# Determination of Polycyclic Aromatic Hydrocarbons in the Water, Soils and Surface Sediments of the Msunduzi River, KwaZulu-Natal, South Africa

UNIVERSITY OF
KWAZULU-NATAL ™
INYUVESI
YAKWAZULU-NATALI

By

Alexis Munyengabe

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

School of Physics and Chemistry

University of KwaZulu-Natal

Pietermaritzburg

South Africa

Supervisors: Dr B. Moodley

Dr A. Mambanda

January 2016

Declaration

I, Alexis MUNYENGABE, hereby affirm that the work reported in this thesis is my own contribution and where other people's work was incorporated it was fully acknowledged by the means of citations. The thesis has not been submitted to any other University for a qualification award.

This work was carried out in the School of Chemistry and Physics, Pietermaritzburg Campus, CAES, University of KwaZulu-Natal.

Signed (candidate)…………………………

………………..day of …………..2016

Signed Co-supervisor………………………..

………………day of ……………2016

Signed Supervisor…………………………..

…………….day of …………….2016

Poster presentations:

- College of Agriculture, Engineering and Science (CAES), UKZN Research day: 22nd September 2015. Poster title: Determination of Polycyclic aromatic hydrocarbons in the water, soils and the surface sediments of the Msunduzi River in KwaZulu-Natal.

- 42nd National Conference of SACI: 29th November-4th December 2015, Durban. Poster title: Determination of Polycyclic aromatic hydrocarbons in the water, soils and the surface sediments of the Msunduzi River in KwaZulu-Natal.

…………………………..…………………………………

Signature

Alexis Munyengabe

Date: …………………………………..

Dedication

To the Almighty God

To my late father

To my mom

To my uncles and aunts

To my brother and sisters

To my friends and family

Acknowledgements

Table of Contents

List of Figures

List of Tables

List of Plates

List of acronyms and abbreviations

AA	Agricultural area
ACY	Acenaphthylene
ANTH	Anthracene
ASE	Accelerated-Solvent Extraction
ASTDR	Agency for Toxic Substances and Disease Registry
°C	Celsius degree
CAES	College of Agriculture, Engineering and Science
CD	Camp's Drift
CHRY	Chrysene
CSIR	Council for Scientific and Industrial Research
CO$_2$	Carbon dioxide
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
DUCT	Duzi-Umgeni conservation trust
DuTV	Du Toit Viljoen Bridge
dw	Dry weight
DWWTP	Darvill Wastewater Treatment Plant
ECD	Electron Capture Detector
EI	Electron Impact

eV   Electron Volt

GC   Gas Chromatography

GC-MS   Gas Chromatography-Mass Spectrometry

g/m³   Gram per cubic meter

GPS   Global Position System

FID   Fluorescence Ionization Detector

FLUO   Fluorene

FT-IR   Fourier transform-infrared cyclotron

HD   Henley Dam

HETP   Height-Equivalent to a Theoretical Plate

HMW-PAHs   Higher Molecular Weight Polycyclic Aromatic Hydrocarbons

HPLC   High-Performance Liquid Chromatography

ID   Internal Diameter

ISO   International Organization for Standardization

JUM   Junction of the Msunduzi and Umgeni Rivers

KC   Equilibrium constant

K*ow***   Octanol-water partition coefficient

KZN   KwaZulu-Natal

LLE   Liquid-Liquid Extraction

LMW-PAHs   Lower Molecular Weight Polycyclic Aromatic Hydrocarbons

LOD   Limit of Detection

LOQ   Limit of Quantification

MAE	Microwave-Assisted Extraction
MAR	Mean Annual Run off
ML	Mega Litre
mg/kg	Milligram per Kilogram
mg/L	Milligram per Litre
MSD	Mass Selective Detector
MT	Msunduzi Town
m/z	Ratio of Mass over Atomic number
µg/g	Microgram per gram
µg/kg	Microgram per Kilogram
µg/L	Microgram per Litre
µg/mL	Microgram per millilitre
NA	Naphthalene
ND	Not Detected
NDA	Nagle Dam
ng/g	Nanogram per gram
ng/L	Nanogram per litre
ng/mL	Nanogram per millilitre
ng/mole	Nanogram per mole
ng/m³	Nanogram per cubic meter
NIST	National Institute of Standards and Technology
NRC, US	National Research Council (US)

PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PHEN	Phenanthrene
PHWE	Pressurized Hot Water Extraction
PLE	Pressurized Liquid Extraction
PMAE	Pressurized Microwave-Assisted Extraction
PMB	Pietermaritzburg
PNAs	Poly-Nuclear Aromatic Hydrocarbons
POPs	Persistent Organic Pollutants
PYR	Pyrene
RSA	Republic of South Africa
RSD	Relative Standard Deviation
RT	Retention Time
SCOT	Support-Coated Open Tubular
SD	Standard Deviation
SE	Soxhlet Extraction
SFE	Supercritical Fluid Extraction
SIM	Selected Ion Mode
SPE	Solid-Phase Extraction
SPME	Solid-Phase Microextraction
TCD	Thermal Conductivity Detector
TLC	Thin Layer Chromatography

USA   United States of America

US EPA   United States Environmental Protection Agency

VPC   Vapour-Phase Chromatography

WCOT   Wall-Coated Open Tubular

WHO   World Health Organization

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants which are known carcinogens. Their presence in the environment has been linked to cancer, neurological and reproductive illnesses to name but a few. Hence it is important to monitor the levels of these PAHs in order to identify areas of high pollution and possible toxic exposure to aquatic and human life. The concentration of seven polycyclic aromatic hydrocarbons (namely naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, pyrene and chrysene) were determined in 28 surface water, 8 wastewater, 26 soil and 26 surface sediment samples from the Msunduzi River. Water samples were extracted using a liquid-liquid extraction technique into dichloromethane and dehydrated with sodium sulfate anhydrous. The soils and surface sediments were extracted with a mixture of dichloromethane and *n*-hexane (1:1 v/v) using the soxhlet extraction technique. The crude extracts were purified by silica gel packed column chromatography. The concentrations of PAHs in the extracts were analysed by GC-MS. The instrument was calibrated using internal standardization (deuterated PAH) and PAH standards. Percentage recoveries of 7 PAHs in the spiked and equilibrated samples varied from 79.16 ± 0.01 to 101.28 ± 0.02 and 80.30 ± 0.02 to 105.56 ± 0.01 for solid and water samples, respectively. The grand average in the summed concentrations of concentrations of the 7 PAHs in the water for all seasons decreased in the order: $\sum[\text{7-PAH}]_{spring} > \sum[\text{7-PAH}]_{summer} > \sum[\text{7-PAH}]_{autumn} > \sum[\text{7-PAH}]_{winter}$ while in the surface sediments was in the order: $\sum[\text{7-PAH}]_{spring} > \sum[\text{7-PAH}]_{autumn} > \sum[\text{7-PAH}]_{summer} > \sum[\text{7-PAH}]_{winter}$ and in the soils was in the order: $\sum[\text{7-PAH}]_{spring} > \sum[\text{7-PAH}]_{autumn} > \sum[\text{7-PAH}]_{winter} > \sum[\text{7-PAH}]_{summer}$. The concentration of PAHs was found to be comparatively higher in the soils and surface sediments than in the water samples.

Chapter I

Introduction

1.1 Source and Occurrence of PAHs in the Environment

Since the onset of the industrial revolution, pollution of the water, soil and sediment matrices is progressively becoming a major problem all over the world (Farmer, 1991; Alloway & Ayres, 1997; Sakari, 2012). Apart from this, the air quality we breathe has also deteriorated mainly as a result of anthropogenic activities (Azhari, 2012; Sakari 2012). The level of pollution has been exacerbated by the increase usage of chemicals in agricultural production and generation of wastes, some of which are persistent and toxic to environmental ecosystems (Alloway & Ayres, 1997). The most well-known pollutants that contaminate water, soils and sediments are the persistent organic pollutants (POPs). These pollutants are mainly generated from the incomplete combustion of organically-derived materials at both domestic as well as industrial scales (Odabasi et al., 1999; Soclo et al., 2000; Wenzl et al., 2006).

Polycyclic aromatic hydrocarbons (PAHs) are a subgroup of POPs and an important class of hydrocarbons, which contain mainly carbon and hydrogen atoms in their structures (Sakari, 2012). Sometimes, PAHs can be found as their alkylated or substituted derivatives, and they can exist in many isomeric forms (Gurjar et al., 2010). However, the most commonly occurring ones do not carry branching substituents on their rings (Wenzl et al., 2006).

Being a subclass of POPs, PAHs are formed frequently from the incomplete combustion of organic wastes and fossil fuels (Azhari, 2012). Polynuclear aromatic hydrocarbons (PNAs) are a major category of PAHs found naturally on the atmospheric particulates, the aquatic and terrestrial environments. They comprise of fused benzene rings in which each sp^2-hybridized carbon shares electrons with at least

two other carbon atoms (Sims & Overcash, 1983). The simplest structure of these compounds is naphthalene ($C_{10}H_8$), which consists of two coplanar six-membered rings sharing an edge. Naphthalene occurs in the environment as a first non-alkylated lower molecular weight PAH (LMW-PAH) *(Okedeyi, 2012)*. This extended aromaticity of PAHs can be through fused planar rings that share their edges in a linear, angular or two-dimensional clustered arrangement (Figure 1.1) (Harvey, 1998; Loganathan & Lam, 2011). In the fused ring system of PAHs, electrons are delocalized within their extended π-molecular orbitals (Harvey, 1998).
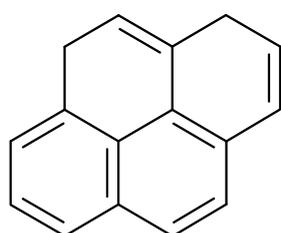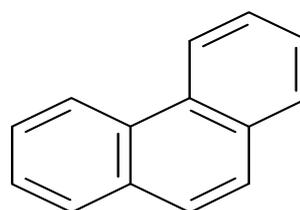
(a) Pyrene   (b) Phenanthrene

Figure 1.1 Examples of naturally occurring PAHs

This makes them more thermodynamically stable against biochemical degradation *(Okedeyi, 2012)*. Because of their chemical stability, and hydrophobic nature, they are considered as environmentally persistent organic compounds of varied structures (isomers) and toxicities, especially those with five or more aromatic rings *(Adrian et al., 1999)*. An increase in the aromatic conjunction and structural angularity both enhance the recalcitrance to biochemical degradation of PAHs in the natural environment (Loganathan & Lam, 2011). This recalcitrance to degradation of PAHs increases as a function of their molecular weight. As a result of their toxicity, chemical stability and hydrophobic nature, PAHs are considered to be higher priority organic pollutants for regular monitoring in the environment (Neff, 1979).

Moreover, due to their persistent characteristics and widespread sources, PAHs are mainly emitted from anthropogenic and natural sources such as biomass and fossil fuel burning into the atmosphere. They can remain over a long period of time and

hence reach even cold and remote areas through atmospheric currents (Sakari, 2012). For example, elevated levels of alternant PAHs was 428±240 g/m^3 and was found in the urban atmosphere of the city of Chicago in a study aimed at determining their air-water exchange and dry deposition levels (Odabasi et al., 1999). The emission sources were attributed to industrial activities within the metropolitan area. In another study, PAHs were also detected in the marine aerosols collected from the West Mediterranean Sea. The source of emissions was suspected to be petroleum rigging and pyrolysis fuels from industries. Their concentrations ranged between 0.25 and 0.45 ng/m^3 (Sicre et al., 1987). Elevated concentrations of PAHs have been detected in the airborne particles sampled as headspace from the São Paulo city in Brazil and compared to levels measured in the scarcely inhabited nearby virgin forests (Vasconcellos et al., 2003).

PAHs in the atmosphere can be dispersed over long distances depending upon prevailing atmospheric conditions. PAHs can be transported as adsorbed chemicals, on suspended particulate matter or as their clustered particulates. This global distribution in the atmosphere in other remote locations has been blamed for the occurrence of PAHs in remote locations with temperate climates where they are easily deposited by precipitation (Loganathan & Lam, 2011; Azhari, 2012). In general, higher molecular weight PAHs (HMW-PAHs) are deposited over shorter dispersal distances and are easily removed by precipitation. The relatively lower molecular weight polycyclic aromatic hydrocarbons (LMW-PAHs) are dispersed over long distances spanning continents (Fernandez et al., 2000). For example, PAHs have been detected in the sediments and biota from Bjørnøya (Bear Island) located in the Norwegian Arctic far away from agricultural and industrial activities (Evenset et al., 2007).

Rose et al. (2004) detected some PAHs in a lake from the Svalbard Archipelago, Norway. Their occurrence in the island sediment was attributed to a long-range transport through the atmosphere since coal combustion from local power stations could not account for the elevated amounts. Research on PAHs in environmental samples such as aerosols as well as marine and lacustrine sediments, wet deposition and sea water has been reported. Also, PAHs have been detected in ice core samples

from Greenland. It was suggested that these organic pollutants were coming from past atmospheric emissions, from fossil fuel combustion and from the Northern hemisphere countries (Kawamura et al., 1994).

