Cape Peninsula
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SPATIO-TEMPORAL DISTRIBUTION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN SOILS IN THE VICINITY OF A PETROCHEMICAL PLANT IN CAPE TOWN

by

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DECLARATION

I, Raissa Andong Omores, declare that the contents of this thesis represent my own unaided work and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

10/10/2016

Signed   **Date**

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are an alarming group of organic substances for humans and environmental organisms due to their ubiquitous presence, toxicity, and carcinogenicity. They are semi-volatile substances which result from the fusion of carbon and hydrogen atoms and constitute a large group of compounds containing two to several aromatic rings in their molecule. Natural processes and several anthropogenic activities involving complete or incomplete combustion of organic substances such as coal, fossil fuel, tobacco and other thermal processes, generally result in the release of the PAHs into the environment. However, the fate of the PAHs is of great environmental concern due to their tendency to accumulate and their persistence in different environmental matrices and their toxicity. Animal studies have revealed that an excessive exposure to PAHs can be harmful. Evidence of their carcinogenic, mutagenic, and immune-suppressive effects has been reported in the literature. In the soil environment, they have the tendency to be absorbed by plants grown on soil being contaminated by the PAHs. It is, therefore, important to evaluate their occurrence levels in different environmental matrices such as soil concentrations.

The concentration, the seasonal variation, the distribution and possible source of the eight US Environmental Protection Agency (USEPA) priority PAHs namely benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(k)fluoranthene, chrysene, benzo(a)anthracene, dibenzo(a,h)anthracene and fluoranthene were investigated in 35 soil and leaf samples. Gas chromatography coupled with flame ionization detector (GC-FID) was used to separate and quantify the concentration of the eight PAHs from the soil and leaf samples collected in the vicinity of a petrochemical plant in Cape Town. The samples were collected during winter and summer.

The sum of the concentrations of the detected PAHs (ΣPAHs) in winter ranged between nd (not detected) and 13.88 mg/kg, with a mean concentration of 4.08 mg/kg and a standard deviations of distribution of 2.64 mg/kg while in summer, ΣPAHs ranged between nd (not detected) and 10.43 mg/kg, with a mean concentration of 0.50 mg/kg and standard deviation of distribution 1.83 mg/kg. The presence and concentration level of the PAHs detected in the soil samples in the vicinity of a petrochemical plant vary significantly under climatic change ($p < 0.05$). The maximum concentrations of individual PAHs in the soil samples were higher in winter compare to the maximum concentrations of the PAHs detected in summer. The diagnostic ratios of the selected PAH compounds showed that

petrogenic processes were the most likely sources of the PAH compounds in the soil samples with minimal pyrogenic contributions. A weak and moderate correlation was found among individual PAHs detected in the soil samples and the soil organic matter (SOM) during summer and winter seasons. The same trends were also observed between the pH of the soil samples and individual PAH concentrations. In winter, the highest value (sum of the PAHs) was observed in the roadside soils, followed by the industrial and residential soils with concentrations of 9.31, 5.37, 5.23 mg/kg, respectively. In summer and winter, the total PAH concentration increased in order of residential soil < industrial soil ≤ roadside soil.

In the leaf samples the sum of the concentrations of the detected PAHs (ΣPAHs) ranged between 10.48 and 76.73 mg/kg, with a mean concentration of 29.47 mg/kg. The mean concentration of the individual PAHs ranges between 0.92 and 7.74 mg/kg. Fluoranthene was found with the lowest concentration (0.92 mg/kg) and DahA with the highest concentration (7.74 mg/kg) in the leaf samples. No significant differences ($p > 0.05$) between unwashed and water-washed leaves with regard to PAH concentrations were observed. The PAH concentrations in the leaf samples were higher than the corresponding soil samples concentration collected at the same site during summer, which indirectly suggested that the PAHs accumulate in the leaves predominantly via atmospheric deposition.

ACKNOWLEDGEMENTS

DEDICATION

This thesis is dedicated to my father Omores Bruno. Thank you, daddy, for your unconditional love, support and encouragement. You are and will always be my hero.

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LIST OF ABBREVIATIONS

ASE	Accelerated solvent extraction
Ant	Anthracene
ATSDR	Agency for Toxic Substances and Disease Registry
BaA	Benz(a)anthracene
BaP	Benzo(a)pyrene
BkF	Benzo(k)fluoranthene
Bpe	Benzo(g,h,i)perylene
Chry	Chrysene
DahA	Dibenz(a,h)anthracene
DCM	Dichloromethane
DHHS	Department of Health and Human Services
DNA	Deoxyribonucleic acid
EFs	Emission factors
EU	European Union
FAO	Food and Agricultural Organization
FID	Flame ionization detector
Fluo	Fluoranthene
GC	Gas chromatography
GC-FID	Gas chromatography -flame ionization detector
GC-MS	Gas chromatography-mass spectrometry
GPC	Gel-permeation cleanup
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
IP	Indeno(1,2,3-cd)pyrene
IPCS	International Programme on Chemical Safety
MAE	Microwave assisted extraction
MPCs	Maximum permissible concentrations
nd	Not detected
PAH	Polycyclic aromatic hydrocarbon
PBT	Persistent bioaccumulative toxic
PCA	Principal component analysis
PFE	Pressurized fluid extraction
pH	Potential hydrogen

Phe	Phenanthrene
PNAs	Poly-nuclear aromatics
POM	Polycyclic organic matter
POPs	Persistent organic pollutants
Pyr	Pyrene
RP	Reversed-phase
DEA	Department of Environmental Affairs
SAS	Statistical analysis system
SCF	Standing Committee on Foodstuffs
SFE	Supercritical fluid extraction
SOM	Soil organic matter
TLC	Thin layer chromatography
UEPA	US Environmental Protection Agency
WHO	World Health Organisation
ppm	Parts per million

LIST OF SYMBOLS

p	Pearson correlation coefficient
R^2	Correlation coefficient
r_s	Spearman's correlation coefficient
t_R	Retention time

GLOSSARY OF TERMS

Adsorption   A process in which solid, liquid or gas molecules become attached to a solid surface by physical attraction or chemical bonding.

Bioaccumulative   The accumulation of a substance, such as a toxic chemical, in various tissues of a living organism.

Biodegradation   The series of processes by which living systems render chemicals less noxious to the environment.

Biomarker   A distinct biochemical, genetic, or molecular characteristic or substance that is an indicator of a particular biological condition or process.

Chromatography   Methods of separation based on the interaction of species with a stationary phase while they are being transported by a mobile phase.

Cuticle   A protecting film covering the epidermis of leaves, young shoots, and other aerial plant organs without periderm. It consists of lipid and hydrocarbon polymers impregnated with wax and is synthesized exclusively by the epidermal cells.

Dry deposition   The deposition of pollutants, including gasses and particulate matter, as they settle out of the atmosphere or are absorbed by plant tissues.

Hydrophobic   Repelling water; insoluble in water; not readily absorbing water.

Lipophilic   Refers to the ability of a chemical compound to dissolve in fats, oils, lipids, and non-polar solvents such as hexane or toluene.

Metabolism   The sum of the physical and chemical processes in an organism by which its material substance is produced, maintained, and destroyed, and by which energy is made available.

Retention time   The time between sample injection on a chromatographic column and the arrival of an analyte peak at the detector.

Soil organic matter   The organic matter component of soil, consisting of plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by soil organisms.

Teratogenicity   The property or capability of producing congenital malformations.

Wet deposition   The transfer of pollutants from the atmosphere to the earth by inclusion or solution in precipitation.

CHAPTER ONE

INTRODUCTION

1.1   Background

Environmental pollution has become one of the most globally stressful challenges that mainly originate from industrialization processes, population growth and globalization (Gillespie, 2002; Khan & Ghouri, 2011). For the past decades, persistent organic pollutants (POPs), often known as polycyclic aromatic hydrocarbons (PAHs), polycyclic organic matter (POM) poly-nuclear aromatic hydrocarbons, poly-nuclear aromatics (PNAs) or poly-nuclear hydrocarbons, have been identified as potential environmental contaminants (ATSDR, 2009). Based on their high stability, the aforementioned class of compounds has been proved resistant to biodegradation. Their accumulation in living organisms and nature significantly impacts human health and the ecosystems (Peng *et al*., 2011; Allan *et al*., 2012). Apart from this, anthropogenic activities (human processes) have also been held responsible for the presence of PAHs in the environment (Arias *et al*., 2010; Abdel-shafy & Mansour 2015). Literature maintained that the discharge of PAHs into the environment usually occurs through thermal processes which might involve complete/incomplete combustion of substances such as coal and similar activities including burning fossil fuel (e.g. wood, oil, and charcoal), garbage, charcoal meat and heating of tobacco (Langenkamp *et al*., 2001; Chen *et al*., 2011). In addition, PAHs compounds consist of two or more benzene rings arranged in various configurations (Abdelaziz *et al*., 2013). Moreover, Mahler *et al*. (2012) reported that there are approximately 200 known PAHs found in different environmental matrices such as water, sediment, soil and atmosphere. However, only sixteen out of the two hundred PAHs have been included in the priority list of pollutants by the US Environmental Protection Agency (USEPA) and European Union (EU). The sixteen prioritized PAHs include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene,

benzo(ghi)perylene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene. Aside from the sixteen toxic compounds previously mentioned, several other benzene based complexes have been classified as poisonous not only to the animal but to humans as well. For instance, US department of Health and Human Services (DHHS) reported that benzo(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene are carcinogenic to animals (ATSDR, 2009).

On the other hand, the International Agency for Research on Cancer (IARC) reported that benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, and indeno(1,2,3-cd)pyrene might also be carcinogenic to humans. Similarly, the US Environmental Protection Agency (USEPA) suggested that benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, and indeno(1,2,3-cd)pyrene could also be carcinogenic substances for humans. Likewise, the Environmental Programs Directorate (2011) recognized anthracene, benzo(a)pyrene, chrysene, fluorene and pyrene as the most common PAHs found in the environment. However, benzo(a)pyrene whose molecular structure is shown in Figure 1.1 has been selected as the standard indicator of carcinogenic PAHs in many countries and organizations (Nadal *et al.*, 2004).
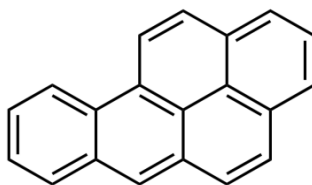
Figure 1.1: Structure of benzo(a)pyrene

Guo *et al.* (2007) reported that when PAHs are deposited in terrestrial environments, they are partitioned into different compartments among which some might be bonded to dust particles and enter the atmosphere. Furthermore, Quiroz *et al.* (2010) reported that PAH containing dust could be deposited on different surfaces especially soils and plants via rain precipitation, fog

(snow), storm water and erosion. They can also be transported to remote locations of the earth such as distant mountains, lakes and in the Arctic and Antarctic regions where they could also be detected far away from their sources of release. The airborne PAHs, especially those with low molecular weight and vapour densities may be transported over long distances (Marti *et al.*, 2008). Numerous studies have been conducted worldwide on PAHs to understand their distribution, fate and effect in the environment (Abbas & Barck, 2005; Ping *et al.*, 2007; Ratola *et al.*, 2010). Therefore it is crucial to assess and update data on the existence and occurrence of these harmful substances in Cape Town (South Africa).

According to El-Shahawi *et al.* (2010), the fate of PAHs is of great environmental concern due to their toxicity and tendency to accumulate and persist in different environmental matrices. However, this depends on several factors such as atmospheric photolysis, sorption, water and lipid solubility, chemical oxidation, vaporization, and microbial degradation (Wick *et al.*, 2011). Furthermore, Lu *et al.* (2011) and Wick *et al.* (2011) posits that the PAHs binds easily to organic materials which make them less susceptible to biodegradation. However, PAHs often degrade naturally in the soil; likewise, their high concentration could impede their decomposition process. In addition, natural and biological degradation processes are very slow because of the structure of the PAH molecules.

Humans, biological organisms, and the environment could be exposed to PAHs through different pathways such as inhalation, breathing of contaminated air during asphalt and coal tar production, in petroleum plants and smokehouses (Choosong *et al.*, 2010; Li *et al.*, 2010). Furthermore, exposure to PAHs might occur by eating grilled or charred meats, drinking contaminated water and contaminated cow's milk. Exposure may also occur when the skin is in contact with contaminated water, air, and the soils which are closer to the hazardous waste sites (WHO, 2000; ATSDR, 2009). Serious hazardous effects have been associated with exposure to PAHs which may be acute or chronic. The acute (short-term) effects include vomiting, eye irritation, nausea, diarrhea, and confusion, while the chronic (long-term) effects include cancer, cataracts, kidney and liver damage, and skin inflammation. Therefore, this study focuses on the monitoring of these harmful substances in the environment. The first step in the identification and quantification of PAHs in soil involves sample preparation which includes sample collection and extraction. To date, the following methods: soxhlet extraction,

automated soxhlet extraction, pressurized fluid extraction, ultrasonic extraction and microwave assisted extraction reported in the literature have been used for extraction of PAHs (USEPA, 2003). The aforementioned PAH extraction methods in soil have been demonstrated to be effective and were approved by the USEPA (Zhao *et al*., 2013). The microwave assisted extraction (MAE) method, described by EPA method 3546 was selected as a suitable technique for this study because it reduces solvent consumption; it has a shorter extraction time, a high recovery and a good reproducibility compared to other methods (Wang *et al*., 2010).

After the extraction process, PAHs can be identified and quantified using various chromatographic techniques such as Tin layer Chromatography (TLC) with UV detection, capillary gas chromatography coupled with mass spectrometry and high performance liquid chromatography (HPLC) with fluorescence or UV detection as suggested by Wu *et al*. (1997) and Pandey *et al*. (2011). Literature reported that gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) have been widely used for analytical purposes (Balachandran *et al*., 2012). Kumar *et al*. (2011) confirmed that GC-MS is more selective while GC-FID has a higher sensitivity. In addition to this, Fernandez *et al*. (2015) indicated that GC-FID has become more popular due to its high selectivity, good precision, and resolution. Therefore, in this study, GC-FID was selected as the best analytical method for identification and quantification of PAHs in soil and leaf samples.

1.2   Problem statement

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemical compounds that are extremely harmful when excessively exposed to humans and environmental organisms. Animal studies have revealed that excessive exposure to PAHs can be harmful. Evidence of their carcinogenic, mutagenic and immune-suppressive effects has been reported (Arfsten *et al.,* 1996; Page *et al*., 2003). Moreover, literature also sustained that some PAHs have been found to be persistent, bioaccumulative, and toxic (PBT) to humans and other organisms (El-Shahawi *et al*., 2010). The persistence and bioaccumulative potential of PAHs is the result of

their tendency to remain in the environment for a long period of time (Jones & Voogt, 1999; Ren *et al*., 2011). Substances with PBT characteristics usually raise concern from an environmental perspective and often pose a threat to humans. When these toxic chemicals are released into the environment, they can rarely be removed due to their accumulation and resistant characteristics that have been claimed to be harmful to plants, animals and ultimately humans (Alani *et al*., 2012). Petrochemical industries are major hot spots for PAH release into the environment. Some refining and production operations have been reported to release a substantial quantity of PAHs into soils and water bodies (Das *et al*., 2008). These substantial quantities enter as process water effluents in nearby industrial sites and as gaseous particulates into the atmosphere. This information is based on reviewed literature concerning the levels and prevalence of the PAHs in many matrices especially soil, water and air in Cape Town (Quin *et al*., 2009; Niewoudt *et al*., 2011; Nekhavhambe *et al*., 2014). In many African cities especially Cape Town, there is a lack of critical environmental exposure levels that guarantee the safety and health of the environment. Therefore, it was important to evaluate their occurrence in different environmental matrices such as soil, sediment and water within industrial sites and communities. Furthermore, this research provides data on PAH content in soils and plants in the vicinity of a petrochemical industry in Cape Town. This data could be useful for future studies and could also be considered as reference background information.

1.3   Aim of the study

The aim of this study was to investigate the spatial and seasonal variations in the concentration of eight PAHs namely: benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(k)fluoranthene, chrysene, benzo(a)anthracene, dibenzo(a,h)anthracene and fluoranthene in soil and leaf samples taken in the vicinity of a petrochemical plant in Cape Town using gas chromatography coupled with flame ionization detection (GC-FID) method.

1.4   Research objectives

The objectives of this research were:

(i)   To determine the presence and the level of eight PAHs: benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(k)fluoranthene, chrysene, benzo(a)anthracene, dibenzo(a,h)anthracene and fluoranthene in the soil and leaf samples.

(ii)   To investigate the spatial and seasonal variations in the concentration of eight PAHs in the soil samples.

(iii)   To determine a correlation between the physicochemical properties (soil organic matter and pH) and the PAH concentration in the soil samples.

(iv)   To investigate the level of the PAHs in both washed and unwashed leaf samples.

(v)   To compare the concentration of the PAHs detected in the soil samples with allowed guideline concentrations, recommended by ATSDR and USEPA.

1.5   Research questions

- Are PAH compounds detectable in the soil and leaf samples around petrochemical industries?
- What are the possible sources of PAHs in the soil samples collected in the study area?
- Is there evidence of seasonal variation of PAHs in sampled soils?
- Are the levels of PAHs in the sampled matrices above or below recommended limits?
- What are the spatial distributions of the PAHs?
- Is there a correlation between pH, soil organic matter and the PAHs levels in the soil?
- Is there a correlation between individual PAH compounds in the soil samples?
- Is there a correlation between PAHs concentration detected in the soil samples and in the leaves of the Port Jackon Willow?
- Can the Port Jackon willow leaves be used as a suitable indicator of pollution of PAHs in the environment?

1.6   Research approach

The research processes used to carry out this study includes sampling, sample preservation, sample preparation and sample analysis.

1.6.1   Sampling

Samples were collected seasonally during winter and summer in 35 sampling locations around the petrochemical industry in Cape Town. The use of a Global Positioning System (GPS) was used to ensure sampling from the same area.

1.6.2   Sample pretreatment

Soil and plant samples were sieved, air-dried and grinded in order to increase their homogeneity and to increase the extractability of the analytes in the soil and plant.

1.6.3   Sample preparation

The two main steps required in sample preparation were:

- **Extraction:** performed to release the analyte from the solid matrix and to transfer them to another medium. Soil and leaf samples were extracted following the EPA method 3546 microwave-assisted extraction.
- **Clean-up*:* It was recommended that sample extracts had to be cleaned prior to analysis due to the presence of a large number of contaminants, which tend to damage the GC column. Florissil-silica clean-up was used as a clean-up technique in this study.

1.6.4   Sample analysis

Instrumental analysis is performed to separate, identify and quantify the individual analytes in the sample. A gas chromatograph (GC-2010 plus Shimadzu) equipped with a flame ionization

detector (GC-FID) and a data processor was used for separation and determination of the PAHs.

1.7   Chapter outline

The current study encapsulates five more chapters presented as follows:

Chapter Two gives an overview of numerous studies with regard to PAHs in different environmental matrices (soil, water, air, plants). It also includes the sources and emission of PAHs, health and environmental effects of PAHs and a review of analytical methods used worldwide to determine PAHs in soil samples.

Chapter Three describes each step of the experimental procedures used in this study. It also provides a clear and complete description of the specific procedure including sampling, extraction by MAE for soil and leaf samples.

Chapter Four presents the results obtained from soil samples collected during winter and summer seasons. A correlation between the physico-chemical properties of soil (SOM and pH) and PAH concentrations are presented. Furthermore, the possible sources of the PAH compounds in the soil samples collected in the vicinity of a petrochemical plant are discussed.

Chapter Five presents the results obtained from the leaf samples collected during the summer season. The results obtained from the comparison between the level of PAHs in unwashed and washed plant leaves are presented. Furthermore, a correlation between PAH concentrations detected in the soil and in the leaves of the port jackon Willow are presented.

Chapter Six provides answers to the research problems. It suggests possible solutions and provides conclusions to this study.

CHAPTER TWO

LITERATURE REVIEW

2.1   Introduction

This chapter presents the studies that have been conducted by various researchers with regards to the presence and levels of PAHs in the environment. The issues discussed include the description of PAHs and their physical and chemical properties which are important in order to have a better understanding of their persistence, their ability to accumulate in environmental media (soil, sediment, water and plants), their toxicity and potential for long-range transport. The chapter also highlights the possible sources of PAHs in the terrestrial and aquatic environment. Furthermore, information on the distribution pathways of PAHs in soil, water, air, and plants, and the factors influencing the spatial distribution patterns and fates of PAHs in the environment are also provided. In addition, information on their health effects on humans and the analytical methods used for the determination of PAHs in different environment matrices are discussed.

2.2   Physical and chemical properties of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are organic substances made up of carbon and hydrogen atoms (Kaszubkiewicz *et al*., 2010). They have various isomeric structural forms characterized by different positioning of benzene rings relative to one another (Wang *et al*., 2012; Godzik *et al*., 2014). In addition, PAHs are solids with low volatility at room temperature. They are high molecular weight, non-polar compounds which can become soluble in organic solvent and slightly soluble in water (Wick *et al*., 2011; Okere & Semple, 2012; Tsibart & Gennadiev, 2013). Polycyclic aromatic hydrocarbons are lipophilic, they exhibit hydrophobic properties and can easily mix with organic solvents. Thus, they can accumulate better in sediments, soil, and oily substances unlike in water or air (Xing *et al*., 2011; Okere & Semple, 2012).

