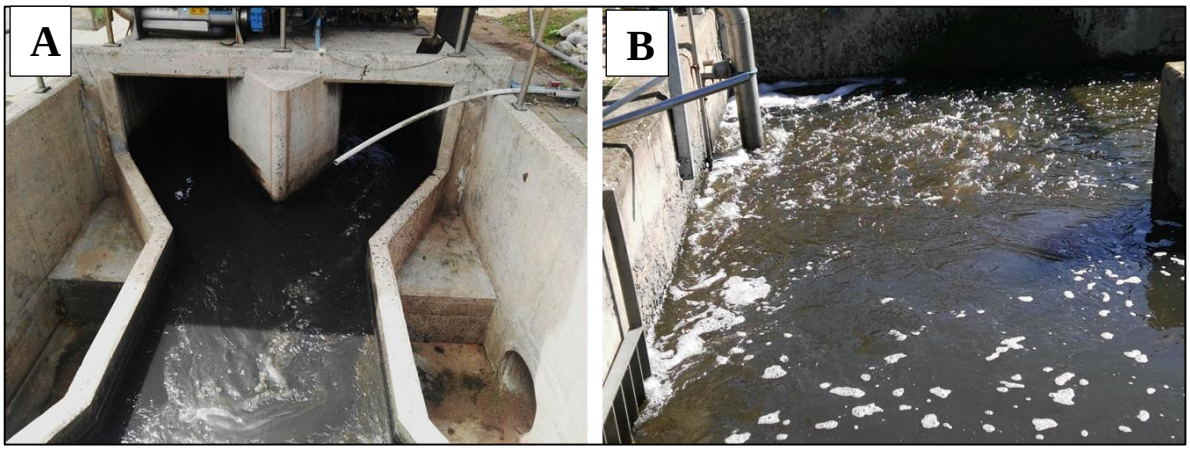

Plate 4. 7 Durban wastewater treatment plant, A): - Influent, B): - Effluent. Coordinates: - Umhlathuzana S 29˚52’32,5 E 29˚52’32.5”.

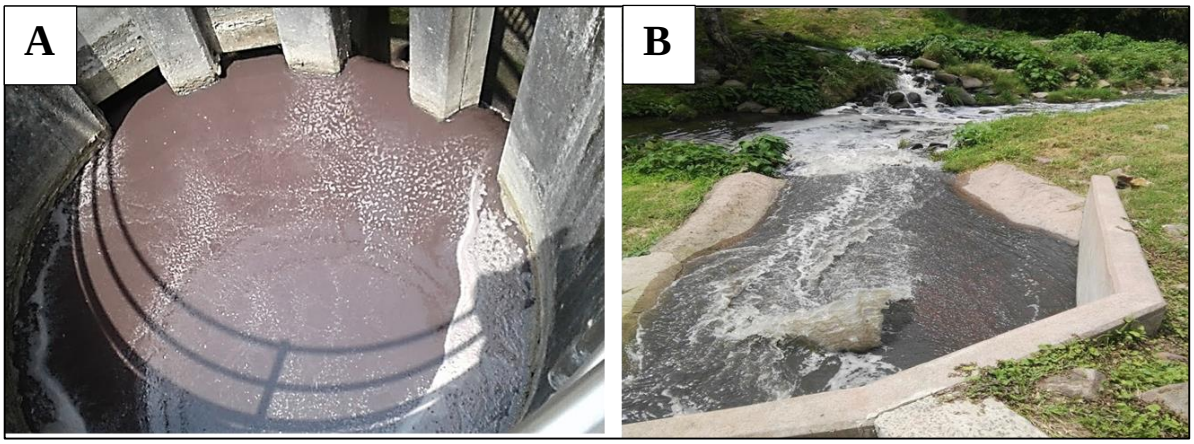

Plate 4. 8 Durban wastewater treatment plant, A): - Influent, B): - Effluent. Coordinates: - Umbilo S 29˚50’47,0 E 30˚53’26,3”.

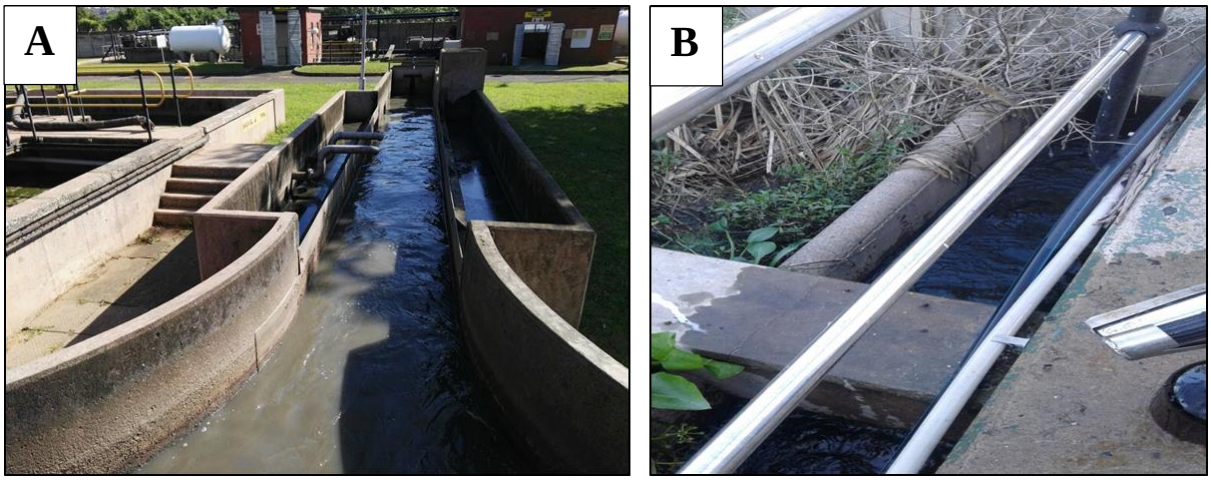

Plate 4.9 Durban wastewater treatment plant, A): - Influent, B): - Effluent. Coordinates: - Northern S 30˚00’24,3 E 30˚55’03,2”.

4.7.3 **Third study area: - Howick**

Cedara agricultural college is located near Howick town, they offer practical based courses on forestry production, crop production, broiler production, beef and piggery production, advanced animal production, goat production and layer production (Naidoo, 2018). These processes have a potential to generate and release persistent organic pollutants (POP) into the environment during harvesting and burning of grass (Jones, 1999). There are no published studies on the assessment of the concentration levels of POPs in this study area. Three sampling points namely H3(1), H3(2), and M(1) were chosen to map out spatial variations in relation to potential pyrogenic source of PAHs near the sampling points that are mainly pyrogenic (Plate 4.10).

Plate 4. 10 M1 dam; sampling point showing a potential pyrogenic source of naphthalene found in water. Coordinates: 29˚33’15.9”S 30˚15’24.6”E.

4.8 **Experimental**

4.8.1 **SPE optimization procedure**

A procedure by Wang *et al*. (2016) on the determination of chlorinated PAHs in water using SPE coupled with GC-MS was used as a build-up procedure and optimized to obtain better percentage recoveries of targeted PAH. Parameters of the SPE technique such as the conditioning solvent, and sample loading volume were optimized. Optimized method was then used to analyse water samples from water samples from the river, dams, and wastewater treatment plants. The Oasis HLB cartridge (60 mg, 3 mL) was washed with 6 ml hexane and conditioning solvents were investigated by comparing recoveries obtained for different solvent combinations of 12 mL (1:1 v/v) (MeOH + H_2O, MeOH + DCM, and MeOH + hexane). Water spiked samples with 7 µg/L of each PAH were investigated at sample loading volumes of 250, 500, 750 and 1000 mL by loading them out into a pre-conditioned cartridge and were then rinsed with 10 mL of 20% methanol in ultra-pure water. The cartridges were then dried for 15 min before eluting with 6 ml *n*-hexane: Dichloromethane (4:1, v/v) eluting solvent. Eluent was then reduced to 1 mL by blowing with nitrogen flow and then transferred into an autosampler vial. The concentrated eluent was taken for GC-MS analysis.

4.8.2 **Ultrasonic optimization (UE)**

A procedure by Banjoo *et al.*, (2005) was used as a build-up procedure and further optimized to obtain better recoveries for individual PAH. Optimization parameters included extraction solvent compositions (*n*-hexane + acetone, *n*-hexane + acetonitrile, and *n*-hexane + dichloromethane), and the extraction time (30, 60, and 90 minutes). Investigation on the extraction solvent was essential to obtain a solvent that will penetrate and break the sediment surfaces, thus allowing efficient extraction of PAHs. The extraction time was also optimized to ensure efficient extraction period.

15 g sediment was spiked with 100 µg/kg of PAHs (50 µL from stock solution) and extracted using 50 mL of different solvent compositions (1:1, v/v) to investigate their effect on the recovery of analytes. The extraction medium was ultrasonicated and allowed to settle, then the extract drawn using a glass pipette. USE optimization was conducted in two 30 minutes cycles, each time using a fresh solvent. The extracts after the two cycles were then combined and evaporated on the rotary evaporator to give a final volume of ≤ 1 mL. Concentrated extracts were then taken to a GC-MS instrument for analysis (Wang et al., 2016, Banjoo and Nelson, 2005).

4.8.3 **Quality assurance**

Quality control is important for appropriate qualitative and quantitative analysis of the compounds. Quality control of the developed SPE and USE methods were investigated in terms of accuracy (recovery test), linearity, specificity, Limit of detection (LOD), limit of quantification (LOQ). LOD is the lowest detectable analyte concentration, whereas the LOQ is the smallest amount of analyte that can be quantified with accuracy and precision (Kanan *et al.*, 2012).

Selectivity and specificity were ensured by using the mass spectrometer as a detector. It selectivity detects only ions of the targeted PAHs (Hinshaw *et al.*, 2010). Specificity of the method was conducted by comparing spiked and non-spiked samples under SIM mode to ensure that the electron ionization detector source on the GC-MS instrument is set to target and identify only the PAHs of interest by using specific m/z values (Lynch, 2017).

As part of the optimization of GC-MS method, it was calibrated regularly with sequential standards containing analyte which were measured. A procedural blank was performed each time a batch of samples was run, this was done to minimize or get rid of matrixes due to column contamination by previous runs and to saturate the column with the solvent. Limit of detection (LOD) and Limit of quantification (LOQ) for each PAH analyte were calculated at concentrations given by 3 and 10 times the standard deviation of signal to noise ratio, respectively. Repeatability of results expressed by %RSD was evaluated by performing analysis of samples in triplicates.

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Chapter Five – Results & Discussion

5.1 Introduction

This research work covers the optimisation of gas chromatography mass spectrometry (GC-MS) as well as solid phase extraction (SPE) and ultrasonic extraction (USE) techniques for liquid and solid samples, respectively. The optimised conditions were then applied to analyse real water and sediment samples. Water samples were collected along Msunduzi River in Pietermaritzburg (PMB), in Cedara agricultural college and in waste water treatment plants around Durban. Finally, seasonal effect on the concentration levels of PAHs are also discussed.

5.2 Physico-chemical parameters of real water samples

The optimized and validated GC-MS-SPE method was then applied to real river and wastewater samples. The quality of water and sediment is often reported by investigating characteristics of total dissolved solids, dissolved oxygens, heavy metals, pH, salinity, conductivity, temperature. Tables 5.4-5.6 shows sample parameters that were recorded in different sampling sites during spring season. There was no significant difference on the sample parameters collected in spring and autumn seasons (Appendix A).

The pH results (Table 5.1-5.3) obtained in all the studied sampling points are comparable and within the standard set by the World's Health Organization (WHO) and South African water quality standards (SAWQS,1996) for drinkable water which is 6.5-8.5. The pH of samples from Cedera dams indicated that the samples were slightly acidic, meaning that water might have been polluted by acidic substances and by the deposition of organic contents (Mobegi *et al*., 2016). pH acidity and alkalinity are dependent on evolution/absorption of HCO_3^-, CO_3^-, and CO_2 sourced by river bedrocks, minerals and organic contaminants from surface runoff (Sahu *et al.*, 1998). The pH of wastewater from the treatment plants as well as river water from Msunduzi River were slightly basic. Microbial activity of aquatic environmental microbes responsible for degrading organic pollutants is activated at neutral pH (Rousk *et al.*, 2009), therefore, the pH results obtained suggested that there was minimal microbial activity since pH is either slightly acidic or basic, thus possible organic pollutants were present in water from almost all sampling points investigated.

Dissolved oxygen values measured in all sampling points are within the minimum permissible standard limits defined by WHO (5 mg/L); meaning that the decomposition and decay of organic matters are minimal (Leena and Choudhary, 2013). However, the other contributing factor would have been the turbidity effect which results from oxygen depletion during the flow of influent from industries and informal settlements (Mobegi *et al.*, 2016).