Long-term exposure to high concentrations of PAHs is linked to different health complications such as infertility, cancer, and neurotoxicity (Vu et al., 2010; Loganathan & Lam, 2011; Azhari, 2012). For example, some PAHs are suspected carcinogens, mutagens and an immunosuppressant to different kinds of organisms (White et al., 1985; Loganathan & Lam, 2011). There are many different PAHs existing in common human-interfaced environments, but benzo [a] pyrene and 7,12-dimethylbenzo [a] anthracene are two of the most studied PAHs threatening human health in most urban built environments. They are feared to biotransform into even more toxic metabolites (Buha, 2011; Azhari, 2012).

Inhalation of PAHs adsorbed on particulates poses a serious risk, which include bronchitis and pulmonary cancers. Higher incidence of nasal cancers has been reported in persons residing close to heavily industrialized locations compared to rural residents (Cerniglia, 1984). Due to the high lipophilicity of PAHs, their bioaccumulation in the body after ingestion or inhalation is significant. In general, elevated concentration levels of PAHs are usually found in adipose tissues of mammals and their renal excretion is low. These tissues can store PAHs for a long period of time releasing them only through metabolic activation by specific enzymes (Cerniglia, 1984; Buha, 2011).

Anthropogenic PAHs enter the aquatic systems through two main sources, namely petrogenic and pyrogenic. LMW-PAHs are emitted from petrogenic sources such as combustion of diesel fuel, kerosene and gasoline and weathering of asphalt surfaces. They can also enter the environment through oil spills from container wreckages, surface runoff, and atmospheric fallouts. On the other hand, HMW-PAHs are emitted from pyrolytic processes (Lee et al., 2009; Nkansah, 2012; Sakari, 2012). Pyrogenic PAHs are dispersed as clusters on nuclei particulates facilitating their dispersal over long distances. They are eventually deposited on terrestrial plants, soil, surface sediments and ocean bottom sediments (NRC US, 1985). In the aquatic systems, higher

concentration levels of LMW-PAHs can be found in the pore water and in the column of surface water itself due to their slight solubility. However, HMW-PAHs, due to their higher hydrophobic nature, are normally transported as bound complexes to fine particles dissolved in organic matter and can be found in elevated concentrations in the sediments and soils (Vu et al., 2010; Loganathan & Lam, 2011). This was confirmed by the study conducted on the water and sediments from the Baltic Sea investigating the pollution level of PAHs. Relatively, higher concentration levels of LMW-PAHs were found in the water than in the sediments while the opposite trend was reported for HMW-PAHs where the surface sediments had elevated levels of PAHs than water. In the environment, the incidences of elevated levels of HMW-PAHs (Witt, 1995; Loganathan & Lam, 2011).

In the sediments, PAHs can be resuspended into the water column *via* bioturbation, degradation or subjection to long-term persistence by threatening the aquatic life (Wong et al., 1995). However, research done on long-term exposure to any concentration levels of PAHs in the water has shown it to cause sub-lethal effects to the aquatic organisms. Thus, it is important to monitor any pollution of PAHs in all compartments of the environment (Witt, 1995).

1.2 Chemical and Physical Properties of PAHs

As pure chemicals, PAHs usually exist as white, pale yellow or colourless solids. Chemical and physical properties of PAHs vary with an increase in their molecular weights (Yu et al., 2011). In the atmosphere, PAHs can react with other contaminants such as chlorine, sulfur dioxide, ozone and nitrogen oxides, producing dinitro- and nitro-PAHs, sulfonated acids, diones, and chlorinated PAHs (Loganathan & Lam, 2011). Based on differences in their physical and chemical characteristics, PAHs can be conveniently classified into two major groups. The first group comprises of the lighter or lower molecular weight PAHs with its members having 2 to 3 fused benzene rings. They range from naphthalene to fluorene. PAHs falling within this first group

are thought to be acutely toxic to mammals. The second group comprises of the heavier PAHs. Members of this group contain 4 to 6 fused benzene rings and range from chrysene to indeno[1,2,3-(c,d)]pyrene.

PAHs have been classified as 16 priority pollutant by the United States Environmental Protection Agency (US EPA) because of their toxic, mutagenic, carcinogenic and teratogenic properties *(Silva et al. , 2007; Wenzl et al., 2006)*. In general, PAHs are highly lipophilic, but some of the LMW-PAHs are slightly soluble in the water and at the same time are more volatile. The HMW-PAHs are nearly insoluble in water and tend to adsorb strongly on the surfaces of particulates or onto non-polar matrices (Neff, 1979; Buha, 2011). Their vapour pressure is very low and decreases with an increase in the number of fused benzene rings, while their resistance to oxidation and reduction reactions increases with an increase in molecular weights *(Manoli & Samara, 1999; Nekhavhambe et al., 2014)*. Some of their physical properties are presented in Table 1.1.

Table 1.1 Physical and chemical properties of selected PAHs

PAHs	Number of rings	Structure	Chemical formula	Molecular weight (mg/mole)	Melting point (^oC)	Boiling point (^oC)	Solubility in water (mg/L)
Naphthalene	2		$C_{10}H_8$	128.1	81	218	3.2
Acenaphthylene	3		$C_{12}H_8$	152.2	92-93	265-275	3.9
Fluorene	3		$C_{13}H_{10}$	166.2	116-117	295	1.7-2.0
Anthracene	3		$C_{14}H_{10}$	178.2	218	340-342	0.8
Phenanthrene	3		$C_{14}H_{10}$	178.2	100	340	1.2 (at 2 ^oC)
Pyrene	4		$C_{18}H_{12}$	202.3	156	393-404	2.3×10^{-3}
Chrysene	4		$C_{18}H_{12}$	228.3	255-256	448	2.8×10^{-3}
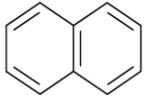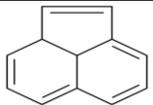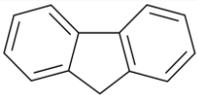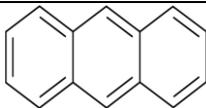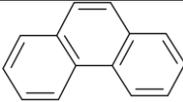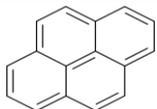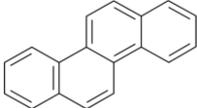
The boiling and melting points of PAHs increase with an increase in the number of fused benzene rings. Their fate in the environment is primarily governed by their chemical and physical properties (Buha, 2011). This also means that at the point of emission through pyrolysis, they resist thermal transformation and degradation into new by-products. They can withstand the high combustion and pyrolysis temperatures in anthropogenic emitters such as thermal power stations, fuel refineries, synthetic fuel reactors, automobile exhausts, domestic burning and incinerators (Manoli & Samara, 1999; Nekhavhambe, et al., 2014). PAHs are known to be resistant to enzymatic degradation in biological systems with a long-term effect of initiating cancers in cells (Gibson et al., 2011; Jazestani, 2011).

It is likely that PAHs are primary causative agents in a significant percentage of human cancers (Harvey, 1982). Indeed, PAHs have been shown to induce tumours on the ears of rabbits by repeated application of coal tar. The mutation has been shown to occur in a pink salmon embryos after the fish were exposed to PAHs, indicating a possibility that exposure of fish to some HMW-PAHs can lead to alteration of genes (Walker, 2008).

Exposure of PAHs that leads to chronic diseases has been reported to cause mutagenic and carcinogenic effects, cataracts, kidney and liver damage and jaundice in marine invertebrates, fish, amphibians, animals and human beings (Neff, 1978; Azhari, 2012). Human exposure to PAHs has also been generally accompanied by high levels of mutated DNA adducts and reproductive defects (Gaspari et al., 2003).

A diverse range of malignancies can be induced by PAHs, relying on the experimental approach. For example, an oral administration of 7,12-dimethylbenzo[a]anthracene to the female Sprague-Dawley rats has shown to cause mammary cancer. On the other hand, an intracellular injection of the same compound in the male Long-Evans rats induced local sarcomas (Harvey, 1982). The toxicological effects of PAHs have been widely reported in fish. For example, when a fish is exposed to sediments with high PAH pollution level, such as 250 mg/kg, they can develop some hepatic tumours (Walker, 2008). As a result, environmental concern over the fate and effects of these contaminants has increased in recent years (Yunker et al., 2002).

Chemical analysis to determine their fate in different receiving environmental components should be regulated. This is necessitated by their persistence, acute toxicity, and potential for bioaccumulation, widespread distribution and carcinogenicity to mammals (Walker, 2008). This means that all industrial and domestic activities, which are known to generate and emit these compounds should be monitored regularly to prevent or minimize pollution of the wider environment.

1.3 Reactivity of PAHs

In general, PAHs are chemically stable under environmental conditions. As such, in the aquatic systems, they are strongly sorbed to clay particles and can resist biochemical conversion to other products or water washing (Douglas et al., 1996). In the atmosphere, they are unaffected by visible radiation, although they are known to undergo photo-induced free radical reactions. The photochemical reactions of PAHs are important in determining their ultimate fate in the atmosphere (Yu, 2002; Lohmann et al., 2009).

Taking naphthalene as an example, when it absorbs an ultraviolet photon, an excited molecular radical cation may be formed. The reactive radical is easily oxidized to form a variety of aromatic carbonyl compounds as shown in Figure 1.2. Alternatively, the radical can dissociate to give a daughter radical accompanied by the loss of a small molecule such as hydrogen gas or a low molecular hydrocarbon. The reactivity of PAHs adsorbed on natural aerosol particles was studied by exposing them to model oxidants simulating their fate in the wider atmosphere (Yu, 2002). LMW-PAHs were found to have higher reactivity compared to HMW-PAHs. This was attributed to higher surface absorptivity onto soil organic matter by HMW-PAHs, which made them unavailable for reactions with model oxidants (Ringuet et al., 2012). For example, naphthalene can easily react with O_3, OH and NOx radicals, forming in each case more reactive and toxic products, some of which are mutagenic. However, photons of high intensity are required to convert PAHs into non-aromatic products as (Yu, 2002; Bente

et al., 2009) shown in Figure 1.2. The photolysis of naphthalene in aqueous solutions normally produces 7-hydroxy-1,4-naphthoquinone (a), 2-formylcinnamaldehyde (b) and 2-carboxycinnamaldehyde (c). It was suggested that compound (a) can be formed *via* the 1,4-naphthoquinone intermediate, and compounds (b) and (c) can be formed from the breaking of the C_1-C_2 bond in naphthalene (Yu, 2002).

(a)   (b)   (c)
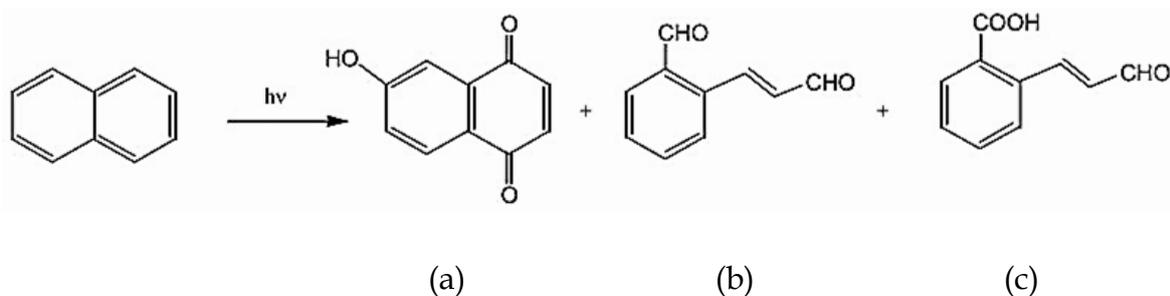
Figure 1.2 The photochemical reaction of naphthalene in aqueous solution (Yu, 2002).

The photochemical reactions of anthracene and benzo[a]anthracene also follow the same decomposition as naphthalene because they both have two sensitive positions in their molecular structures: positions 9 and 10 in anthracene and positions 7 and 12 in benzo[a]anthracene (Yu, 2002).

In addition, lower molecular weight PAHs can undergo chemical reactions characteristic of aromatic hydrocarbons such as electrophilic substitution, oxidation and reduction under harsh laboratory experimental conditions (Keyte et al., 2013). Oxidation and reduction reactions partially decompose the extended conjugation of the fused benzene rings. It has been established that resistance to oxidation and reduction tends to decrease with increasing molecular weight *(*Harvey, 1991*)*.

PAHs can also undergo different reactions by opening their rings to form PAH-diol epoxides which are very highly reactive and mutagenic. These PAH-diol/epoxides can have an impact on the normal cell replication when they react with DNA to produce adducts that can initiate the carcinogenic processes (Jerina et al., 1980; Zhong et al., 2010; Muñoz & Albores, 2011).