Furthermore, PAHs are divided into two main categories: low molecular weight PAHs composed of fewer than four rings (e.g., naphthalenes, phenanthrenes and anthracenes) and high molecular weight PAHs composed of four or more rings (e.g chrysene, benzo(b)fluoranthene) (Wick *et al*., 2011). The PAHs which have three rings or less are found in the vapour phase in the environment, while the PAHs with four and five rings are distributed between particles and the vapour phase depending on their temperature (Wick *et al*., 2011). In addition, the PAHs with six and more rings are found exclusively in the particle state (Oishi, 2013).

The physical and chemical properties of PAHs depend on their molecular weight and the number of rings (Wick *et al*., 2011; Tsibart & Gennadiev, 2013). The low molecular weight PAHs are highly volatile, slightly soluble in water and less carcinogenic. They were found to be acutely toxic to marine organisms (Stogiannidis & Laane, 2015). The average concentration level of naphthalene in saltwater, fresh water, and groundwater system was found to be 429.7, 111.9 and 329.3 ng/L respectively (Environment Agency, 1995). On the other hand, the high molecular weight PAHs tend to be more carcinogenic and can be persistent in the environment due to their low volatility, their resistance to leaching and their resistance to oxidation (Wick *et al*., 2011). Some PAHs such as benzo(a)pyrene, benzo(k)fluoranthene, and benzo(b)fluoranthene contain the same number of rings, but a different configuration of rings which may lead to differences in the properties of these compounds (Skupinska *et al*., 2004). An example of the structural representation of such PAHs with the same number of rings is illustrated in Figure 2.1.

Benzo(a)pyrene   Benzo(k)fluoranthene   Benzo(b)fluoranthene
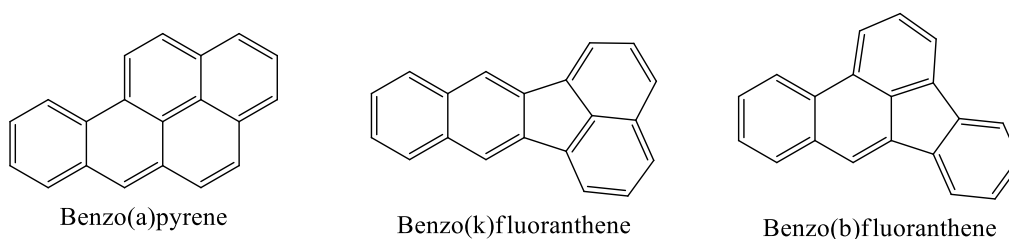
Figure 2.1: Representations of the PAHs with five aromatic rings

Information on the chemical and physical properties of 16 USEPA's priority PAHs are presented in Table 2.1 while Figure 2.2 shows the chemical structures of the PAHs.

Table 2.1: Physico-chemical properties of the 16 USEPA's priority pollutant PAHs

Chemical Name	Molecular formula	Molecular weight (g/mol)	Melting point (^{o}C)	Boiling point (^{o}C)	Vapour pressure (pa)	Solubility in water at 25 ^{o}C (mg/L)
Naphthalene	$C_{10}H_8$	128.2	81	218	11.87	31.00
Acenaphthylene	$C_{12}H_8$	152.2	95	96	3.87	16.10
Acenaphthene	$C_{12}H_{10}$	154.2	93	275	0.50	3.80
Fluorene	$C_{13}H_{10}$	166.2	116	295	0.43	1.90
Phenanthrene	$C_{14}H_{10}$	178.2	100	340	9.07×10^{-2}	1.10
Anthracene	$C_{14}H_{10}$	178.2	218	342	3.40×10^{-3}	0.05
Fluoranthene	$C_{16}H_{10}$	202.3	107	384	1.08×10^{-3}	0.26
Pyrene	$C_{16}H_{10}$	202.3	147	404	5.67×10^{-4}	0.13
Benzo(a)anthracene	$C_{18}H_{12}$	228.3	159	400	2.05×10^{-5}	0.01
Chrysene	$C_{18}H_{12}$	228.3	255	448	1.04×10^{-5}	0.0015
Benzo(b)fluoranthene	$C_{20}H_{12}$	252.3	168	168	1.07×10^{-5}	0.0015
Benzo(k)fluoranthene	$C_{20}H_{12}$	252.3	216	480	1.03×10^{-8}	0.008
Benzo(a)pyrene	$C_{20}H_{12}$	252.3	179	310	6.52×10^{-7}	0.0038
Benzo(ghi)perylene	$C_{22}H_{12}$	276.3	273	550	1.33×10^{-8}	2.6×10^{-6}
Indeno(1,2,3-cd)pyrene	$C_{22}H_{12}$	276.3	164	530	1.87×10^{-8}	0.06
Dibenzo(a,h)anthracene	$C_{22}H_{14}$	278.4	262	262	2.80×10^{-9}	0.0005

(Adapted from Wick *et al*., 2011)
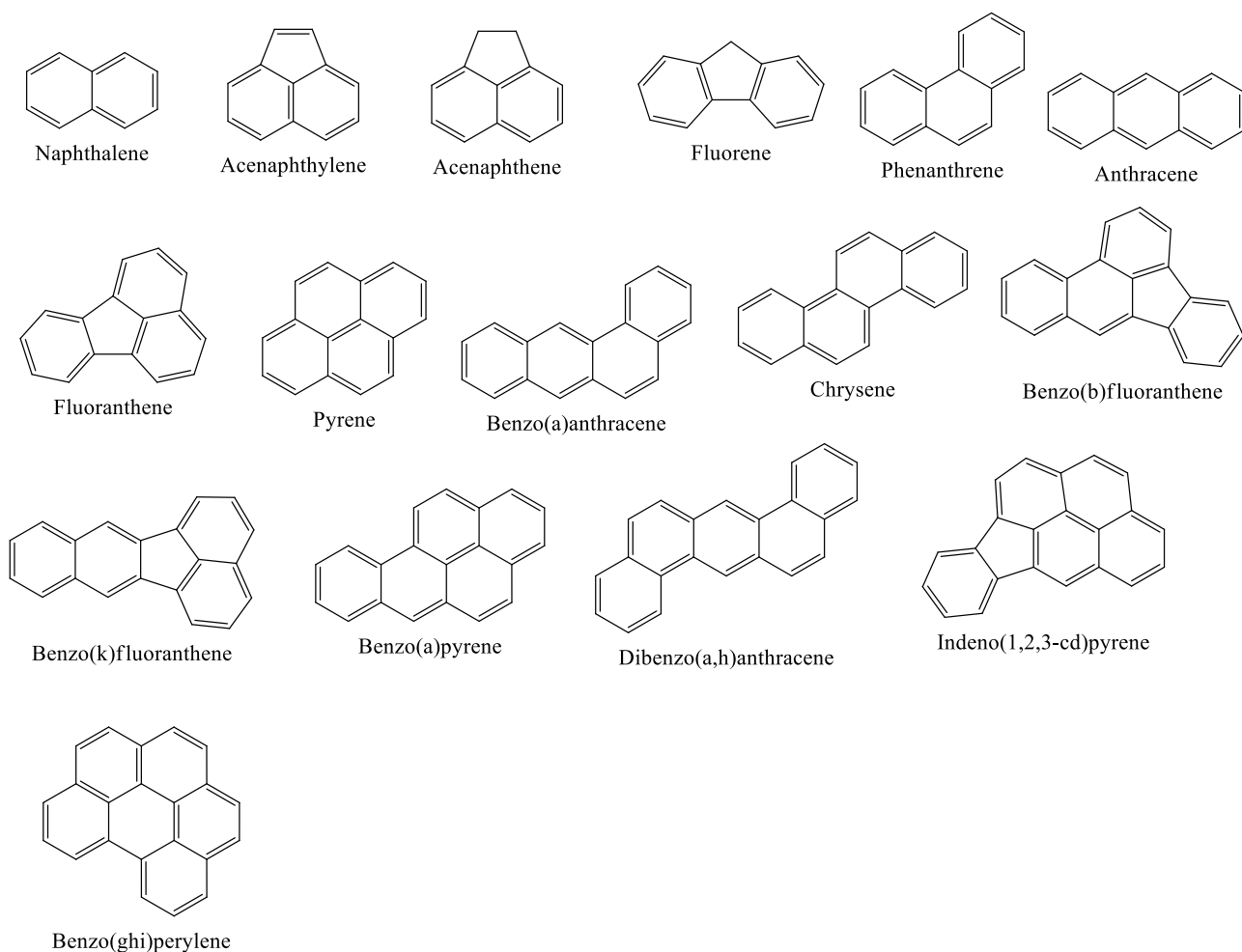
Figure 2.2: Structures of the USEPA's 16 priority pollutant PAHs

2.3   Fate of polycyclic aromatic hydrocarbon in the environment

Upon release into the environment, PAHs may undergo a number of different processes such as volatilization, absorption, leaching, erosion, adsorption, diffusion, biological and chemical degradation (Wick *et al*., 2011). Table 2.2 shows the movement and fate of organic substances such as PAHs in the environment.

Table 2.2: Movement and fate of polycyclic aromatic hydrocarbons in the environment

Process	Consequences	Factor
Transfer (processes that relocate PAHs without altering their structure)		
Volatilization	Loss of PAHs due to evaporation from soil, plant, or aquatic ecosystems.	Vapour pressure, wind speed, temperature
Absorption	Uptake of PAHs by plant roots or animal ingestion. Polycyclic aromatic hydrocarbons usually do no transfer into above ground biomass from the soil.	Cell membrane transport, contact time, susceptibility, plant species
Leaching	Translocation of PAH either laterally or downward through soils	Water content, macropores, soil texture, clay and organic matter content, rainfall intensity, irrigation
Erosion	Movement of PAH by water or wind action	Rainfall, wind speed, size of clay and organic matter particles with adsorbed PAH on them
Degradation (processes that alter the PAH structure)		
Biological	Degradation of PAHs by microorganisms, biodegradation, and cometabolism	Environmental factors (pH, moisture, temperature, oxygen), nutrient status, organic matter content, PAH bioavailability, microbial community present, molecular weight of PAH (LMW or HMW)
Chemical	Alteration of PAHs by chemical processes such as photochemical (i.e. UV light) and oxidation-reduction reactions	High and low pH, structure of PAH, intensity and duration of sunlight, exposure to sunlight, and same factors as for microbial degradation
Sequestration (processes that relocate PAHs into long-term storage without altering structure)		
Adsorption	Removal of PAHs from bioavailable pools through interaction with soils and sediments	Clay and organic matter content, clay type, moisture
Diffusion	Diffusion of PAH into soil micropores where it is unavailable for microbial degradation	Hydrophobic nature of micropores and PAH

(Adapted from Wick *et al*., 2011)

2.3.1 Photochemical transformation

Polycyclic aromatic hydrocarbons can undergo photochemical transformation and the resulting products are often more carcinogenic and mutagenic than the original PAH compounds (Yu, 2002). They can react with atmospheric pollutants such as SO_2, HNO_3, NO_2, OH radical and O_3 (Yu, 2002; Zimmermann *et al*., 2013; Jariyasopit *et al*., 2014). Polycyclic aromatic hydrocarbons react with O_3 to form direct acting mutagens such as dione and epoxides (Chu *et al*., 2010). In addition, when they react with NO_2, they form

dinitro-PAHs and nitro-PAHs (Zimmermann *et al*., 2013). When PAHs react with SO_2 they can yield sulfonic acid, and can also undergo photo-oxidation by ultra-violet rays from the sun to yield mutagenic and carcinogenic reaction products namely aldehydes, phenols, dihydrodiols, ketones, and acids (Srogi, 2007). Endoperoxides are the common products of photolysis. When PAHs undergo secondary reactions they can form diones (Jorgensen, 2010). Figure 2.3 illustrates the photo- oxidation of benzo(a)pyrene in aqueous solution.
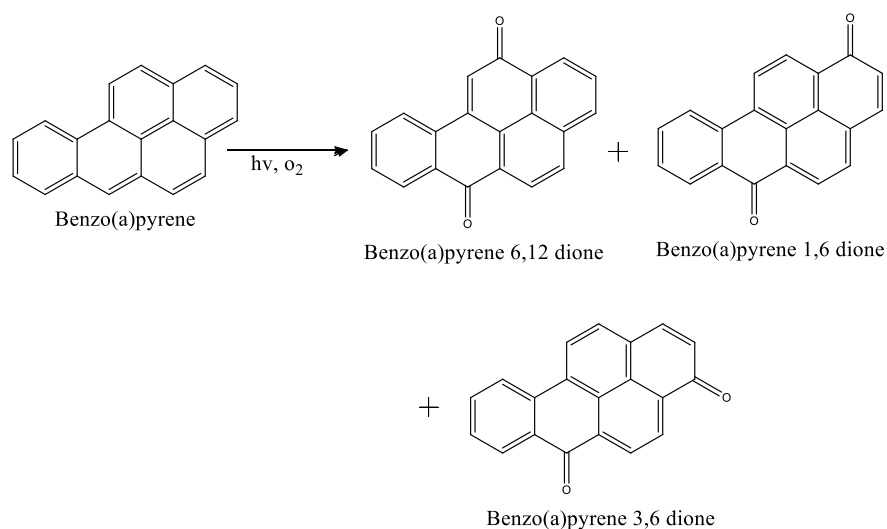
Figure 2.3: Photo-oxidation of benzo(a)pyrene in aqueous solution

Abdel-shafy *et al*. (2015) found that high molecular weight PAHs are more resistant to photo-oxidation than low molecular weight PAHs. For instance, benzo(a)anthracene, anthracene and phenanthrene were found to be more sensitive to photo-oxidation, whereas benzo(a)pyrene and pyrene were relatively resistant to photodegradation.

2.3.2   Biological transformation

Microorganisms such as bacteria and fungi present in the soil, sewage, and water were found to be capable of transforming and degrading PAHs (Das & Chandran, 2010). Microbial metabolism of PAHs may result in either; complete or incomplete hydrocarbon degradation depending on the environmental condition (pH, dissolved oxygen and temperature) and the type of PAH involved (Vila *et al*., 2015). Low molecular weight

PAHs tend to oxidize completely into inorganic products such as CO_2 and H_2O, while the heavier PAHs will degrade partially to yield various oxygenated metabolites (Ubani *et al*., 2013). During metabolism, some PAHs are excreted out of the body and this process is usually referred to as a detoxification process (Yu, 2002). However, some PAHs will generate reactive intermediates which are capable of forming covalent adducts with the DNA leading to mutagenicity and tumorigenicity (Yu, 2002).

2.4   Sources and emission of polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons are produced mainly from natural sources and anthropogenic activities (Jiao *et al*., 2013; Oishi, 2013; Godzik *et al*., 2014). Some of the natural sources include petrogenic, accidental burning (forest and grass fires), volcanic activity and biogenic sources (Okere & Semple, 2012; Stogiannidis & Laane, 2015). Anthropogenic sources of PAHs can be classified into three groups: mobile emissions, stationary sources (domestic and industrial sources) and agricultural activities (Byeong-Kyu and Van, 2010).

2.4.1   Natural sources

Polycyclic aromatic hydrocarbons can enter the environment through natural processes that cannot be controlled by humans, for instance, forest and grass fires which may be initiated by lightning strikes (Byeong-Kyu & Van, 2010). Petrogenic PAHs involve the transformation of organic matter into petroleum and other fossil fuels such as coal (Saber *et al*. 2006). They are formed at low temperatures (100-300 ^{o}C) and over a long period of time (Stogiannidis & Laane, 2015). Oil seeps also contribute to the level of PAHs in the environment (He *et al*., 2014; Wickliffe *et al*., 2014). Recent global estimates of crude-oil seepage rates suggest that about 47% of crude oil currently entering the marine environment is from natural seeps, while the remaining 53% result from leaks and spills during the extraction, refining, transportation, storage, and utilization of petroleum by humans (Kvenvolden & Cooper, 2003).

Although they constitute a minor proportion of total sources, biogenic PAHs are produced by chlorophyllous plants, bacteria, and fungi (Bakhtiari *et al*., 2009). Some PAHs such as

benzo(b)fluoranthene, naphthalene and phenanthrene can originate from termite activity or vascular land plants (Bakhtiari *et al*., 2009; Tobiszewski & Namiesnik, 2012). Benzo(a)pyrene can be biosynthesized by certain bacteria and plants (Peters *et al*., 2005).

2.4.2   Stationary sources (domestic and industrial sources)

2.4.2.1   Domestic sources

Domestic sources of PAHs include the incomplete/complete burning of oil, coal, gas, wood, charbroiled foods and other organic substances such as tobacco and char broiled meat (Mao *et al*., 2016). The cooking and heating processes are the main domestic sources of PAHs (Byeong-Kyu & Van, 2010) while the burning of wood to warm houses or residences is considered as the largest source of PAHs in the United States (ATSDR, 2009). Liu *et al*. (2009) found that PAH emissions from cooking account for approximately 32.8% of total indoor emission. In addition, PAHs released from cigarette smoke is a principal source of PAHs in indoor environments (Hoh *et al*., 2012). Several studies have shown that the amount of PAHs in the indoor air of smoke residences tend to be higher than those of non-smoking residences (Byeong-Kyu & Van, 2010). Furthermore, a report by the World Health Organization (2002) revealed that more than 75% of people living in India, China, and South East Asia cook daily using heat sources involving the burning of solid fuels such as wood, while 50-75% of people in parts of Africa and South America cook daily using the same medium. This high proportion of wood burning users contributes greatly to the presence of PAHs in the environment (Bari *et al*., 2010). Kim *et al*. (1999) investigated the total emission factors (EFs) from a solid fuel such as wood fuel, charcoals, and coal briquettes in a stove for domestic combustion. They found that the burning of wood as fuel released the highest emissions with a total of 18 PAHs, where 11 out of the 18 PAHs are genotoxic, while in terms of emission factors (EFs), charcoal released the lowest amount of PAHs.

2.4.2.2 Industrial sources

Polycyclic aromatic hydrocarbons are released via emissions during petroleum cracking and refining and during chemical manufacturing in industrial activities, such as petrochemical industries (Orecchio, 2010; Xiao *et al*., 2014; Singh *et al*., 2016). Other

industrial sources include aluminum production facilities, cement manufacturing, coke production (as part of iron and steel production), creosote, wood preservation, municipal incinerators, rubber tire power generation, waste water treatment plant, asphalt and bitumen industries. The release of PAHs from industrial processes may be from the burning of fuels such as gas, oil, and coal (Byeong-Kyu & Van, 2010). Several studies have reported the presence of PAHs in sewage sludge which frequently occurs at very high concentration levels (Sanchez-Trujillo *et al*., 2014; Hung *et al*., 2015). The study conducted by Li *et al*. (2008) in Zhejiang province of China shows that PAHs have become one of the principal pollutants in the sludge of waste water treatment plants. The authors reported that levels of the sum of nine PAHs found in the sludge (13.87 mg/kg to 61.86 mg/kg dry weight) were above the value recommended by the European Union.

2.4.3   Mobile Sources

In urban areas, mobile sources account for the major release points of PAH emissions (Tobiszewski *et al*., 2012). These organic pollutants are emitted from automobiles (vehicular exhaust fumes), such as cars, railways, ships, aircrafts, and other motorized vehicles. According to Ravindra *et al*. (2008), PAH emissions during rail transportation were caused by the use of diesel and electric locomotives. Jiao *et al*. (2013) reported that the PAH concentration in soils at roadsides of heavy traffic (726-1026 ng/g) were significantly higher than those at roadsides of light traffic (340-538 ng/g). The emissions of PAHs from the exhausts of motor vehicles were associated with the use of fossil fuel such as petrol, coal, diesel, gasoline and lubricant oil. Moreover, the type of fuel used in motor vehicle engines significantly determines the type of PAHs that will be released and their toxicity. He *et al*. (2010) indicated that diesel engines using diesel/biodiesel and their blends can greatly reduce total emissions of PAHs by 19.4 and 13.1%, respectively. Abrantes *et al*. (2009) reported that the total emission factor of PAHs from light-duty vehicles using ethanol fuel ranged from 11.7 to 27.4 µg/km unlike those vehicles using gasohol (41.9 to 612 µg/km). Furthermore, vehicular emission was found to be the major source of PAHs in Malaysia because it contributed to about 82% of PAHs in the atmosphere (Azhari *et al*., 2011). The authors found that In Kuala Lumpur cities, vehicular emission was also the dominant source of PAHs in the atmosphere.

2.4.4   Agricultural sources

Polycyclic aromatic hydrocarbons emissions from agricultural sources are produced during open burning of brushwood, straw, moorland and heather, and stubble (Agarwal *et al*., 2009; Guo *et al*., 2011). In many industrialized countries, the application of sewage sludge to agricultural land is a major means for disposal of wastes (Kelessidis *et al*., 2012). Applying sewage sludge to land will generally increase the PAH content of agricultural soils. In many of the European Community (EC) member states, agricultural land receives over 30% of the total sludge produced. Therefore, there is a potential accumulation of PAHs in these soils. In the UK, approximately 70% of sludge produced is deposited on land for an economic reason (Chale-Matsau, 2005). In the US the use of sludge as a soil amendment is widely practiced where more than 60% of the 6.2 million dry metric tons of sludge produced annually are applied to land (Harrison *et al*., 2006). In the EU, the disposal of sludge by land filling accounted for 40% of the waste, followed by spreading on land which accounted for 37% of the sludge produced (Christensen *et al*., 2004). In 1990, the sewage sludge production in South Africa was estimated to be 318 million tons per year (Wade *et al*., 2005). Thus, the application of sludge for agricultural purposes and disposal contributes immensely to the presence of PAHs in the soil.