Conductivity is a measure of the ionic charge/ change in concentration of water due to both anion and cation species present within the water body. Electrical conductivity of water is ascribed as a direct measurement of dissolved ions in water. However, polar organic and inorganic matter do not actively contribute to the conductivity yet they are an important component of total dissolved solids(TDS) (Wright and Hamilton, 1982). The obtained conductivity values vary from 33.3-1371 µS/cm (Table 5.1-5.3). Significantly high conductivity values were recorded in wastewater treatment influent plants due to high pollution load of wastewater influent water compared to rivers and dams. According to the WHO, the threshold limit for drinkable water is up 700 µS/cm. All the sampling point's water conductivity falls within the acceptable limit, except for Amanzimtoti treatment plant. This is due to high concentration of ionic species in wastewater, both of organic and inorganic nature released matters from industries and households around the area (Wright and Hamilton, 1982). Values are comparable to those from Ughanda (208 to 1884 µS/cm) and Nakivubo stream water (Sekabira *et al*., 2010).

Total dissolved solids (TDS) depict the number of dissolved matters (both charged and polarisable particles) in water or sediments. In general, high levels of TDS correlates with the concentration of trace metals and organic pollutants (Curran *et al*., 2000). There is a directly proportional relationship between TDS and the conductivity because most particles that are suspended in water and sediment have a potential to conduct electricity (Moodley, 2014). In addition, TDS also gives information about the available oxygen in the water; thus, high levels of TDS may signify oxygen depletion, leading to minimum microbial growth and activity. Results in Table 5.3 show a good correlation between TDS concentrations in the influent and effluent. Since the influent is untreated wastewater from industries and households containing multiples solids is expected to have high TDS concentrations. However, the effluent should have lower concentrations of TDS because of removal by treatment

Table 5. 1 Chemical parameters for sediment samples collected in Cedara in spring season.

	Cedara dams			
	Parameters	H3 (1)	H3(2)	M (1)
	pH	6.4	7.01	6.81
	Conductivity (µS/cm)	101.4	78.1	33.3
	Water T (˚C)	22.4	23.2	22.4
Coordinates	South	29˚32'50.8"	29˚32'46.5"	29˚33'16.2"
	East	30˚16'29.9"	30˚16'35.5"	30˚15'23.2"

Table 5. 2 Physico-chemical parameters for Msunduzi river samples in spring season.

	Msunduzi river sampling points			
	Parameters	College Road	Campsdrift	Bishopstowe	YMCA
	pH	8.32	7.98	8.08	7.97
	Conductivity (µS/cm)	264	254	326	263
	Ambient T (˚C)	23.4	23.4	23.3	23.2
	Water T (˚C)	22.4	22.9	21.5	21.8
Coordinates	South	29˚36'53.6"	29˚37'40.5"	29˚36'21.5"	29˚36'48.5"
	East	30˚22'43.2"	30˚21'43.6"	30˚24'27.0"	30˚23'16.8"

Table 5. 3 Chemical parameters of wastewater samples in spring season.

		WWTP Sampling Sites			
	Parameters	Amanzimtoti	Umhlathuzana	Umbilo	Northern
Influent	pH	7.59	7.60	7.77	7.56
	Conductivity (µS/cm)	1318	1089	835	745
	Water T (˚C)	23.5	23.1	23.7	23.6
	Salinity (psu)	0.66	0.53	0.39	0.38
	TDS (ppm)	667	540	398	384
	DO (mg/L)	0.81	0.5	3.02	3.45
Coordinates	South	30˚00'29.7"	29˚52'32.5"	30˚00'24.3"	29˚47'40.0"
	East	30˚54'54.9"	30˚52'58.3"	30˚55'03.2"	30˚59'58.1"
Effluent	pH	7.53	7.10	7.56	7.22
	Conductivity	1371	673	683	683
	Water T (˚C)	23.1	23.6	22.9	23.4
	Salinity (psu)	0.68	0.33	0.33	0.33
	TDS (ppm)	688	338	331	341
	DO (mg/L)	3.54	0.4	4.31	2.74
Coordinates	South	30˚00'24.3"	29˚52'40.0"	29˚50'47.0"	30˚00'24.3"
	East	30˚55'03.2"	30˚53'04.9"	30˚53'26.3"	30˚55'03.2"

5.3 **Optimization of gas chromatography-mass spectrometer (GC-MS)**

In this study, GC-MS instrument conditions were optimized to obtain analysis conditions that would allow detection and good separation of targeted PAH compounds at reasonable retention times. This was done by changing the analysis temperature and injecting 1ppm standard mixture of the analytes of interest. Table 5.4 shows the initial build-up conditions (Munyengabe *et al*, 2016) and the final optimized GC-MS conditions and Figure 5.1 shows the chromatogram obtained under optimum conditions.

Table 5. 4 Optimization of GC-MS instrument conditions.

Experiment	**Instrument conditions**
Initial (split mode, SCAN)	✓ Injection temperature: 300 ˚C ✓ Initial temperature: 40 ˚C ✓ 1st ramp: 120 ˚C rate: 25 ˚C/min ✓ 2nd ramp: 160 ˚C rate: 10 ˚C/min ✓ Final ramp: 300 ˚C
Final (splitless mode, SIM)	✓ Injection temperature: 300 ˚C ✓ Initial temperature: 40 ˚C rate: 8 ˚C/min ✓ 1st ramp: 50-200 ˚C rate: 25 ˚C/min ✓ Held isotherm: 20 min, 200 ˚C ✓ Final ramp: 200-300 ˚C rate: 10 ˚C/min

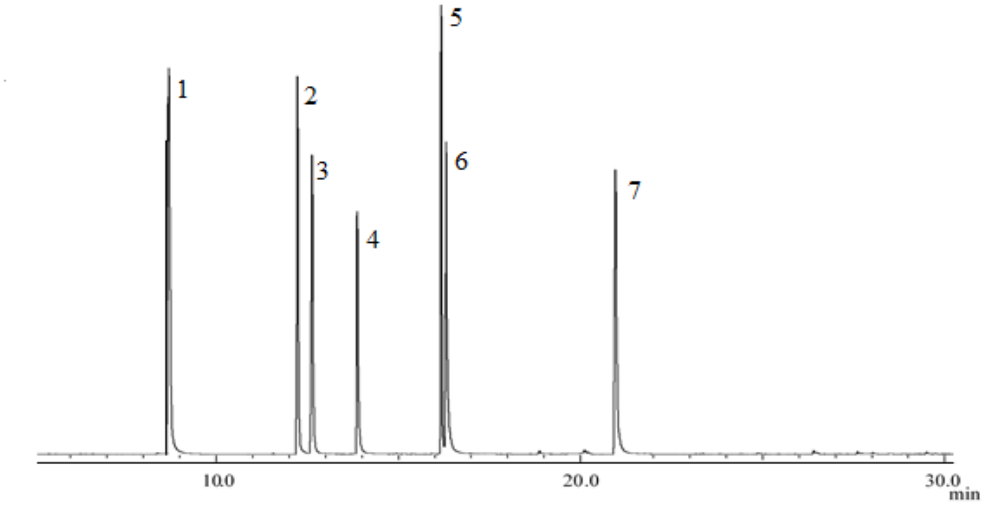

Figure 5. 1 A typical TIC chromatogram for 1 mg/L of PAHs mixture obtained using optimized SPE-GC-MS conditions, where 1 = naphthalene; 2 = acenaphthylene; 3 = acenaphthene; 4 = fluorine; 5 = phenanthrene; 6 = anthracene; 7 = pyrene.

5.4 **Calibration of GC-MS method**

Performance of the optimized method was evaluated through regression equations, which showed good linearity from 0.2-1.0 mg/L with correlation coefficients (R^2) ranging from 0.9968 to 0.9999 (Figure 5.2 and Table 5.5) and were all passing through the origin.

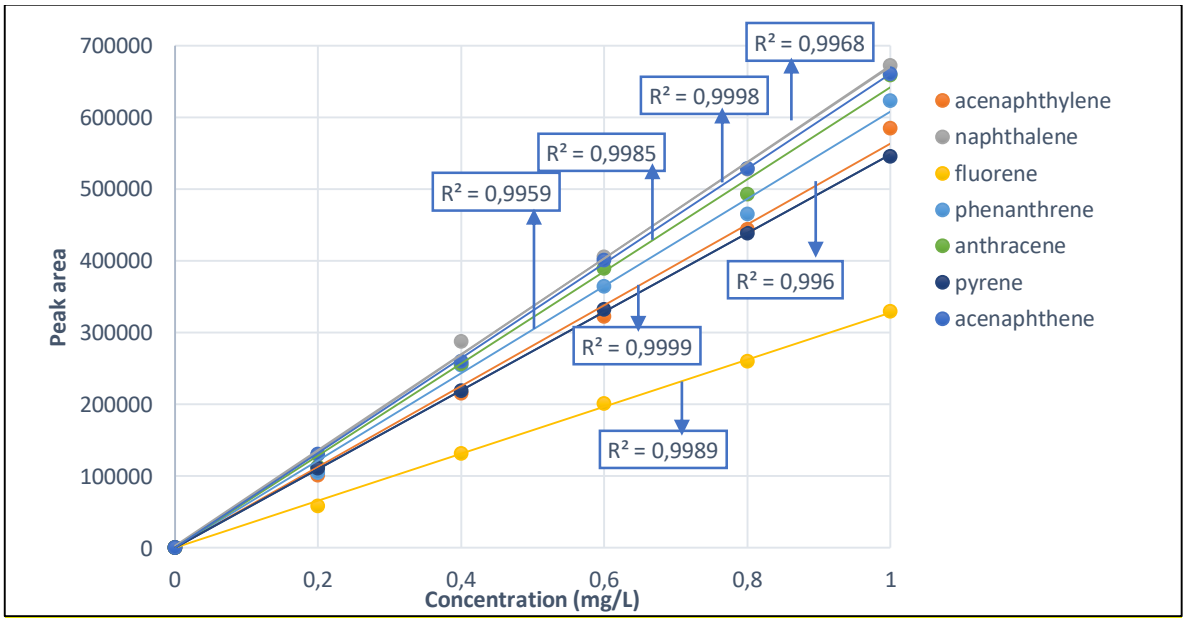

Figure 5. 2 Typical calibration curves of PAHs analysed using GC-MS instrument

Table 5.5 Linear range, calibration equations and correlation co-efficient of a GC-MS instrument.

PAHs	Linear range (mg/L)	Standard curve	Coefficients (R^2)
Naphthalene	0.2-1.00	y= 655095x	0.9968
Acenaphthylene	0.2-1.00	y= 563283x	0.9960
Acenaphthene	0.2-1.00	y= 671752x	0.9998
Fluorene	0.2-1.00	y= 328040x	0.9989
Phenanthrene	0.2-1.00	y= 607545x	0.9959
Anthracene	0.2-1.00	y=641521x	0.9985
Pyrene	0.2-1.00	y=547901x	0.9999

A procedural blank was also performed before running any batch of samples in order to minimize or check for memory effects due to column contamination by previous runs (Wang *et al*., 2016). None of the PAH compounds were detected on the spectra of the blanks. In addition, the GC separation was done by setting the column at temperatures high enough to vaporize the PAHs but not high enough to decompose them (Maliszewska-Kordybach, 1993). A correlation between the molecular weight and the retention time was observed, whereby higher molecular weight compounds were eluted with longer the retention times (Figure 5.1). This is due to the higher molecular weight compounds are being retained more on the stationary phase then low molecular weight compounds due to increased hydrophilic interactions (Snow, 2006).

5.5 **Optimization of SPE conditions**

To enhance the SPE extraction efficiency, parameters such as the conditioning solvents, sample loading volume and eluting solvent need to be optimized before application to real samples. A method by Wang *et al*., (2016) was adopted and further optimized using Oasis HLB cartridges. Wang *et al*. optimized a method for the determination of chlorinated PAHs using SPE coupled with GC-MS, however in this study non-chlorinated PAHs were used as targeted PAHs, thus optimization of their method was important. Deionized water was spiked to have a concentration

of 7 µg/L of each targeted PAHs that was used as spike samples to optimize SPE parameters. The Oasis HLB cartridges was first cleaned with n-hexane, then conditioned, while other parameters were kept constant. This was done to investigate a best solvent partition equilibrium (MeOH + H_2O, MeOH + DCM, and MeOH + hexane) that will yield the highest recoveries of the analyte. Sample loading volumes (250, 500, 750 and 1000 mL) was also investigated to ensure that maximum retention of the analyte into the activated sorbent pores is achieved without overloading the cartridges (Lord and Bojko, 2012). Further optimization of other SPE parameters such as the appropriate washing solvent and elution solvent were insignificant since high recoveries for all the targeted PAH compounds has already been obtained.

5.5.1 **Effect of conditioning solvent on extraction efficiency**

Conditioning of the cartridge is one of the significant steps of SPE procedure. The conditioning solvent helps on activating the polar hooks binding groups (hydrophilic-lipophilic balanced) of the sorbent, thus increasing the surface area for the sorbent to interact with the analyte. Conditioning with a solvent can also remove soluble matrices adsorbed on the packaging material that may interfere with the cleaning up process (Butler, 2012). Effective adsorption of the analyte is dependent on the type of solvent or solvent composition selected for conditioning. Different solvent compositions which included MeOH + H_2O, MeOH + DCM, and methanol + hexane were tried as conditioning solvents in separate experiments (Figure 5.3). The MeOH + H_2O composition gave highest recoveries (61-96%) for all the compounds of interest compared to other compositions when hexane: dichloromethane (4:1, *v*/v) was used as an eluting solvent. In this chromatographic mode, water is considered a weaker wetting solvent compared to hexane and dichloromethane and thus helps establish a more homogenous hydrophilic-lipophilic balanced and solvent (MeOH + H_2O) equilibrium, for subsequent elution by a non-polar solvent such as hexane (Zeitsch, 2000). Hence, this composition activated the cartridge better than the other tested compositions. Therefore, allows effective binding of analytes. MeOH + H_2O was then chosen as the optimum conditioning solvent (Maldaner and Jardim, 2012, Wang *et al.*, 2016).