1.4 Source Apportionment of PAHs

The emission of PAHs into the atmosphere can be grouped into at least five sources, namely domestic (fuel, wood, coal, liquid paraffin, biomass), mobile (aircraft, ships, rail wagons, road vehicles), industrial (electricity generation, mining, manufacturing, aluminium production, iron and steel foundries), agricultural production (open burning of brushwood and straw among others) and natural (forest fires, volcanic eruptions and degradation of biological materials) (Suess, 1976; Rubailo & Oberenko, 2008). Then again, these sources of PAHs can be categorised into two major groups, namely anthropogenic (these include pyrogenic and petrogenic sources) and lithogenic (which are natural).

LMW-PAHs tend to be emitted predominantly from petrogenic processes while HMW-PAHs are mainly produced during pyrogenic activities (Lee, Kim & Kim, 2009; Nkansah, 2012). When combustion of organic matters occurs in a limited supply of air, the products cool rapidly and small organic substances may condense to form new chemicals, which include higher molecular pyrolytic PAHs (Neff et al., 2005). The main anthropogenic activities that produce PAHs pyrolytically are domestic or commercial burnings, weathering of creosote, tar-applied surfaces, coal-tar production, industrial waste, vehicular exhaust from diesel, and petrol engines and burning of waste (WHO, 2003).

Researchers have used the magnitude of calculated concentration ratios of certain individual PAHs in environmental matrices to characterise possible sources and emission pathways. For example, the magnitude of concentration ratios such as [Anth]/[Phen] and [Anth]/([Anth]+[Phen]) have been used to distinguish petrogenic sources from pyrogenic ones. When the magnitude of [Anth]/[Phen]) is less than 0.10 and that of [Anth]/([Anth]+[Phen]) is less than 0.40 in several matrices (sludge, dust particulates, sediments, pore water, etc.), the source of emission of PAHs into the environment is usually of petrogenic output. Conversely, if the ratio of [Anth]/[Phen]

is greater than 0.10 and even that of [Anth]/([Anth]+[Phen]) is greater than 0.40; the source of PAHs will be attributed to pyrogenic activities such as pyrolysis of crude oils and coal *(Silva et al., 2007)*. Under pyrolytic conditions, a significant amount of anthracene is formed in the emitted aerosols, which is released into the atmosphere and finally deposited in the terrestrial as well as aquatic environments (Budzinski et al., 1997; Chen et al., 2012). This increases its molar fraction in the emitted aerosols. Unlike, most other organic chemicals in the environment, PAHs are not released in their pure form. Reasonably, because PAHs consist of thousands of structures originating from at least three sources, they always occur in the environment as complex mixtures, not as pure compounds *(Ravindra, Mittal & Van Grieken, 2001; Burgess, Ahrens & Hickey, 2003)*.

1.5 Exposure and pathways of PAHs

PAHs are present in atmospheric, aquatic and terrestrial ecosystems at concentration levels that depend on the location, nature of the anthropogenic sources of emission and the prevailing weather situations. As a result of this, exposure of humans to PAHs is expected to be higher in the atmosphere of an urban-built environment, including its water receiving amenities *(Jenkins, Tomkins & Guerin, 2000; Tao et al., 2006)*.

There are mainly three different routes by which humans may be exposed to PAHs leading to active assimilation in their bodies. These are ingestion of contaminated food and water, dermal contact and direct inhalation of polluted air including tobacco smoke *(Ravindra et al., 2008; Jazestani, 2011)*. Higher amounts of benzo[a]pyrene and other varieties of PAHs have been detected in tobacco smoke (Albers, 2002). However, it is difficult to accurately quantify exposure, due to the wide variety of the types of PAHs in the contaminated environments *(Jenkins et al., 2000; Albers, 2002)*. Due to higher concentration levels of PAHs in the emissions from domestic combustion of solid fuels, PAHs have been linked to lung cancer and other related diseases, especially in developing countries (Straif et al., 2006).

Some studies suggested that PAHs can inhibit the sustainable growth of diatoms in deep oceans and may even affect the global cycle of carbon (Zhang & Tao, 2009). Even terrestrial plants can also assimilate PAHs from the atmosphere by wet or dry deposition on their leaves, and from soils through their roots and translocate them to other plant parts thus making them more available in the food chain. Their uptake rates are generally governed by many factors such as concentration, aqueous solubility, physical and chemical states as well as soil type *(*Tao et al., 2006*)*. PAH-induced phytotoxic effects are rare. However, certain plants contain substances that can protect against PAH effects whereas others can synthesize PAHs that perform as growth hormones *(*Tao et al., 2006*)*.

1.6 Environmental Fate

Pyrogenic and petrogenic sources release airborne dust and particulate matter contaminated with PAHs into the atmosphere. The adsorbed PAHs can be dispersed over long-range distances with the possibility of polluting pristine and remote located areas, depending on the prevailing weather conditions (Baek et al., 1992 and Gurjar et al., 2010). Several studies have been reported on the occurrence of PAHs detected in some remote ecosystems and mountainous areas or crater lakes, in the Arctic and Antarctic regions, which are locations that are far away from possible human-related sources of emission *(*Quiroz, Grimalt & Fernández, 2010; Jazestani, 2011*)*. This means that PAHs emitted from Asia can influence the atmospheric concentrations of PAHs in the Arctic region and similarly PAHs from the USA can end up in the Antarctic region (Zhang & Tao, 2009). This is possible because of their long residence time in the atmosphere due to their chemical stability (Jazestani, 2011). However, PAHs can be washed off from the atmosphere through precipitation and atmospheric fallout into receiving aquatic systems (frost, fog or snow) (Baek et al., 1992).

Once deposited in the marine environment (surface water, rivers and oceans) *via* dry or wet deposition, PAHs partition easily onto hydrophobic phases such as organic

matter or sediments (Loganathan & Lam, 2011). The sedimentation of PAHs stabilises them in the solid phase and concentrates those that are slightly soluble in water and in the pore waters of the sediments. This forms the base source of PAHs to marine organisms which can reach lethal levels through bioaccumulation in marine predators (Baek et al., 1992; Lohmann et al., 2009).

In the aquatic systems, PAHs can photodegrade, volatilize, biodegrade, oxidize and bind to small organic particles suspended in water. For example, PAHs present in soils may undergo volatilization, adsorption, chemical oxidation, accumulation in plants and photolysis even though microbial degradation is the major transformation process. Moreover, PAHs can also enter ground waters and can be transported within the aquifer (Baek et al., 1991; Yu et al., 2011).

PAHs occurring in the aquatic ecosystems can adsorb tenaciously on the alluvial, sediments and suspended organic particulate matter, forming stable chemisorbed complexes. Consequently, this makes them readily bioavailable to aquatic microorganisms at the base of the food chain (Ahangar, 2010). For example, benthic invertebrates play an important role in the transfer of contaminants such as PAHs from sediments through bioturbation and in turn, the PAHs are taken up by high order feeders such as fish and other aquatic creatures (Palmqvist et al., 2006).

Through this bioaccumulation, organisms at the top of the food chain are exposed to a high concentration of PAHs, which can be lethal (Clements, 1992). Surface adsorption can also stabilise chemisorbed-PAH complexes on suspended and micro-sized organic particulates in the atmosphere. This facilitates their long-range dispersal leading to global atmospheric pollution. However, PAHs can stay in the adsorbed forms (e.g. on sediments or suspended particulate matters) since they are non-volatile compounds. Consequently, their higher concentration levels have been recorded in some coastal waters, soils, aquatic organisms, food materials and sediments of rivers and lakes near agricultural areas, urban and industrial cities in many parts of the world (Silva et al., 2007; Martins et al., 2014). A total concentration of 15 PAHs were found in soil samples taken from locations from different continents. Their concentrations spanned over 5 orders of magnitude. The concentration levels in the

soils decreased in the order: locations in Europe > North America > Asia > Oceania > Africa > South America. Locations susceptible to higher atmospheric deposition inputs tended to have higher concentrations *(*Nam, Sweetman & Jones, 2009*).*

PAHs emitted into the atmosphere can be transformed through photo-oxidation, chemical oxidation into stable or even in more toxic forms. In the biological systems, PAHs can be metabolized into more toxic forms or accumulated to levels that are lethal to organisms (Cerniglia, 1993).

Both environmental persistence and genotoxic properties increase as PAH molecular weights increase. Those with many fused benzene rings are the sensitizers of cancers, leading to the development of cancers in mammals and are of toxicological and environmental concern (Cerniglia, 1993). Possible sources of emission and the fate of some PAHs in the wider environment are presented in Table 1.2.

Table 1.2 Source of emission and fate of PAHs in the environment

Analyte	Sources of emission and fate of selected PAHs
Naphthalene	It is a toxic compound in the water-soluble fraction of crude and fuel oils but is a non-carcinogenic PAH. It is generated from pyrolysis of coal tar and petroleum refinery products. It is chemically stable, but can be decomposed by ultraviolet radiation to form more toxic compounds. In mammals, it destroys red blood cells and it causes confusion, nausea, vomiting, diarrhea, jaundice and cataracts in animals *(Jazestani, 2011)*.
Acenaphthylene	It is formed from combustion of crude oil, coal tar, dyes, plastics and pesticides, creosote, burning of agricultural wastes, produced by vehicle exhausts, hazardous wastes, wildfires, roofing tar, and heavy oils (Manoli et al., 2004). It can target fat tissues like kidney, liver and fat.
Fluorene	It is combustible and commonly found in dye, pesticides, and plastics and is produced from a byproduct of coal–conversion and energy related industries (Manoli et al., 2004).
Anthracene	It is formed from pyrolytic processes and occurs as a minor contaminant in wastewater effluents from coal gasification and liquefaction processes. It is a stable and persistent PAH in the environment due to its slow kinetics to biodegradation. In the aquatics, it tends to bind strongly to sediments and other particulate matters. Its small concentrations can leach into underground waters and this presents a potential contamination problem. It tends to bioaccumulate in aquatic animals and is highly toxic to wildlife. It is a non-carcinogenic PAH, but it may cause respiratory irritation. It may cause long-term adverse effects in the aquatic environment (Morrison et al., 1992; McMurry & Simanek, 2007) .
Phenanthrene	It is a constituent of exhaled cigarette ashes and smoke. It is a non-carcinogenic and non-mutagenic PAH and is toxic to mussels, gastropods, crustaceans, fish marine and diatoms *(Ouyang, 2006)*.
Pyrene	It is a possible mutagenic and a carcinogenic PAH, and toxic to microalgae *(Clément et al., 2005)*. It originates from oil seepages and oil spills. It is mainly formed from the incomplete combustion of oil products and. Being a perfused PAH, it is much more resonant stabilized PAH than fluoranthene, its five-membered rings containing isomer *(Petersen et al., 2009)*. In the body, pyrene can induce a kidney disease, nephropathy, and some slight changes in the blood as well increase the weight of the liver.
Chrysene	It is found in creosote and is used as a wood preservative. It is formed during the burning or distillation of crude oil, coal and plant material. It is thought to be a human carcinogen *(Ravindra, Sokhi & Van Grieken, 2008)*.

1.7 Justification of the Study

It is suspected that the Msunduzi River receives and conveys contaminated water with potentially toxic organic and inorganic compounds *via* sporadic bursting of sewer pipes (laden with domestic fecal matter), the urban surface runoff and intentional discharge of treated sewage effluent from the city of Pietermaritzburg. Amongst the discharged pollutants, PAHs may be included which are known to be carcinogenic, mutagenic and teratogenic to humans, animals, plants and aquatic life. In addition, domestic burning, seasonal veld fires and industrial emissions in and around the city can significantly transfer PAHs into the atmosphere which is further washed off by precipitation into receiving rivers.

Ordinarily, the concentration levels of environmental persistent organic pollutants in South Africa are not well-known, although the country is economically and industrially developed in Africa (Nieuwoudt et al., 2011). Even though some research has been done on selected POPs especially PCBs and pesticides such as DDT, there is very little information about the occurrence of PAHs in the South African environment (Nieuwoudt et al., 2011; Gakuba et al., 2015).

The PAHs that have been designated as priority contaminants by the US EPA are mainly of the petrogenic or pyrogenic source. This implies that PAHs are mostly part of the crude product at low levels or some may be formed during processes such as crude oil refining, burning of fossil fuels and petrochemical processes, or as a result of an incomplete combustion of organic matters, domestic heating, power generation, incineration, vehicle exhaust emissions, or natural fires (Masih & Taneja, 2006). These sources arise in South Africa, either distributed in suburban and rural areas or concentrated in industrial zones. Exposure of humans to PAHs can lead to elevated levels of reproductive deficiencies, an increased risk of cancer, and DNA mutation or other adverse health effects. It is therefore very important to control their concentration levels in the South African environment, mainly in areas where human health may be harmfully affected by the occurrence of these compounds (Nieuwoudt et al., 2011).