2.5   Source identification of polycyclic aromatic hydrocarbon

Several PAH ratios of selected compounds have been used as a tool for identifying and assessing pollution emission sources in the environment (Tobiszewski *et al*., 2012; El-Mubarak *et al*., 2014; Katsoyiannis *et al*., 2014). These ratios distinguish PAH emission originating from biomass (coal burning), petroleum products, and petroleum combustion. To differentiate the pyrogenic and petrogenic sources the low molecular weight PAHs (LPAHs) to the high molecular weight PAHs (HPAHs) ratio (ΣLPAHs/ΣHPAHs) has been successfully used in many studies (Orecchio, 2010; Xing, 2011; Tsibart and Gennadiev, 2013; Jamhari *et al*., 2014). The ratio of ΣLPAHs/ΣHPAHs < 1 is an indication of pyrogenic source whereas, the ratio of ΣLPAHs/ΣHPAHs > 1 is an indication of petrogenic sources. Furthermore, besides the ratio of ΣLMPAHs/ΣHMPAHs, some typical PAH isomeric ratios such as Fluo/Pyr, Phe/Ant, BaP/Bpe, BaA/(BaA + Chry), An/(Phe + Ant) and Fluo/(Fluo + Pyr) have also been widely used to identify the pyrogenic and petrogenic sources of PAHs (Chen *et al*., 2012; Tobiszewski *et al*., 2012; Yim *et al*.,

2014). The ratio anthracene to anthracene plus phenanthrene Ant/(Ant + Phe) lower than 0.1 implies a petrogenic source while the ratio higher than 0.1 indicates fuel combustion source (Zhao *et al*., 2014). A fluoranthene to fluoranthene plus pyrene, Fluo/(Fluo + Pyr) ratio lower than 0.4 indicates a petrogenic source. A ratio higher or equal to 0.4-0.5 indicates pyrogenic sources (Ekpo *et al*., 2012). When the Fluo/(Fluo + Pyr) ratio is higher than 0.5, it indicates biomass and coal sources (Yim *et al*., 2014). A benzo(a)anthracene to benzo(a)anthracene plus chrysene BaA/(BaA + Chry) ratio from 0.20 to 0.35 is considered as either petroleum or combustion sources. A ratio higher than 0.35 originates from combustion sources while a ratio lower than 0.2 implies petrogenic sources (Chen *et al*., 2012; Qinal *et al*., 2012).

Indeno(1,2,3-cd)pyrene to indeno(1,2,3-cd)pyrene plus benzo(g,h,i)perylene (IP/IP + Bpe) ratios lower than 0.20 indicate a petroleum source. A ratio between 0.20 and 0.50 indicates a source from liquid fossil fuel (vehicle and crude oil) combustion while the ratio higher than 0.50 indicates the PAHs originate from grass, wood and coal combustion (Tobiszewski *et al*., 2012). When the ratio of benzo(a)pyrene to benzo(a)pyrene plus chrysene BaP/(BaP + chry) falls between 0.07-0.24 it implies biomass and coal sources (Ekpo *et al*., 2012). A value of 0.49 indicates gasoline source and 0.73 is attributed to diesel source for the ratio of benzo(a)pyrene to benzo(a)pyrene plus chrysene BaP/(BaP + chry) (Rajput & Lakhani, 2012).

2.6   Distribution and pathway of polycyclic aromatic hydrocarbons in different environmental matrices

Polycyclic aromatic hydrocarbons are redistributed between the gaseous and particle phases in the atmosphere (Klilare *et al*., 2014). Subsequently, they are deposited on solid surfaces through dry deposition sticking to the ground, buildings, trees, cars or through wet deposition (acid rain, fog, and snow) (Guo *et al*., 2007; Harmens *et al*., 2013). They can also be found in drinking water and/or sediments. However, the airborne PAHs can be transported over long distances before deposition (Klilare *et al*., 2014). The main pathway for the accumulation of PAHs onto vegetation and soil is through gaseous deposition. PAHs can also be adsorbed into plants through roots from soil and water uptake (Sun, 2010; Wick *et al*., 2011).

2.6.1   Polycyclic aromatic hydrocarbons in soil

In recent years, concern about PAH contamination in soils has increased considerably (Villar *et al*., 2006; Marti *et al*., 2008 ; Indeka *et al*., 2009; Banger *et al*., 2010; Bortey-Sam *et al*., 2014). Only a few studies have been conducted to determine the levels, presence and possible sources of persistent organic pollutants, including PAHs in South Africa, despite the fact that it is one of the largest economies in Africa (Das *et al*., 2008; Nieuwoudt *et al*., 2011; Chimuka *et al*., 2015). A study conducted in South Africa reported that the total concentration of PAHs in soil ranged from 44 to 39 000 ng/g and the results were similar to those reported in other countries (Nieuwoudt *et al*., 2011). In 2012, Okedeyi reported that the total PAH concentration in soil in the vicinity of coal power plants in South Africa ranged from 9.73 to 61.24 µg/g. The author found that the concentrations of PAHs were above the recommended value by the Agency for toxic Substances and Disease Registry.

Several studies have revealed that petrochemical industries are the major hot spots for the release of PAHs into the environment (Marti *et al*., 2008; Liu *et al*., 2010; Abdollahi *et al*., 2013). Literature also showed that soils in the vicinity of heavy transportation, residential and industrial activities often contain high concentrations of PAHs (Abbas & Barck, 2005; Ping *et al*., 2007; Karaca, 2016). The study conducted by Karaca (2016) reported that the total PAH concentration ranged between 8 and 4970 ng/g in soil from Bursa, Turkey. The author highlighted that that highest PAH concentrations were measured in soil samples taken from traffic, residential, and industrial areas.

The concentration levels of PAHs in soils have increased in the past 100-150 years because of growing industrial activities (Wick *et al*., 2011; Al-Dabbas *et al*., 2015). A Canadian soil survey revealed that the concentration of PAHs near industrial areas was up to 1000 µg/g and those measured in unpolluted areas were between 0.005 and 0.1 µg/g. In addition, the concentration of PAHs measured in soil near high traffic ranged from 2 to 5 µg/g (WHO/IPCS, 1998; Zeng *et al*., 2008). Other studies also showed that the concentration of PAHs in urban industrial soils can be 10-100 times higher than those in isolated soils (Wild & Jones, 1995). Peng *et al*. (2016) investigated the sources and distribution of PAHs in the soils of Beijing. Their paper highlighted that the total PAH concentration in suburban soil was higher compared to the rural soil (321.8 and 219.2 5 ng/g, respectively). The highest concentration of PAHs in soil is believed to result mainly

from chemical industries and residential locations (Abdel-shafy *et al*., 2015). In 2002, a monitoring study carried out in Spain to assess the impact of the release of PAHs from petrochemical and chemical companies on the environment and human health was investigated. The study has reported that residential and chemical manufacturing areas showed the highest mean values of PAHs (146.4 and 113.9 ng/kg, respectively), while the petrochemical and unpolluted zones showed the lowest mean values of PAHs levels (37.5 and 84.3 ng/kg, respectively) (Nadal *et al*., 2004). Another study conducted in Spain showed that industrial and urban sites contain the highest mean contamination of PAHs with the least amount in the soil of rural areas (465, 433 and 233 ng/g, respectively) (Ratola *et al*., 2010). Furthermore, a study conducted around a petrochemical plant in Płock (Spain) showed that although the refinery has reduced the level of its hydrocarbon emission, it still remains an emission source of PAHs into the soil (Indeka *et al*., 2009).

2.6.2   Polycyclic aromatic hydrocarbons in plants

Polycyclic aromatic hydrocarbons accumulate in plants mainly via atmospheric deposition and can also be taken up by the root system when pollutants are deposited into the soil (Wick *et al*., 2011). Consequently, they can be translocated to other plant parts such as developing shoots or above ground foliage (Calderon-Preciado *et al*., 2012). In addition, low molecular PAHs tend to be absorbed more readily compared to high molecular PAHs (Augusto *et al*., 2013).

Pozo *et al*. (2012) reported that the level of PAHs in the vegetation of urban areas was 10 times higher than in rural areas. Literature has reported that vegetables that grow in polluted areas may contain up to one hundred times higher levels of total PAHs than those growing in unpolluted environments (Cazier *et al*., 2016). Bakker *et al*. (2000) investigated the level of PAHs in the leaves of two different plant species, Plantago major and grass leaves, in the vicinity of an oil refinery in Belgium. The authors found that Plantago major leaves had the highest PAH compared to grass leaves (300.8 and 2 μg/g, respectively). The difference in PAH concentration was related to the characteristic of the plant such as the age of the leaves, leaf area and aerodynamic surface roughness. Furthermore, Lin *et al*. (2006) found that the PAH concentration in plants are generally greater in above-ground parts of vegetables than in the underground parts. The author reported that the concentration of PAHs in above-ground plants were not in any way related to levels of

PAHs in the soil due to variation in the atmospheric deposition and subsequent adsorption. However, the study conducted by Sun and Zhou (2016) showed a significantly positive correlation between benzo(a)pyrene contents in leaves from the Tagetes patula and Mirabilis jalapa plants and soil benzo(a)pyrene concentrations.

The use of tree leaves has been suggested by many authors as indicators to evaluate the atmospheric PAH levels in urban areas (Rodriguez *et al*., 2012; Nicola *et al*., 2016). They found that such bio-monitoring was efficient and cost effective because it is easier to collect leaves than air samples. Environmental conditions can also influence the rate of accumulation of PAHs on leaf surfaces. According to Ray *et al*. (2012) when the ambient temperature is low during the winter season, gaseous PAHs partition into vegetation while during the summer season, some PAHs volatilize back into the atmosphere.

2.6.3   Polycyclic aromatic hydrocarbons in air

The atmosphere can be contaminated by PAHs through the process of combustion such as air from volcanoes, forest fires, residential wood burning, and exhaust from automobiles. Some PAH particles can evaporate into the air from soil or surface waters. In 2004, approximately 530000 tons of 16 EPA PAHs were emitted into the atmosphere worldwide. However, China had the highest emission with about 114000 tons, followed by India with about 90000 tons, and the United States with about 32000 tons (Zhang *et al*., 2009). In 2010, about 191.5 tons of four PAHs namely benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene were emitted into the atmosphere in Germany. Approximately 93% of these emissions are produced from small and medium-size combustion units in households and businesses, 5% are produced from industrial processes and the remaining proportion which is less than 1% is produced from large combustion plants and traffic (German Federal Environment Agency, 2012). Many studies have also reported that the levels of PAHs in the urban air were 10 times greater than those found in rural areas (WHO, 2000). This fact was confirmed by the background levels of 16 priority PAHs found in the ambient air according to the toxicological profile of the Agency for Toxic Substances and Disease Registry. In this report, ambient air contained 0.02-1.2 ng/m^3 of PAHs in rural areas and 0.15-19.3 ng/m^3 of PAHs in urban areas (ATSDR, 2009).

2.6.4   Polycyclic aromatic hydrocarbons in water

PAHs enter water bodies through atmospheric deposition and the direct release of substances through petroleum spills, municipal wastewater treatment plants, industrial discharges, storm water runoff, landfill leachate and surface runoff (Langenkamp *et al*., 2001). According to National Primary Drinking Water Regulations, there are two major sources of PAHs in drinking water. Contamination of raw water supplies may be due to natural and man-made sources, leachate from coal tar and asphalt linings in water storage tanks and distribution lines. Most PAHs tends to be adsorbed by particulate matter due to their low solubility in water. For this reason, they are usually found in low concentrations in water bodies. A few studies conducted in South Africa have reported on the level of PAHs in the aquatic environment. For instance, Edokpayi *et al*. (2016) found that the total PAH concentrations in water collected from wastewater effluent in Vhembe district, South Africa ranged from 13.174 to 26.382 mg/L. Similarly, Nekhavhambe *et al*. (2014) reported that the total levels of PAHs in river and surface run-off collected in Limpopo province, South Africa, ranged from 29.2 to 3064.8 µg/L.

2.6.5   Polycyclic aromatic hydrocarbons in food

The major sources of PAHs include smoking, drying and cooking food processing which involved high temperatures (Essumang *et al*., 2012; Aaslying *et al*., 2013; Nisha *et al*., 2015; Singh *et al*., 2016). In barbecued meat, 130 µg/kg of PAHs has been reported (Standing Committee on Foodstuffs, 2001). In addition, fish and fishery products can be contaminated with PAH due to environmental pollution which is caused by crude oil accident seeps. Contamination of vegetable oil with PAH usually occurs during technological processes especially in direct fire drying where combustion products may come into contact with the oil seeds or oil (SCF, 2001; Jing Chen *et al*., 2014; Jiang and al., 2015). The study conducted by Olatunji *et al*. (2014) in Cape Town, South Africa showed that the concentration of benzo(a)pyrene, benzo(k)fluoranthene,   indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene in smoked meat was higher compared to those found in grilled and boiled meat samples. Similarly, the study conducted in Ghana by Essumang *et al*. (2012) highlighted the danger of consuming smoked fish. The authors found a high accumulation of PAHs in smoked fish compared to non-smoked fish.

2.7   Factors influencing the spatial distribution pattern of PAHs in soil

The distribution and fate of PAHs in soil can be influenced by factors such as soil type, soil organic matter, soil pH, distance to emission source and meteorological conditions (temperature, rainfall, wind direction, wind speed, atmospheric pressure, relative humidity) and population (Davie-Martin *et al*., 2015; Marques *et al*., 2016).

2.7.1   Effect of soil organic matter and potential hydrogen on the concentrations of PAHs in soil

The relationship between the PAH concentration and soil organic matter (SOM) had been reported by various authors (He *et al.,* 2010; Wang *et al*., 2013; Wang *et al*., 2010; Xiao *et al*., 2014). According to Obrist *et al*. (2015), PAHs accumulate mainly in the organic layers of soils. Based on the data presented by Tahir *et al*. (2014) and Xiao *et al*. (2014) the distribution of PAHs in soil was found to correlate positively with soil organic matter content ($p < 0.01$). For instance, it was reported that peat, which is a partially carbonized form of vegetable matter, was often used as fertilizer and forest soils, causing a higher PAH in the soil which is not agricultural soils (Wild and Jones, 1993). Based on the study conducted by Wang *et al*. (2012), the concentration of individual and total PAHs in soils were positively correlated with SOM content in the North China Plain. Similarly, Zhang *et al*. (2006) reported that the value of SOM varied greatly among 49 soil samples collected in the Guiyu area of China. The SOM from those soil samples ranged from 3.7% to 14.3% with a mean of 7.8%. From the results, a positive correlation was found between the individual and the total PAH concentrations in soil except for naphthalene and acenaphthylene. In other environmental studies, the same trends have been observed. These findings indicated that SOM content was an important factor governing the concentration of PAHs in soils. This can be explained by the fact that organic matter is a dominant sorption medium for organic compounds in soils. However, other studies reported a poor correlation between PAH concentrations and organic carbon (Heywood *et al*., 2006; Oen *et al*., 2006; Zhang *et al*., 2006; Cai *et al*., 2007; Wang *et al*., 2007).

In the study conducted by Jiao *et al*. (2013), it was observed that no significant correlation existed between soil pH and ΣPAHs. Similarly, Peng *et al*. (2011) reported that the pH value in the soils of urban green space ranged from 7.19 to 8.31. The authors found that

PAH mobility and accumulation in the soil were not affected by the soil pH. However, others studies have shown some correlation. It was found that the level of PAHs in a very acidic soil was higher compared to a non-acidic soil. This could be attributed to the fact that acidic soils are unfavorable for the development of soil microorganisms which are essential for PAH biodegradation (Soti *et al*., 2015).

2.7.2 Distance to emission source

Previous studies have shown a positive correlation between the distance to emission source and the spatial distribution of PAHs in the soil. It was found that the concentration of PAHs in the surface soil were decreasing with increasing distance from the industrial areas, with the remote areas showing the lowest levels (Rodriguez *et al*., 2012). Indeka *et al*. (2009) reported that PAHs tend to decrease in concentration as they move further from their initial point source. The authors also reported that the concentration of PAHs in the soils sampled from points nearest to the plant (1 km and 3 km) were higher than those at a longer distance (6 km, 12 km, 18 km).

2.7.3 Meteorological conditions

Numerous studies have shown a relationship between PAH concentrations in the soil and seasonal variation (Nouia *et al*., 2013; Zang *et al*., 2013; Hussain *et al*., 2015). Li *et al*. (2013) reported that PAH levels in winter samples were higher than summer samples. The authors also found that PAH concentrations in winter varied from 9176 to 63573 ng/g whereas, PAH concentrations in summer varied from 6875 to 27766 ng/g.

The seasonal variation may be attributed to different meteorological parameters such as temperature, relative humidity and wind (speed and direction). Such meteorological parameters may control volatilization, degradation, and volatilization of pollutants (Li *et al*., 2013). In the study conducted in China, factors such as higher temperature, photolysis, and radical degradation have been noted to contribute to the lesser concentration of PAHs in the soil during summer (Wang *et al*., 2010). Wind speed and direction significantly influence the diffusion of PAHs in the atmosphere. In addition, they have been found to affect the distribution and accumulation of PAHs in a terrestrial environment (soil, water,

plant). The study conducted by Nam *et al*. (2003) showed that the distribution of PAHs was significantly affected by the wind direction. They also found that the prevailing northwest wind was the main factor in the dispersal of PAHs in the agricultural soils in Seoul (Korea).

2.8   Route of exposure to polycyclic aromatic hydrocarbons

Humans, animals, and other biological species can be exposed to PAHs through ingestion, inhalation, and dermal contact (Kim *et al*., 2013; Domingo *et al*., 2015; Yu *et al*., 2015; White *et al*., 2016). The major route of exposure to PAHs in the general population is through breathing ambient and indoor air (Yu *et al*., 2015). Polycyclic aromatic hydrocarbons concentrations in air can vary from less than 5 to 200000 ng/m^3 (ATSDR, 2009). Inhalation of the compounds in tobacco smoke is the primary route of exposure to PAHs in the majority of the US population. Smoking one cigarette can result in an intake of 20-40 ng of benzo(a)pyrene (Rengarajan *et al*., 2015). In addition, it was reported that smoking one pack of unfiltered cigarettes per day leads to exposure of 0.7 µg/day of benzo(a)pyrene, and smoking a pack of filtered cigarettes per day leads to the release of 0.4 µg/day (Sullivan and Krieger, 2010). Drying and cooking methods which involved high temperatures such as grilling, roasting, charcoal-broiled foods and frying are a major source generating PAHs (Jedrychowski *et al*., 2012; Lee *et al*., 2016).

In barbecued meat, a concentration of about 130 µg/kg of PAH had been reported (Standing Committee on Foodstuffs, 2001). Fish and fishery products can be contaminated with PAHs due to environmental pollution which can be caused by accidental spillage of crude oil. Furthermore, contamination of vegetable oil with PAHs usually occurs during processes like direct drying where combustion products may come into contact with the oil seeps or oil (SCF, 2001). Polycyclic aromatic hydrocarbons may be in groundwater near disposal sites where construction wastes are buried. As a result, people living in this area may be exposed by drinking this water (WHO, 2000). Polycyclic aromatic hydrocarbons can also be absorbed through the skin by handling contaminated soil and bathing in contaminated water. Polycyclic aromatic hydrocarbons and their metabolites are reportedly found in breast milk since they move through the placenta (Crepeaux *et al*., 2014). Figure 2.5 illustrates the diverse exposure pathways of PAHs in terrestrial and biological systems.
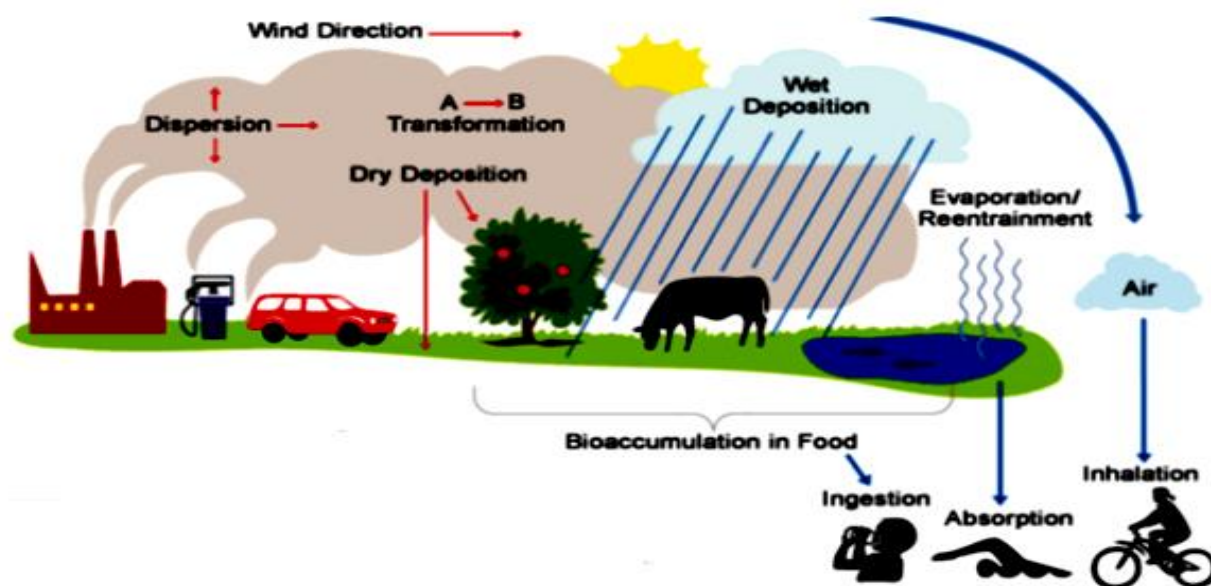
Figure 2.4: Pathways of terrestrial and biological system exposure to PAHs

(Adapted from http://www.in.gov/idem/risk/)

2.9   Health effects

Harmful effects of PAHs depend on numerous factors such as the route of exposure, the duration of exposure, the dose, immune state of heath, personal habits (nutritional status, lifestyle), gender and age (ATSDR, 2009; Kim *et al*., 2013). Exposure to PAHs may result in short-term side effects such as vomiting, eye irritation, nausea, diarrhea and confusion (ATSDR, 2009). Some PAHs such as benzo(a)pyrene, anthracene and naphthalene were found to be skin sensitizers because they cause an allergic skin response in animals and humans (ATSDR, 2009; Cross *et al*., 2010). The long-term health effects of PAHs include cancer, cataracts, kidney damage, liver damage, skin inflammation, breathing problems and lung function abnormalities. Naphthalene has been found to cause the breakdown of red blood cells if inhaled or ingested in large amounts (ATSDR, 2009). Table 2.3 summarizes the effects of some PAHs on humans.