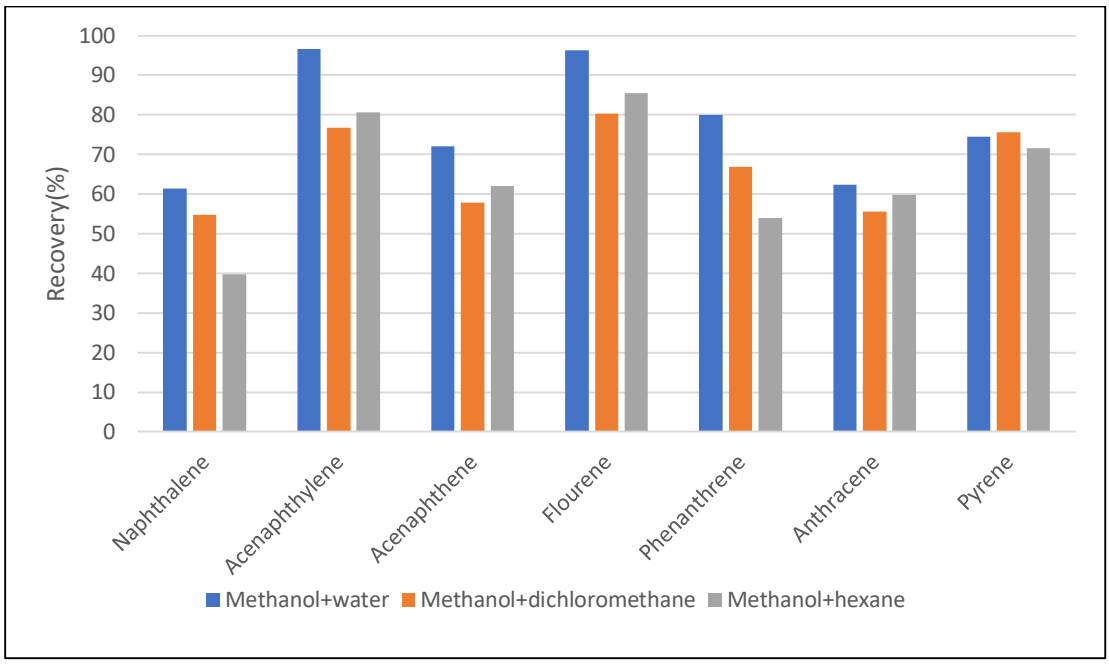

Figure 5. 3 Effect of conditioning solvent on extraction efficiency;
Sample loading volume (1000 mL), solvent (20% methanol+ water) and eluting solvent (4:1 hexane: dichloromethane).

5.5.2 **Effect of sample loading volume on extraction efficiency**

A range of parameters are known to influence SPE extraction efficiency during sample loading such as the breakthrough volume, the presence of inorganic ions, pH, etc. (Henderson *et al.*, 1991). Therefore, it is imperative that these parameters are investigated to ensure good recoveries. The effect of sample loading volume was investigated using 250 – 1000 mL spiked water sample, while maintaining the other parameters constant (MeOH+H_2O, 20% MeOH in water, and hexane: dichloromethane (4:1, *v/v*)). The PAHs recoveries obtained with 250 mL sample volume were very low, but they increased when the sample loading volume increased from 250 to 500 mL (Figure 5.4). The correlation between the sample loading volume and the analyte recoveries can underscores fact that when a large volume of the phase containing the analyte is used, there are more chances of trapping of more analyte to the activated sites of sorbent (Amsterdam, 1993). However, there is limit to this amount that can be held for enrichment and hence to the volume of sample to be loaded. The results show that saturation effect starts to show for loading volumes greater than 500 mL. This could be due to the breakthrough volume effect whereby the sorbent gets saturated with too much analyte and water making the analytes (PAHs) to be weakly bound to the activated sites of the adsorbent. A scientific explanation of the breakthrough volume phenomenon would be an excessive sample loading volume that results in a shift of the adsorption/desorption equilibrium. This means that

the analyte desorption from the sorbent occurs when the excessive loading volumes are used, thus leading a loss of adsorbate (Xie *et al.*, 2003). Sibiya *et al.*, **(**2012) also reported similar observations on the optimisation of SPE sample loading volumes between 100 to 200 mL and good recoveries were observed at 100 mL, indicating that the breakthrough volume was reached at 100 mL sample volume. The highest recoveries (86-120%) were reached at 500 mL sample volume and it was taken as the optimum volume.

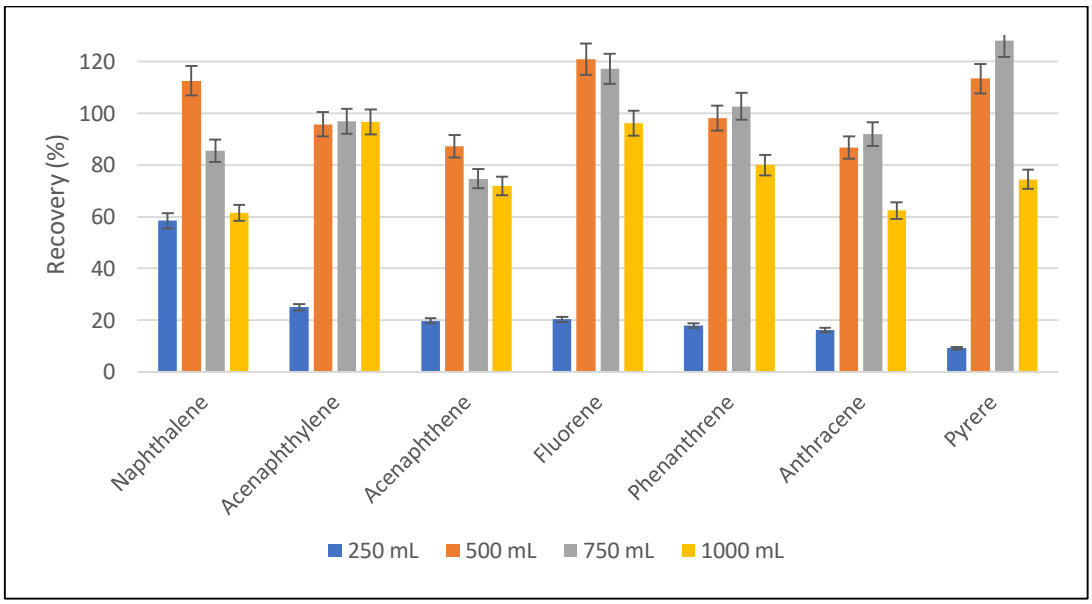

Figure 5. 4: Effect of sample loading volume on extraction efficiency; Conditioning solvent (MeOH+H_2O), washing solvent (20% methanol+ water) and eluting solvent (4:1 hexane: dichloromethane).

5.6 **Validation of SPE extraction method**

During method development and optimization, precision and accuracy of the developed method are the key parameters considered. In this study, spiked water samples were used to evaluate the accuracy of efficiency of SPE in recovering PAH analytes. 500 mL distilled water samples spiked with a mixed standard consisting of the seven targeted PAH compounds with final concentration of 7 µg/L were loaded on the Oasis HLB cartridges and washed with 20% methanol in water and eluted with hexane: dichloromethane (4:1, *v/v*). Table 5.6 shows the recovery results obtained for all seven PAH analytes. The analysis was conducted in triplicate to obtain average recoveries.

The LODs and LOQs were calculated at concentrations given by 3 and 10 times the standard deviation of the blank, respectively. The LODs obtained ranged from 0.016-0.17 µg/L, and the LOQs ranged from 0.050-0.53 µg/L. The recoveries obtained in this study were between 86-120% and are comparable to the results reported by Wang *et al.*, (2016) for the extraction of PAHs from water samples using SPE. However, in this study 500 mL of sample loading volume was used instead of 1000 mL as reported in that study. The recoveries obtained in this study fall within the acceptable analytical range of 80-120% (Betz *et al*., 2011, Edokpayi *et al*., 2016).

Table 5. 6 LOD, LOQ and recoveries obtained for the analysis of PAH using SPE.

PAHs	LOD (µg/L)	LOQ (µg/L)	% Recovery and RSD (n=3)
Nap	0.06	0.14	112.6±0.07
Ace(y)	0.11	0.32	107.4±0.2
Ace	0.17	0.53	87.2±0.3
Flu	0.06	0.18	120.9±0.1
Phe	0.12	0.38	98.2±0.2
Ant	0.11	0.32	86.7±0.2
Pyr	0.016	0.05	113.4±0.02

*Nap = Naphthalene, Ace(y) = Acenaphthylene, Ace = Acenaphthalene, Flu = Fluorene, Phe = Phenanthrene, Ant = Anthracene, Pyr = Pyrene

5.7 Application of the optimised GC-MS-SPE method

5.7.1 Application to wastewater samples

The optimized conditions of SPE were applied to investigate concentrations of PAHs in wastewater samples collected from Umhlathuzana, Amanzimtoti, Umbilo and Northern works wastewater treatment plant (Table 5.7). Most of the PAHs were detected and their concentrations were below the acceptable concentration limit which is 3.0 mg/L as recorded in Table 2.2. The obtained PAH concentration results are shown in Table 5.7 The total concentration levels of PAHs in Durban effluent (0.251-0.908 µg/L) are very low compared to the results recorded in Vhembe district of Limpopo province, where the total concentration levels of PAHs were recorded to be 13.174-26.38 mg/L on the effluent (Edokpayi et al., 2016). Acenaphthene and anthracene were not detected in wastewater in all sampling sites (Figure 5.5). This could be due to their low aqueous solubility and thus poor detection in water. The concentrations of naphthalene were high in Marianridge and Amanzimtoti sampling sites, however, they were below the limit of quantification in other sampling sites. High concentration of naphthalene (7.74 µg/L) in water corresponds to it high relatively aqueous solubility (Table 2.1). It was therefore expected that naphthalene was more likely to be detected in wastewater (Gakuba *et al*., 2015).

Although fluorene has a low aqueous solubility, high concentrations of fluorene (7.15 µg/L) were recorded in Marrianridge and Shallcross influents; this could be due to high concentration of colloidal organic matter suspended in water where it could be adsorbed with an octanol/water partition constant (which is 4.48 (Table 2.1)). Fluorene's log K_{ow} value indicates that fluorene has an affinity to bind in suspended solids and total organic carbons (TOC) to form suspended colloidal particles (Bégué and Bonnet-Delpon, 2008). Therefore, highly contaminated water (influent) with suspended soils and organic material is more likely to contain fluorene in the presence of a fluorene source.

The Marrianridge influent samples had high concentrations of PAHs with naphthalene and fluorene being significant contributors to the total PAHs concentration of each treatment plant (Figure 5.6). Naphthalene has a wide range of applications, these include it use as a critical ingredient in the production of azo-dyes, plasterboards, concrete, phthalic plasticisers and tanning agents (Rowland and Yost, 2010). Since Marrianridge is under reconstruction development programme (RDP) by the housing department of South Africa, where the use of concrete and plasterboards is utilised, it is presumed that through rainwater runoff, naphthalene

produced during this development enters treatment plant streams and thus affecting the water quality (EThekwini, 2016).

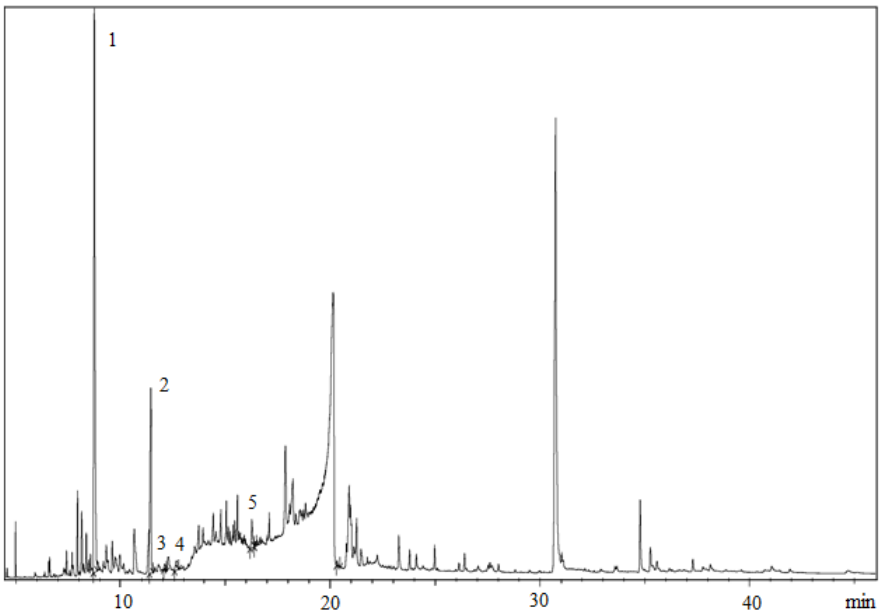

Figure 5. 5 A typical TIC chromatogram of Marianridge wastewater influent sample where 1 = Naphthalene;2 = Acenaphthylene; 3= Acenaphthene; 4 = Fluorene; 5 = Phenanthrene.