A literature review has shown that no study has been reported on the concentration levels of PAHs along the Msunduzi River, KwaZulu-Natal, South Africa. Data is available only on the analysis of pharmaceutical residues in the water and sediments, heavy metals in sediments and PCBs in the soil and milk samples from nearby farmlands located within the industrial catchments in KwaZulu-Natal. Other different studies on concentration levels of PCBs in the pore water, water and sediment of the Umgeni River, chlorinated hydrocarbon pesticides and chlorophenols at Isipingo Estuary have also been reported (Batterman et al., 2009; Grobler et al., 2010; Agunbiade & Moodley, 2014; Gakuba et al., 2015).

However, some isolated studies on PAHs in the soils taken from the Isipingo River, Umgeni River and Durban Bay (Umbilo, Umhlatuzana and Amanzimyana Rivers) have been described (Vogt, 2014). No data has been reported on the pollution levels of PAHs in the soils and water along the Msunduzi River. However, a study has been conducted to quantify PAHs in the treated sludge, collected from a wastewater treatment plant discharging treated effluent water back into this river (Cele, 2010). Meanwhile, there is a growing degree of ecological pollution, and it is, therefore, necessary to establish a data source on the occurrence of seven selected PAHs around KwaZulu-Natal. This study was conducted on five selected LMW-PAHs and two HMW-PAHs (see Table 1.2). This will add knowledge to the lack of information and to add a new database of these PAHs in environmental matrices of this country as a signatory member of the Stockholm convention in 2001. The selection of these PAHs was based on their solubility and availability in water, toxicological, carcinogenic, mutagenic and teratogenic effects on human beings and wildlife animals using the Msunduzi River water. These 7 PAHs were selected from 16 PAHs classified as priority pollutants to be regularly monitored in the environment since they can undergo bioconcentration and biomagnification processes. Moreover, the modification and development of modern analytical techniques and measures are essential to easily assess the concentration levels of different classes of PAHs. Thus, the results from this study will be an important component of the database on the incidence of PAHs along the Msunduzi River. They will also be useful and beneficial for future policy making by the city municipality and for making recommendations

to the people relying on the river for their daily source of water as well to the pollution reduction of PAHs.

1.8 Scope of the Study

The key questions to be solved in this study were the following: given the Msunduzi River's location, its catchment area and course of flow, were the soils, waters and surface sediments contaminated from PAHs? If yes, were the following designated PAHs, namely naphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, chrysene and pyrene present and at what concentration levels do they occur? If these PAHs were present, were their emission sources coming from petrogenic or pyrogenic processes?

1.9 Aims and Objectives

The aim of this study was to determine the concentration levels of seven selected PAHs in wastewater, soil and surface sediment samples from the Msunduzi River, KwaZulu-Natal Province.

The objectives of this study were:

i. To investigate the presence of the selected PAHs in water, soils and surface sediments in the Msunduzi River basin using GC-MS.
ii. To quantify the concentration levels of PAHs in the water, soil and sediment matrices from the Msunduzi River using GC-MS.
iii. To identify the major sources of PAHs contaminating this river.

Chapter outline

The summary below provides the plan of the chapters in this study and the procedure that was followed. It also provides the main sections that are contained by each chapter.

Chapter I provides the background on the occurrence of PAHs in the environment and the purpose of this investigation. It presents the issues of PAHs. The hypotheses, aim, justification and objectives of the whole study are also presented in this chapter. It also describes the ecological effects, toxicity and the sources of PAHs. The chapter also analyses the relevant chemical, physical and environmental properties of PAHs as well as the primary hazards and human exposure to PAHs.

Chapter II reviews some extraction techniques and instrumental methods of analysis of semi and non-volatile organic pollutants.

Chapter III presents the methodology, site description and experimental procedures used in the study. It also indicates the techniques used during sample collection, their extraction and analysis procedures.

Chapter IV examines the analysis of the data obtained during this work. It also compares the outcomes with national concentration levels of PAHs. It presents seasonal variability of PAH concentration levels in this study, source apportionment of PAHs and discussions about the obtained results. Chapter V presents the conclusion and recommendation of the whole study.

Chapter II

Literature review

2.1 Extraction Techniques and Instrumental Methods Used in the Analysis of Semi- and Non-volatile Organic Pollutants

2.1.1 Pre-treatment of environmental samples

The distribution of any analyte in a solid sample is complex and non-uniform, unlike non-viscous liquids in which analytes are homogeneously distributed. For this reason, solid samples should be prepared in a fine powder form (Bendicho et al., 2012). This ensures that the analytes are uniformly distributed, thus allowing better extraction efficiency. The preparative treatments also reduce the gross field samples into a reasonable size for ease of handling during the extraction and analysis (Khan et al., 2005).

The treatments may include centrifugation to remove the pore water from sediments, air-drying, sieving, size reduction and further grinding or blending to reduce the particle size of the solid matrices (Khan et al., 2005; van Zuydam, 2009; Bendicho et al., 2012). The commonly used techniques to reduce the size of pre-sieved gross samples are coning and quartering. During coning and quartering, the sample is heaped using a steel cross splitter and split into quadrants of equal sizes (Rubio & Ure, 1993). The opposite or diagonal quadrants are collected together while the other two are discarded as shown in Figure 2.1. These steps are repeated on a subsequently heaped subsample until the required size of the sample is attained (Rubio & Ure, 1993; Gerlach et al., 2002).
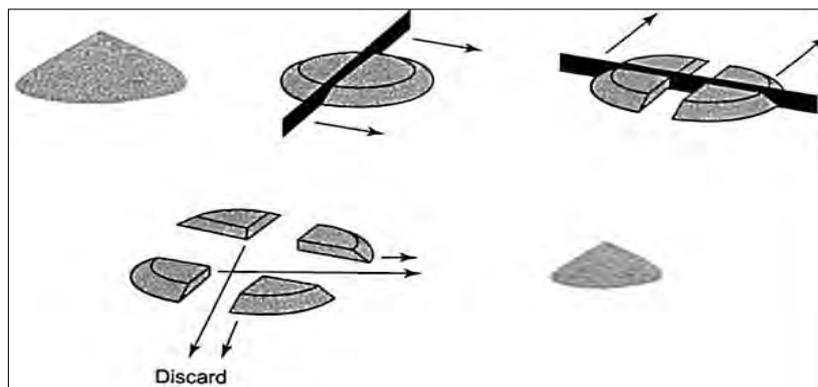
Figure 2.1 Coning and quartering techniques (Gerlach et al., 2002).

The other known technique used to reduce the size of solid samples is a mortar and pestle. The dried sample is placed in the mortar and ground using a pestle to homogenize it (Khan et al., 2005). This increases the extractability of the analytes by increasing the surface area in the sample (Mandingo et al., 2015). After grinding, to get a desired fine powder, the sample can be sieved (Wischmann et al., 1996; Mandingo et al., 2015).

2.1.2 Methods for extracting persistent organic pollutants from environmental samples

The choice of the methods for transferring organic pollutants from solid and liquid matrices into another medium (organic solvent) depends on the nature of the sample as well as the volatility of those pollutants (Khan et al., 2005). It also depends on the cost, availability of equipment in the laboratory and their effectiveness (Berset et al., 1999; Khan et al., 2005; Sibiya, 2012).

There are several methods in which a mixture of semi and non-volatile organic pollutants can be extracted from solid and liquid matrices for subsequent separation and analysis by chromatography (Khan et al., 2005; Bendicho et al., 2012). These include liquid-liquid extraction (LLE) (Habaki & Egashira, 2015), soxhlet extraction (Bendicho et al., 2012; Carro et al., 2012), mechanical shaking (Berset et al., 1999),

sonication (Bendicho et al., 2012), solid-phase extraction (SPE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE) (Lau, Gan & Ng, 2010), matrix dispersion and CO_2 supercritical fluid extraction (SFE) (Dzyuba & Bartsch, 2003). These extraction techniques can be grouped into three major categories:

- Nonthermal techniques such as LLE, agitation at room temperature, and blending of plant and animal materials (Habaki & Egashira, 2015).
- Thermal techniques such as soxhlet (USEPA, 1996; Lopez-Avila, Benedicto & Bauer, 1998) and atmospheric microwave-assisted extraction (aMAE) (Chee et al., 1996; Camel, 2001).
- Pressurized techniques such as pressurized microwave-assisted extraction (pMAE), pressurized hot water extraction (pHWE) (Oluseyi et al., 2011) and CO_2 supercritical fluid extraction (Yunker et al., 2002).

Of these techniques, LLE and soxhlet extraction (SE) are frequently chosen as the best extraction methods for analysis of PAHs, PCBs, pesticide residues and other POPs from liquid and solid matrices, respectively (Berset et al., 1999). They have better extraction efficiency, and low cost of equipment and operation compared to pressurized techniques (Luque de Castro & Garcia-Ayuso, 1998; Ramos et al., 2000). Only the techniques which were used in this study, i.e. the LLE and SE will be reviewed in detail.

Choice of solvents

Extraction of PAHs from different environmental matrices is accomplished using organic solvents whose polarity ranges from medium to low (Juhani et al., 2004; Khan et al., 2005). Examples are dichloromethane (DCM) (Juhani et al., 2004), toluene, chloroform, diethyl ether, and *n*-alkanes such as *n*-hexane, isooctane, pentane and cyclohexane (Khan et al., 2005). Medium-polar solvents such as DCM or toluene, or mixtures of polar and non-polar solvents are the preferred ones (Sun et al., 1998).

Toluene is more suited for extracting thermally stable organic pollutants due to its high boiling point. DCM is preferred for extracting fused aromatic compounds (Juhani et al., 2004). The polarity of the collector solvent can be tuned by using binary and even ternary mixtures. Examples of mixed solvents are *n*-hexane/DCM, *n*-hexane/DCM/ether and isooctane/DCM (Yunker et al., 2002; Khan et al., 2005).

In this study, *n*-hexane and DCM were chosen as effective solvents for extracting PAHs from water, soils and sediments due to their low boiling points, medium to non-polar characteristics and their capacity to dissolve a wide range of organic compounds (Filser et al., 1997; Juhani et al., 2004; Rossberg et al., 2006).

2.1.2.1 Extraction of PAHs from water samples

PAH compounds have low aqueous solubility due to their higher hydrophobicity, and therefore they easily adsorb onto organic particulate matter and sediment particles in contact with the water. Their concentration levels in water are generally low. Reliable analytical measurements of PAHs require robust extraction methods such as LLE, SPE, and SPME to transfer them into compatible solvents for subsequent separation by chromatography (Khan et al., 2005). Solvents such as dichloromethane, *n*-hexane, 2-propanol, cyclohexane, acetonitrile, and acetone are commonly used to extract PAHs (Juhani et al., 2004; Yang et al., 2015). Only the technique which was utilized in this work, i.e. the LLE will be reviewed in detail.

Liquid-liquid extraction (LLE) technique

In this technique the organic compounds in the feed solution (usually water) are transferred to an immiscible liquid (organic solvent). After shaking to mix the phases together in a separator funnel (Figure 2.2), the analytes are allowed to partition between the two phases according to their solubility.
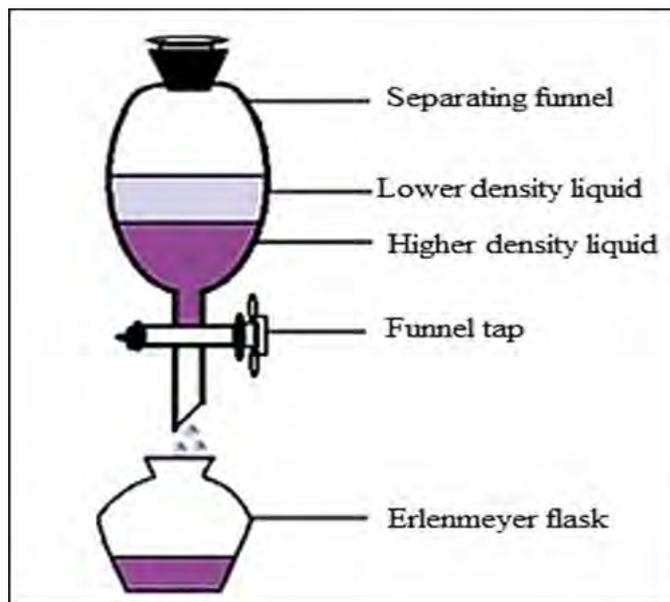
Figure 2.2 Liquid-liquid extraction (Mohrig et al., 2014).

Sometimes, the pH of the mixture should be controlled to optimize the extraction efficiency. In addition, a salting-out electrolyte such as NaCl can be added in order to expedite the separation of the two immiscible phases by preventing formation of emulsions (Müller, Fattore & Benfenati, 1997).

When the two phases are totally separated, the organic phase containing the target analytes is collected for further analysis. To ensure that all the target compounds in the feed solution are completely extracted, the feed solution has to be extracted several times (at least three). This increases the fraction of the analytes in the collector solvent. The extracts from each batch are combined before they are dried with dehydrating agents such as sodium sulfate, magnesium sulfate or aluminium sulfate. After dehydration, the excess solvent is removed to concentrate the analytes in the extract using a rotary vacuum evaporator.