Table 2.3: Effects of selected polycyclic Aromatics Hydrocarbons on humans

PAHs	Effects
Anthracene	Toxic, skin sensitizer, eye irritation, nausea, vomiting, diarrhea, and confusion.
Acenaphthylene	Eye irritation, Toxic, Carcinogenic, heart malformations, childhood
Benzo(a)anthracene	Asthma, skin irritations.
Benzo(a)fluoranthene	Toxic
Pyrene	Toxic , eye irritation
Benzo(a)pyrene	Carcinogenic, mutagenic, birth defects, a decrease in body weight, toxic, skin irritants, malformations, childhood asthma eye irritation, nausea, vomiting, diarrhea , and confusion.
Chrysene	Toxic, Carcinogenic, kidney and liver damage and jaundice cataracts.
Benzo(k)fluoranthene	Toxic, Carcinogenic ,Tumors of the gastrointestinal tract and lungs
Benzo(j)fluoranthene	Toxic, Tumors of the breast, lungs. Toxic, Carcinogenic. Toxic, Skin
Benzo(b)fluoranthene	Irritants, Breakdown of red blood cell, heart malformations,
Naphthalene	Childhood asthma, eye irritation, nausea, vomiting, diarrhea, and confusion.
Dibenzo(a,h)anthracene	Carcinogenic, toxic, kidney and liver damage and jaundice cataracts. Increase in mammary tumors in rat kidney and liver damage and jaundice cataracts.
Indeno(1,2,3-cd)pyrene	Skin sensitizer, eye irritation, nausea, vomiting, diarrhea and confusion.

(Adapted from Okenyi *et al*., 2016)

2.9.1   Teratogenicity

Animal studies conducted on mice have shown that the ingestion of high levels of PAHs such as benzo(a)pyrene, naphthalene and benzo(a)anthracene during pregnancy resulted in birth defects and decreased body weight in the offspring (Rengarajan *et al*., 2015). Based on the Center for Children's Environmental Health reports, the exposure to PAH pollution during pregnancy might cause birth defects such as low birth weight, small head circumference, damaged DNA, low IQ, growth retardation, premature delivery, heart malformation and childhood asthma (WHO, 2000).

2.9.2   Carcinogenicity

Reactive PAH intermediates which are toxic metabolites have become one of the major health concerns (Kan *et al*., 2012; Yang *et al*., 2012; Man *et al*., 2013; Ciarrocca *et al*., 2014; Moorthy *et al*., 2015). Such products can bind to the cellular proteins and DNA (Yang *et al*., 2012). The biochemical disruption and cell damage were found to cause mutations, developmental malformations, tumors and cancer (Man *et al*., 2013). In many studies, 1-hydroxypyrene, which is a metabolite of pyrene, has been used as a biomarker for human exposure (Herbstman *et al*., 2012; Ciarrocca *et al*., 2014).

Polycyclic aromatic hydrocarbons are reported to be probable carcinogens for human and animals (ATSDR, 2009). Animals studies involving individual PAHs mostly using benzo(a)pyrene have also shown carcinogenic effects. In laboratory studies, animals exposed to some PAHs over long periods have developed some forms of lung cancer from inhalation, stomach cancer from ingesting and skin cancer from skin contact (Sforzini *et al*., 2015). Studies conducted on workers exposed to a substantial amount of mixtures of PAHs during coke production, oil refining, and bituminous products have shown an increase in skin and lung cancers and also bladder and gastro-intestinal cancers (Li *et al*., 2013; Jongeneelen *et al*., 2014).

US department of Health and Human Services (DHHS) reported that benzo(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene are known animal carcinogens. The International Agency for Research on Cancer (IARC) also reported that benzo(a)anthracene and benzo(a)pyrene probably have carcinogenic properties and identified benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene and indeno(1,2,3-cd)pyrene as possible human carcinogens. In the same view, the US Environmental Protection Agency listed benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene as probably carcinogenic to humans. The classification of the priority hazardous PAHs reported in the literature is presented in Table 2.4.

Table 2.4: Classification of PAHs by EPA, IARC, and DHHS in terms of their carcinogenicity

PAHs	EPA	IARC	DHHS
Acenaphthylene	Not classifiable	Not classifiable	Not classifiable
Acenaphthene	Not classifiable	Not classifiable	Not classifiable
Fluorene	Not classifiable	Not classifiable	Not classifiable
Phenanthrene	Not classifiable	Not classifiable	Not classifiable
Anthracene	Not classifiable	Not classifiable	Not classifiable
Fluoranthene	Not classifiable	Not classifiable	Not classifiable
Pyrene	Not classifiable	Not classifiable	Not classifiable
Benz(a)anthracene	Probable human carcinogen	Probable human carcinogen	Animal carcinogen
Chrysene	Not classifiable	Not classifiable	Not classifiable
Benzo(b)fluoranthene	Probable human carcinogen	Probable human carcinogen	Animal carcinogen
Benzo(k)fluoranthene	Probable human carcinogen	Probable human carcinogen	Not classifiable
Benzo(a)pyrene	Probable human carcinogen	Probable human carcinogen	Animal carcinogen
Benzo(ghi)perylene	Not classifiable	Not classifiable	Not classifiable
Indeno(1,2,3-cd)pyrene	Probable human carcinogen	Probable human carcinogen	Animal carcinogen
Dibenz(a,h)anthracene	Probable human carcinogen	Not classifiable	Animal carcinogen
Benzo(e)pyrene	Not classifiable	Not classifiable	Not classifiable
Benzo(j)fluoranthene	Not classifiable	Probable human carcinogen	Probable human carcinogen

(Adapted from ASTDR, 2009)

2.10   Legislations and regulations

There are various regulations that stipulate the allowed limit of PAHs in specific products and in the environment. These standards have been set by several organizations such as World Health Organization (WHO), US Environmental Protection Agency (USEPA), Food and Agricultural Organization (FAO), International Agency for Research on Cancer (IARC) and European Union (EU). However, regulations on the acceptable levels of PAHs in different environmental matrices in South Africa have not yet been established. Only the guideline value for BaP in the soil for residential and informal residential (0.71 and 0.34

mg/kg, respectively) has been set by the Department of Environmental Affairs, South Africa (DEA, 2010). In the Netherlands, the maximum permissible concentration (MPCs) for PAHs in the soil is 0.26, 7.50, 2.40, 10.70, 0.25 and 0.51 mg/kg for BaP, BghiP, BkF, Chr, BaA and Fluo, respectively. The US Environmental Protection Agency (2011) regulatory limits for the concentration of carcinogenic or mutagenic polycyclic aromatic hydrocarbons in the soil are 0.15; 0.01; 0.15; 1.50; 0.15, 0.01 and 0.15 mg/kg for BaA, BaP, BbF, BkF, Chr, DahA and IPyr, respectively.

2.11   Review of analytical methods

2.11.1   Extraction recovery from different environmental matrices

Extraction protocols are used to transfer the sorbed PAHs from the solid phase into organic solvents (Lau *et al*., 2010). There are several types of extraction methods that can be used to extract PAHs from different environmental matrices. USEPA recommends a number of different methods for the extraction of organic compounds from liquid or solid matrices. Those methods include soxhlet extraction, which is one of the early methods of PAH extraction. The soxhlet extraction method is reported to be efficient for solid samples and a relatively inexpensive technique and filtration of the extract is not required. However, this method is limited by a time duration which is often long (18-24 hours). In addition, soxhlet extraction requires large volumes of solvent and evaporation of the solvent after extraction is mandatory (Frenna *et al*., 2013).

Liquid-liquid extraction technique is excellent for samples containing particulates (of up to 1% solids) that cause emulsion. In addition, it provides more efficient extraction of analytes that prove difficult to extract, with either soxhlet or sonication extraction (Vazquez-Roig *et al*., 2015). Solid-phase extraction (SPE) is relatively fast and uses small volumes of solvent. However, it is difficult to filter resulting mixtures, thus reducing the extraction efficiency (Kanchanamayoon & Tatrahun, 2008). Recently, supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and accelerated solvent extraction (ASE) have been introduced and they have been reported to be very efficient for the extraction of PAHs. Pressurized Fluid Extraction (PFE) is equivalent to Soxhlet extraction although the technique uses less solvent and is much faster. The disadvantage of this method is that it requires the use of expensive apparatus (USEPA, 2003). In MAE, organic

solvents or a mixture of solvents used for the extraction of an organic compound and the sample are irradiated by microwaves. The MAE has more advantages because it requires a short extraction time, reduced solvent use and simple instrumentation. In addition, MAE can be utilized at a higher temperature and the loss of volatile substances during microwave irradiation is also avoided. However, the solvent needs to be physically removed from the sample matrix prior to analysis which is the main limitation. The high pressure used in MAE poses safety (explosion) risks and analyte clean-up is required (Routray *et al*., 2012; Zuloaga *et al*., 2012).

2.11.2 Clean-up method

Sample clean-up is performed to remove co-extracted compounds that could interfere with results of the subsequent analysis (Wick *et al*., 2011). In addition, the clean-up is done to separate different classes of analytes prior to analysis. Table 2.5 lists some of the available clean-up methods and provide a brief description of the type of clean-up procedure recommended by the EPA method.

Table 2.5: Some selected methods used for clean-up of polycyclic aromatic hydrocarbons

Method No.	Method Name	Cleanup Type
3610	Alumina Cleanup	Adsorption
3620	Florisil Cleanup	Adsorption
3630	Silica Gel Cleanup	Adsorption
3640	Gel-Permeation Cleanup (GPC)	Size-Separation
3650	Acid-Base Partition Cleanup	Acid-Base Partitioning
3660	Sulfur Cleanup	Oxidation/Reduction
3665	Sulfuric Acid/Permanganate Cleanup	Oxidation/Reduction

(Adapted from https://www.epa.gov/sites/production/files/2015-12/.../3600c.pdf)

2.11.3 Instrumental analysis

Instrumental analysis is performed to separate, identify and quantify individual components in the sample. Capillary gas chromatography coupled with mass spectrometry is a widely used method for the determination of PAHs (Jung *et al*., 2013; Luzardo *et al*.,

2013; Aragon *et al*., 2015). High performance liquid chromatography (HPLC) with fluorescence or UV detection can also be utilized for quantitative and qualitative determination of PAHs (Fernandez *et al*., 2015).

Capillary gas chromatography has a high resolving power, but disadvantaged in that most PAHs can be decomposed on exposure to high temperature during the separation and those isomeric PAHs are difficult to separate.The GC-MS is a preferred procedure due to its superior selectivity and sensitivity over the HPLC method (Balachandran *et al*., 2012). The HPLC method has a lower resolving power when compared to GC. However, HPLC remains an ideal means of fractionation of PAHs for subsequent analysis by spectroscopic techniques. Reversed-phase (RP) columns can be used in the HPLC system method in order to separate several PAH isomers that may be difficult to separate by the GC procedure. The application of spectrofluorimetric detectors results in greater sensitivity and selectivity (Chiu *et al*., 1997).

2.12   Summary of Chapter Two

On the whole, polycyclic aromatic hydrocarbons are organic substances that are formed and released into the environment mainly via natural and anthropogenic processes (Abdel-shafy *et al*., 2015). Over the last few decades, many countries have experienced rapid growth in population and urbanization (Khan & Ghouri, 2011; Al-Dabbas *et al*., 2015). The aforementioned factors have been shown to contribute to the increase of those toxic substances into the environment. Numerous studies have been done in developed countries with regard to PAHs in the environment, unfortunately, only a few studies have been conducted to determine the levels, presence and possible sources of persistent organic pollutants, including PAHs in South Africa despite the fact of being one of the largest economies in Africa (Nieuwoudt *et al*., 2011; Chimuka *et al*., 2015). From the literature review, exposure to PAHs occurs by means of ingestion, inhalation and thermal exposure (Choosong *et al*., 2010). For the past decade, it had been found that excessive exposure to PAHs can be harmful. Evidence of their carcinogenic, mutagenic, and immune-suppressive effects has been reported (ATSDR, 2009). Wick *et al*. (2011) have reported that PAHs can undergo a number of processes when released into the environment. The authors highlighted that PAHs can be transferred into another media via volatilization, absorption, leaching and erosion, undergo sequestration via diffusion and adsorption processes and

undergo degradation through biological and chemical processes. He *et al*. (2010), Wang *et al*. (2013) and Soti *et al*. (2015) have reported that soil properties (SOM and pH) can be the factors influencing the spatial distribution pattern of PAHs in soil. The authors have reported that there was a positive correlation between PAHs and organic matter since PAHs accumulate mainly in the organic layers of soils. On the other hand, other studies have reported a poor correlation between PAH concentrations and soil organic matter (Jiang *et al*., 2009). The study conducted by Jiao *et al*. (2013) showed that there were no significant correlations between soil pH and PAHs which contradicted the finding of Soti *et al*. (2015).

In the present study, PAH compunds in soil in the vicinity of a petrochemical plant in Cape Town was investigated. Beside the soil properties, another factor that had been found to influence the spatial distribution of PAHs in soil were the meteorological conditions. Li *et al*. (2013) reported that PAHs levels in winter soil samples were higher than in summer soil samples. One of the main objectives of the present study was to investigate the seasonal variation of PAHs in soil in the vicinity of a petrochemical plant in Cape Town**.** Over the past decade, soil samples have been used to assess the presence and level of PAHs in the environment. However, the present study has investigated the use of the Port Jackson Willow as a possible biomonitor for the presence of PAHs in soil.

CHAPTER THREE
EXPERIMENTAL AND ANALYTICAL METHODOLOGIES

3.1   Introduction

This chapter describes the experimental procedures and analytical techniques used in this study. It also provides a description of the specific procedure used for the identification and quantification of the selected PAHs in soil and leaf matrices: sample collection, sample extraction, sample clean up and finally analysis by gas chromatography (GC-FID).

3.2   Description of the study area

This study was conducted in Cape Town. Cape Town is a port city on South Africa's southern west coast. It is located at latitude 33.55 °S and longitude 18.25 °E. Cape Town has a population of about 3,750,000 people according to the 2014 census figure released by Statistics South Africa. Based on the world weather information service, Cape Town has a Mediterranean climate with a dry, warm summer and a mild, wet winter. Winter months in the city average a maximum of 18 °C and a minimum of 8 °C. On the other hand, summer months average a maximum of 26 °C and a minimum of 16 °C. The total annual rainfall in the city averages 515 millimeters. In winter the wind is generally strong and comes from the northwest while in summer it varies between southeast and southwest.

3.3   Materials and method

3.3.1   Standards and calibration solutions

The polycyclic aromatic hydrocarbons, namely benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(l,2,3-cd)pyrene, benzo(a)anthracene, chrysene, dibenzo(a,h)anthracene and fluoranthene (neats), were purchased from Sulpelco, Bellfonte, PA, USA. A 100 ppm stock solution of each of the PAH was prepared by

dissolving 0.01 g in 4:1 mixtures of high purity n-hexane and dichloromethane (Sigma-Aldrich, Germany). The stock solutions were stored in a volumetric flask at -4 °C. Working calibration solutions were subsequently prepared by serial dilution of the stock solutions in a five-point working range of 10-50 mg/L.

3.3.2   Chemicals, solvents and materials

High purity solvents including n-hexane (99.5%), dichloromethane (99.5%), acetone (99.8%) and acetonitrile (99.9%) were purchased from Sigma-Aldrich (Germany). Sodium sulphate was purchased from Merck (South Africa), silica gel and florissil were purchased from Merck (Germany). All glassware used in this work was washed with soap and soaked in a prepared nitric acid solution for 24 hours and then washed with distilled water and oven-dried, the glasswares were washed again with acetone before use. The equipments used for the determination of the PAHs in the soil and leaf samples include the following: Shimadzu GC-2010 equipped with a flame ionization detector and chemsite software. A crison GLP 21+ pH-meter (Lasec, South Africa) was used for measuring the pH of the soil samples. A MLS200S was used for the extraction of soil and leaf samples. A Centrifuge (Roto-uni 11/BHG), Shaker (Orbi-shake labotec), and a Metler Toledo PB 303 balance (Metler Toledo, Zurich, Switzerland) were also used for sample preparation.

3.3.3   Instrumentation and chromatographic conditions

A gas chromatograph (GC-2010 plus Shimadzu) equipped with a flame ionization detector (GC-FID) and a data processor was used for separation and determination of the eight PAHs. Parameters such as carrier gas flow rate, injection volume, oven temperature program parameter, flame ionization detectors temperature, column temperature, and split injection time and make-up gas flow rate, were optimized before analysis. An HP-5% Phenyl Methyl Siloxane column (30 m length and 0.32 mm ID 0.25 μm film thickness) was used for separation of the analytes. The injection volume was 1.0 μL, (Syringe size: 10.0 μL). The injector temperature was set at 270 ^{o}C and used as split mode and the split ratio was 1. The detector temperature was set at 320 ^{o}C. Helium was used as the carrier gas and makeup gas at a flow of 30 mL/min at a pressure of 200 kpa. Hydrogen flow rate was 40 mL/min; air flow rate was 400 mL/min. The column flow was 1.99 mL/min, linear

velocity was 46.8 cm/s, purge flow was 3 mL/min. The oven program was 40 ^{o}C for 5 min, 200 ^{o}C hold for 10 min. The column temperature program was 170 °C hold for 4 min, a ramp to 250 °C at 15 °C/min with 5 min hold time and a final ramp to 300 °C at 10 °C with a 5 min hold time. The total run time was 24.33 min.

3.3.4   Sample collection

Top soil and leaf samples were collected from different sampling sites around a major petrochemical industry in Cape Town. The different sampling points from where the samples were collected at each of the sampling sites were marked, and their geographical reference point observed and recorded using a GPS (model X76). The geographical coordinates of the sampling points are presented in Table 3.1.

Table 3.1: Description of the different sampling sites and their GPS coordinates

Site No	Map reference	Site No	Map reference
Site 1	S33 51.888 E18 29.886	Site 19	S33 52.572 E18 34.382
Site 2	S33 51.044 E18 29.689	Site 20	S33 51.721 E18 33.864
Site 3	S33 50.829 E18 31.752	Site 21	S33 52.187 E18 32.436
Site 4	S33 50.888 E18 31.326	Site 22	S33 51.624 E18 32.598
Site 5	S33 50.238 E18 31.507	Site 23	S33 50.906 E18 32.421
Site 6	S33 48.605 E18 32.528	Site 24	S33 50.749 E18 32.889
Site 7	S33 49.245 E18 32.326	Site 25	S33 49.936 E18 33.068
Site 8	S33 49.919 E18 31.619	Site 26	S33 49.993 E18 32.691
Site 9	S33 50.053 E18 31.680	Site 27	S33 49.318 E18 33.541
Site 10	S33 50.049 E18 31.421	Site 28	S33 49.414 E18 34.748
Site 11	S33 50.290 E18 30.640	Site 29	S33 44.879 E18 32.533
Site 12	S33 49.828 E18 30.279	Site 30	S33 45.291 E18 31.500
Site 13	S33 49.604 E18 30.978	Site 31	S33 45.930 E18 32.867
Site 14	S33 48.328 E18 31.013	Site 32	S33 46.294 E18 32.356
Site 15	S33 48.038 E18 30.436	Site 33	S33 46.466 E18 32.834
Site 16	S33 48.301 E18 29.239	Site 34	S33 49.572 E18 32.482
Site 17	S33 47.877 E18 30.143	Site 35	S33 50.662 E18 32.066
Site 18	S33 48.029 E18 31.274		

The sampling was carried out during summer (March) and during winter (August) 2014. In winter, the average temperatures recorded ranged between 7.5 ^{o}C and 17.8 ^{o}C. The average precipitation during the period was 82 mm. During summer, the average ambient atmospheric temperatures ranged between 14.2 ^{o}C to 25.4 ^{o}C, with an average precipitation of 20 mm.