Table 5.7 Concentration levels PAH compounds collected from wastewater treatment plants from Durban area and their relative standard deviation (n=3).

Seasons	PAHs	Umhlathuzana		Northern treatment works		Amanzimtoti	Umblilo
		Concentration (µg/L, ±SD)		Concentration (µg/L, ±SD)		Concentration (µg/L, ±SD)	Concentration (µg/L, ±SD)
		Shallcross Influent	Marrianridge Influent	Influent	Effluent	Influent	Effluent
Autumn	Nap	nd	0.27±0.014	0.81±0.01	nq	2.4±0,01	0.36±0.04
	Ace(y)	nd	nd	nq	nq	0.45±0.02	nq
	Ace	nq	nq	nd	nd	Nq	nd
	Flu	2.71±0.08	0.56±0.03	0.13±0.02	0.17±0.01	Nd	0.41±0.04
	Phe	0.97±0.03	nq	1.19±0.02	nq	0.16±0.001	nd
	Ant	nd	nq	nq	nq	Nq	nd
	Pyr	nd	0.078±0.04	0.037±0.005	nd	0.071±0.02	nd
Spring	Nap	-	nd	nd	nd	nq	nd
	Ace(y)	-	nd	nd	nd	1.13±0.01	nd
	Ace	-	nd	nd	nd	0.42±0.03	nd
	Flu	-	1.03±0.05	0.54±0.01	nq	0.14±0.02	nd
	Phe	-	0.19±0.04	nq	nq	1.30±0.01	nd
	Ant	-	nd	nd	nd	nd	nd
	Pyr	-	nd	nd	nq	nd	nd

*Nap = Naphthalene, Ace(y) = Acenaphthylene, Ace = Acenaphthalene, Flu = Fluorene, Phe = Phenanthrene, Ant = Anthracene, Pyr = Pyrene, nd = not detected, nq = not quantified

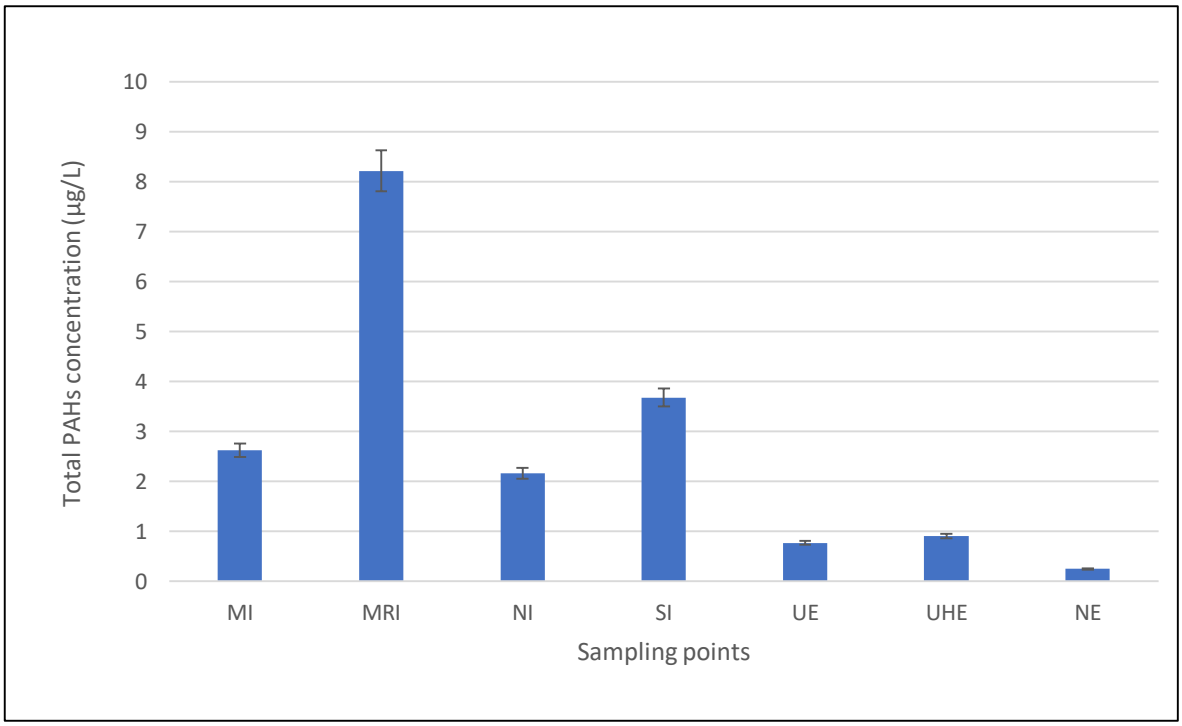

Figure 5. 6 Total concentration of PAHs in waste water treatment plants from Durban.

Moreover, the results showed that the total concentrations of each PAH in the combined treatment plants effluents contributed 44% Naphthalene alone followed by fluorene (38%) (Figure 5.8). According to the United States Environmental Protection Agency (USEPA), naphthalene may also be produced through incomplete combustion of fuels, manufacturing of mothball, creosote, and side stream smoke (Preuss *et al*., 2003). Occurrence of PAHs in samples from Durban region is possibly due to combustion petroleum fuels at urban fills and household discharge of waste as well as industrial dumps. The lowest total concentrated PAH in Durban treatment plants was measured for pyrene (0.19±0.005 µg/L), while highest concentrated PAH in water was recorded for Naphthalene (7.74±0.08 µg/L). This might be due to emissions from sources related urban developmental and transformation projects occurring in Durban which result in overpopulation of the Durban area, thus increasing chances of leakage of sewage discharge and leaching from agricultural and industrial activities. Also, poor management of solid waste through landfills in Durban causes sporadic fires that may emit PAHs **(**EThekwini, 2016**)**. Thus, it was expected that the levels of commonly produced, highly soluble PAHs would be present in wastewater through rain run-off.

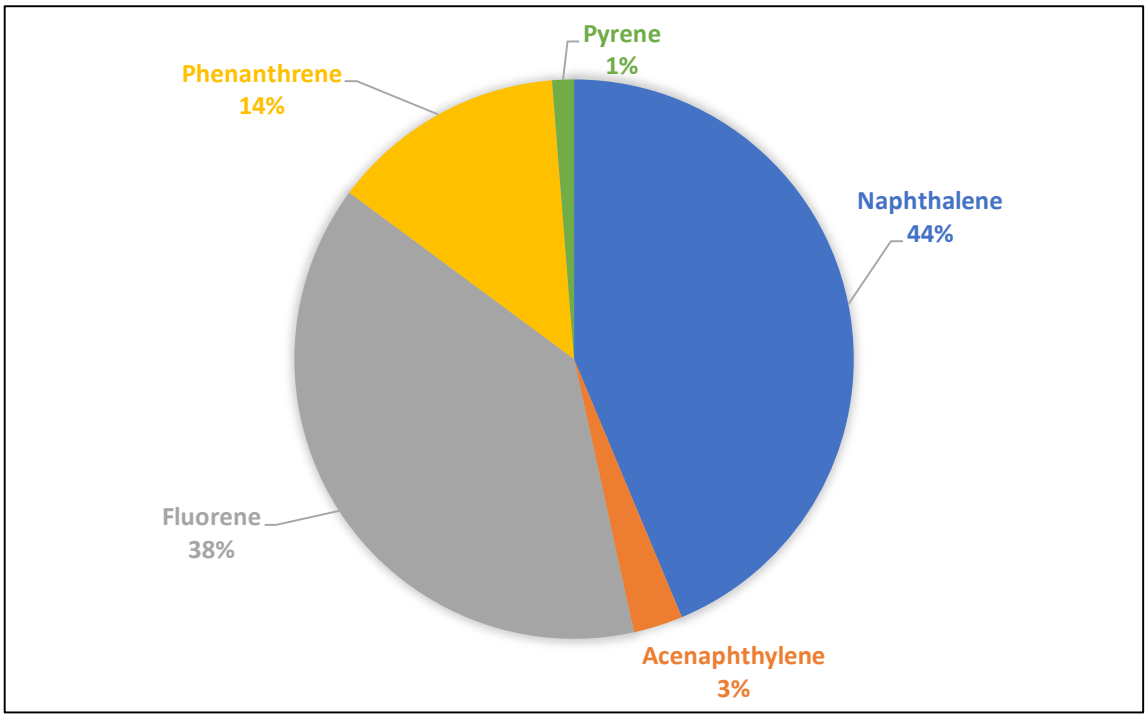

Figure 5. 7 Percentage contribution of individual PAHs to the total PAHs concentration in wastewater from treatment plants in Durban.

To investigate seasonal effect, a study of PAHs in wastewater treatment plants was conducted in two seasons (autumn and spring). The concentration levels of PAHs were higher during autumn season (0.037-2.71 µg/L) compared to the spring season (0.14-1.30 µg/L). This trend is contrary with the expectation that during the spring season, the concentration levels of PAHs are supposed to be higher due to loads of waste being washed off from surface run-off (Nakajima *et al*, 1995). This could indicates that the major sources of PAHs may be petrogenic such as fuel processing and steelwork industries rather than surface water run-off and the petrogenic source contribution to river or wastewater contamination is not seasonal but depends on the fuel processing and steelwork activities that occurred prior to sampling (Wlodarczyk-Makula, 2005). Higher concentrations of PAHs were observed in autumn in Amanzimtoti treatment plant which could mean that wastewater was highly contaminated by petroleum products from the industries surrounding the Amanzimtoti area.

The PAH concentration levels obtained in spring season and autumn season in this study are low compared to results obtained in Limpopo Vhembe District 13.174-29.382 mg/L. These high concentrations in Vhenda were due to localised anthropogenic activities around the sampling

points, such as fired brick-making, wood creosote treatment plants, refuse, paint industries, and burning of tyres (Edokpayi *et al*., 2016).

5.8 **Comparison of the levels of PAHs obtained from wastewater effluents around South Africa and worldwide**

There are limited studies that have been conducted on the levels of PAHs on the effluents in South Africa. Table 5.8 shows results that were obtained by other researchers in South African provinces (KwaZulu-Natal and Limpopo) and the United Kingdom (Jordan). The concentration levels of PAHs in Pietermaritzburg (KZN) were found to be between 4.6-37.70 ng/L. These concentration levels were lower compared to the levels obtained in Durban (KZN) (78-560 ng/L) and in UK (71-756 ng/L). This might be due to lots of anthropogenic catchment activities, such as urbanization, industries, agriculture and household activities in both Durban and Jordan since these are tourism destinations (Moodley, 2014).

Table 5. 8 Comparison of the levels of PAHs obtained from wastewater effluents around South Africa and in the world

	Concentration levels of PAHs in WWTP effluent (ng/L)			
	KZN (PMB)	Limpopo (Vhenda)	Jordan (UK)	This study KZN (Durban)
Naphthalene	4.67	$4.7x10^3$	-	360
Acenaphthylene	90.78	nd	-	nd
Acenaphthene	-	nd	-	nd
Fluorene	22.32	$2.20x10^6$	71	560
Phenanthrene	37.70	$1.72x10^6$	342	nd
Anthracene	21.41	nd	89	nd
Pyrene	20.28	nd	254	78
∑PAHs	228.84	$88.50x10^6$	756	100
References	(Munyengabe *et al*., 2016)	(Edokpayi *et al*., 2016)	(Alawi *et al*., 2018)	

*nd = not detected, PMB= Pietermaritzburg, UK= United Kingdom, KZN= KwaZulu-Natal.

5.9 **Application to Msunduzi river water samples.**

Only naphthalene and pyrene were detected in all samples along Msunduzi River sampling points. A typical TIC of an extracted sample from College road bridge is shown Figure 5.8. The total PAH concentrations ranged from not detectable levels to 10.38±0.001 µg/L at College road bridge sampling point followed by 9.04±0.001 µg/L at YMCA (Table 5.9). The concentrations of individual PAHs were generally below acceptable limits of 3.0 mg/L. Occurrence concentrations of naphthalene in these sampling points may be due to automobile exhaust systems, industrial activities and illegal dumping of waste along these sampling points (Plate 4.1 and Plate 4.3). Pyrene was present in all sampling points along Msunduzi River and the concentration levels were ranging from 0.33±0.01 to 0.53±0.01 µg/L and were below the acceptable levels.

Possible sources of naphthalene and pyrene could be incomplete combustion of organic material in automobile auxhaust systems, coal, oil spills from industries, production of optical brighteners and dyes. Illegal dumping and burning of waste by Manor informal settlements (Plate 4.1), industrial activities by petroleum refinery companies (Somta and Hulamin) and traffic along uMsunduzi river (Plate 4.3) might have resulted in the presence of waste containing compounds from incomplete combustion of organic material generating pyrene was detected in water sample. Water samples at the College road bridge had the highest concentrations of naphthalene (10.4 µg/L) compared to other sampling points, because of carry over contamination by upstream industries at Campsdrift (Plate 4.1).