LLE is a low-cost technique and has shorter extraction times. However, it requires large volumes of toxic and flammable solvents. Disposal of such liquid wastes finally result in contamination of the environment. Usually, the formation of emulsions and

lack of automation of this technique reduce the sample processing throughput (Manoli & Samara, 1999). Despite these disadvantages, LLE is the most commonly used method for extracting POPs from water samples due to its better extraction efficiency (EPA Method 3510-C, EPA, 1996).

For example, Titato & Lanças (2005) used the LLE technique to extract PAHs {naphthalene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene and dibenzo(a,h)anthracene} from water samples using DCM as the collector solvent. Bercaru et al. (2006) used the LLE technique to extract PAHs from water samples with *n*-hexane. Jong et al. (2006) used LLE for extracting PAHs from water samples reporting LODs of 0.01 µg/L using GC-MS. Furthermore, Pena et al. (2009) used the LLE technique to extract PAHs from rain water and raw wastewater using DCM.

2.1.2.2 Extraction of PAHs from solid samples

PAHs are strongly adsorbed on suspended particulate matter and sediments, their extraction process from these matrices requires a longer period of time in order to transfer these organic pollutants into a collector solvent.

Examples of techniques which are usually applied to extract PAHs from solid matrices are soxhlet extraction, microwave-assisted extraction and accel erated solvent extraction (Wang et al., 2007). Only the soxhlet extraction technique (Khan et al., 2005) will be reviewed in detail as this is the extraction technique used in this study for sediments and soils.

Soxhlet extraction (SE)

Figure 2.3 shows the main components of a soxhlet extractor. This technique is routinely used to extract semi and non-volatile organic compounds from solid matrices.
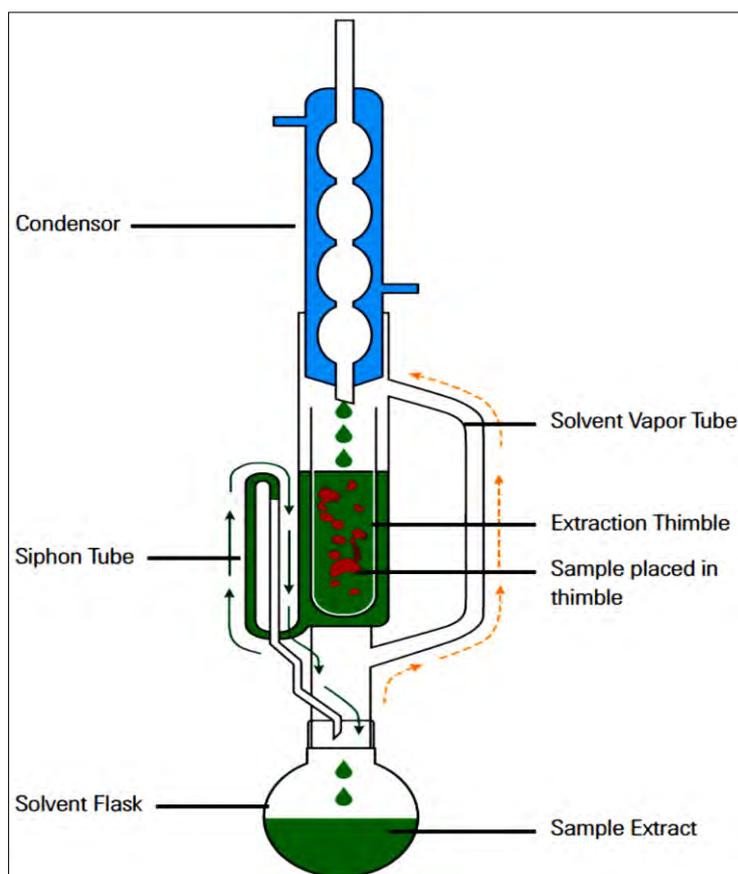
Figure 2.3 Example of a soxhlet extractor and its main components (Majors, 2013).

Soxhlet extraction technique is a US EPA standard method as well as an ISO standard method for extracting PAHs from soils, plants, sediments and sludge (Guerin, 1999). This technique showed to have higher extraction efficiency for trace organic pollutants (Guerin, 1999; Banjoo et al., 2005).

A test material containing the compound to be extracted is wrapped in a filter paper and placed into a porous cellulose thimble and PAHs are extracted at the reflux temperature of the solvent for several hours or days (Guerin, 1999).

Solvents such as *n*-hexane and dichloromethane (DCM) have shown a higher extraction efficiency of PAHs from solid matrices (Juhani et al., 2005; Jensen, 2007; Kanchanamanyoon & Tatrahun, 2008).

As shown in Figure 2.3, the extraction solvent is placed into a round bottom flask attached to a soxhlet extractor and a condenser (Carro et al., 2012). On heating the solvent with an isomantle, the condensed solvent fills up the main chamber, offering an intimate contact for extracting analytes from the sample. When full, the extracted mixture is siphoned down *via* a side arm and this cycle is repeated many times (Luque de Castro & Garcıa-Ayuso, 1998; Khan et al., 2005). Each of the vaporization-condensation cycles brings a fresh front of solvent while the extracted solute which is relatively non-volatile remains in the boiling flask (Luque de Castro & Garcıa-Ayuso, 1998). After the extraction, the crude extract containing PAHs is concentrated to a smaller volume using a rotary vacuum evaporator.

The advantages and disadvantages of soxhlet extraction are summarized below.

Advantages:

- Apparatus and equipment for this technique are cheap.
- Easy and safe to handle
- It is a continuous extraction technique lending it to higher extraction efficiency (Lau, Gan & Ng, 2010).

Disadvantages:

- It uses large amounts of toxic organic solvents (Luque de Castro and Garcia-Ayuso, 1998; Blanco et al., 2000; Suchan et al., 2004).
- Longer extraction times are required (Blanco et al., 2000; van Zuydam, 2009).

Despite its disadvantages, soxhlet extraction is still the most widely used method for extracting trace levels of analytes from sediments and soils (Lau, Gan & Ng, 2010) due to its full and longer contact time between the sample and solvent (EPA Method 3540-C) (EPA, 1996c).

For example, Reimer and Suarez (1995) used a soxhlet extraction method to extract native PAHs from soils and the recoveries ranged between 64 and 96%. Saim et al. (1997) used this technique to extract PAHs from the soils. Ünlü & Alpar (2006) also

used this technique for the extraction of PAHs from seabed sediments from the Gemlik Bay (Southwestern Marmara Sea). The concentration of 14 PAH compounds in sediment samples collected from 61 locations were in the range of 50–13480 ng/g.

Furthermore, Sibiya (2012) used this extraction technique to extract PAHs (naphthalene, fluoranthene, acenaphthene, and pyrene) from sediment samples collected around the city of Johannesburg, using a mixture of *n*-hexane and dichloromethane. The concentration obtained ranged from 90 to 1670 µg/kg in sediments collected from two different locations.

2.1.2.3 Purification of crude extracts by column chromatography

Column chromatography is one of the most useful methods for separating and purifying crude extracts containing organic analytes after their extraction from solid and liquid samples (Zdráhal et al., 2000; Saponaro et al., 2002). Polar stationary phases are commonly used to remove off polar co-extracts using a solvent system that is comparatively non-polar (Sloan et al., 2004; Prycek et al., 2007). Common stationary phases include silica gel, alumina, florisil, calcium phosphate, starch and calcium carbonate of particle size ranging from 50 to 200 µm (Zdráhal et al., 2000). The stationary phase or a solid adsorbent is usually packed in a vertical glass column (Bocca et al., 2003; Sloan et al., 2004). The mobile phase is added from the top and allowed to flow down the column by either gravity or external pressure (Figure 2.4).
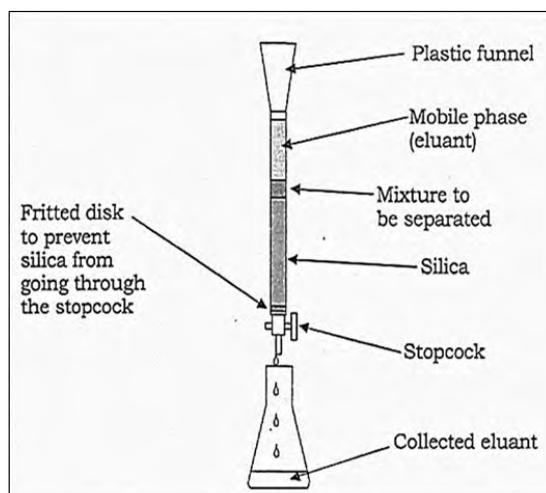
Figure 2.4 The diagram showing the main parts of column chromatography (Nicolas, 2014).

Analytes and co-extracts are partitioned based on differential adsorption on the surface of the adsorbent (Zdráhal et al., 2000). The rate at which the analytes in the mixture are separated normally depends on the polarity of the solvent and the surface activity of the adsorbent (Bodzek et al., 1997). If the polarity of the solvent is very low and the surface activity of the adsorbent for the analytes is very high, automatically a slow separation occurs but gives a good separation. Conversely, if the polarity of the solvent is high and the surface activity of the adsorbent is low, the separation is rapid but results in poor separation and co-elution of analytes with matrix interferences. As the mixture is eluted, the active polar groups cooperatively interact with the polar groups on the sorbent and weakly with the target analytes such as PAHs which are relatively non-polar (Saponaro et al., 2002). The analytes with greater adsorption power are adsorbed more on the column. Analytes can be collected separately in different fractions by adding more solvent onto the column. The weakly adsorbed components are eluted more rapidly. If the analytes or their derivatives are coloured, they can be collected separately as purified fractions.

Excess solvent is removed from the purified extracts through rotary evaporation. Column chromatography has been widely used as a purification tool in the analysis of persistent organic pollutants (Sun et al., 1998; Saponaro et al., 2002). Lough & Wainer (1995) purified the crude extracts of PAHs using a short chromatographic column packed with silica gel and alumina. Furthermore, Prycek et al. (2007) also used this technique to purify their crude extract for PAH analysis.

2.2 Chromatographic Methods of Separating POPs

The difficulties of resolving POPs from environmental samples coupled to their occurrence at lower concentration demand effective analytical procedures which commensurate with high sensitivity (Manoli & Samara, 1999). During the last few

years, several methods have been used for separating semi and non-volatile organic compounds (e.g. PAHs) present in the mixture. These include high-performance liquid chromatography (HPLC) and gas chromatography (GC) after extraction *via* techniques such as LLE and SPE (Shen, 2005). GC and HPLC are analytical methods which separate compounds based primarily on their differential partitioning between the stationary and mobile phases (Bailon et al., 2000). The separated compounds are detected using sensitive, specific and compatible detectors. Only GC will be reviewed in detail.

2.2.1 Gas chromatography (GC)

A gas chromatograph comprises of a supply of an inert gas, associated flow controller, heated injector port, temperature-programmable oven, analytical column, detector and data recorder (Pavia et al., 2006) as shown in Figure 2.5.
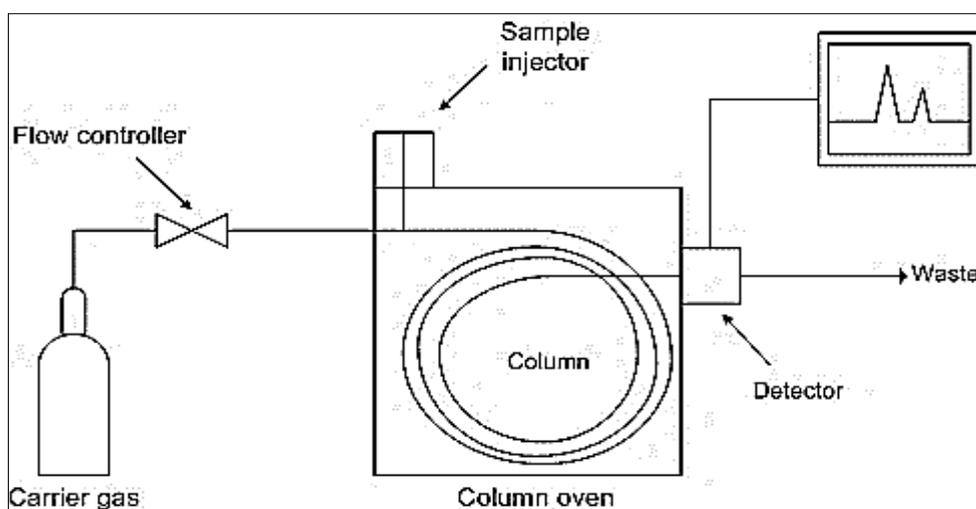
Figure 2.5 Block diagram of a gas chromatograph (Zhao & Barron, 2012).