3.3.4.1   Soil sample collection

Thirty-five soil samples were collected in the vicinity of a petrochemical plant in Cape Town shown in Figure 3.1.
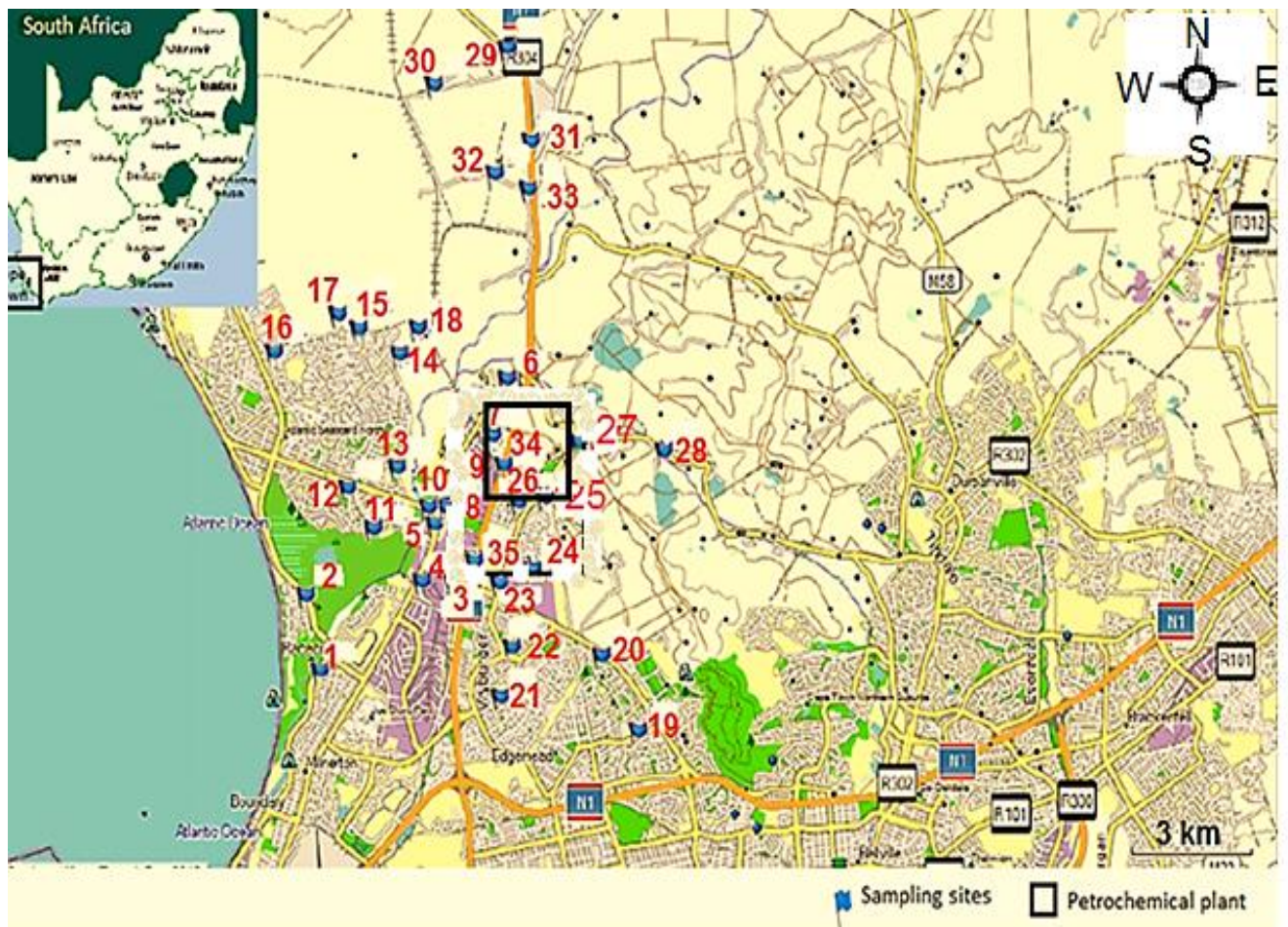
Figure 3.1: Map of the sampling sites location in the vicinity of a petrochemical plant in Cape Town

The distribution of the sampling sites was as follows: ten (10) soil samples were collected close to the road, 19 soil samples were collected in the residential area and 6 in the

industrial area. About 500 g of surface soil (0-5 cm depth) was collected with the aid of a stainless steel trowel and transferred in polyethylene bags. In the laboratory, the soil samples were dried at room temperature and sorted to remove impurities such as stones and detritus materials. The dried soil samples were crushed in an agate mortar and sieve through a 2 mm mesh for homogeneity and removal of contaminants. Finally, the soil samples were kept in aluminum foil and stored at room temperature and protected from solar light until analysis.

3.3.4.2 Leaf sample collection

The *Acacia Saligna* commonly known as the Port Jackson willow (Figure 3.2) was selected over other plant species that are found in Cape Town because of its abundance.

Figure 3.2: Picture of the Port Jackson Willow

The Port Jackson willow is a member of the Fabaceae family originating from southwest Australia. In South Africa, it is found in KwaZulu-Natal, and the Western and Eastern Cape provinces. The plant leaves were sampled in summer at the 35 sampling points. Leaf samples were selected according to size, shape, and age in order to obtain a homogeneous sample. The leaf samples were collected in a polyethylene bag. Once in the laboratory, the homogeneous leaf samples were divided into two batches. The first batch was washed using tap water and the other batch remained unwashed. The two batches were air dried at room temperature. After being dried, the leaf samples were ground, put in aluminium foil and stored at room temperature where they were protected from solar light until analysis.

3.3.5   Physico-chemical analysis

3.3.5.1   Determination of soil pH

Soil pH was determined using the ASTM D 4972 method (ASTM, 2013). Five grams of soil sample was weighed out into a 50 mL beaker, followed by the addition of 10 mL of distilled water and two drops of 1 M $CaCl_2$. The mixture was stirred in the shaker for 30 min. The mixture was then allowed to settle for 20 min. The pH of the supernatant was measured and recorded using a calibrated pH meter (Crison GLP 21+, Lasec, South Africa).

3.3.5.2   Determination of soil organic matter

The organic content was determined using the ASTM D 2974 method in which 1 g of air-dried soil was burnt in a furnace at 440 $^{\circ}$C for 24 hours (ASTM, 2000). The soil sample was reweighed after burning to obtain the mass of burnt soil. The organic matter was determined by the difference in the mass of the burnt soil from the mass of the dried soil, expressed as a percentage.

3.3.6   Sample preparation

3.3.6.1 Soil samples

For the analysis of the PAHs, the soil samples were extracted following the EPA microwave-assisted extraction method 3546. Ten grams of the soil sample was weighed in triplicate and transferred into a beaker, followed by the addition of a mixture of 5 mL DCM and 20 ml hexane. The mixtures were stirred for 2 hours in the shaker to equilibrate. The recommended extraction conditions used were: temperature: 100 ^{o}C, pressure: 150 psi, time at temperature: 20 min, cooling: to room temperature, filtering/rinsing: with same solvent system. After extraction, the extracts were centrifuged at room temperature for 20 min at 2000 rpm in order to separate the solid phase and liquid phase.

3.3.6.2 Leaf samples

Leaf samples from the different sampling sites were separated into three batches. The first batch of leaves was washed with tap water, the second batch with a polar solvent (DCM) and the last batch remained unwashed. The washing procedure was done in order to determine the distribution of the PAHs deposited on the leaves since PAHs compounds may be adsorbed to the leaf surface, in the cuticular wax or diffuse into the interior of the leaf. Five grams of dried leaf powder was weighed out into a beaker, followed by the addition of a mixture of 5 mL DCM and 20 mL hexane. The mixtures were stirred for 2 hours in a vibro shaker to equilibrate then extracted with microwave-assisted extraction.

3.3.6.3 Clean-up of the samples

It is recommended that sample extracts are cleaned prior to analysis due to the presence of a large number of contaminants which could damage the GC column. In addition, the cleanup process minimizes interference during analysis. The column was prepared as follows: Cotton was stocked to the bottom of the column and then filled with 1 g of silica gel, 0.5 g of florisil followed by another 1 g of silica gel and then topped with 0.25 g sodium sulphate anhydrous. Each column was pre-washed and activated by eluting 20 mL of 4:1 mixture of n-hexane and dichloromethane (DCM) at a flow rate of 1.0 mL/min. The concentrated extract was then loaded onto the column and eluted with about 80 mL of a

mixture of n-hexane and dichloromethane (DCM). The volume of the eluates was brought to 2 mL using a rotary evaporator under reduced pressure.

3.3.7 Statistical Analysis

Statistical analysis of the results was performed using the statistical software package SAS system version 9.1. Samples in which the PAHs were not detected (nd) were assumed to be equal one-half of the detection limit (nd $= \frac{1}{2}$ LOD). The correlation between variables was performed by the Spearman test. For spearman's correlation coefficient (r_s) values ranging between 0.00-0.19, 0.20-0.39, 0.40-0.59, 0.60-0.79 and 0.80-1.00 are interpreted as very weak correlation, weak correlation, moderate correlation, strong correlation and very strong correlation, respectively. A multivariate analysis of the results was carried out. Principal component analysis (PCA) was used to demonstrate the seasonal variation and spatial distribution in the concentration of the PAHs in soil samples as well as the relationship between the variables. The number of components extracted from the variables was determined according to Kaiser's rule, which retains only components with with Eigenvalue > 1. Statistical significance of the difference was determined by the one-way analysis of variance (ANOVA) for the data that are normally distributed and for data that are not normally distributed, the Kruskal-Wallis test was used. For all statistical tests, a significance level of 0.05 was used ($p < 0.05$).

3.3.8 Quality control and quality assurance

100 ppm stock solutions of each of the eight PAHs: benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(l,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(k)fluoranthene, fluoranthene and chrysene, were prepared in a 100 mL volumetric flask separately. 10 mg of each PAH standards were weighed out and transferred quantitatively into a 100 mL volumetric flask and dissolved in about 10 mL of 4:1 mixture of n-hexane and dichloromethane (DCM). From each stock solution 10, 20, 30, 40 and 50 mg/L calibration standard solutions were prepared. The test of reliability on PAHs concentration data was checked against spiked soil samples in recovery studies carried out in replicates. The results obtained showed procedural consistencies in selected PAH standard extraction from the spiked soils. The extraction efficiency of the selected PAHs

obtained from 20 g soil samples ranged from 93.02% to 100.04%. The values of the percentage recovery of each PAH compounds falls within the generally acceptable recovery 100 ± 5%. The limit of detection and limit (LOD) (defined as S/N > 3), of individual PAHs, were between 0.0006 and 0.0023 mg/kg.

CHAPTER FOUR

RESULTS AND DISCUSSION FOR SOIL ANALYSIS

4.1   Introduction

This chapter presents the results of the validation method and the results obtain from the soil samples collected in the vicinity of a petrochemical plant in Cape Town during winter and summer seasons.

4.2   Analytical method validation

4.2.1   Chromatograph of the standards

The chromatogram of the standard cocktail mixture of the eight selected PAHs is presented in Figure 4.1
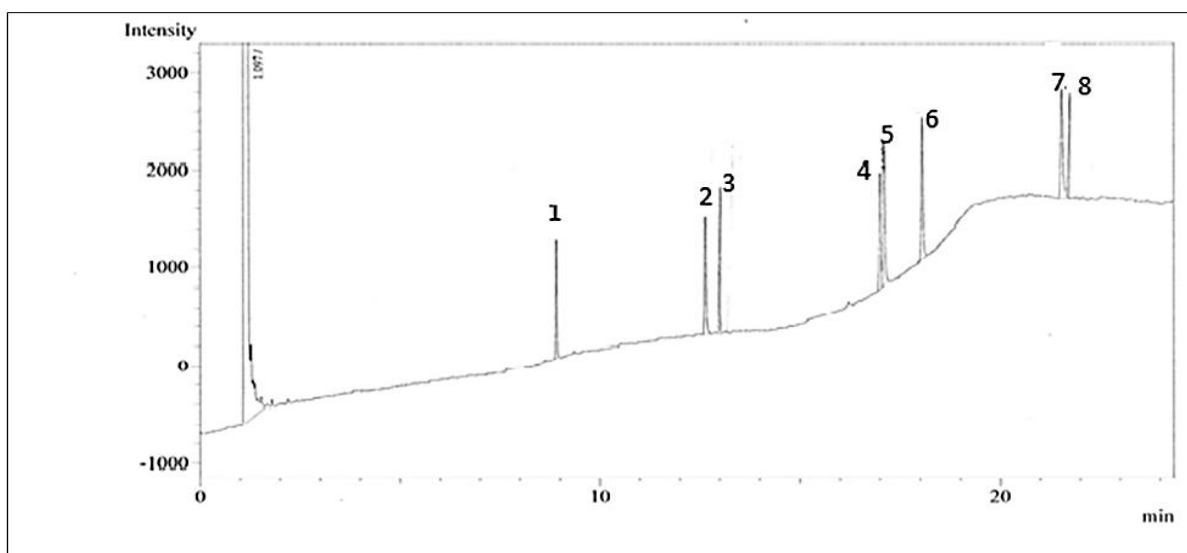
Figure 4.1: Chromatogram of the target PAHs fluoranthene (1), benzo(a)anthracene (2), chrysene (3) benzo(b)fluoranthene (4), benzo(k)fluoranthene (5), benzo(a)pyrene (6), indeno(l,2,3-cd)pyrene (7) and dibenzo(a,h)anthracene (8) using GC-FID

The chromatogram showed the elution time of the selected PAH standards. The retention times (t_R) of the PAH fraction were: 9.00 min, 12.70 min, 12.80 min, 17.10 min, 17.20 min and 18.10 min, 21.50 min and 21.60 min respectively for fluoranthene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(l,2,3-cd)pyrene and dibenzo(a,h)anthracene (Table 4.1).

Table 4.1: Retention times of the target PAHs

S / No	PAH	Retention time (t_R) (min)
1	Fluoranthene	9.00
2	Benzo(a)anthracene	12.70
3	Chrysene	12.80
4	Benzo(b)fluoranthene	17.10
5	Benzo(k)fluoranthene	17.20
6	Benzo(a)pyrene	18.10
7	Indeno(l,2,3-cd)pyrene	21.50
8	Dibenzo(a,h)anthracene	21.60

The selectivity of the method showed good separation and the peaks of the eight PAH fractions were distinct, although chrysene and benzo(a)anthracene; benzo(b)fluoranthene and benzo(k)fluoranthene; and dibenzo(a,h)anthracene and indeno(l,2,3-cd)pyrene were separated in the order of one minute.

4.2.2 Instrument Response (Calibration)

The method's linearity was evaluated by plotting the instrument response for the chromatographic peak area against the standard's concentration (Figure 4.2). The calibration standards, the target PAHs, 10, 20, 30, 40 and 50 mg/L concentrations were obtained by serial dilution from 100 ppm stock solution of each of them
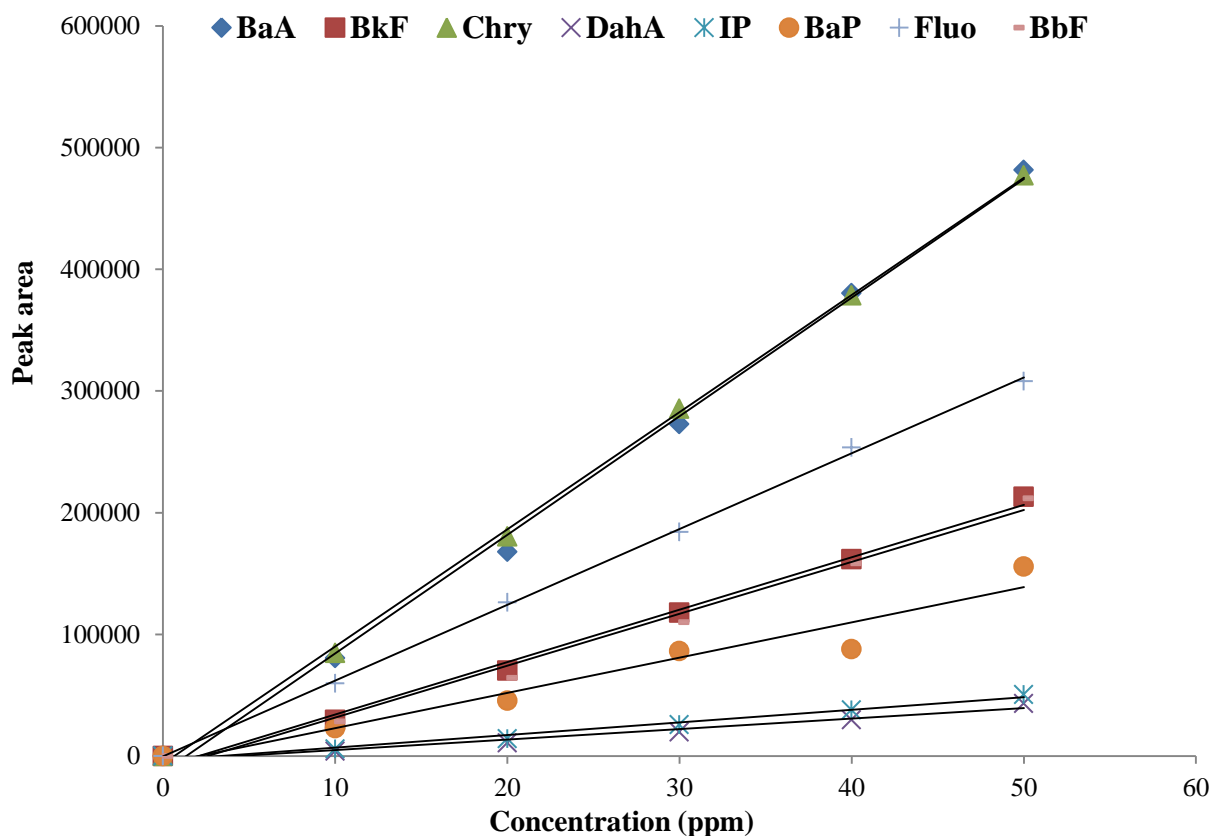
Figure 4.2: Calibration   standard curve of the target PAHs (dibenzo(a,h)anthracene, chrysene, benzo(a)anthracene, benzo(a)pyrene, indeno(l,2,3-cd)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and fluoranthene) showing peak area against concentration

The results in Table 4.2 present the correlation coefficient (R^2) values for the instrument response (peak area) to increasing analyte concentrations were 0.9842, 0.9997, 0.9998, 0.9934, 0.9946, 0.9949, 0.9986 and 0.9988, for dibenzo(a,h)anthracene, chrysene, benzo(a)anthracene, benzo(a)pyrene, indeno(l,2,3-cd)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and fluoranthene, respectively. The R^2 values obtained for each of the calibration curves showed that the correlation between relative peak area and concentration were close to unity, and reproducible within the concentration range selected, since the regression coefficient R^2 values of the selected PAHs were > 0.9800 using GC-FID. Thereafter the concentrations of the analytes were estimated by external calibration.

Table 4.2: Regression equation and correlation coefficient of the target PAHs

PAH	Concentration of working standard range (mg/L)	Correlation coefficient (R^2)			Regression Equations
Dibenzo(a,h)anthracene	10-50	0.9842	y	=	9834.00x - 13665
Chrysene	10-50	0.9997	y	=	10145.00x - 27597
Benzo(a)anthracene	10-50	0.9998	y	=	3401.80x - 15924
Benzo(a)pyrene	10-50	0.9934	y	=	1135.30x - 7184.3
Indeno(l,2,3-cd)pyrene	10-50	0.9946	y	=	4609.70x - 23636
Benzo(b)fluoranthene	10-50	0.9949	y	=	4583.10x - 18961
Benzo(k)fluoranthene	10-50	0.9986	y	=	6240.10x - 756.9
Fluoranthene	10-50	0.9988	y	=	10145.00x - 13665

4.2.3 Limit of detection and quantification

The limit of detection (LOD) refers to the minimum concentration that can be detected with suitable precision and accuracy at a known confidence level while the limit of quantification (LOQ) refers to the lowest amount of analyte in a sample that can be quantitatively determined with precision and accuracy under the stated experimental conditions. Table 4.3 shows the detection and limits quantification for the selected PAHs used in the study.

Table 4.3: Method and instrumental limit of detection and limit of quantification of the target PAHs

PAH	Method		Instrument	
	LOD (mg/kg)	LOQ (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
Dibenzo(a,h)anthracene	0.0023	0.0069	0.0115	0.0345
Chrysene	0.0003	0.0009	0.0015	0.0045
Benzo(a)anthracene	0.0006	0.0019	0.0030	0.0095
Benzo(a)pyrene	0.0173	0.0519	0.0865	0.2595
Indeno(l,2,3-cd)pyrene	0.0013	0.0040	0.0065	0.0200
Benzo(b)fluoranthene	0.0013	0.0039	0.0065	0.0195
Benzo(k)fluoranthene	0.0007	0.0021	0.0035	0.0105
Fluoranthene	0.0006	0.0019	0.0030	0.0095

Based on the residual standard deviation of the response and the slope, the limit of detection and limit of quantification of each PAH were calculated as three and ten times of the standard deviation divided by the slope, respectively. The limit of detection for the method ranged from 0.0003 mg/kg for chrysene to 0.0173 mg/kg for benzo(a)pyrene and the limit of quantification ranged from 0.0009 mg/kg for chrysene to 0.0519 mg/kg for benzo(a)pyrene. The limit of detection for the instrument ranged from 0.0015 mg/kg for chrysene to 0.0865 mg/kg for benzo(a)pyrene and the limit of quantification ranged from 0.0045 mg/kg for chrysene to 0.2595 mg/kg for benzo(a)pyrene. Samples with values less than the detection limit were recorded as not detected (nd).

4.2.4   Recovery

The efficiency of the recovery of the PAHs were evaluated by spiking 0.10, 0.20 and 0.40 mg/L soil samples with a mixture of six PAHs, e.i. dibenzo(a,h)anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and fluoranthene followed by extracted using microwave assisted extraction (MAE) into a 1:4 mixture of Hexane:DCM. The percentage recovery was thereafter calculated. The extraction efficiency of the selected PAHs obtained from 20 g soil samples ranged from 87.71% to 100.04% (Table 4.4).