Table 5. 9 Concentration levels of PAHs in Msunduzi river water samples.

	Concentration (µg/L)							
	Nap	Ace(y)	Ace	Flu	Phe	Ant	Pyr	Total
WOH	2.04±0.01	nd	nd	nd	nd	nd	0.53±0.01	2.57±0.02
COL	10.38±0.01	nd	nd	nd	nd	nd	0.46±0.01	10.84±0.01
CAM	6.73±0.02	nd	nd	nd	nd	nd	0.41±0.01	7.14±0.01
YMCA	9.04±0.01	nd	nd	nd	nd	nd	0.44±0.01	9.48±0.01
BIS	2.58±0.05	nd	nd	nd	nd	nd	0.33±0.01	2.91±0.01
Total	30.77						2.17	32.94

*WOH = Woodhouse, COL= College road, CAM= Campsdrift, BIS= Bishopstowe, nd = not detected

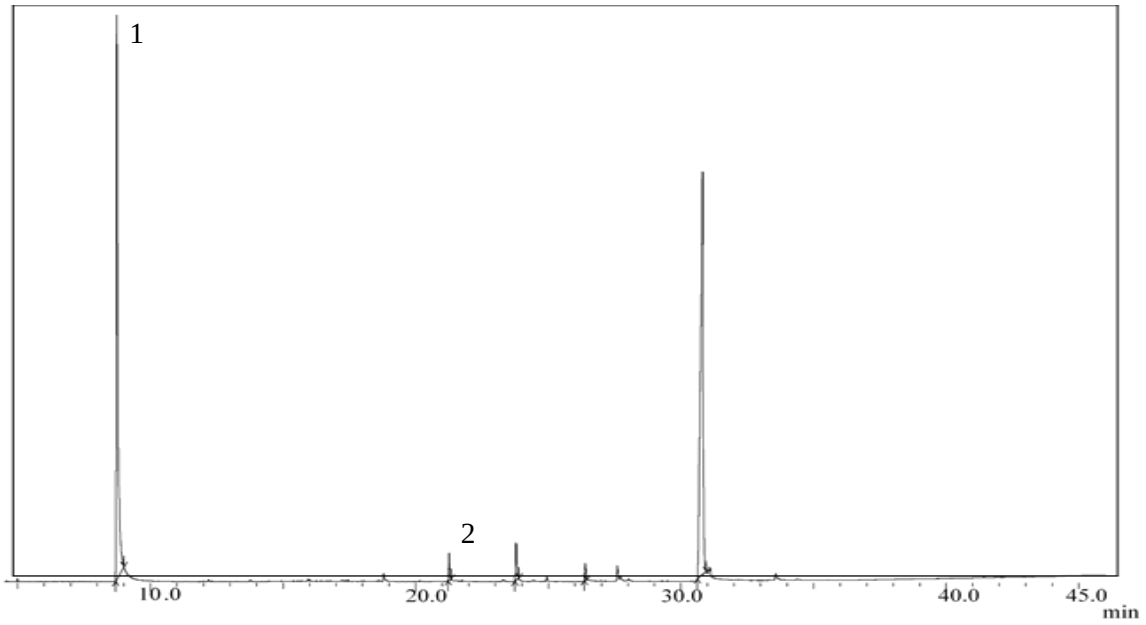

Figure 5. 8 A typical TIC chromatogram of College road water sample where 1 = naphthalene; 2 = pyrene (I.D. = 0.25 mm, film thickness = 0.25 µm, Japan)).

5.10 **Comparison of the levels of PAHs obtained from river water around South Africa**

There are limited results on studies of PAHs in South Africa. Table 5.10 shows the results that were obtained by other researchers in cities around South Africa (Johannesburg, Vhenda and Pietermaritzburg). The concentration range of PAHs in Pietermaritzburg (KZN) river water was from 2.04-530 ng/L and were similar to those obtained by (Moodley, 2014)where the concentrations were reported to range between 0.67-689.63 ng/L and they were lower compared to the concentration levels obtained in Vhenda (Limpopo) (222-2480 ng/L). However, the results obtained in this study were slightly higher than the results obtained in Johannesburg (28.5-615.7 ng/L) by Sibiya *et al.*, 2012. The high concentrations in different cities might be due to anthropogenic activities happening within and around different cities. On the other hand, the presence of these compounds in different cities indicate the importance of their monitoring as a result of their adverse human and environmental effects.

Table 5. 10 Comparison of the levels of PAHs in river water obtained around South African cities.

Sites	Concentration levels (ng/L)								
	Sampling points	Nap	Ace(y)	Ace	Flu	Phe	Ant	Pyr	∑PAHs
Pietermaritzburg (Munyengabe *et al.*, 2016)	Camp's Drifts	36.6	314.97	-	279.02	211.64	99.25	nd	3177.2
	Du Toit Viljoen Bridge	14.13	81.2	-	13.97	22.32	nd	5.06	
	Msunduzi and Umgeni river junction	61.25	689.63	-	627.95	156.43	228.3	nd	
	Msunduzi town	0.67	59.4	-	60.93	94.84	23.65	nd	
Johannesburg (Sibiya *et al.*, 2012)	Blaauwpan	128.5	-	406.6	-	615.7	-	28.5	955.1
	Hennops River	46.5	-	75.8	-	53.5	-	28.5	
	Jukskei River	64.7	-	136.8	-	74.0		35.7	
Vhenda (Edokpayi *et al.*, 2016a)	Mvudi River	222	537	579	2480	126	256	1138	7736
	Nzhelele River	458	765	423	2	177	nd	573	
This Study	Woodhouse road	2.04	nd	nd	nd	nd	nd	530	2200.8
	College road	10.38	nd	nd	nd	nd	nd	460	
	Camp's Drifts	6.73	nd	nd	nd	nd	nd	410	
	YMCA	9.04	nd	nd	nd	nd	nd	440	
	Bishopstowe	2.58	nd	nd	nd	nd	nd	330	

Note: - = not analysed, nd = not detected, Nap = Naphthalene, Ace(y) = Acenaphthylene, Ace = Acenaphthene, Flu = Fluorene, Phe = Phenanthrene, Ant = Anthracene, Pyr = Pyrene

5.11 **Application to Cedara dam water samples.**

Cedara sampling points were chosen due to potential pyrogenic sources such as vegetation removal, crop farming, broiler production, and livestock farming around the area. Table 5.11 shows concentration levels of the PAHs in the water samples. In all the sampling points, only naphthalene was detected, however, its concentration levels (2.46-3.52 µg/L) were very low and were lower than WHO's set minimum risk level of 3 mg/L acceptable limits. The presence on naphthalene could be due to its relatively higher solubility constant at room temperature (Table 5.11). Its origin could be related to use in agriculture, where it is used as a moth repellent to protect crops from destruction. However, about 89% of naphthalene is produced by wood burning related to land preparation (Dahbia *et al*., 2017). The slightly higher concentration of naphthalene recorded in M1 dam could be due to the burning of grass near the sampling point for land preparation (Plate 4.9). The absence of the other PAHs could be due to the absence of sources that produce these specific PAHs, or they might be present at very low concentrations levels to be detected. However, small peaks were observed in the chromatogram for Cedara sample. To check if those small peaks were not targeted PAHs, baseline function and molecular ion peak were observed, and it was confirmed that they were due to sample impurities (Figure 5.9).

Table 5. 11 Concentration levels of PAHs in Cedara dams.

	Concentration (µg/L)						
	Nap	Ace(y)	Ace	Flu	Phe	Ant	Pyr
H3(1)	3.0±0.04	nd	nd	nd	nd	nd	nd
H3(2)	3.27±0.04	nd	nd	nd	nd	nd	nd
J1	2.46±0.05	nd	nd	nd	nd	nd	nd
M1	3.52±0.04	nd	nd	nd	nd	nd	nd
Total	12.25						

Note: nd = not detected, Nap = Naphthalene, Ace(y) = Acenaphthylene, Ace = Acenaphthene, Flu = Fluorene, Phe = Phenanthrene, Ant = Anthracene, Pyr = Pyrene.

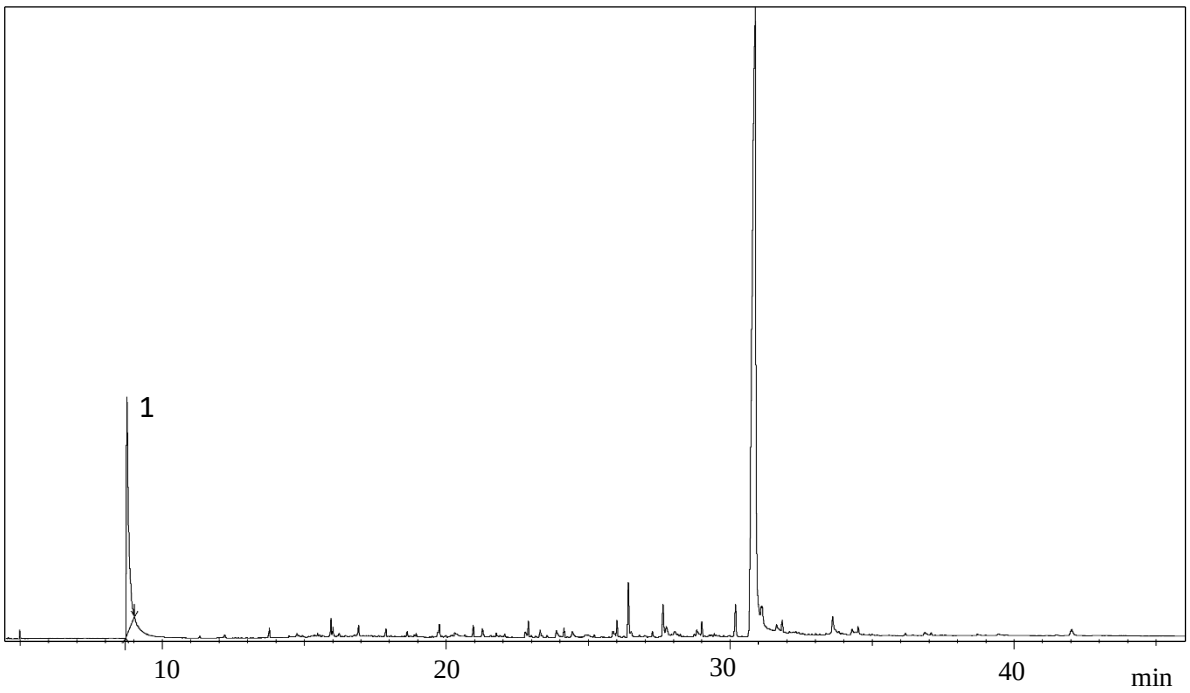

Figure 5. 9 A typical TIC chromatogram of M1 sampling point where 1 = naphthalene.

5.12 **Optimization of ultrasonic extraction method (USE) conditions**

In this study, ultrasonic extraction was chosen based on its merits such as the instrument availability, and the use of less solvent volume, shorter extraction time and multiple analysis in a single instrumental run. The ultrasonic extraction method was optimized for parameters such as the extraction solvent, solvent volume, and extraction time. Ultrasonic method optimization for the extraction of PAHs was conducted through spiking environmental sediments with 100 µg/kg of PAHs standard solution. Moreover, multiple studies using ultrasonic have shown good recoveries ranging from 60-114% of most targeted PAHs in soils and sediments (Martinez et al., 2004, Song et al., 2002, Barco-Bonila et al., 2009). However, some researchers have reported poor recoveries for low molecular weight (LMW) PAHs such as naphthalene (23-65%) when ultrasonic extraction is being employed and also uses large volumes of extraction solvents (Miége et al., 2003, Cavalcante et al., 2008, Barco-Bonila et al., 2009). Therefore, optimisation of ultrasonic condition parameters was significant since most of the targeted analytes in this work were LMW-PAHs.

A Build-up procedure by Banjoo *et al.*, (2005) was adopted in this study and further optimized. USE of PAHs from sediments was first optimized for extraction solvents (hexane-acetone/ hexane-dichloromethane/hexane-acetonitrile), for solvent volume (50,100 and 150 mL) and extraction time (30, 60, and 90 min) as will be discussed in detail in subsections 5.11.1-3. The optimized extracting parameters were applied to evaluate the accuracy (i.e. the percentage recoveries of the targeted PAHs in sediments as described below).

A solvent-sediment mixture was ultrasonicated using an ultrasonic bath (50-60 Hz) at room temperature. The extraction was conducted in time intervals of two cycles and then solvent extracts were combined. A mass of 15g of dried sediment was spiked with 100 µL of a mixed standard of PAHs and added 100 mL of hexane-acetone and then ultrasonicated for 60 minutes. The extracts were pre-concentrated using a rotary evaporator to about 1.0 mL before analyses by GC-MS. The results obtained are shown in Figure 5.10. A non-spiked sample was treated as a blank to check if there was no contribution of the sample concentration on the total recovered concentration of the targeted PAHs. A contribution to naphthalene and pyrene from the samples was observed, and those were subtracted for investigating USE extraction efficiencies (Appendix A).