In order to separate analytes on a GC column, the analytes should be easily vaporized and thermally stable (Khan et al., 2005). An aliquot of the extracted sample is introduced through a syringe *via* a self-sealing rubber septum into the heated injector port set at a temperature of around 200 °C. The heated sample is then introduced into

the analytical column without or with splitting it with the aid of flowing inert gas (carrier gas). Some examples of carrier gases that are usually used are nitrogen, helium, and argon (Stashenko & Martínez, 2014). In this study, pure helium was used as the carrier gas for the GC-MS.

On the analytical column, the analytes are separated by partitioning them on an immobilized viscous liquid/wax (called stationary phase). The different rates of partitioning between the stationary phase in the column and the analytes in the carrier gas cause each one of them to exit from the analytical column at a different time, known as the time of retention. The stationary phase is housed in a temperature-programmable oven. Temperature control of the liquid stationary phase plays a significant role in the separation of the mixture of analytes due to the differences in their boiling points.

Most modern GC instruments use fused capillary columns to separate out the analytes into individual compounds. An example of a capillary column is shown in Figure 2.6.

Figure 2.6 An example of a capillary column (Zhao & Barron, 2012).

A capillary column is flexible, has an internal diameter of a few tenths of a millimeter and is between 15 and 60 m in length. The capillary column can be wall-coated open tubular (WCOT) or support-coated open tubular (SCOT) (Stashenko & Martínez, 2014). For the wall-coated column, the liquid stationary phase is directly coated on the walls of the column. In the SCOT column, the inner wall of the capillary is lined with

a thin layer of finely divided, inert, solid support material such as diatomaceous earth, onto which the stationary phase is adsorbed (Hübschmann, 2015).

In general, the SCOT columns are less effective than the WCOT columns in the separation of analytes from complex mixtures (Sibiya, 2012). However, both capillary columns have a significantly higher separation efficiency than packed columns (Stashenko & Martínez, 2014).

Modern GC capillary column stationary phases use synthetic and high boiling materials such as polysiloxane, polyoxyethylene or polyester to partition analytes. They offer a film thickness ranging between 0.1 and 2.5 μm when they are heated (Armarego & Chai, 2003). Thinner films offer higher resolutions while limiting the amount of stationary phase that can be loaded on a capillary column.

The polarity and hence the composition of the stationary phase is critical in the successful separation of analytes especially congeners. For example, the separation of PAHs can be performed on low polarity columns such as DB-5MSUI, DB-5MS, VF-5MS, Rtx-5Sil-MS, and ZB-5MS. In this work, a 30 m (I.D. = 0.25 mm; film thickness = 0.25 μm) 5Sil-MS: {1,4-bis(dimethyl siloxy)phenylene dimethyl polysiloxane} was used to separate the PAHs in the extracted samples. This column is a low polarity cross-bond between the aliphatic siloxanes and the phenylene groups.

The resolving power of the analytical column is governed by the van Deemeter equation,

$$HETP\ = A + \frac{B}{u} + (Cs\ +\ Cm).u.$$

In this equation, HETP is the height equivalent to a theoretical plate of the analytical column, A is the eddy-diffusion parameter and is related to channelling through a non-ideal packing. B is the diffusion coefficient related to the dispersion of the analytes due to longitudinal drag. C is a term related to the resistance to the mass transfer of coefficient of the analytes between stationary and mobile phases while u is the linear flow rate of the carrier gas (van Deemter et al., 1956).

Optimum separation is possible through a careful control of the linear flow rate of the carrier gas. In general, if the longitudinal flow rate is high, then diffusion of analytes within the stationary phase becomes eddy leading to band broadening, tailing and fronting of peaks (Zhao & Barron, 2012).

In an open capillary column, the A term is equal to zero due to minimum tortuous channelling of analytes. Hence, separation on a capillary column is faster than in a packed column. High separation efficiency, which is equivalent to a low HETP value is achieved only at an optimum flow rate of the carrier gas (Rick, 2015).

The separated analytes are swept past a detector, which can be universal or selective for a particular class of analyte. Examples of commonly used detectors are flame ionization detector (FID) (Magdic & Pawliszyn, 1996), electron capture detector (ECD), thermal conductivity detector (TCD) (Kishi et al., 2015) and mass spectrometer detector (MSD) (Aguilar et al., 1998; Stashenko & Martínez, 2014).

An example of a selective detector is an ECD. This detector is highly sensitive for the organo-halides, nitrates, nitriles, peroxides, anhydrides and organometallics and very useful for the analysis of POPs such as PCBs and organochlorines. It can detect these compounds in complex environmental samples down to 50 femtograms (Zhao & Barron, 2012). TCD is a non-specific detector usually used for the analysis of nitrogen, phosphorus, and sulfur-containing compounds. FID is a non-selective detector mainly used for detecting hydrocarbons such as PAHs (Khan et al., 2005). However, it is not as sensitive as other detectors such as MSD (de Saint Laumer et al., 2015).

An MSD is a universal detector suitable for identification and quantification of trace and ultra-trace levels. It has a fast response, wider linear range and is highly sensitive to almost all classes of analytes. Of these detectors, only mass spectrometry will be reviewed in detail.

2.2.2 Gas chromatography-mass spectrometry (GC-MS)

Analytes separated from the gas chromatograph are passed on to the inlet of a mass spectrometer for ionization and detection. The technique is called gas chromatography-mass spectrometry (GC-MS). Modern GC-MS instruments have the GC microcapillary columns directly coupled to the inlet of the mass spectrometer (Randall & Wahrhaftig, 1978). A schematic diagram of a GC-MS is shown in Figure 2.7.
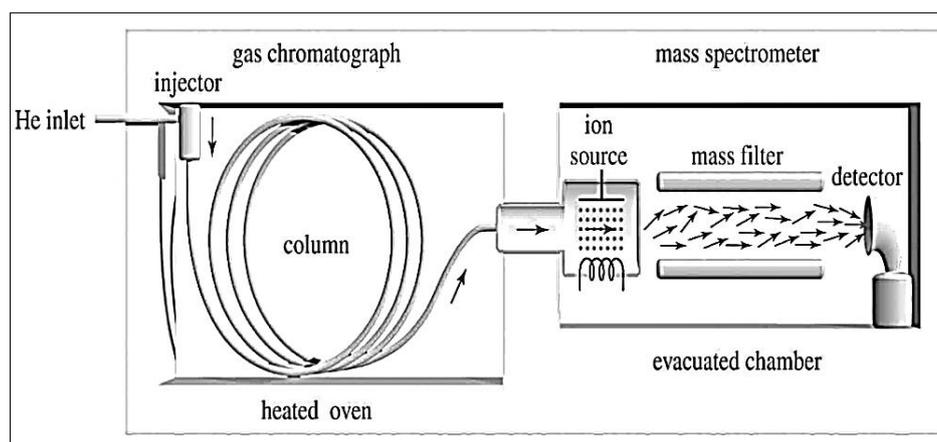
Figure 2.7 Modular representation of a GC-MS (Wade, 2003)

GC-MS combines the separation power of a microcapillary column and the high sensitivity of mass spectrometry for the identification of a compound's mass to charge ratio and quantification (Goates et al., 1987).

To enable direct coupling, a low volume amount should be introduced into the ionization source of the MS. Within the ionization source, rough mechanical pumping applied between the sampling and skimmer cones reduces pressure at the exit of the capillary column down to 10^{-2} Torr (Randall & Wahrhaftig, 1978). Additional pumping from turbo pumps ensures that only ions are introduced into the analyzer operated at near-vacuum ($< 10^{-7}$ Torr). The effluent from the GC can be ionized by a beam of energetic electrons or reagent gas such as methane, ammonia and isobutene. The ions that are generated are accelerated into the analyzer through ion optic grids at a negative gradient potential (Randall & Wahrhaftig, 1978).

2.2.2.1 Ionization sources

Electron impact ionization (EI)

Electron ionization (also called hard ionization) forms ions from sample vapour passed from the GC capillary column through a series of collisions with an energetic beam of electrons (70 eV) introduced orthogonally to the path of the gaseous effluent (Smith, Udseth & Kalinoski, 1984). This ionizes the molecules and also fragments them into their characteristic daughter ions. Since the fragmentation pattern of the analytes depends on the energy of the electron beam, a standard ionisation energy of 70 eV is used for ionisation, so that the experimental spectral data can be easily compared with standard mass spectral data provided by the US-National Institute of Standards and Technology (NIST) mass library. The disadvantage of electron ionization is that sometimes the peaks due to the molecular ion peak can be missing completely due to excessive fragmentation.

Chemical ionization (CI)

Chemical ionization produces ions from the effluent of the capillary column through a series of chemical reactions between a reagent gas and the molecules of the analytes (Munson & Field, 1966; Chhabra et al., 2015). Most commonly used reagents are methane, ammonia and isobutene. The introduction of reagent gas within the ion source slightly raises the pressure (10^{-2} Torr). Collisions between the reagents create stable reagent ions that ionize the molecules of the analytes. Just like in electron ionization, small amounts of effluent should be passed from the capillary column to the ion source (Smith, Udseth & Kalinoski, 1984).

Since lower energy is used compared to that employed in EI sources, molecular ion peaks are usually observed in the mass spectral data, which allows direct identification of the compounds.

2.2.2.2 Mass analysers

There are many types of analysers which are used to separate and focus ions passed from the ion source. These include sector-based analysers (magnetic and electrostatic analysers), time of flight (TOF), and filter-based {ion trap, quadrupole and Fourier transform-infrared (FT-IR) cyclotron} (Shah et al., 1989).

For routine GC-MS work, a quadrupole is used for separating ions either as a stand-alone analyser or in a tandem incorporating a collision cell between two quadrupole analysers (QqQ). In this study, an instrument of a single quadrupole analyser was used to separate the ions.

A quadrupole analyser comprises four parallel cylindrical (metal) rods. An alternative radio frequent current is passed at diagonally related rods, super-imposed to a DC voltage, whose magnitude is ramped with time (de Hoffman et al., 2003). This analyser filters the ions based on their *m/z* ratio and stability of their paths in the oscillating electric field that are applied to the rods (de Hoffman et al., 2003). As a result, only an ion of a specific *m/z* value is passed to the detector at a time, while those with unstable trajectories are neutralized on the poles of the quadrupole.

A variation to the quadrupole analyser is an ion trap. In the ion trap, the ions are trapped inside the cavity and injected into the detector one at a time (Fountain et al., 1994). The ions can be analysed in two modes, namely full scan mode and selected ion monitoring mode.

Full scan (FS) mode

Full scan mode is useful in analysing qualitatively and quantitatively the unknown compounds in a sample by considering all peaks within a spectrum. It offers more data than the SIM mode, during the confirmation of the compounds in a sample. During instrument method development, it is better to first investigate the test

solutions in an FS mode in order to define the retention times and the mass of each fragment before using the SIM mode (McLafferty et al., 1974).

Selective ion monitoring (SIM) mode

The performance of a GC-MS instrument in SIM mode permits for the detection of the specific analytes with increased relative sensitivity to the FS mode. In SIM mode, the electron multiplier detector only monitors the mass/charge values of the target ions rather than considering all masses over a wide range (Steingass et al., 2015).

Usually, two to four *m/z* values are monitored per compound, and the ratios of these ions are unique to the target analyte. In order to increase the sensitivity, the mass scanning rate and retention times must be adjusted. Lower detection limits can be obtained within the SIM mode than the FS mode for a quantitative analysis because the sensitivity is ten to hundred times better (Steingass et al., 2015).

2.2.2.3 Electron-multiplier detectors (EMDs)

In an MS, an electron-multiplier is often used as a detector of ions that have been separated by the mass analyser. These EMDs can be of the continuous-dynode type, where electrons are swerved by a strong magnetic field (Allen, 1947). When the ions hit one end of the detector, primary electrons are emitted and directed to continuously hit the surface of the dynode resulting in the amplification of electrons and hence the current output. The input end of the EMD is set at a negative voltage relative to its output end. The inside surface of the continuous dynode is coated with a micro thin film of semi-conducting substance (glass doped with Pb). At the endpoint, a separate electrode (anode) remains necessary to collect the multiplied electrons (Herox & Hintergger, 1960).

Thus, the EMD with a higher amplification gain allows ultra-trace levels of compounds ($<10^{-12}$ g or even $< 10^{-15}$ g) to be detected routinely using an MSD (Allen, 1947; Herox & Hintergger, 1960).

2.3 Preparation of Calibration Curves Using the Internal Calibration Method

Accurate quantification of trace levels of compounds such as PAHs requires that the GC-MS instrument is calibrated using external standards of each target analytes. A deuterated internal standard can be added at a constant amount to the calibrating solution to normalize variations that occur during the analysis of standards and samples. A stock solution of each analyte is diluted to 5 concentrations by pipetting out suitable aliquots of each compound in a common volumetric flask to prepare a mixed standard solution.