Table 4.4: Result of determination and recoveries of real soil samples spiked with six PAH standards

Spike (mg/L)	Recovery (%)					
	BkF	**BbF**	**BaP**	**DahA**	**Fluo**	**BaA**
0.10	94.59	94.26	93.02	87.71	98.66	97.34
0.20	98.16	97.92	97.33	93.98	99.85	99.29
0.40	99.14	99.06	98.49	96.71	100.04	99.85

Fluo: fluoranthene; BaP: benzo(a)pyrene; BkF: benzo(k)fluoranthene; DahA: dibenzo(a,h)anthracene; IP: indeno(1,2,3-cd)pyrene; Chry: chrysene; BaA: benzo(a)anthracene; BbF: benzo(b)fluoranthene

4.3   Physical and chemical properties of the soil and their effect on the PAH concentration in soil samples

4.3.1   Effect of soil organic matter on the PAH concentration in soil samples

Soil organic matter (SOM) is known to play an important role in the environmental partitioning, store, and longevity of PAHs and might also significantly influence the distribution of the PAHs in the soil samples (He *et al.,* 2010; Wang *et al.*, 2013). The percentage SOM in the 35 soil samples ranged from 0.10 to 8.77% with a mean value of 2.74% and from 0.20 to 7.61% with a mean value of 2.91% in winter and summer, respectively (Appendix A). In summer, a weak correlation ($r_s \leq 0.39$) was found between SOM and the PAH concentrations. On the other hand, a moderate positive correlation (r_s values between 0.40 and 0.59) was observed between SOM and Chry ($r_s = 0.47$) and BbF ($r_s = 0.42$) during winter (Appendix B). A weak correlation between the SOM and PAH concentrations in summer suggests that soil samples were perhaps contaminated to different levels, probably due to easy degradation of the PAHs. Another possible reason can be due to the fact that the PAH emission point source could be low even if the SOM is high (Wang et al., 2012). However, the moderate correlation in winter can be explained by the fact that during the winter season, PAH degradation is expected to slow down. These results are consistent with finding reported in other studies conducted (Heywood *et al.*, 2006; Oen *et al.*, 2006; Zhang *et al.*, 2006; Cai *et al.*, 2007). Wang *et al.* (2010) reported that a very weak correlation should be expected between SOM and PAHs concentration in a soil subjected by continuous input of fresh PAH contamination.

4.3.2   Effect of pH on the PAH concentrations in soil samples

Soil pH is an important factor controlling the mobility and accumulation of organic substances such as PAHs in the soils. Studies found that the PAH concentrations were generally higher in acidic soils compared to non-acidic soil. This can be explained by the fact that acidic soils are unfavourable for the development of soil microorganisms which are essential for PAHs biodegradation (Soti *et al.*, 2015). The pH values from the 35 sampling sites ranged from 4.63 to 7.79 with a mean value of 7.28 and from 6.07 to 8.20 with a mean value of 6.67 in winter and summer, respectively (Appendix A). In summer, a weak positive correlation was found between pH and PAH concentrations ($r_s \leq 0.39$) while

in winter a moderate negative correlation exists for Fluo (r_s = - 0.45) and Chry (r_s = - 0.41). The remaining PAHs showed a weak correlation (Appendix B). A weak correlation indicates that the pH did not have a significant influence in the distribution of the PAH compounds in the soil.

4.4   Concentration of PAH in top soils

The concentration levels of the investigated PAHs in the different soil samples were variable. The PAHs Fluo, BaP, BkF, Chry, IP, BbF, DahA, and BaA were detected in the soil samples collected from the different sampling sites around Cape Town. The mean concentrations, standard deviations of distribution, minimum and maximum values for the concentrations of the selected PAHs detected in soil collected in the vicinity of a petrochemical plant in Cape Town during winter and summer are presented in Table 4.5.

Table 4.5: Summary statistics for PAH mean concentrations in soil samples (mg/kg)

Type	Winter			Summer		
of PAHs	Minimum	Maximum	Mean ± SD	Minimum	Maximum	Mean ± SD
Fluo	nd	0.62	0.20 ± 0.23	nd	0.55	0.02 ± 0.09
IP	nd	3.92	1.64 ± 0.65	nd	2.34	0.15 ± 0.48
Chry	nd	1.38	0.36 ± 0.16	nd	0.35	0.01 ± 0.04
BaP	nd	3.69	1.11 ± 0.55	nd	1.70	0.11 ± 0.31
BkF	nd	1.35	0.60 ± 0.47	nd	0.94	0.03 ± 0.15
BaA	nd	1.07	0.08 ± 0.23	nd	nd	nd
BbF	nd	1.84	0.09 ± 0.35	nd	1.88	0.08 ± 0.31
DahA	nd	nd	nd	nd	2.69	0.10 ± 0.44
∑ PAHs	nd	13.88	4.08 ± 2.64	nd	10.43	0.50 ± 1.83

SD: standard deviation; Fluo: fluoranthene; BaP: benzo(a)pyrene; BkF: benzo(k)fluoranthene; DahA: dibenzo(a,h)anthracene; IP: indeno(1,2,3-cd)pyrene; Chry: chrysene; BaA: benzo(a)anthracene; BbF: benzo(b)fluoranthene.

The sum of the concentrations of the detected PAHs (ΣPAHs) ranged between nd and 13.88 mg/kg, with a mean concentration of 4.08 mg/kg and a standard deviations of distribution of 2.64 mg/kg in winter while in summer ΣPAHs ranged between nd and 10.43 mg/kg, with a mean concentration of 0.50 mg/kg and standard deviation of distribution 1.83 mg/kg. In winter the mean concentration of the individual PAH compounds ranged from nd to 1.64 mg/kg with DahA not detected and IP having the highest mean value (1.64 mg/kg) while in summer, the mean concentration of the individual PAHs range from nd to 0.15 mg/kg with BaA not detected and IP having the highest mean value (0.15 mg/kg). Benzo(a)anthracene was not detected in winter. This may be attributed to the the physical and chemical properties of the PAH. On the other hand, dibenzo(a,h)anthracene was not detected in summer. This could perhaps be as a result of the tendency for volatilization of dibenzo(a,h)anthracenene from the soil during the warm season, occasioned by an increase in temperature.

All the individual PAH compounds detected in soil samples except for fluoranthene were above the maximum allowable concentration (MACs) recommended by ATSDR (2006) and USEPA (2011). The regulatory limit for the amount of carcinogenic and toxic polycyclic aromatic hydrocarbons in soils are 3.00, 0.15, 1.50, 0.01, 1.50, 0.15, 0.15, 0.01 mg/kg for Fluo, IP, Chry, BaP, BkF, BaA, BbF, and DahA, respectively. These results are consistent with findings reported in the study conducted by Nieuwoudt *et al*. (2011) in central South Africa. The author reported that the total PAH concentration of soil varied from 0.04 to 39.00 mg/kg. In the study conducted in urban soils of UK, the sum of the concentrations of the detected PAHs ranged from 4 to 67 mg/kg, with a mean concentration of 18 mg/kg (Vane *et al*., 2014). Similarly, Peng *et al*. (2011) reported the total concentration of PAHs in the soils of Beijing ranged from 0.09 to 13.14 mg/kg with a mean concentration of 1.23 mg/kg. The study conducted by Masih and Taneja (2006) reported the average concentration of total PAH in soil samples was 12.10 mg/kg and the ranged between 3.10 mg/kg and 28.50 mg/kg.

4.4.1   Relationship between individual PAHs in soil samples

The relationship between the concentration of the PAHs in soil samples collected in winter and summer was investigated. In general, it was found that PAHs with structural similarities, closeness in solubility and molecular weight correlate well among themselves

and will, therefore, be affected in a similar way (Farant & Gariépy, 1998). In this study, the correlation analysis for the pairs of PAH compounds in summer showed a strong positive relationship between IP and BaP ($r_s = 0.71$), IP and BbF ($r_s = 0.72$), IP and DahA ($r_s = 0.72$), Chry and BkF ($r_s = 0.82$), Chry and BbF ($r_s = 0.72$), Chry and DahA ($r_s = 0.62$), BaP and BbF ($r_s = 0.74$), BkF and DahA ($r_s = 0.76$), BbF and DahA ($r_s = 0.67$). On the other hand in winter, Fluo and Chry ($r_s = 0.68$), BaP and IP ($r_s = 0.71$), BaP and BbF ($r_s = 0.75$), BkF and Chry ($r_s = 0.68$) were positively correlated while BaP and Chry ($r_s = -0.56$), Fluo and BkF ($r_S = -0.62$) were negatively correlated (Appendix B). According to Farant and Gariépy (1998), the correlation of the concentrations of benzo(a)pyrene with that of other PAH compounds in a given sample usually exceeded 0.50. This is the reason why it is usually selected as a marker for PAH exposure in the environment.

4.4.2   Seasonal effects on concentration levels of PAHs in soil samples

In this study, the presence and concentration of the PAHs detected in soil samples vary significantly under climatic change ($p < 0.05$). The maximum concentrations of individual PAHs in soil samples were higher in winter while in summer most of the individual PAHs were not detected in most of the sampling sites (Figure 4.3).
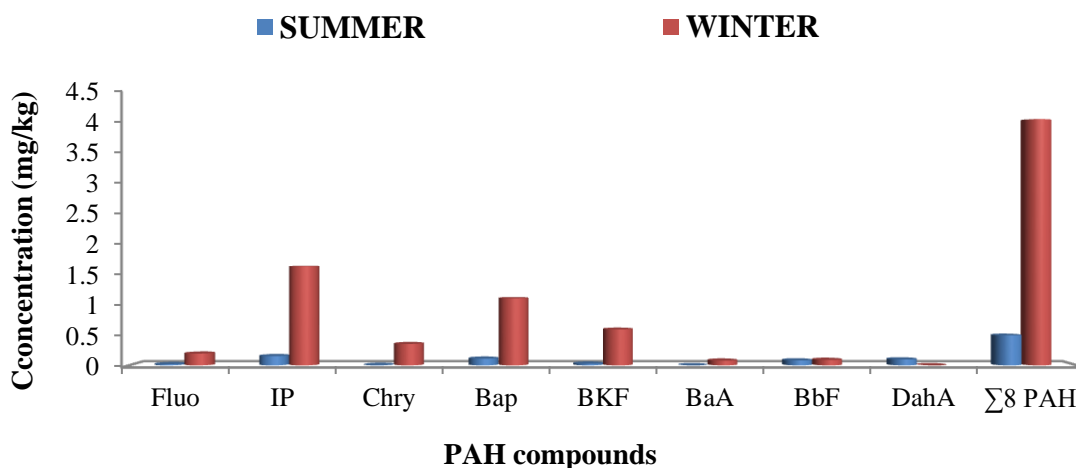
Figure 4.3: Average concentration of individual PAHs in soil samples during winter and summer

The results are consistent with the findings of Masih and Taneja (2006), Khillare *et al*. (2014) and Kumar *et al*. (2015) who reported that the concentration of PAHs detected in soil samples were higher in the winter season and lower in the summer season. In this study, the summer was characterized by an average temperature ranging from 14.2 ^{o}C to 25.4 ^{o}C with an average precipitation of 20 mm. The above temperature might have favoured volatilization of the PAHs from soil to another media as well the biodegradation of the PAHs in the soil. Moreover, at elevated temperature, photochemical decomposition rates of the PAHs in the atmosphere increases and could result in decreased deposition of the pollutant on the soil surface (Khillare *et al*., 2014). On the other hand, during winter the season was characterized by an average temperature ranging from 7.5 ^{o}C to 17.8 ^{o}C with an average precipitation of 82 mm. According to Ahangar (2010), the microbial breakdown of PAHs is decreased during winter which might have contributed to the higher concentration of PAHs during this season. In addition, dry deposition and wet deposition of PAHs on the soil surface might have also contributed to the level of PAHs in soil. According to Khillare *et al* (2014), the higher concentration of PAHs in the winter season could also be attributed to higher emissions (biomass and coal burning) leading to higher rate of deposition of PAHs onto the soil.

4.4.3   Seasonal variation and spatial distribution of PAH concentration in soil samples at different sampling sites during winter and summer

The mean concentrations of the PAH compounds collected in the 35 soil samples collected during winter and summer are presented in Table 4.5. The PAH compounds in the soils varied from one sampling site to another. Fluo, BaP, IP, Chry, BaA, BkF and BbF were detected in 51, 86, 46, 97, 17, 83 and 9% of the soil samples in winter, respectively while in summer DahA, Fluo, BaP, IP, BbF, BkF and Chry were detected in only about 25, 6, 14, 26, 14, 8 and 6%, respectively. The decrease in the presence of the PAHs may be attributed to the increase of volatilization of the PAHs from the soil during summer. This was consistent with the findings of Masih and Taneja (2006) who reported that the maximum mean concentration of the PAH compounds was higher in the soil samples during the winter season and decreased in the summer season.

Table 4.6: Mean concentrations of PAHs (mg/kg) in winter and summer soil samples in sampling sites 1 to 35 (RE: Residential area, IN: Industrial area, RO: Roadside)

Sampling sites	Seasons	Fluo	BaP	IP	Chry	BaA	BkF	BbF	DahA	∑PAHs
Site 1 RE	Winter	0.45	1.08	1.61	0.33	nd	nd	nd	nd	3.47
	Summer	nd	1.03	nd	nd	nd	nd	nd	nd	1.03
Site 2 RE	Winter	nd	1.08	1.50	0.32	nd	nd	nd	nd	2.90
	Summer	nd	1.01	1.52	nd	nd	0.88	nd	nd	3.41
Site 3 RE	Winter	0.45	1.04	1.48	0.33	nd	nd	nd	nd	3.30
Site 4 RE	Winter	0.45	nd	1.49	0.37	nd	nd	nd	nd	2.31
	Summer	nd	nd	nd	nd	nd	nd	nd	nd	nd
Site 5 RO	Winter	0.60	1.99	1.80	0.96	1.02	1.25	1.6	nd	9.31
	Summer	nd	nd	nd	nd	nd	nd	nd	nd	nd
Site 6 RO	Winter	0.45	1.20	1.71	0.38	nd	0.97	nd	nd	4.71
	Summer	nd	nd	nd	nd	nd	nd	nd	nd	nd
Site 7 RE	Winter	0.46	1.01	2.15	0.51	nd	1.10	nd	nd	5.23
	Summer	nd	1.00	1.45	nd	nd	nd	nd	nd	2.45
Site 8 RO	Winter	0.45	1.31	1.65	0.43	nd	0.99	nd	nd	4.83
	Summer	nd	nd	nd	0.35	nd	nd	nd	nd	0.35
Site 9 RO	Winter	0.46	1.26	1.95	0.36	nd	0.96	nd	nd	4.99
	Summer	nd	nd	1.69	nd	nd	0.92	nd	1.13	3.74
Site 10 RO	Winter	0.47	1.44	1.78	0.46	nd	1.05	nd	nd	5.20
Site 11 RE	Winter	nd	1.14	1.79	0.32	nd	0.91	nd	nd	4.16
	Summer	nd	nd	1.45	nd	nd	nd	nd	nd	1.45
Site 12 RE	Winter	nd	1.15	1.72	0.33	nd	0.90	nd	nd	4.10
Site 13 RE	Winter	nd	1.14	1.52	0.32	nd	0.92	nd	nd	3.90
	Summer	nd	nd	1.48	nd	nd	nd	nd	nd	1.48
Site 14 RE	Winter	nd	1.05	1.49	0.31	nd	0.91	nd	nd	3.76
Site 15 RE	Winter	nd	1.04	1.73	0.32	nd	0.88	nd	nd	3.97
Site 16 IN	Winter	nd	1.11	1.49	0.35	0.58	1.01	nd	nd	4.54
	Summer	nd	nd	nd	nd	nd	nd	nd	nd	nd
Site 17 RE	Winter	nd	1.04	1.48	0.31	nd	0.89	nd	nd	3.72
Site 18 RE	Winter	0.45	1.04	1.51	0.32	nd	nd	nd	nd	3.32
Site 19 RE	Winter	nd	1.05	1.70	0.30	nd	0.87	nd	nd	3.92
	Summer	nd	1.02	nd	nd	nd	nd	nd	1.08	2.10
Site 20 RE	Winter	0.45	1.11	1.51	0.33	nd	0.89	nd	nd	4.29
Site 21 RE	Winter	nd	1.03	1.78	0.30	nd	nd	nd	nd	3.11
Site 22 RE	Winter	nd	1.14	1.78	0.31	nd	0.92	nd	nd	4.15
Site 23 IN	Winter	nd	1.04	1.45	0.32	nd	0.90	nd	nd	3.71
Site 24 RE	Winter	nd	1.01	nd	0.31	nd	nd	nd	nd	1.32
Site 25 RE	Winter	nd	1.30	2.09	0.34	nd	0.91	nd	nd	4.64
Site 26 RE	Winter	nd	1.17	2.64	0.34	nd	0.88	nd	nd	5.03
Site 27 RO	Winter	nd	1.31	2.02	0.38	nd	0.90	nd	nd	4.61
Site 28 IN	Winter	0.45	1.34	1.97	0.40	nd	0.92	nd	nd	5.08
Site 29 RO	Winter	0.45	2.78	1.89	0.67	nd	1.00	nd	nd	6.79
	Summer	nd	1.10	1.48	nd	nd	nd	1.11	1.11	4.80
Site 30 RO	Winter	nd	1.16	2.05	0.33	nd	0.89	nd	nd	4.43
Site 31 IN	Winter	nd	1.09	1.75	0.33	nd	0.90	nd	nd	4.07
	Summer	nd	1.14	1.47	nd	nd	nd	1.13	1.13	4.87
Site 32 IN	Winter	0.45	1.19	2.48	0.34	nd	0.91	nd	nd	5.37
	Summer	nd	1.01	1.48	nd	nd	nd	1.09	1.09	4.67
Site 33 IN	Winter	0.52	1.05	1.47	0.37	nd	0.94	nd	nd	4.35
	Summer	0.55	1.70	2.34	0.30	nd	nd	1.88	1.88	8.65
Site 34 RO	Winter	0.45	1.50	2.01	0.33	nd	0.95	nd	nd	5.24
	Summer	0.55	1.02	1.60	nd	nd	0.90	1.10	1.10	6.27
Site 35 RO	Winter	0.45	1.32	2.56	0.42	0.57	0.92	nd	nd	6.24
	Summer	nd	1.03	nd	nd	nd	nd	nd	nd	1.03

The concentration of IP, BaP and BkF were higher at all the sampling sites during winter compared to the other PAHs. This can probably be explained by the fact that they are more abundant in the environment. The sum of the PAHs at the road side in winter ranged from 4.43 (site 30) to 9.31 mg/kg (site 5), from 2.31 (site 4) to 5.37 mg/kg (site 31) for the soil samples collected at the industrial area and from 1.32 (site 24) to 5.23 mg/kg (site 7) for the soil samples collected at the residential area. On the other hand, the soil samples collected at the road side during summer ranged from nd to 6.27 mg/kg (site 34), from nd to 8.65 mg/kg (site 33) for the soil samples collected at the industrial area and from nd to 3.41 mg/kg (site 2) for the soil samples collected at the residential area. The total PAH concentration increased in order of residential soil < industrial soil ≤ roadside soil.

From the spatial distribution, it was observed that residential areas located in the northwest of the petrochemical plant contributed least PAH except for site 7 (5.23 mg/kg) located at the residential area with heavy traffic and biomass combustion. The highest PAH concentration at the road side suggested that vehicular emissions probably contribute the most to the level of the PAHs in the soils followed by the industrial emissions during both winter and summer. These results are in agreement with the finding of Rafael *et al. (*2014) who reported that the levels of the PAHs in a vicinity of a petrochemical plant were due to vehicular exhausts from both diesel and gasoline engines. Similarly, results were also reported by Yu *et al.* (2013) where the highest concentrations of the PAHs were observed at the roadsides (0.96 mg/kg), followed by industrial areas (0.71 mg/kg) and residential areas (0.52 mg/kg). Among the soil samples collected at the roadside, the highest concentration were recorded for site 10 (5.20 mg/kg), site 34 (5.24 mg/kg), site 35 (6.24 mg/kg), site 29 (6.79 mg/kg) and site 5 (9.31 mg/kg). The spatial distribution of the PAH compounds showed that the concentrations of PAH at some sites were lower close to the petrochemical plant. Wind direction and speed were found to affect the spatial distribution and accumulation of PAHs in the terrestrial environment (Nam *et al*., 2003). In Cape Town, the wind is predominantly from the northwest in winter while in summer it varies between southeast and southwest. Most locations with higher concentrations were located in southwest and southeast areas of the petrochemical plant and the locations with lower PAH concentrations were mainly distributed near the northwest area of the petrochemical plant. Those results suggested that the distribution of PAHs could possibly be affected by the wind direction.