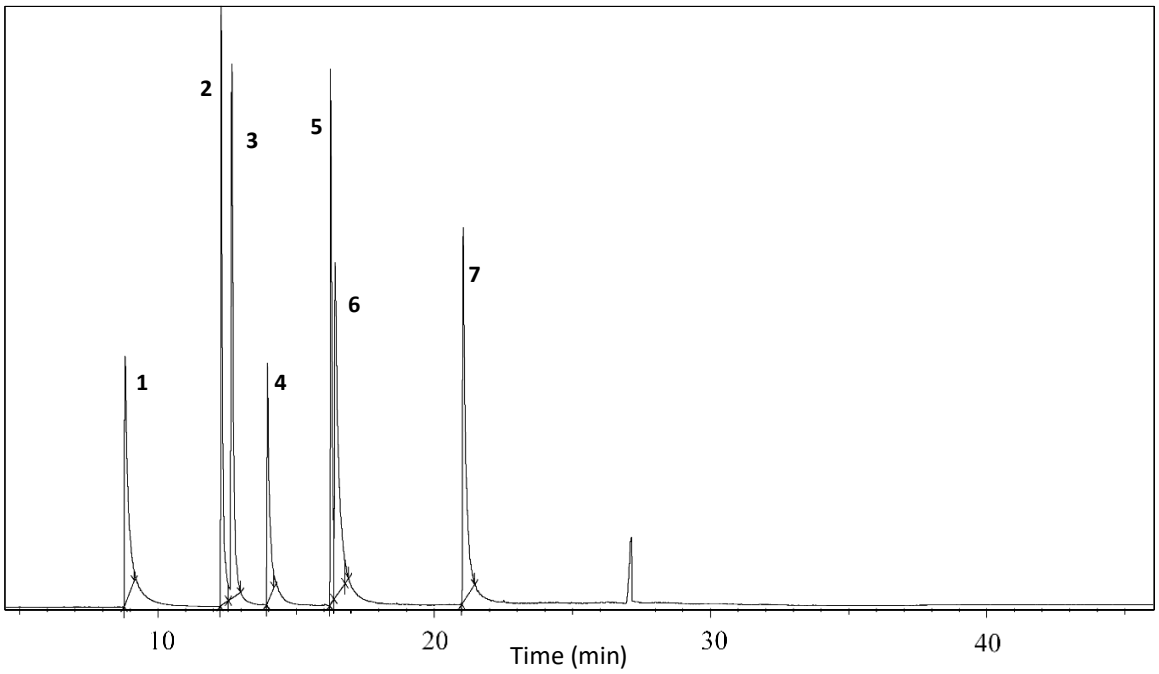

Figure 5. 10 A TIC chromatogram (SIM mode) of 100 µg/kg spiked Campsdrift bottom sediment where 1 = Naphthalene; 2 = Acenaphthalene; 3 = Acenaphthylene; 4 = Fluorene; 5 = Phenanthrene; 6 = Anthracene; 7 = Pyrene.

5.12.1 **The effect of solvent composition on extraction efficiency of the USE method**

Three solvents combinations, namely hexane-acetone, hexane-acetonitrile and hexane-dichloromethane. The solvent combinations were chosen based on their dielectric constants (a measure of solvent's degree to absorb microwave energy) and were used as equimixtures. The results obtained showed that n-hexane-acetone was the most suitable and gave the highest recoveries (83-118%) for all targeted PAHs except naphthalene. It was followed by n hexane-acetonitrile mixture (60-116%). The composition of n-hexane-dichloromethane gave lowest recoveries for all the compounds (Figure 5.11). This is due to lower polarity hence the lower extraction strength for dichloromethane when compare to acetone. Acetone is a more polar solvent compared dichloromethane. This allows it to break down soil aggregates more efficiently, thus enhancing van de Waals forces (dipole-dipole interactions) between an analyte and extraction solvent which enables high extraction efficiency (Ncube *et al.*, 2018). Naphthalene recoveries were low in all extraction solvent compositions; thus, further optimisation of the method was imperative. Composition of n hexane-acetone was then adopted as the optimum extraction solvent since it gave high recoveries for almost all PAHs analytes.

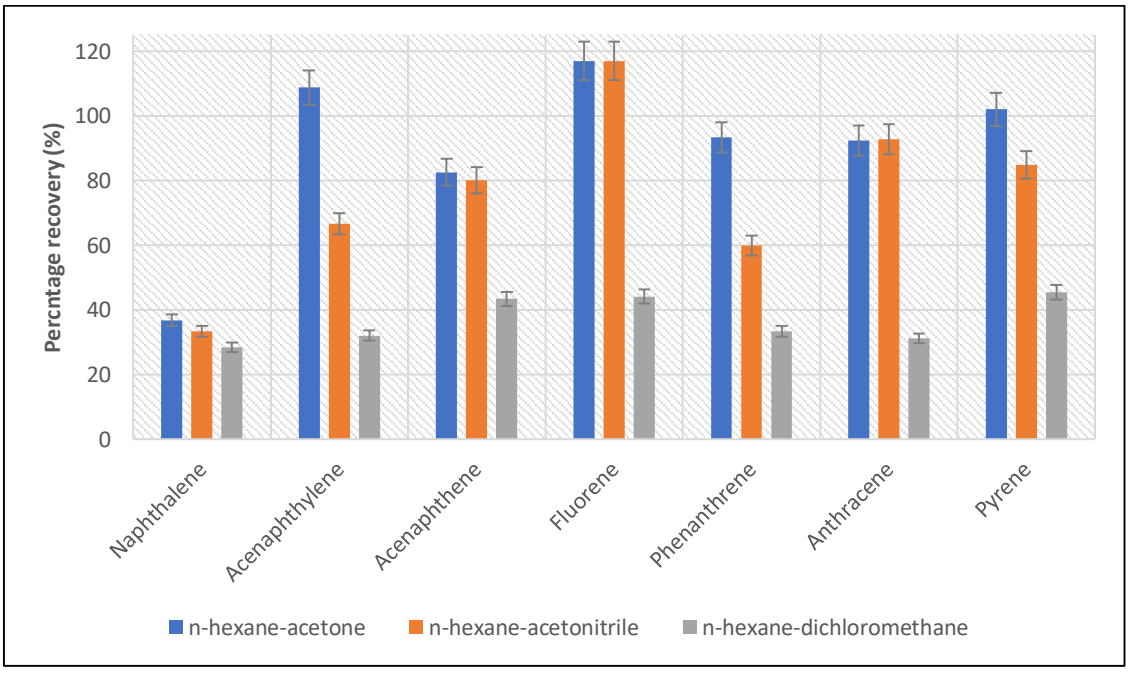

Figure 5. 11 Effect of extraction solvent composition on extraction efficiency; Extraction volume (1000 mL) and extraction time (60 minutes).

5.12.2 **Effect of solvent volume on extraction efficiency**

The following volumes 50, 100 and 150 mL were investigated using initial build-up method conditions. The percentage recovery of PAHs increased with an increase in the solvent volume from 50 to 100 mL for all the compounds except naphthalene. As the solvent volume is increase more analyte is dissolved in the solvent, thus leading to the enhancement of extraction efficiency. However, the decreases in extraction efficiency was observed as the solvent volume was further increased to 150 mL for most of the compounds (Figure 5.12). This phenomenon can be explained by the establishment of dissolution equilibrium of targeted analytes between the extraction solvent and the walls on the reaction container, therefore reducing the recovery of the analytes (Chemat and Esveld, 2013; Liao *et al*., 2016). 100 mL was taken as an optimum volume, because most PAHs had high recoveries (37-117%) compared to other volumes. However, naphthalene recovery was still very low, thus further optimization was needed.

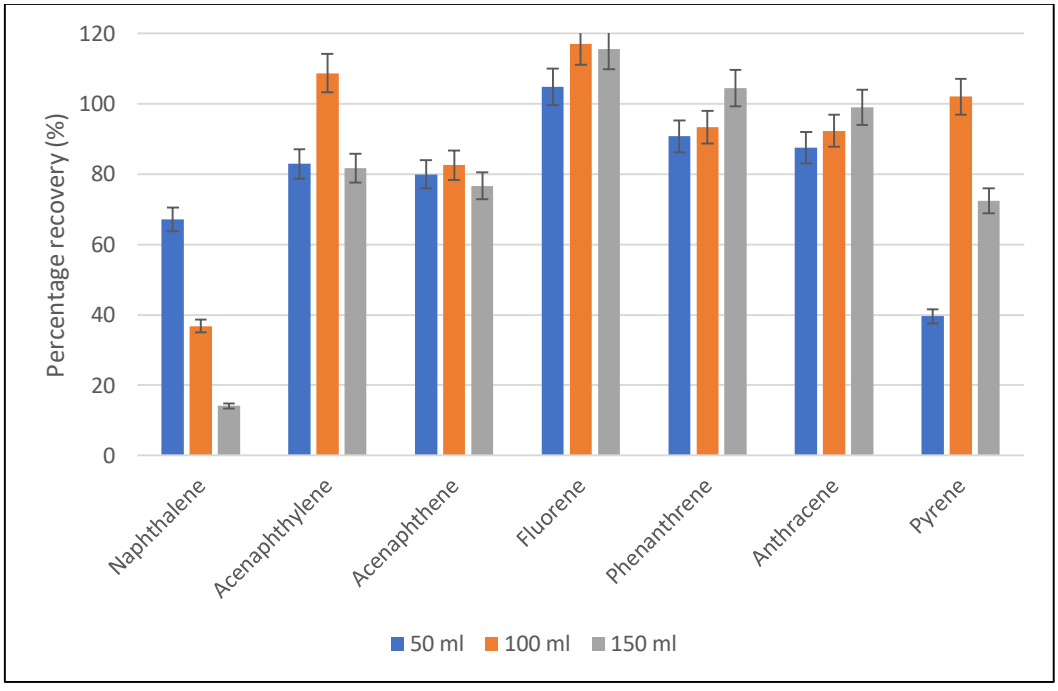

Figure 5. 12 The effect of extraction solvent volume on extraction efficiency. Extraction solvent(n-hexane-acetone) and extraction time (60 minutes).

5.12.3 **Effect of extraction time on extraction efficiency**

The effect of extraction time on the extraction efficiency was studied using 30, 60 and 90 minutes. A noticeable trend in the rapid increase of extraction yield was observed for all PAHs except naphthalene as the extraction time was prolonged from 30 to 60 min (Figure 5.12). When more time is given to ultrasonicate the sediment, more disruption of sediment PAHs aggregates occurred. This is equivalent to increasing the surface contact between the extraction solvent and the sediment sample leading to better extraction efficiency (Liao *et al*., 2016). However, a significant decrease on the extraction yield was observed from 60 to 90 minutes for all compounds which could be due to perturbation of mass transfer as a results of very long time exposure of the analytes to cavitation effect caused by ultrasonic waves (Carail *et al*., 2015, Assami *et al*., 2015). Also, in general, the increase of the extraction time result in an improved quantity of analyte extracted from the sediment, though long-time exposure of semi-volatile PAHs to ultrasonic waves might result in analyte degradation due to long exposure to cavitation effects (Mandal *et al*., 2007). LMW-PAHs can be easily degraded by cavitation effects; thus it is not ideal to use ultra-sonication for them. A sonication time of 60 min was then selected as the suitable extraction solvent for the targeted PAHs, due to good recoveries for almost all targeted PAHs (83-117%) except for naphthalene. In this study, naphthalene was therefore omitted for quantification due to it instability (i.e. decomposition) when ultrasonic extraction is being employed (Miége *et al*., 2003).

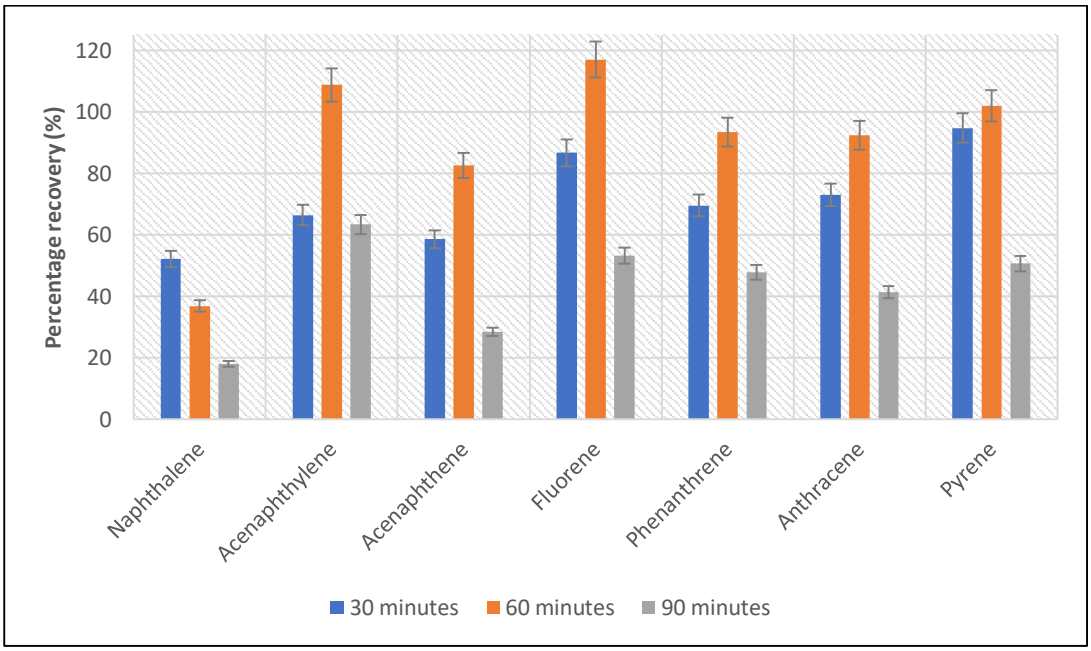

Figure 5. 13 Effect of time on the extraction of targeted PAHs;
Extraction solvent (n-hexane-acetone) and solvent volume (100 mL).