To account for instrumental variations, an internal standard is added at a constant amount in each diluted mixed standard (Khan et al., 2005). The internal standard should preferably be non-natural PAHs, which is not found in samples and do not co-elute with the target PAHs (Khan et al., 2005). Several deuterated PAHs have proved to be worthy for the GC-MS instrument as well as for the HPLC analyses. For instance, the phenanthrene-d10, naphthalene-d8, chrysene-d12 and the perylene-d12 may also be used for the GC-MS analysis whilst the fluoranthene-d_{10}, perylene-d12, phenanthrene-d10 and the 6-methylchrysene can be used for HPLC analysis (Wise et al., 1995). In this study, naphthalene-d8 was used as an internal standard (Khan et al., 2005).

The dilute calibration solutions are run three times on the GC-MS instrument. The amount of each standard present in the chromatogram is normally proportional to the area under the peak (Jiji, Cooper & Booksh, 1999). The ratio of peak areas of PAH standards over that of internal is used to plot the calibration curve. The calibration curve must be linear over a wider concentration range and should have low RSD values, not exceeding at least 5%.

2.4 Reliable Approach to Quantification of Trace Level Amounts of Analytes from Environmental Samples

In order to quantify the amount of the target analytes, the real samples must be spiked with PAHs as well as their deuterated (isotopes) standards (Khan et al., 2005). The spiked solutions with a known concentration are normally run several times (at least three) on the GC-MS instrument to record their chromatograms as well as the mass spectra. The concentration of each separated analyte is directly proportional to its chromatographic peak area (Koltsakidou et al., 2015). The concentration is obtained by applying a linear equation: $y = ax + b$ produced from each calibration curve (as identified in section 2.3), where y symbolises the ratio of the peak areas of the internal standard and the analyte and x represents the amount of the analyte, b is an intercept and a is the slope. If the spiking is done at a constant level, then the concentration of the analyte detected by the instrument (and native to the sample) is estimated by subtracting the added amounts from the amount deduced from the analysis of the spiked split. This approach eliminates chances of detecting false positives for trace level analysis and especially towards the detection limits of the technique.

Since the effects (mobility and toxicity) of PAHs show up as a class of compounds rather than individual compounds, their concentration in each sample can be summed, averaged and expressed as a range.

2.5 Applications of GC-MS Method for the Analysis of PAHs and Other Related POPs

The gas chromatograph separates the components of a mixture providing a chromatogram while the mass spectrometer provides their mass spectra and intensity of ions from fragmentation of the compound. By combining the GC and MS techniques, the analytes can be qualitatively and quantitatively determined.

As a result, a GC-MS instrument has been applied to analyse the active elements and the analytes in different subjects such as drug discovery, fire investigation, ecological study, explosive investigation and the identification of the unknown samples (Dong et al., 2012; Pang et al., 2015). In addition, it can identify the trace elements in different materials that were previously believed to have decayed beyond the identification (Girard, 2013). Furthermore, GC-MS has also been used in the last decade to analyse some POPs such as PAHs in different environmental matrices.

For example, Guillén & Errecalde (2002) have determined naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene in aquatic species using GC-MS analysis. The obtained concentration levels of PAHs ranged between 0.5 and 5 ng/g dry weight.

El-Nemr et al. (2007) extracted PAHs from sediments using *n*-hexane followed by dichloromethane. The determination and quantification of PAHs in the sediments were performed by GC-MS. The total PAH concentrations ranged from 88 to 6338 ng/g dry weight.

Guo et al. (2009) extracted PAHs in sediment samples by the soxhlet extraction technique and separated and quantified them using a GC-MS instrument. The concentrations varied between 6 and 46 µg/g dry weight.

Petersen et al. (2009) investigated the distribution of PAHs in superficial sediments of the western Mediterranean Sea using GC-MS. The PAH concentrations ranged between 1 and 20500 ng/g dry weight in the sediments.

Al-Rashdan et al. (2010) used soxhlet to extract PAHs from bread baked from white wheat flour, bread baked from brown wheat flour and sandwich bread baked from white wheat flour. After purification and concentration of the extract, the analysis was accomplished using GC-MS instrument. The total PAH concentrations varied between 1 and 280 µg/kg dry weight.

Dong et al. (2012) extracted industrial harbour sediments and the determination and quantification of PAHs in sediment were performed by GC-MS with the aid of

deuterated PAH internal standards and surrogate standards. The total concentration levels of the 16 PAHs ranged between 4425 and 51261 ng/g, with an average concentration of 13196 ng/g dry weight.

In this study, liquid-liquid and soxhlet extraction techniques have been chosen as the techniques for the extraction of PAHs in Msunduzi River water, and soils and surface sediments, respectively. The identification and quantification were performed by GC-MS method.

Chapter III

Methodology

3.1 Understanding the Study Area and its Impacts on the Pollution of the Msunduzi River from Persistent Organic Pollutants

3.1.1 Description of KwaZulu-Natal Province

KwaZulu-Natal (KZN) is the most populated province of South Africa. It has an estimated population of 10.5 million people (SSA, 2014). It is located between geographical coordinates of 29º 0′ 0″ S and 31º 0′ 0″ E. It is approximately 95000 km² and has a coastal climate and a long shoreline with the Indian Ocean *(DRAFT & HSRC, 2010; Mbatha, 2012)*. KZN has several districts which include eThekwini and uMgungundlovu *(Bruckner et al., 2002; Lewu & Mavengahama, 2011)*. Durban and Pietermaritzburg (PMB) are the largest cities in the province.

KZN has three topographic areas, namely the lowland along the Indian Ocean, the Natal Midlands and the mountainous regions of the Drakensberg and Lebombo near Lesotho *(Fairbanks & Benn, 2000)*. The climate of the province varies due to its complex topography. The coast is subtropical while inland regions are generally colder. Durban has an average annual rainfall of 1000 mm from spring to autumn with an annual temperature of 25 ºC while Pietermaritzburg receives about 695 mm of rain per year with an annual temperature of 24ºC *(Fairbanks & Benn, 2000)*.

3.1.2 Location of sampling sites in KZN Province

The Msunduzi River basin stretches across the uMgungundlovu District. It is a major tributary of the Umgeni River, the main supply of fresh water to the Durban

Metropolitan (Simpson & Pillay, 2000). The catchment area of the river basin is about 875 km² and covers a distance of 115 km to the Umgeni River *(Commission, 2002; Gemmell & Schmidt, 2013)*. It serves as a major source of drinking water for rural communities residing along the length of the river and irrigation of agricultural crops and gardens (Gemmell & Schmidt, 2013).

The Msunduzi River water is also used for recreational sports. Its confluence (or source) is at the Elandskop near Bulwer (29° 48′ 0″ S and 29° 46′ 0″ E, 1 500 m above sea level). It flows eastward to the Henley Dam, Edendale and past the urban built area of Pietermaritzburg. Because of the deterioration of urban sewer infrastructure and upsurge growth in the population of the city, urban amenities are stretched out, resulting in sporadic pollution of raw sewer into storm water drains of the river basin. In addition, surface runoff from asphalt-surfaced roads, effluents from industries and the burning of organic matters may find their way into the river.

Due to its proximity to the industries which includes energy production, mining, manufacturing, aluminium production, iron and steel foundries, the Msunduzi River may also receive some of the effluent wastes emanating from these industries *(Kaplinsky & Morris, 1999; Sutton, 2008)*. The river then winds past Camp's Drift (characterized with a slow flow rate), where it is also suspected to receive domestic and industrial wastes which worsen its pollution. At the Du Toit Viljoen Bridge and the N3 Freeway, pollution is likely to come from traffic exhaust fallouts and surface runoff from different urban areas.

At the Darvill wastewater treatment plant (DWWTP) site, some of the treated effluents from this plant are discharged back into the Msunduzi River (Plate 3.5). This plant treats about 81 ML of sewerage wastes per day *(Mabaso et al., 2003)*. Because South Africa is characterized by low and highly variable rainfall (arid to semi-arid country), all discharged wastes and other contaminants remain in the river for a long period of time. This increases their concentration levels, especially in sediments and soils along the bank of the river *(Richard & Poccard, 1998; Gemmell & Schmidt, 2013)*.

Around the agricultural and Msunduzi town areas, the river is again highly characterized by a low flow rate, which can enhance the deposition of pollutants in the sediments. It slowly winds up before draining into the Umgeni River. The Nagle and Inanda dams are located upstream and downstream respectively, of the Umgeni River and samples were collected at the Nagle Dam and the joining point of the Msunduzi and Umgeni Rivers to make a comparative reference with sampling sites located on the Msunduzi River.

Figure 3.1 is a map of the KZN Province showing the six sampling sites along the Msunduzi River, one site on the Umgeni River (i.e. Nagle Dam) and one site at their junction at which samples for this study were collected.
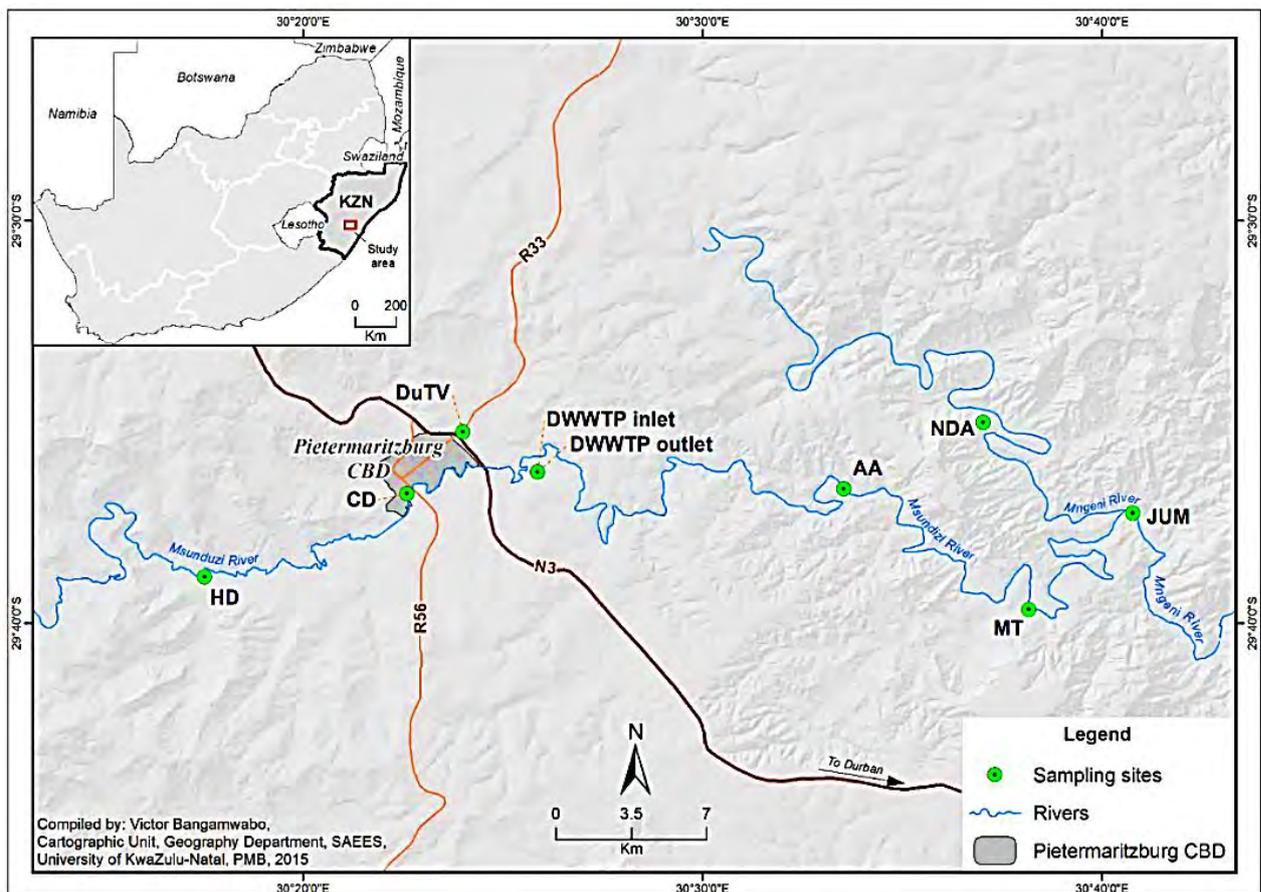
Figure 3.1 The map of the KZN Province showing six sampling sites along the Msunduzi River, one site on the Umgeni River (Nagle Dam) and one site at their junction.

3.1.3 Detailed descriptions of sampling sites

Henley Dam outlet area (HD)

Henley Dam is located at a latitude 29º 37' 45.3" S and longitude 30º 14' 39.5" E (Figure 3.1). It lies in the upper catchment of the river at an average altitude of 926 m. Its water is generally clear and clean compared to other downstream sites (RHP, 2015). The contamination of PAHs in this dam can possibly arise from surface runoff and atmospheric fallouts. This dam was constructed to support small-scale agriculture in the upper portion of the catchment, with emphasis on irrigation, recreation and subsistence fishing (RHP, 2015).