4.5   Principal component analysis

To further demonstrate the seasonal variation and spatial distribution in the concentration of the PAHs in soil samples as well as the relationship between the ten variables (pH, SOM, BaP, BaA, BkF, IP, DahA, Chry, BbF, Fluo) an analysis based on the principal component method (PCA) was employed. Principal component analysis is a statistical tool that enables the transformation of the original large multivariable data set into a minimum number of factor loading (Ray *et al*., 2012). The principal components with Eigen values > 1 were chosen. Figure 4.4 shows the first three components with Eigen values greater than 1 in summer (a) and winter (b). The eigenvalues indicate that three components provide a good description of the data.
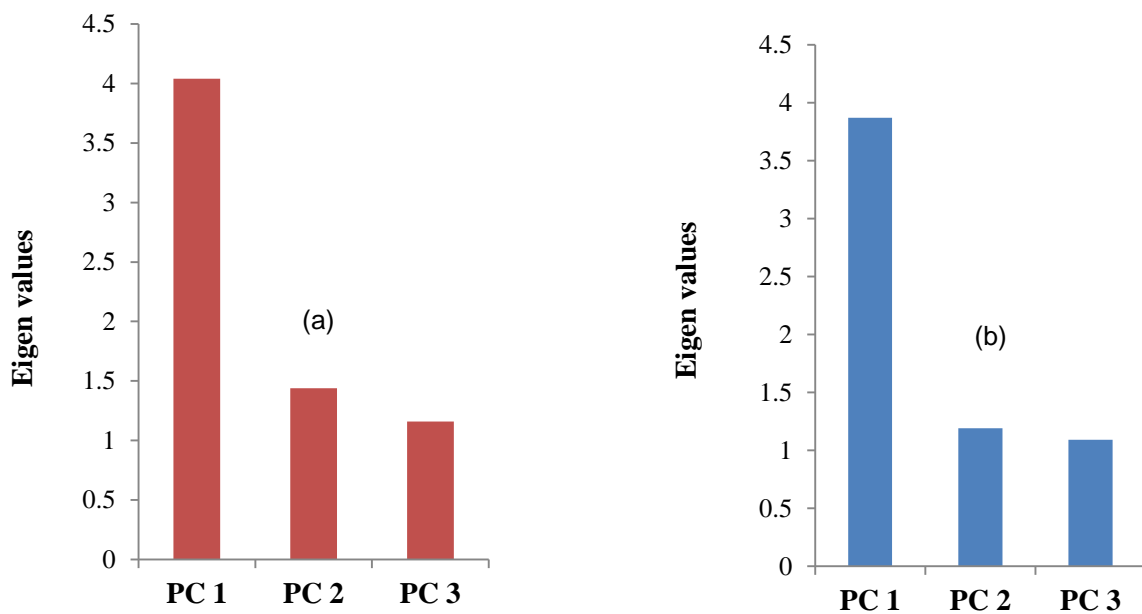
Figure 4.4: Plot of the first three principal components versus the Eigen values in summer (a) and winter (b)

The concentration of the original data for each variable and the three principal components as well as Eigen values, percentage variance and percentage cumulative in summer and winter are presented in Table 4.7. In summer the three principal components account for 73.75% of the variance obtained. Principal component one explained 44.93% of the variance and is positively correlated with IP, BbF, and DahA. Principal component two explained 15.96% of the variance and is negatively related with Chry and BkF and

positively correlated with the pH. Principal Component three explained 12.86% of variance shows a strong correlation with SOM and a negative correlation with pH.

Table 4.7: Correlation coefficients between variables and the first three principal components

Variables	Principal components Summer			Principal components Winter		
	1	2	3	1	2	3
pH	0.06	0.42	- 0.51	- 0.23	0.64	- 0.26
SOM	- 0.12	- 0.21	0.61	0.33	- 0.05	- 0.03
Fluoranthene	0.28	0.25	- 0.21	0.23	- 0.46	0.26
Indeno(1,2,3-cd)pyrene	0.43	0.16	0.06	0.26	0.35	0.61
Chrysene	0.32	- 0.54	- 0.19	0.44	0.02	- 0.18
Benzo(a)pyrene	0.35	0.35	0.39	0.39	- 0.15	- 0.22
Benzo(k)fluoranthene	0.34	- 0.50	- 0.21	0.29	0.33	0.46
Benzo(a)anthracene	nd	nd	nd	0.35	0.35	- 0.31
Benzo(b)fluoranthene	0.41	0.16	0.29	0.40	0.01	- 0.32
Dibenzo(ah)antracene	0.45	- 0.07	0.02	nd	nd	nd
Eigen values	4.04	1.44	1.16	3.87	1.19	1.09
% of variance	44.93	15.96	12.86	46.38	15.67	12.10
Cumulative %	44.93	60.89	73.75	43.96	56.18	68.28

The relationships between individuals PAH compounds, SOM, and pH variables are well represented the loading plot in Figure 4.5. Soil organic matter did not have an impact on individual PAH compounds in summer that could be attributed to the volatilization of the PAH compounds during summer. Non-acidic soil pH was found to be favourable for the microbial activities regarding the degradation PAH compounds. From the loading plot BaP and Fluo, IP and BbF and Chry are clustered together and are positively correlated with component two. This suggests that BaP, Fluo, IP and BbF vary with pH and therefore were greatly affected by the microbial degradation. On the other hand, BkF and Chry variables are clustered and were negatively correlated with component two and did not vary with soil pH suggesting a lesser impact on microbial activities. Benzo(a)anthracene was not detected in soil samples during summer, therefore, the vector was not included in Figure 4.5.
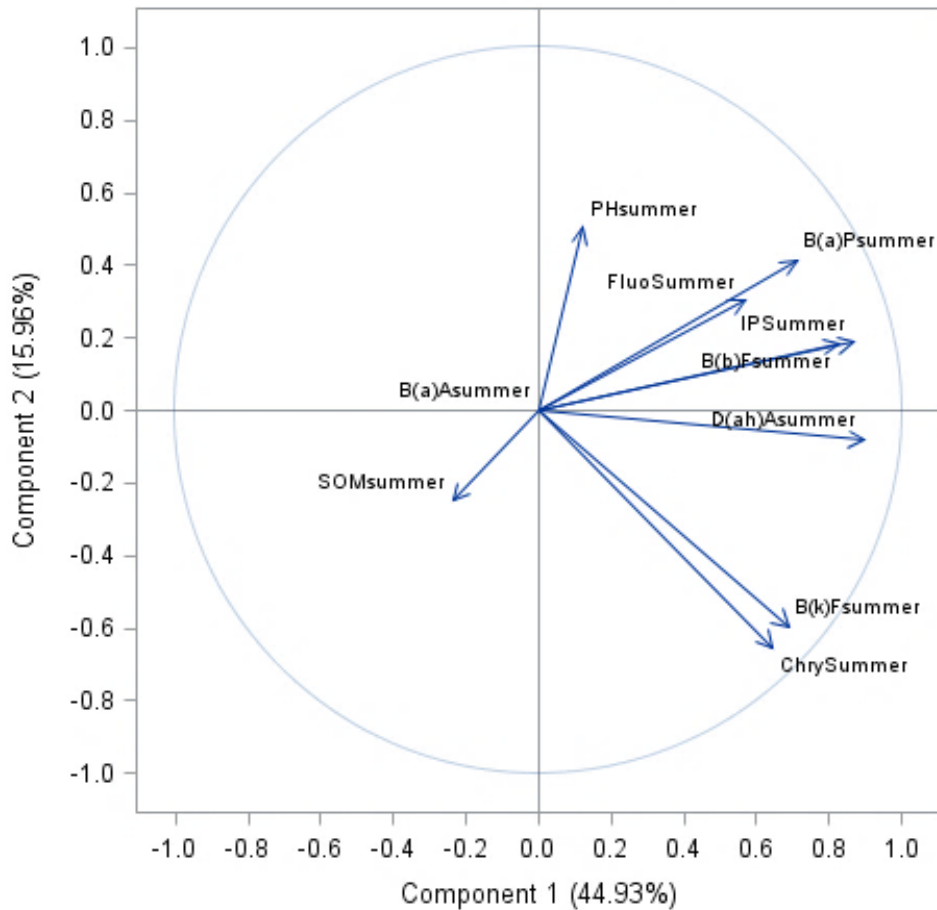
Figure 4.5: PCA projection of the eight PAHs, SOM and pH variables in summer

In winter the three principal components explain 68.28% of variance obtained. Principal component one represents 46.38% of the explained variance level. The second and third principal components represent 15.67% and 12.10% of the variance, respectively. Principal component one is mainly a contribution of Chry and BbF. Component two is positively correlated with the pH and negatively correlated with Fluo. The third principal component increases with two variables: Fluo and BkF. The plot of the first principal component versus the second principal component is shown in Figure 4.6. From the loading plot none of the individual PAH compounds were affected by the soil pH in winter while the SOM and the individuals PAH compound were positively correlated with principal component one. This suggests that the PAHs and SOM vary together. This might be due to the low volatilization, decreases in microbial breakdown during winter. Also dry and wet deposition might increase the accumulation of the PAH compound in the soil.
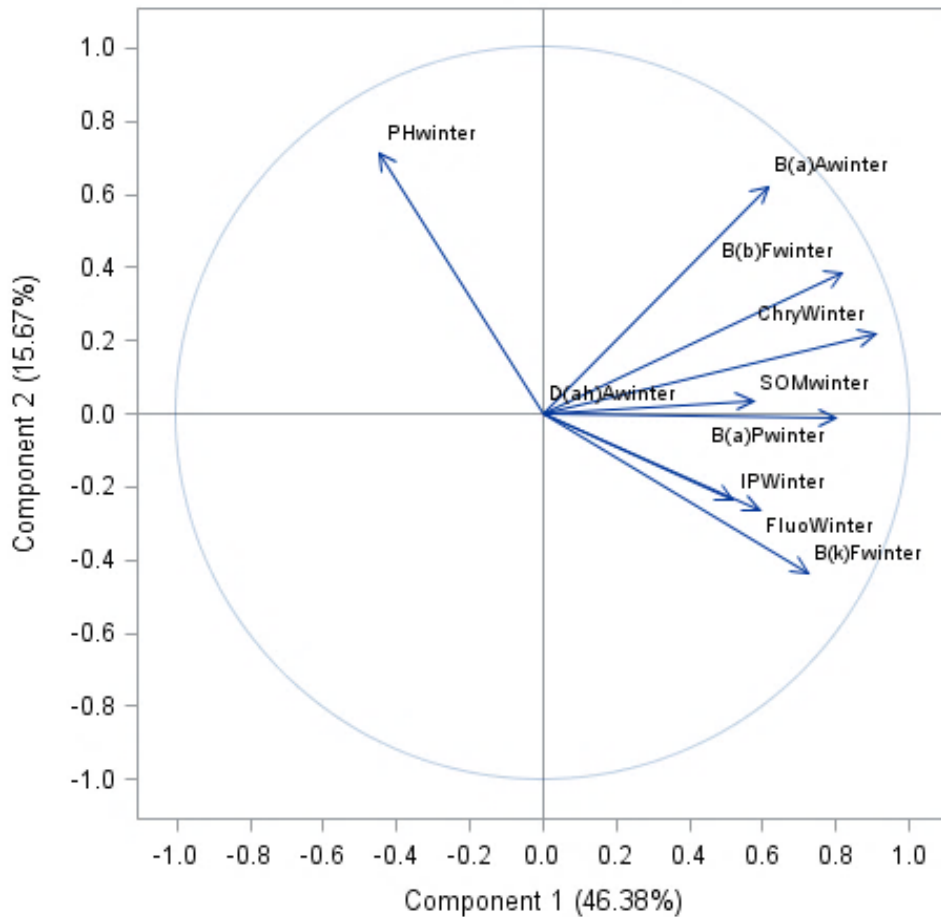
Figure 4.6: PCA projection of the eight PAHs, SOM, and pH variable in winter

The loading plot was useful because it shows the relationship between the variables in the space of the first two components. Figure 4.7 gives a visual representation of the seasonal variations and spatial distribution of the PAHs in soil samples at the 35 sampling locations. The PAHs detected in summer are clustered together and the PAHs detected in winter are also clustered together which confirmed the seasonal effect on the PAHs concentrations in the soil samples. Most of the PAHs were found at low concentration in the summer season when compared to the winter season. From the biplot in Figure 4.7, the distribution of the PAHs compounds in winter was highly concentrated in the soil samples collected at the road side (site 5, site 29, site 35, site 10 and site 8), residential area (site 7) and industrial area (site 28). This indicates that the PAHs detected in those soil samples were probably originated from similar sources. While in summer the PAHs were highly concentrated in the soil samples taken at the roads side (site 9 and site 34) and industrial areas (site 33, site 32 and site 31).
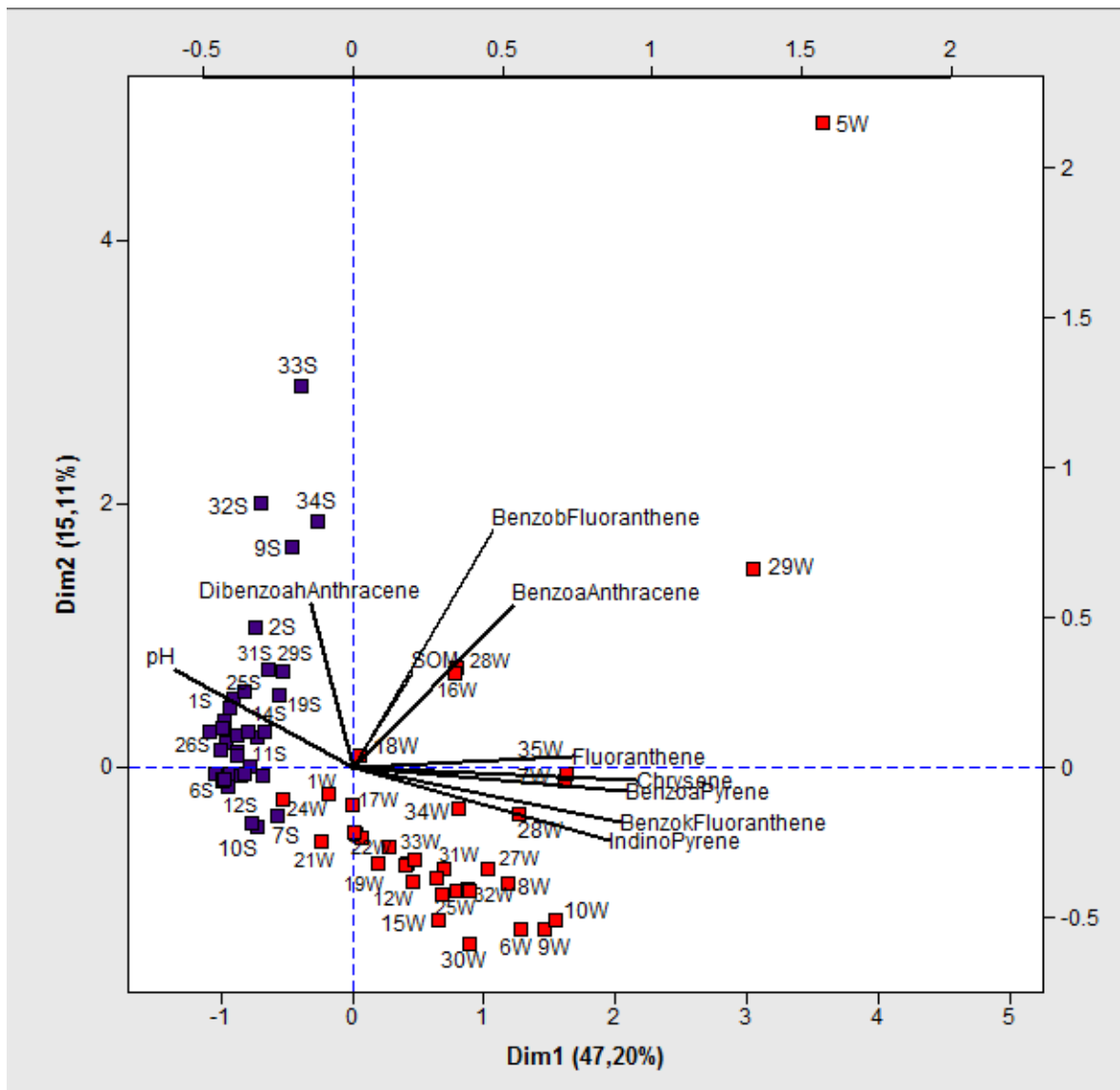
Figure 4.7: A biplot showing the variables and the observations

4.6 Probable sources of PAHs in soil in a vicinity of a petrochemical plant

4.6.1 Diagnostic ratios

The potential sources of PAHs in different environmental media can be identified using the ratios between pairs of the concentration of individuals PAHs. The literature values presented in Table 4.8 were used to characterize possible sources of the PAHs in this study.

Table 4.8: Literature diagnostic ratios of PAHs

PAHs ratios	Value of ratios and indicate for possible source		Reference
BaP/(BaP + Chry)	0.07 - 0.24	Biomass	Chen *et al.*, 2005
	0.73	Petrogenic	Khalili *et al.*, 1995
BaA/(BaA + Chry)	< 0.2	Petrogenic	Yunker *et al.*, 2008
	> 0.35	Biomass	Hwang *et al.*, 2003
BbF/BkF	0.8 - 1.1	Biomass	Abbas and Brack, 2005
	1.1 - 1.5	Petrogenic	
	2.5 - 2.9	Smelters	

The ratio of BaP/(BaP + Chry) and the possible sources of PAHs in the 35 soil samples during winter are presented in Table 4.9. In winter, BaP/(BaP + Chry) ratio ranged from 0.66 to 0.78. About 97% of the sampling sites exhibited the typical attribute of petrogenic combustion. In summer, the ratio BaP/(BaP + chry) could not be calculated because benzo(a)pyrene and chrysene were not detected in most of the sampling sites presumably due to the higher rate of degradation of the PAHs. The two PAHs were only detected in sampling site 33 during summer and the ratio BaP/(BaP + chry) was 0.85, which suggested that PAHs originated from a petrogenic source.

Table 4.9: BaP/(BaP + chry) diagnostic ratios for soil samples in winter and summer

Sampling sites	BaP/(BaP + Chry)	Possible source	Sampling sites	BaP/(BaP + Chry)	Possible source
Site 1	0.77	petrogenic	Site 19	0.77	Petrogenic
Site 2	0.77	petrogenic	Site 20	0.77	Petrogenic
Site 3	0.76	petrogenic	Site 21	0.77	Petrogenic
Site 5	0.68	petrogenic	Site 22	0.79	Petrogenic
Site 6	0.76	petrogenic	Site 23	0.77	Petrogenic
Site 7	0.66	petrogenic	Site 24	0.77	Petrogenic
Site 8	0.75	petrogenic	Site 25	0.79	Petrogenic
Site 9	0.78	petrogenic	Site 26	0.78	Petrogenic
Site 10	0.76	petrogenic	Site 27	0.78	Petrogenic
Site 11	0.78	petrogenic	Site 28	0.77	Petrogenic
Site 12	0.78	petrogenic	Site 29	0.81	Petrogenic
Site 13	0.78	petrogenic	Site 30	0.78	Petrogenic
Site 14	0.77	petrogenic	Site 31	0.77	Petrogenic
Site 15	0.76	petrogenic	Site 32	0.78	Petrogenic
Site 16	0.76	petrogenic	Site 33	0.74	Petrogenic
Site 17	0.77	petrogenic	Site 34	0.82	Petrogenic
Site 18	0.77	petrogenic	Site 35	0.77	Petrogenic

Besides the BaP/(BaP + chry) ratio, the BaA/(BaA + Chry) and BbF/BkF ratios were also used as a tool for identifying pollution emission sources in the soil sample in winter (Table 4.10). The ratios BaA/(BaA + Chry) were 0.52, 0.62 and 0.58 at sampling points 5 (road side), 16 (industrial area) and 35(road side) respectively, which suggest that the PAHs are from biomass combustion. The BbF/BkF ratio was 1.35at sampling site 4 during winter, which is indicative of a possible petrogenic source.

Table 4.10: BaA/(BaA + Chry) and BbF/BkF diagnostic ratios for soil Samples in winter

	Sampling sites	Ratio values	Possible source
BaA/(BaA + Chry)	Site 5	0.52	Biomass combustion
	Site 16	0.62	Biomass combustion
	Site 35	0.58	Biomass combustion
BbF/BkF	Site 4	1.35	Petrogenic

<center>**CHAPTER FIVE**</center>

<center>**RESULTS AND DISCUSSION FOR LEAF ANALYSIS**</center>

5.1   Introduction

The reception of contaminants by plant surfaces is a result of aerial deposition and this contributes greatly to the contaminant load in plant species (Li *et al*., 2010). However, there may be variation in the retention of different contaminant fractions, depending on the physical and chemical properties of the PAHs and the physiological characteristics of the plants. The plant cuticle has been found to be an important outer layer consisting of lipid-polymer impregnated with waxes (Bargel *et al*., 2013). Due to its lipophilic properties, the cuticles have been found to act as an accumulation compartment for lipophilic organic pollutant such as PAHs (Bakker *et al*., 2001). Plant leaves with greater lipid concentration have been shown to accumulate significantly more PAHs (Kuang *et al*., 2015). In order to select a suitable plant as markers of exposure, Slaski *et al.* (2000) recommended the use of plants having large surface area and covered with thick, waxy cuticles. The objective of this study was to investigate the use of *Acacia Saligna* commonly known as the Port Jackson willow as a possible biomonitor for the presence of PAHs in the environment for future studies. The Port Jackson willow was selected over plant species because of its abundance in the Western Cape.

5.2   Control experiment

The mean concentrations of the individual PAH compounds and the percentage reduction between unwashed and water-washed leaves and the percentage reduction between unwashed and DCM-washed leaves are presented in Table 5.1. Water was found ineffective for removing PAHs trapped in the lipid layer of the leaf due to its hydrophobic properties while DCM was found effective for removing the PAHs. A Kruskal-Wallis test shows a significant difference in the PAH concentrations between leaves washed with

<center>63</center>

water and the leaves washed with DCM ($p < 0.05$). The percentage reduction between unwashed and washed leaves (calculated as the percentage ratio between unwashed and washed concentration to the unwashed leaf concentrations) were 11.61, 21.83, 2.02, 11.96, 15.53, 9.09 and 2.80% for Chry, BaP, BkF, IP, DahA, BaA, and BbF, respectively. While the percentage reduction between unwashed and DCM washed were 63.87, 61.41, 49.12, 68.07, 60.27 and 81.82% for Chry, BaP, BkF, IP, DahA, BaA, and BbF, respectively. Fluoranthene was not detected in the leaf samples. The reason could be that at elevated temperature, the PAH exists in the gaseous phase and therefore can directly penetrate in the inner leaf compartment via stoma. However during the hot and dry season the plant closes its stoma in order to control water loss as suggested by De Nicola et al. (2008) and when the stoma are closed, the gaseous PAH will not be able to enter the plant leave via the stoma.