5.13 **Validation of USE method**

The USE method was validated for its accuracy (recovery tests) in determining the PAHs concentrations in sediment samples. Analytical parameters such as LOD and LOQ were also determined. In this study, field samples were spiked at a single concentration level and USE applied to evaluate recoveries of PAHs in the sediments. Table 5.12 shows percentage recoveries of PAHs by the USE method. This method gave poor recovery for naphthalene and could not be used to quantify low molecular weight PAHs such as naphthalene.

Table 5. 12 LOD, LOQ and recoveries studied for the analysis of PAH on the USE instrument.

PAHs	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%); RSD (n=3)
Naphthalene	0.02	0.13	36.8±0.10
Acenaphthylene	0.05	0.16	108.7±0.01
Acenaphthene	0.09	0.27	82.5±0.03
Fluorene	0.03	0.09	117±0.03
Phenanthrene	0.06	0.20	93.3±0.02
Anthracene	0.05	0.16	92.3±0.03
Pyrene	0.008	0.02	102.0±0.01

The LODs and LOQs were calculated at concentrations given by 3 and 10 times the signal to noise ratio and the standard deviation, respectively. The LODs obtained ranged from 0.0082-0.0874 µg/kg, and the LOQs ranged from 0.0248 – 0.265.0 µg/kg, this simply meant the method could detect trace concentration levels of PAHs as it was expected in the field sample. The recoveries obtained in this study (82.5 – 108.7%) are higher than those published by Banjoo *et al.*, (2005) where the same USE conditions were used (75.5-93.3%), thus making this study method an optimum method. The recoveries obtained in this study fall within the acceptable analytical range of 80-120% (Betz *et al.*, 2011, Edokpayi *et al.*, 2016).

5.14 Application of optimised ultrasonic extraction method to Msunduzi sediment samples

The optimised procedure was employed on the determination of PAHs in sediment samples from different sampling points along Msunduzi River and Cedara Dam. Table 5.13 shows the concentration levels obtained in sediment samples along Msunduzi River. Of the targeted PAHs, acenaphthene and fluorene were not detected in all the sediment samples. The total concentration of targeted PAHs in Msunduzi River sediments was found to be 118 µg/kg and all the individual PAHs were lower than the acceptable limits of 3.0 mg/kg. Pyrene was detected in all the sediments with the highest concentration of 42 µg/kg recorded for to sediments from Bishopstowe area. This contributed 44.6% to the overall concentration (94.13 µg/kg) of pyrene in sediments from the selected four sampling points. A possible reason for high concentrations of pyrene in sediments could be due to its low aqueous solubility (0.13 mg/L) compared to the rest of the considered PAHs, and thus less likely to be absorbed in water (Gakuba *et al.*, 2015). As a result, it is adsorbed onto the surface of sediments; thus, high concentrations are expected on sediments as observed in this work.

Phenanthrene and acenaphthylene were detected in Bishopstowe bottom sediment sample. Phenanthrene is produced through incomplete combustion, industrial exhaust system, tobacco smoke, fossil fuel, pesticides production, explosives and dyes (Speight, 2017). Acenaphthylene is used to make dyes and pigments that can be employed to produce dye for clothes. It is also used to make soaps, pesticides as well as plastics. Therefore, the presence of phenanthrene and acenaphthylene in Bishopstowe sampling might be due to illegal dumping of waste into the river by informal settlements of Manor residences situated about 300 meters from Msunduzi River as well as the industries such as The Gutter company located in about 400 meters from the river.

The higher concentration levels of pyrene in bottom sediments from College road (12.4 µg/kg) and Bishopstowe (31.9 µg/kg) could be due to that at Bishopstowe there are many potential sources of PAHs (i.e. informal settlements and industries, and illegal dumping of waste (Plate 4.4), whereas in College road there were two potential sources identified (i.e. illegal dumping of waste and automobile exhausts systems crossing the bridge (Plate 3.3).

Bishopstowe bottom sediment had high concentrations and a greater number of PAH compounds compared to Bishopstowe top sediment (Table 5.13). A possible reason for this observation might be due to hydrophobic character of detected compounds which favours intense absorption to the bottom sediments (Baldyga *et al.*, 2004). Another possible reason for less compounds

detected in Bishopstowe top sediments is the fact that PAH undergo photodegradation when exposed to UV light, since the top sediments are exposed to UV radiation (He *et al.*, 2015).

Table **5. 13** PAH concentration levels in Msunduzi sediment samples.

	Concentration (µg/kg)					
	Ace(y)	Ace	Flu	Phe	Ant	Pyr
COL (T)	nd	nd	nd	nd	nd	7.85±0.01
COL (B)	nd	nd	nd	nd	nd	12.38±0.06
BIS(B)	3.5±0.1	nd	nd	12.4±0.06	2.83±0.2	31.9±0.002
BIS(T)	nd	nd	nd	nd	4.7±0.1	42.0±0.002
Total	3.5			12.4	7.5	94.1

*****Note: COL(T)= College road top, COL(B)= College road bottom, Bis(B)= Bishopstowe bottom, BIS(T)= Bishopstowe (Top).

5.15 **Application to Cedara sediment samples**

Table 5.14 shows the results for concentration levels of PAHs in Cedara Agricultural College sediment samples. Of the targeted PAHs, only pyrene was detected at H3(1) (2.84 µg/kg) and M1 dam (3.93 µg/kg) and its concentration levels was below maximum allowable limits which are 3.0 mg/L. The concentrations of Pyrene were very low and could be produced by a variety of source, such as automobile exhaust systems during harvest or vegetation of crops. However, major source of pyrene might have been from creosote wash off into these dams (Plate 4.10).

Table 5. 14 Concentration levels of PAHs in sediments from Cedara sampling points.

	Concentration (µg/kg)					
	Ace(y)	Ace	Flu	Phe	Ant	Pyr
H3(1)	nd	nd	nd	nd	nd	2.84±0.03
H3(2)	nd	nd	nd	nd	nd	nd
M1(B)	nd	nd	nd	nd	nd	nd
M1(T)	nd	nd	nd	nd	nd	3.93±0.02
Total						6.77

*Ace(y) = Acenaphthylene, Ace = Acenaphthene, Flu = Fluorene, Phe = Phenanthrene, Ant = Anthracene, Pyr = Pyrene. Nd= not detected.

5.16 **Comparison of the levels of PAHs obtained from sediments around South Africa and worldwide**

There are limited studies that have been conducted on the levels of PAHs on sediments in South Africa. Table 5.15 summarises results that were reported by other researchers in South Africa and worldwide. The concentration levels of PAHs recorded at Vhenda (SA) were found to be between 500-21600 µg/kg. These concentration levels were higher compared to the levels obtained in USA (0.0049-0.0680 µg/kg) and in Japan (9.11-321 µg/kg) and in this study (Pietermaritzburg) SA (2.83 – 42.0 µg/kg) are much lower (>10^3) than those reported. High concentrations of PAHs in Vhenda were justified by the presence of many petrogenic sources such as petroleum products, fuel combustion, and wool combustion.

Table 5. 15 Comparison of the levels of PAHs in sediments obtained worldwide.

PAHs	maximum concentration levels of PAHs on sediments (µg/kg)			
	South Florida (USA)	Vhenda (SA)	Chiba (Japan)	In this study (PMB, SA)
Naphthalene	0.0228	500	-	-
Acenaphthylene	-	1514	-	3.5
Acenaphthene	-	1135	-	-
Fluorene	0.0296	21600	9.11	-
Phenanthrene	0.0680	778	153	12.4
Anthracene	0.0049	540	66.8	2.83
Pyrene	0.0454	961	321	42.0
References	Jaward *et al.*, 2012	Edokpayi *et al.*, 2016	Onozato *et al.*, 2016	

*SA= South Africa, USA= United State of America, PMB= Pietermaritzburg

5.17 **Comparison of PAH concentration levels in water and sediment samples**

PAHs were measured in wastewater, river water, dam water and sediment samples around KwaZulu-Natal major cities and in an agricultural area in autumn and spring seasons. Naphthalene was found to be the most commonly detected PAH in most sampling locations for water samples with total concentration of 7.74 µg/L, in wastewater, 30.77 µg/L in river water and 12.25µg/L in dam water. Concentrations of naphthalene were high in river water due to naphthalene's high solubility in water and the presence of many potential anthropogenic source around the river. The individual PAH concentration levels ranged from 0.33 - 10.38 µg/L in river water, 0.037 – 2.71 µg/L in wastewater and 2.84 – 42.0 µg/L in sediments. The total concentration of PAHs is much higher on sediment compared to water, because of PAHs have low solubility in water, thus adsorbs into the surfaces of sediments (Edokpayi *et al*., 2016).

5.18 **References**

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Chapter Six - Conclusion and Recommendations

6.1 **Conclusion**

The aim of this study was to develop and apply solid phase extraction (SPE) coupled to GC-MS method for the analysis of PAHs in liquid samples and ultra-sonication extraction (USE) couples to GC-MS method for the analysis of PAHs in solid samples. Optimization of method parameters has been conducted to improve the extraction efficiency as well as detection of the analytes before analysis of real samples.

The optimum SPE which gave good recoveries (80-120%) were 6 mL methanol followed by 6 ml ultra-pure water as conditioning solvent. 500 mL as sample loading volume spiked with the analytes to make the final concentration of 7 µg/L. 20% methanol in 10 ml ultra-pure water as washing solvent and 6 ml *n*-hexane: dichloromethane (4:1, v/v) was used as an eluting solvent.

The optimum USE which gave good recoveries (82-117%) were 100 ml of hexane + acetone (1:1, v/v) as extraction solvent, 15g as sample mass. The extraction was conducted in time intervals of two cycles for 60 minutes and then solvent extracts were combined.

The development and application of SPE or USE coupled to GC-MS has been demonstrated for the analysis of PAHs in water and sediment samples at trace levels. The concentrations obtained ranged from 0.33-10.38 µg/L in river water samples 2.46-3.52 µg/L in dam water, 0.037- 2.71 µg/L in wastewater samples, and 2.84-42.0 µg/L in sediment samples.

In wastewater samples, almost all PAHs were detected, however, they were below allowable concentration limits. In river water and sediment samples, only naphthalene and pyrene were detected and were below allowable concentration limits. In dam water, only naphthalene was detected, and it was below allowable concentration levels.

The concentration levels of PAHs obtained in river and dams in KwaZulu-Natal are within acceptable concentration levels, however, continuous monitoring of PAHs in KwaZulu Natal is imperative for creating a database on PAHs in South Africa and for regulatory purposes. There

are limited studied on PAHs in water and sediments around South Africa, however, obtained levels of PAHs in this study were comparable with the published data from other researchers.

6.2 **Recommendations for future work**

- Addition of more compounds into the library of targeted PAHs, so that a more valid conclusion can be drawn on the presence of PAHs in river and sediment samples.
- Study of all seasons for a broad picture on the concentration of PAHs in different seasons.
- Conduct a study that will involve analysis on fish and soil samples, together with wastewater, river water, sediments to have an overview on the PAH levels in the environment.
- Further optimization of the ultrasonication method, to improve recovery of naphthalene.
- Investigate the effect of photo oxidation on the sediments and the effect of microbial activity on the levels of PAHs.
- Investigate the effect of PAHs on aquatic animal's immune system.
- Target specific industrial waste to analyze the presence of PAHs, for monitoring purposes and to draw a proper conclusion, instead of speculating based on literature.

Appendix A

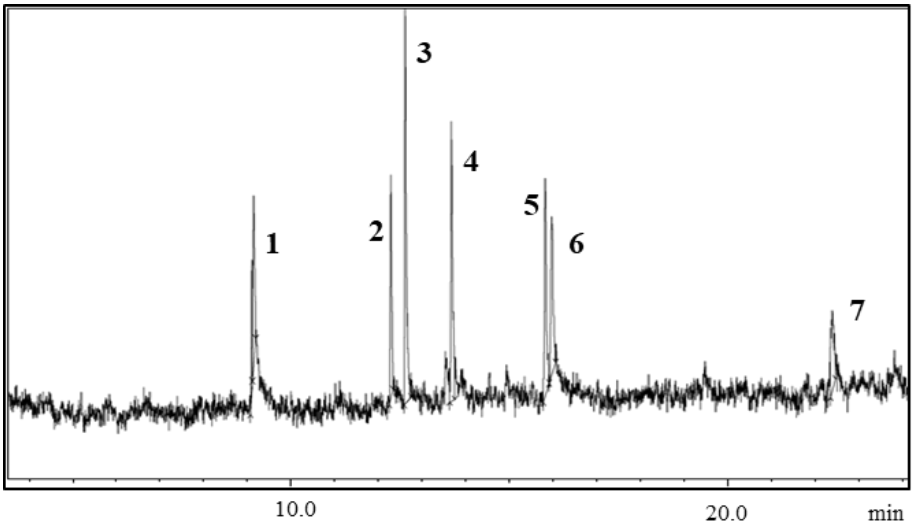

Figure A1 Shows TIC chromatograms of a 0.4 mg/L standard sample analyzed and processed with the initial method conditions. Identification number: - 1= naphthalene, 2= acenaphthylene, 3= acenaphthene, 4= fluorene, 5= phenanthrene, 6= anthracene, 7= pyrene.

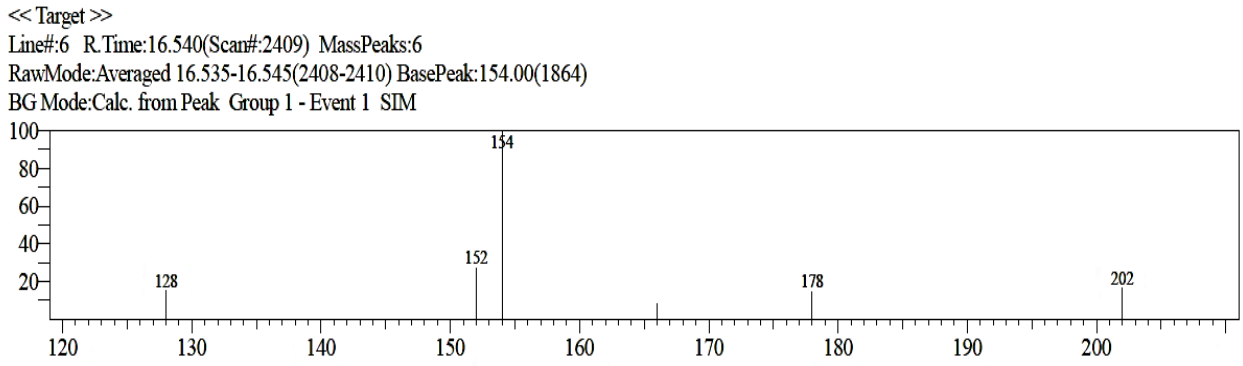

Figure A2 Shows a GC-MS library search resulting an identification of Naphthalene, Acenaphthylene, Acenaphthene and Pyrene in Northern wastewater treatment plant.

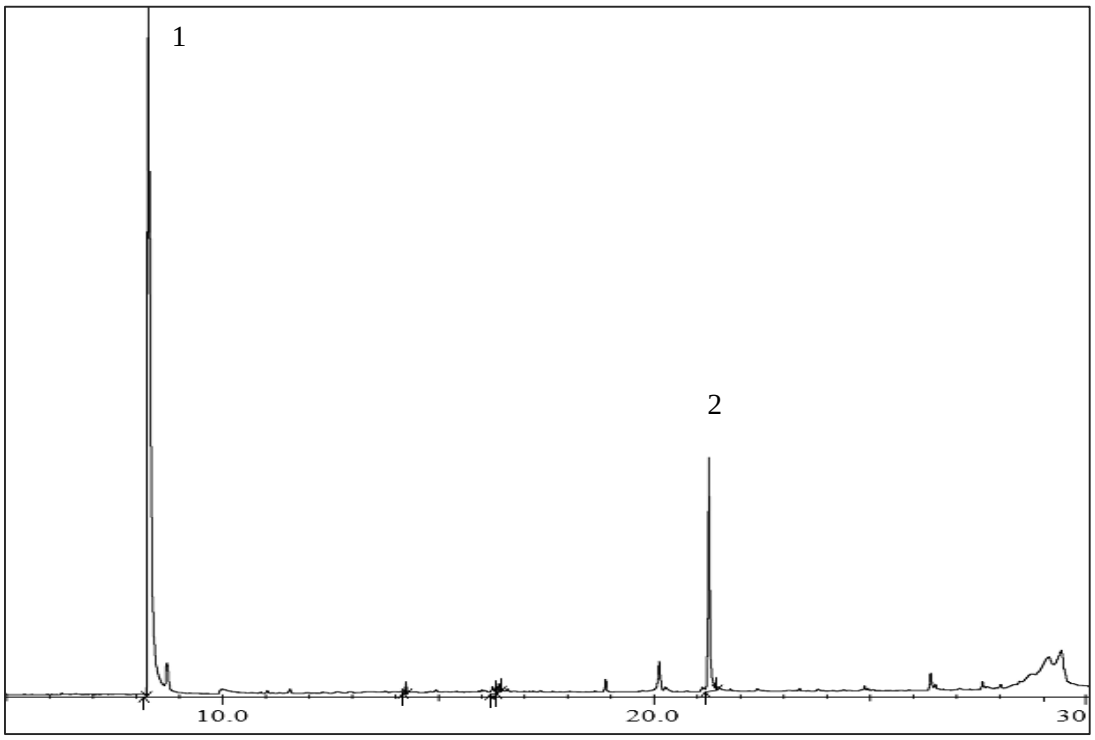

Figure A3 TIC chromatogram of a non-spiked Campsdrift bottom samples used for ultrasonication optimization, where .1= naphthalene; 2= pyrene.

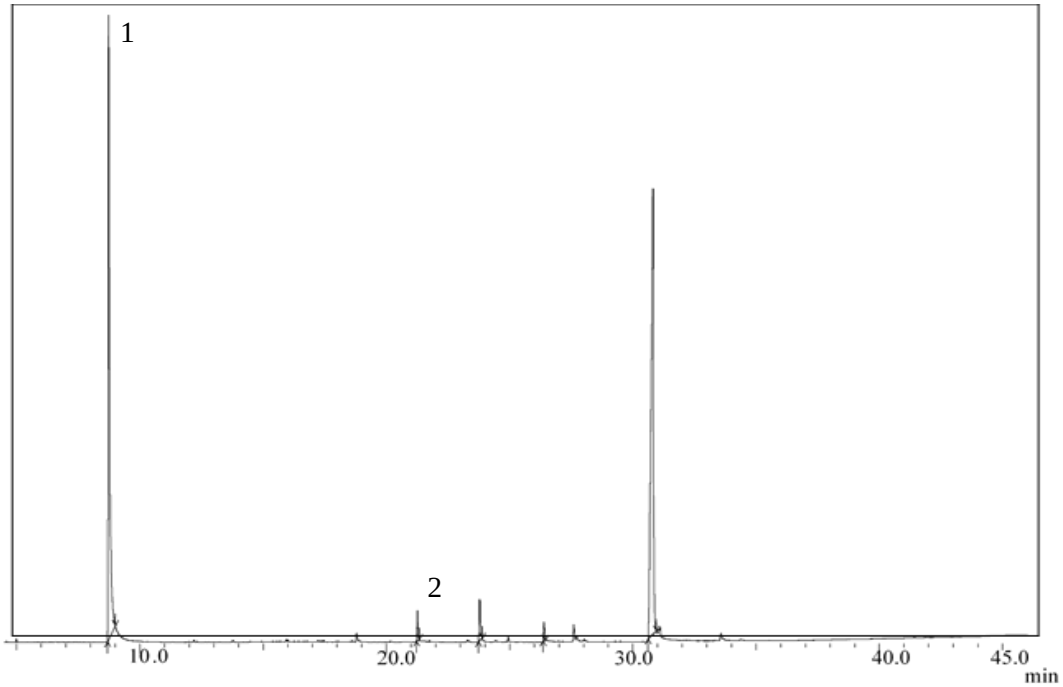

Figure A4 A typical TIC chromatogram of Pietermaritzburg Umsunduzi river; College road sampling point where 1 = naphthalene; and 2 = pyrene.

Table 1a Physico-chemical parameters of Durban wastewater treatment plants in Autumn season.

	Parameters	Amanzimtoti	Umhlathuze	Northern	Umbilo
Influent	pH	7.529	7.51	7.661	7.458
	Conductivity (µS/cm)	1290	683	862	786
	Water T (˚C)	24.6	25.3	24.7	24.8
	Salinity (psu)	0.64	0.33	0.45	0.38
	TDS (ppm)	636	331	445	394
	DO (mg/L)	0.83	0.28	0.17	0.24
Coordinates	South	30˚00'29.7"	29˚52'32.5"	29˚47'40.0"	30˚00'24.3"
	East	30˚54'54.9"	30˚52'58.3"	30˚59'58.1"	30˚55'03.2"
Effluent	pH	7.784	7.837	7.021	7.662
	Conductivity	1279	783	692	754
	Water T (˚C)	24.7	24.9	24.3	25.2
	Salinity (psu)	0.66	0.37	0.35	0.40
	TDS (ppm)	653	361	352	388
	DO (mg/L)	2.72	2.66	0.27	2.34
Coordinates	South	30˚00'24.3"	29˚52'40.0"	30˚00'24.3"	29˚50'47.0"
	East	30˚55'03.2"	30˚53'04.9"	30˚55'03.2"	30˚53'26.3"

Table 2a Effect of conditioning solvent on SPE extraction efficiency.

PAHs	Percentage recovery (%)		
	MeOH + H2O	MeOH + DCM	MeOH + hexane
Naphthalene	61.5	54.7	39.8
Acenaphthylene	96.6	76.7	80.6
Acenaphthene	72	57.9	62.1
Fluorene	96.3	80.4	85.4
Phenanthrene	80	66.9	54
Anthracene	62.4	55.5	59.7
Pyrene	74.5	75.6	71.6

Table 3a Effect of sample loading volume on extraction efficiency

PAHs	Extraction solvent volume			
	250 ml	500 ml	750 ml	1000 ml
Nap	58.5	112.6	85.6	61.5
Ace(y)	25.1	95.8	97	96.6
Ace	19.8	87.2	74.7	72
Flu	20.4	120.9	117.3	96.3
Phe	17.9	98.2	102.7	80
Ant	16.2	86.7	91.9	62.4
Pyr	9.2	113.4	128.1	74.5

Table 4a Effect of extraction solvent composition on USE extraction efficiency.

PAH	Recovery (%); RSD (% n=3)	Recovery (%); RSD (% n=3)	Recovery (%); RSD (% n=3)
	n-hexane-acetone	n-hexane-acetonitrile	n-hexane-Dichloromethane
Naphthalene	37.2	33.3	28.4
Acenaphthylene	108.7	66.6	32
Acenaphthene	82.5	80.1	43.4
Fluorene	117	117	44.1
Phenanthrene	93.3	59.9	33.4
Anthracene	92.3	92.7	31.2
Pyrene	102	84.8	45.4

Table 5a The effect of extraction solvent composition volume on USE extraction efficiency.

PAH	Recovery (%); RSD (% n=3)	Recovery (%); RSD (% n=3)	Recovery (%); RSD (% n=3)
	50 ml	100 ml	150 ml
Naphthalene	67.2	36.8	14.1
Acenaphthylene	82.9	108.7	81.7
Acenaphthene	79.9	82.5	76.6
Fluorene	104.8	117	115.6
Phenanthrene	90.7	93.3	104.4
Anthracene	87.5	92.3	99
Pyrene	39.6	102	72.4

Table 6a Effect of time on the extraction of targeted PAHs on the USE.

PAH	Recovery (%); RSD (% n=3)	Recovery (%); RSD (% n=3)	Recovery (%); RSD (% n=3)
	(30 min)	(60 min)	(90min)
Naphthalene	52.2	36.8	18
Acenaphthylene	66.3	108.7	63.3
Acenaphthene	58.5	82.5	28.4
Fluorene	86.6	117	53.2
Phenanthrene	69.5	93.3	47.8
Anthracene	72.9	92.3	41.3
Pyrene	94.7	102	50.6

Appendix B

Sample calculations

Preparation of stock solution

Require concentration = 100 µg/mL

Pure standard mass = concentration x volume

= 100 µg/mL x 100 mL

= 10 000 µg

= 10 mg →

Preparation of standard (0.2 mg/L)

$C_1V_1 = C_2V_2$

= [(0.2 mg/L)(10 mL)] / 100 µg/mL

= 20 µL →

SPE & UE calculation: Sample recovery (%) from the peak areas and calibration curves.

Acenaphthylene

Y = 563283x Peak area = 2293948 Spiking Conc. = 7 µg/L

2293948= 563283x

= 4.07 mg/L →

Recovery (%) = $[C_AV_A/C_DV_D]$ x 100%

= [(4.07mg/L)(0.9 mL) / (0.007 mg/L)(500 mL)] x 100 %

= 104.7 % →

Where. C_A= Concentration in the acceptor phase. V_A= Volume in the acceptor phase. C_D= Concentration in the donor phase. V_D= Volume in the donor phase.

Instrument Limits of Detection (LOD) = 3.3 (standard error/x-variable)

= 3.3 (9405.99/579485.43)

= 0.0536 →

Instrument Limit of Quantification (LOQ) = 10 x LOD

= 0.536 →

Sample Calculation of the initial sample concentration (**500** mL)

Phenanthrene

Y = 607545x Peak area = 45376

45376= 563283x

= 0.0806 mg/L in 1.0 mL →

Initial concentration (500 mL) = C_2V_2 / V_1 x percentage recovery

= [(0.0806 mg/L) (1.0 mL)/ (500 mL)] x 98.2%

= 0.000149 mg/L

= 0.158 µg/L →