Table 5.1: Mean concentrations (mg/kg) of the PAH compounds and the percentage reduction between unwashed and water-washed and DCM-washed leaves.

PAH	Unwashed	Washed with DCM	Washed with water	Percentage reduction DCM (%)	percentage reduction Water (%)
Fluo	nd	nd	nd	nd	nd
Chry	0.78	0.28	0.69	63.87	11.61
BaP	6.51	2.51	5.09	61.41	21.83
BkF	1.99	1.01	1.95	49.12	2.02
IP	7.74	2.47	6.81	68.07	11.96
BaA	0.22	0.19	0.20	13.64	9.09
DahA	7.89	3.14	6.67	60.27	15.53
BbF	1.43	0.26	1.39	81.82	2.80
∑PAHs	26.54	9.86	22.78	62.87	14.17

Tarzaghi *et al*. (2013) found that when deposited on the leaves, PAHs can first accumulate on the leaves surface with subsequent migration into the cuticular wax (lipid layer) of the leaf and inside the plant. Water could only partially remove the PAHs deposited on the surface of the leaves but not the PAHs incorporated into the cuticular wax of the leaves where they are physically protected and therefore difficult to be removed by water washing while DCM was able to remove the PAHs found on the surface and the lipid layer of the leaves at the same time. Figure 5.1 shows the effect of water-washed and DCM-washed on

the leaf samples collected at the two sampling sites. The control experiment confirmed the fact that plant leaves can be sampled during raining seasons without fear that the rain will wash away the PAHs located on the lipid layer of the leaves.
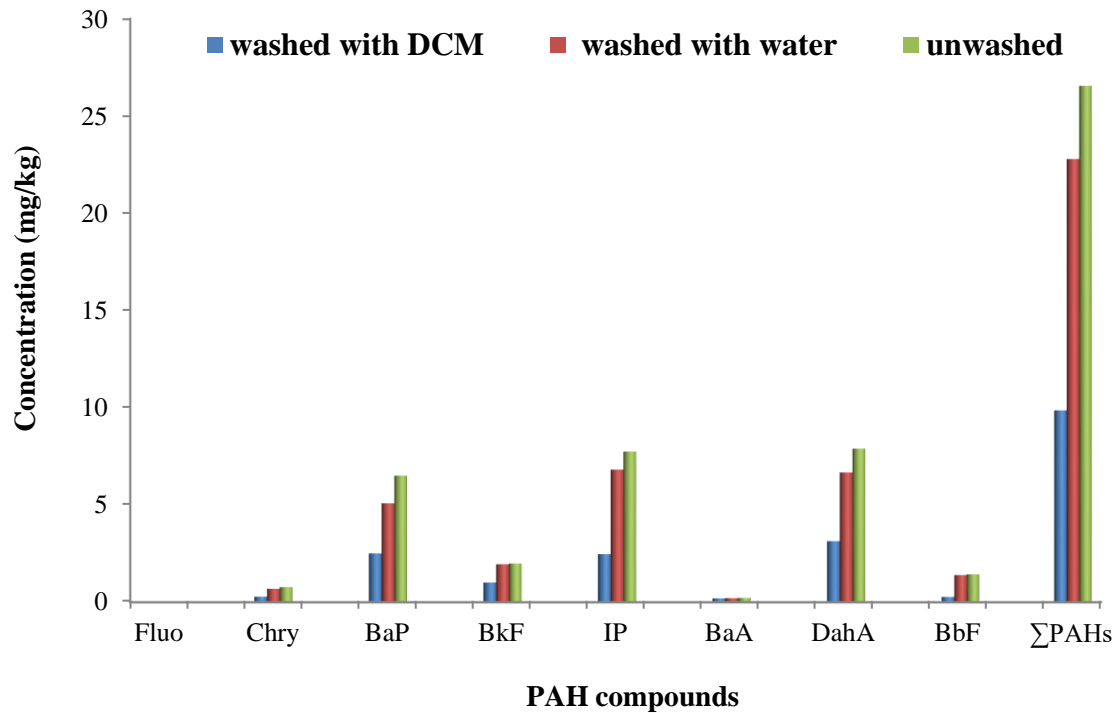
Figure 5.1: Effect of water-washed and DCM-washed on the leaf samples collected at the two sampling sites

5.3   Analysis of the leaf samples

5.3.1   Concentration of PAHs in leaf samples

The concentration of the investigated PAHs in the different leaf samples were variable. Fluo, BaP, BkF, Chry, IP, BbF, DahA, and BaA were detected in the leaf samples collected from the 35 different sampling sites around Cape Town in summer. The mean concentrations, standard deviations of distribution, median concentrations, minimum and maximum values of the selected PAHs in the leaves are presented in Table 5.2. The sum of the concentrations of the detected PAHs (ΣPAHs) ranged between 10.48 and 76.73 mg/kg, with a mean concentration of 29.47 mg/kg and a median concentration of 26.51 mg/kg. The mean concentration of the individual PAHs ranged from 0.92 and 7.74 mg/kg

with fluoranthene having the lowest mean concentration (0.92 mg/kg) and with DahA having the highest mean concentration of (7.74 mg/kg). The high concentration of benzo(a)pyrene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene in leaf samples can be explained by the fact that these PAHs are more abundant in nature compare to the other PAHs. This largely account for the reason why they are often used as biomarkers for environmental pollution.

Table 5.2: Summary statistics for PAHs in leaves

PAH	Mean ± SD	Median	Minimum	Maximum
Fluo	0.92 ± 0.66	1.27	nd	1.59
BaP	5.42 ± 2.55	4.49	1.95	13.01
BkF	3.45 ± 0.81	3.18	1.56	6.95
Chry	1.25 ± 0.35	1.14	nd	2.13
IP	5.85 ± 1.44	5.48	2.60	10.01
BbF	4.03 ± 2.73	3.71	nd	26.11
DahA	7.74 ± 2.12	7.24	4.37	14.39
BaA	0.81 ± 0.96	nd	nd	2.54
ΣPAHs	29.47 ± 11.62	26.51	10.48	76.73

5.3.2   Effect of washing on the concentrations of PAHs in leaf samples

The mean concentrations of the selected PAHs detected in unwashed and washed leaves sample collected in the vicinity of a petrochemical plant in Cape Town during summer are presented in Table 5.3. The sum of the concentrations of the detected PAHs (ΣPAHs) reported was 30.69 ± 12.63 and 28.26 ± 9.65 mg/kg for unwashed and washed leaves, respectively. The mean concentration of the PAHs in unwashed leaves ranged from 0.96 mg/kg for benzo(a)anthracene to 7.95 mg/kg for dibenzo(a,h)anthracene. On the other hand, the mean concentration of the PAHs in washed leaves ranged from 0.67 mg/kg for benzo(a)anthracene to 7.53 mg/kg for dibenzo(a,h)anthracene. Based on the control experiment, it was expected that washing the leaves with water will not remove a large amount of PAHs deposited on the leaves. The percentage reductions between unwashed and washed leaves ranged from 1.10% for benzo(a)pyrene to 30.21% for benzo(a)anthrance.

The Kruskal-Wallis test shows that no significant difference between unwashed and water-washed leaves ($p > 0.05$). Other studies have reported similar results. Giridhar and Krishna (2011) found that deposited PAHs in vegetables were difficult to remove using water washing procedure. They found that less than 25% of the PAHs were washed off from lettuce, spinach, and tomatoes leaves. The study conducted by Lodovici *et al*. (1998) showed that PAHs were strongly bound to the lipid layer of the leaf. They concluded that PAHs deposited on the surface of leaves was not washed away during heavy rain. Librando *et al*. (2002), Lehndorff and Schwark (2004), De Nicola *et al*. (2007) and Tarzaghi *et al*. (2013) have reported similar results showing that PAHs deposited on the leaf surface were adsorbed into the lipid layer of the leaves, where they are physically protected.

Table 5.3: Mean concentrations of PAHs (mg/kg) in unwashed and washed leaves

PAH	Unwashed / washed	Mean ± SD	Percentage reduction (%)
Fluo	Unwashed	1.04 ± 0.63	24.04
	Washed	0.79 ± 0.68	
BaP	Unwashed	5.45 ± 2.53	1.10
	Washed	5.39 ± 2.61	
BkF	Unwashed	3.62 ± 0.82	1.86
	Washed	3.29 ± 0.77	
Chry	Unwashed	1.27 ± 0.38	3.94
	Washed	1.22 ± 0.32	
IP	Unwashed	5.91 ± 1.45	9.12
	Washed	5.80 ± 1.45	
BbF	Unwashed	4.49 ± 3.77	20.49
	Washed	3.57 ± 0.72	
DahA	Unwashed	7.95 ± 2.04	5.28
	Washed	7.53 ± 2.20	
BaA	Unwashed	0.96 ± 1.01	30.21
	Washed	0.67 ± 0.90	
ΣPAHs	Unwashed	30.69 ± 12.63	
	Washed	28.26 ± 9.65	

5.4 Relationship between PAH concentration in leaf and soil samples

The correlation between the PAH concentrations detected in soil samples and the PAHs concentrations detected in unwashed leaf samples were investigated (Table 5.4).

Table 5.4: Correlation between individual PAH concentrations detected in leaf and soil samples

PAHs detected in the leaves	PAHs detected in soils							
	Fluo	IP	Chry	BaP	BkF	BaA	BbF	DahA
Fluo	- 0.08	- 0.06	- 0.09	0.07	- 0.04	nd	0.03	0.06
IP	- 0.05	0.01	- 0.07	- 0.10	- 0.09	nd	- 0.06	- 0.05
Chry	0.01	0.04	- 0.01	0.07	0.05	nd	0.06	0.07
BaP	- 0.01	- 0.01	0.16	- 0.09	0.08	nd	0.03	0.06
BkF	0.06	0.18	0.19	0.22	0.19	nd	0.27	0.13
BaA	- 0.02	0.19	- 0.11	0.25	0.06	nd	0.11	0.11
BbF	0.01	0.01	0.01	0.02	0.01	nd	0.01	0.01
DahA	0.10	0.11	- 0.05	0.00	- 0.01	nd	- 0.05	- 0.05

Correlation analysis of PAH concentrations in leaf and soil samples showed a very weak relationship. The correlation coefficient (r_s) were - 0.08, 0.01, - 0.01, - 0.08, - 0.09, 0.19, 0.01 and - 0.05 for Fluo, IP, Chry, BaP, BkF, BbF and DahA, respectively. A significant difference $(p < 0.05)$ was observed between the PAHs in soil samples and the PAHs in leaf samples. Individual PAH concentrations in the leaves sample was higher than the corresponding soil concentrations collected at the same sites during summer which indirectly suggested that PAHs accumulate in the leaves predominantly via atmospheric deposition (Figure 5.2). The results are in the agreement with the results found by Li *et al*., 2010. The author reported that the concentrations of PAHs in plant leaves were not correlated to levels of PAHs in the soil due to variation in the atmospheric deposition and subsequent adsorption. Based on the result of this study the use leaf samples might be considered to be more suitable as an indicator of the PAH pollution in the environment compare to soil samples. Significant number of soil samples showed no presence of PAHs, while the corresponding leaves sample showed the presence of PAHs. This might be due to the fact that leaves have the ability to accumulate PAHs, whereas in the soils PAHs can degrade via microorganisms, volatilize and leach.
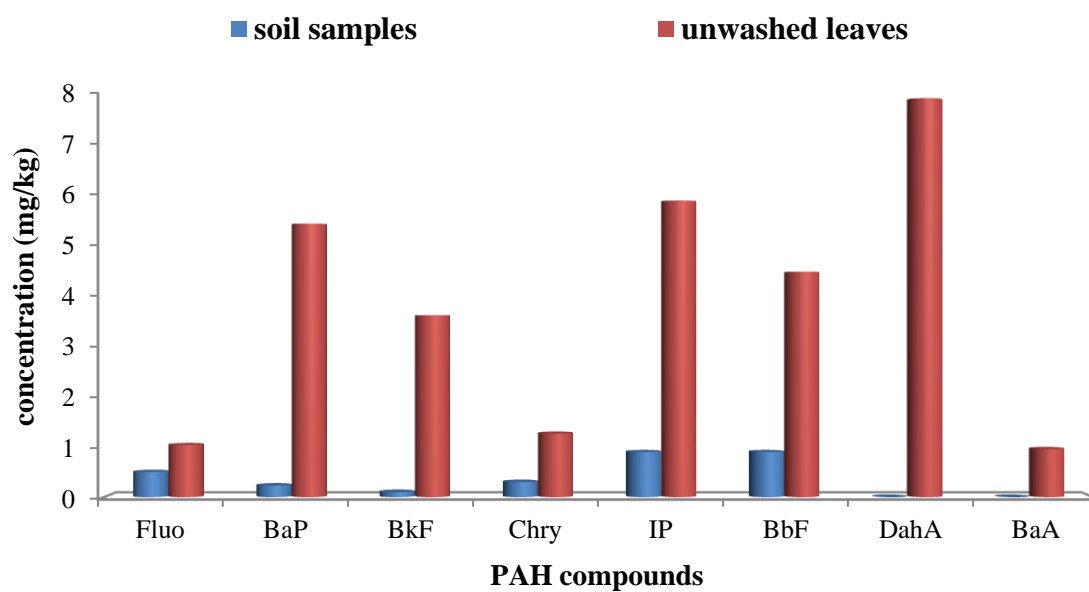
Figure 5.2: PAH concentrations in unwashed leaf and soil samples in summer

CONCLUSION AND RECOMMENDATIONS

6.1 General conclusions

The objective of this study was to investigate the spatial and seasonal variations of eight PAHs namely: benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(k)fluoranthene, chrysene, benzo(a)anthracene, dibenzo(a,h)anthracene and fluoranthene in soil and leaf samples in the vicinity of a petrochemical plant in Cape Town using Gas chromatography coupled with a flame ionization detector (GC-FID). Samples were collected at the roads side, residential and industrial areas.

In winter, the sum of the concentrations of the detected PAHs (ΣPAHs) ranged between nd and 13.88 mg/kg, with a mean concentration of 4.08 mg/kg while in summer, ΣPAHs ranged between nd and 10.43 mg/kg, with a mean concentration of 0.50 mg/kg. Polycyclic aromatic hydrocarbons were found to exceed the recommended limits in the soil samples. There was a significant variation in the levels of PAHs in sampled soil during winter and summer ($p < 0.05$). The levels of the PAHs were higher in soil samples collected in winter compared to the soil samples collected in summer. In winter, the low microbial activity of PAHs, as well as dry and wet deposition of PAHs on the soil surface, might have contributed to the higher level of PAHs in soil compare to summer.

In winter, the highest value for the sum of the eight PAHs was observed in the roadside soils, followed by the industrial and residential soils, with concentrations of 9.31, 5.37, 5.23 mg/kg, respectively. In summer on the other hand, the highest value was observed in the industrial soils, followed by the roadside soils and resedential soils with concentrations of 8.65, 6.27, 3.41 mg/kg, respectively. The spatial distribution of the PAH compounds in the sampled soils showed that the sampling sites close to the petrochemical complex were not necessarily the ones with the highest concentration as expected. The sum of the eight PAHs was the highest for soil samples collected at the roads side suggesting that vehicular emissions were a significant contributor to the level

of the PAHs in the environment. Moreover, the diagnostic ratios of the selected PAHs suggest that the PAHs predominantly originate from a petrogenic source except the soil samples collected at site 5, 16 and 35 where PAHs were probably originating from biomass combustion.

The results of a correlation test showed that a weak correlation ($r_s \leq 0.39$) was found between SOM and the PAH concentration in summer while a moderate correlation (r_s values between 0.40 and 0.59) was observed between SOM and some of the PAH concentrations during winter. A weak correlation between SOM and the PAHs should be expected in the soil subjected to continuous input of PAHs. A strong correlation ($r_s >$ 0.60) between individual PAH compounds in summer and winter was found suggesting that the PAH compounds might originate from similar sources. A lack of correlation between soil pH and the individual PAHs compounds during summer could indicate that the soil pH had no influence on the distribution of the PAHs in the soil.

The sum of the concentrations of the detected PAHs in the leaves was 30.69 ± 12.63 mg/kg for the unwashed leaves and was 28.26 ± 9.65 mg/kg for the washed leaves. No significant difference between unwashed and water-washed leaves with regard to PAH concentrations was observed ($p > 0.05$). A possible reason for that could be due to the fact that after deposition on the leaf surface, PAH compounds could migrate into the cuticular waxes, where they are physically protected. Both washed and unwashed leaves showed a high concentration of benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(b)fluoranthene and dibenzo(a,h)antracene compared to the other PAHs which could possibly be due to the fact that those PAHs are found in abundance in nature.

In the soil samples, the mean concentration of the individual PAH compounds ranged between nd and 0.15 mg/kg, whereas in the leaf samples the mean concentration of the individual PAH compounds ranged between 0.96 and 7.95 mg/kg. A comparaison between the PAH concentration in the soils and in the leaves showed that there were significant differences ($p < 0.05$) between the PAH concentration detected in unwashed leaves and the PAH concentration detected in soil samples during summer. This suggested that PAHs might accumulate in leaves predominantly via atmospheric deposition and not through uptake from the soil via the root of the plant. This study showed that the Port Jackson

Willow leaves can be used as a suitable biomarker for the presence of PAHs in the environment.

6.2   Recommendations

- For future work, the Port Jackson Willow leaves can be used as a biomarker for the presence of PAHs in the environment.
- Further studies to evaluate the use of the Port Jackson Willow as a biomarker for other organic and inorganic pollutants.
- Continued monitoring of PAHs in soil has to be conducted in order to ascertain environmental safety.
- Further studies are required to confirm the petrogenic sources of the PAHs in soil samples around petrochemical plant in Cape Town and to determine which levels are acceptable in soils and plants since their levels in South Africa have not been specified yet.

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APPENDICES

Appendix A: Physicochemical parameter of soil samples in winter and summer

Sampling sites	Winter		Summer	
	SOM (%)	pH	SOM (%)	pH
Site 1	3.07	6.74	5.15	8.08
Site 2	1.20	7.26	1.72	7.59
Site 3	1.46	6.03	1.58	7.30
Site 4	1.60	6.72	2.09	8.16
Site 5	4.93	7.16	7.61	7.36
Site 6	1.25	5.82	2.98	7.62
Site 7	4.80	6.40	2.14	6.48
Site 8	1.66	6.87	3.94	6.73
Site 9	3.07	5.38	1.51	6.97
Site 10	2.74	5.79	1.40	6.07
Site 11	3.31	5.92	1.21	8.20
Site 12	1.41	7.19	6.30	6.68
Site 13	1.20	6.85	2.72	7.68
Site 14	1.78	7.28	7.36	6.45
Site 15	1.21	6.03	0.20	7.74
Site 16	3.43	6.97	2.51	6.95
Site 17	2.40	7.60	1.22	7.36
Site 18	6.14	7.13	0.63	7.47
Site 19	0.81	6.96	2.78	6.67
Site 20	2.09	6.52	4.09	7.78
Site 21	0.10	7.33	0.69	7.29
Site 22	1.06	7.79	0.65	7.45
Site 23	1.49	6.92	5.38	7.71
Site 24	0.66	7.18	0.89	6.30
Site 25	3.83	6.42	2.07	7.74
Site 26	7.07	7.63	4.53	7.30
Site 27	3.94	6.40	3.57	7.78
Site 28	8.57	6.55	5.83	6.95
Site 29	8.77	4.63	3.54	7.26
Site 30	1.48	5.73	0.79	7.45
Site 31	2.76	6.76	2.01	7.74
Site 32	1.18	6.96	3.07	7.49
Site 33	1.67	6.50	1.51	7.52
Site 34	3.66	7.63	3.34	7.27
Site 35	0.15	6.53	4.76	6.23

Appendix B: Spearman Correlation coefficient (r_s) of individual PAHs , SOM and pH in soil samples in winter (values in bold) and summer

	PH	SOM	FLUO	IP	CHY	BAP	BKF	BBF	DAHA	BaA
PH	1.00									
SOM	- 0.14 **- 0.19**	1.00								
FLUO	0.15 **- 0.45**	- 0.22 **- 0.31**	1.00							
IP	0.11 **- 0.15**	- 0.12 **- 0.35**	0.45 **-0.23**	1.00						
CHY	- 0.15 **- 0.41**	- 0.03 **- 0.47**	0.32 **- 0.59**	0.46 **- 0.54**	1.00					
BAP	0.06 **- 0.30**	0.07 **- 0.31**	0.30 **- 0.31**	0.71 **- 0.71**	0.20 **- 0.56**	1.00				
BKF	- 0.07 **- 0.32**	- 0.08 **- 0.33**	0.26 **- 0.62**	0.56 **- 0.42**	0.82 **-0.68**	0.34 **0.42**	1.00			
BBF	- 0.04 **- 0.17**	- 0.04 **- 0.42**	0.35 **- 0.45**	0.72 **- 0.20**	0.52 **- 0.43**	0.75 **- 0.23**	0.42 **- 0.40**	1.00		
DAHA	0.02 **nd**	- 0.10 **nd**	0.43 **nd**	0.72 **nd**	0.62 **nd**	0.56 **nd**	0.76 **nd**	0.67 **nd**	1.00	
BaA	nd **- 0.07**	nd **- 0.31**	nd **- 0.14**	nd **- 0.31**	nd **- 0.37**	nd **- 0.33**	nd **- 0.18**	nd **-0.43**	nd **nd**	1.00