Camp's Drift area (CD)

 At Camp's Drift near the central business district of Pietermaritzburg (29º 36' 47" S and 30º 22' 36" E, Figure 3.1), the Msunduzi River is heavily polluted by effluent from domestic, industrial and commercial activities. Due to the rapid growth of the city, informal settlements have mushroomed rapidly along the banks of the Msunduzi River. This has worsened the contamination of its water from sporadic raw sewage as evidenced from Plate 3.1 (DUCT, 2006).

There are possibilities that raw fecal matter and other domestic wastes could be leached into the river from unprotected pit latrines and nearby bushes. Notably, burning of wood and tires is common in these informal settlements since they use them as a means of survival providing heat energy especially during the winter season.

Plate 3.1 Photo of Camp's Drift showing previous water pollution

Du Toit Viljoen (DuTV) Bridge

This sampling site is located under the N3 Freeway, which conveys large volumes of automobile traffic to and from Durban. It is located close to the city centre of Pietermaritzburg at coordinates 29º 35' 33.3" S and 30º 23' 58.56" E (Figure 3.1). At this point of the river, fumes from vehicle exhausts are likely to contaminate the river with organic pollutants, including PAHs through atmospheric fallouts and deposition. Other significant sources of PAHs may be municipal wastes, and possible illegal dumping and natural veld fires as shown in Plate 3.2 (DUCT, 2006; Essay UK, 2013).

Plate 3.2 Pollution along the Msunduzi River at DuTV Bridge (Photo taken on 26 May 2014).

Darvill wastewater treatment plant (DWWTP) area

This site (located at 29° 35' 33.3" S and 30° 23' 58.56" E) is a main sewage treatment plant for the city of Pietermaritzburg (Figure 3.1). It is about 1 km away from the Msunduzi River and is located between the residential areas of Hayfields and Sobantu (Sikhakhane, 2001). It was recently upgraded to a full biological nutrient removal plant with a handling capacity of 81 ML of domestic and industrial waste per day (Baker et al., 2012). DWWTP (with an approximate estate area of 665 000 m^2) is a host to a bird sanctuary. Some of the treated wastewater are discharged into the Msunduzi River, which empties into the Umgeni River and the Inanda Dam (Sikhakhane, 2001). Plate 3.3 shows the discharge of treated water from DWWTP into the Msunduzi River. Samples were taken at the inlet and outlet canals of DWWTP.
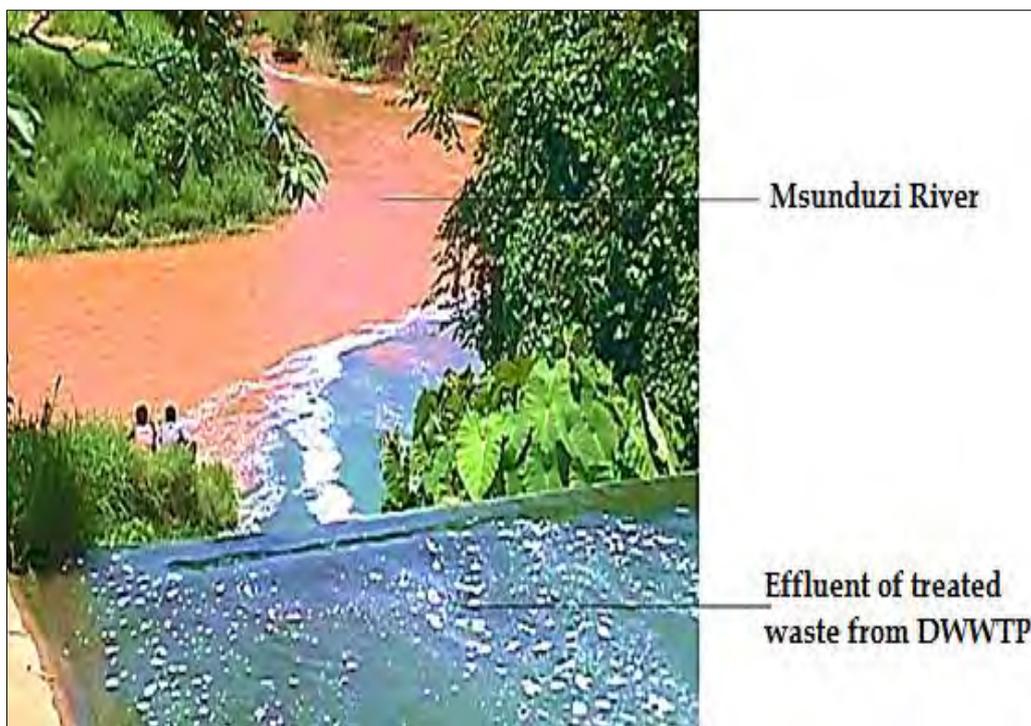
Plate 3.3 Effluent from DWWTP into the Msunduzi River (CSIR. RHP, 2015)

Agriculture area (AA)

This sampling site is located at coordinates 29º 36' 40" S and 30º 33' 32" E (Figure 3.1). Agriculture is the mainstay of the KZN province as it provides the livelihood to millions in the province. The land around this site is used for sugar cane plantations, horticulture, and pasture production as well commercial forestry. The rest is used for subsistence farming. Most rural settlements lack proper sanitation amenities and have no access to clean water. They rely on water from dams and rivers for their daily needs (Thawala, 2010). People from the rural areas use the Msunduzi River water for bathing, washing, and fishing. Some of these activities can pollute the water of the river, causing different diseases *(*Ngcobo, Dladla & Worth, 2002*)*.

Msunduzi town area (MT)

This site (29º 36' 40" S and 30º 33' 32" E) is located near the Msunduzi town (Figure 3.1). In this town, the major industries with potential to impact on the river include aluminum smelters, textile and footwear factories as well as food processing plants (KZN-Top Business, 2015). Also important is the impact of the nearby Vulindlela informal settlement, situated southwest of the town, as well as illegal dumping of household waste occasionally occurs within the area. It is also suspected that dumping of industrial and municipal waste is rampant, which is leached into the river, releasing toxic chemicals (Altman et al., 2002*)*.

Junction of Umgeni and Msunduzi Rivers (JUM)

This site is at the junction point of the Msunduzi and the Umgeni Rivers (GPS coordinates: 29 º 37′ 16″ S and 30 º 40′ 46″ E) (Figure 3.1). At this junction, the river flows at a much higher flow rate characterized by high deposition. In addition, a lot of organic loads enter the river through surface runoff from other smaller rivers. Upstream of this junction, but within its lower reaches, some sections of the river have been canalized in order to improve drainage capacity as well as to reduce flooding.

As a result, a lot of the natural course way of the river around this area has been lost *(Altman et al., 2002).*

Nagle dam (NDA) area

This site is localized at coordinates 29 $^{\circ}$ 35′ 1″ S and 30 $^{\circ}$ 37′ 1″ E on the Umgeni River (Figure 3.1). It was constructed in 1950 and serves as the main water supply to households and local industries as well as agricultural irrigation. It has a catchment area of 2 545 km² and has a total capacity of 39.3 million m³ of water (DWA, 2009).

3.1.4 Sampling plan

In order to establish the occurrence of polycyclic aromatic hydrocarbons (PAHs) along the Msunduzi River basin, samples of each matrix, water or wastewater, soil and surface sediments were collected at 8 different sampling sites as shown in Figure 3.1. The collected samples are considered as relevant matrices for studying the occurrence of persistent organic pollutants such as PAHs in aquatic ecosystems (*Chen et al., 2004*). The samples were collected during the following dates: 26-27th May, 26-27th August 2014, 15-16th January and 29th September-02nd October 2015.

The details of the samples which were collected at each site are presented in Table 3.1. The GPS coordinates were utilized to locate the sampling sites in order to collect samples at the same sampling sites in subsequent sampling trips (*Bevis et al., 1992*).

In summary, samples were collected from 8 sampling sites covering the important sections of the Msunduzi River. The sampling sites can be conveniently classified into three categories. The HD site was chosen to represent the upper section of the river. The CD, DuTV Bridge and DWWTP sites were chosen to represent the middle reaches of the river. They are located in the urban area of Pietermaritzburg where the river is at a high risk of receiving organic pollutants such as PAHs due to the associated anthropogenic activities (commercial, domestic and industrial). The AA, MT, and JUM sites were chosen to represent the lower reaches of the river. The NDA is the only

sampling site located on the Umgeni River. This site was selected for comparative reasons. The details of the geographical coordinates, types of samples and impacting activities done at each site are indicated in Table 3.1.

Sample collection and storage

Physical parameters such as conductivity (Portable Conductivity-Meter, SCHOTT Handy lab LF12, Germany), pH, ambient and water temperatures (Model IQ150, Handheld pH/mV/Temperature-Meter, Hach Company, USA) were recorded on site.

All samples were collected in amber glass bottles, which had been pre-washed with water and soap, then rinsed with distilled water and *n*-hexane *(Petrick et al., 1996)*. Surface water and wastewater samples were filled into 1 litre bottles fitted with screw caps lined with Teflon. Surface sediments and soils (500 g) were collected into bottles using a stainless steel grab sampler. Soon after collection, all samples were placed in a cooler box and transported to the laboratory where they were stored in a refrigerator at 4 $^{\circ}$C until further analysis.

Table 3.1 Sampling sites showing the activities along the Msunduzi River, the site codes, and the coordinates.

Sites and codes	Coordinates		Sample	Activities at the sampling sites
	South	East		
Henley Dam (HD)	29º 38′ 51″	30º 17′ 32″	Water, soil and sediment	Domestic activities
Camp's Drifts (CD)	29º 36′ 47″	30º 22′ 36″	Water, soil and sediment	Domestic and commercial activities
Du Toit Viljoen (DuTV) Bridge	29º 35′ 15″	30º 24′ 00″	Water, soil and sediment	Domestic and commercial activities
Darvill wastewater treatment plant (DWWTP) Inlet	29º 36′ 15″	30º 25′ 52″	Water and wastewater	Influent of treated domestic waste from the city of Pietermaritzburg.
Darvill wastewater treatment plant (DWWTP) Outlet	29º 36 ′15″	30º 25′ 52″	Water and wastewater	The effluent of treated domestic waste from the city of Pietermaritzburg.
Agricultural area (AA)	29º 36′ 40″	30º 33′ 32″	Water, soil and sediment	Agricultural and domestic activities
Msunduzi Town (MT)	29º 39′ 40″	30º 38′ 10″	Water, soil and sediment	Agricultural and domestic activities.
Junction of the Msunduzi and Umgeni Rivers (JUM)	29º 37′ 16″	30º 40′ 46″	Water, soil and sediment	Agriculture and surface water/rivers
Nagle Dam (NDA)	29º 35′ 1″	30º 37′ 1″	Water, soil and sediment	Dam and agricultural activities

3.1.5 Experimental

3.1.5.1 Extraction of PAHs

Liquid-liquid extraction of PAHs from the water and the wastewater

All water samples were extracted without being filtered in order to quantify the concentration levels of PAHs that plants, humans, and animals are exposed to when they directly drink or utilize it. The LLE technique (separating funnel) was used for the extraction of PAHs from these water samples (EPA Method 3510-C (EPA, 1996a)).

The extraction of PAHs in the collected samples and their spiked duplicates followed the same procedure. A 50 mL aliquot of dichloromethane (chromatographic grade) was added to 500 mL of river water/wastewater, in a separating funnel.

Naphthalene-d8 (2.5 mL, 100 mg/L) was also added to the mixture as a surrogate standard. The mixture was shaken for a period of 10 minutes with intermittent release of the pressure. This allowed enough contact between the PAHs in the aqueous phase and the collecting organic solvent. The two phases were allowed to separate out on a retort stand. After separation, the organic layer was collected into a receiving flask. Two more aliquots (30 mL and 20 mL, respectively) were added to the water portion and the extraction process was repeated. The combined organic layers were dried with 20 g of sodium sulfate anhydrous and filtered. The volume of the dried extracts was reduced to 5 mL using a rotary vacuum evaporator (Model Heidolph Laborota 4000, Heidolph North America) (Prycek et al., 2007).

Sample pre-treatment

The soil and surface sediment samples were air-dried in the dark at room temperature. The dried soils and surface sediments were ground using a mortar and pestle. They were then passed through a 2 mm sieve to remove rocks and plant material. The obtained samples were also pulverized with a mortar and pestle, passed through a

sieve (< 150 µm) and homogenized (Lopez-Avila et al., 1994). The powders were kept in amber glass bottles that were pre-washed and rinsed with distilled water and *n*-hexane. The samples were stored in sample cabins (room temperature) until further analysis *(*Dong et al., 2012*;* Rissato et al., 2006*)*.

Soxhlet extraction of PAHs from the surface sediments and the soils

The soxhlet extraction technique was chosen for the extraction of PAHs from the soil and surface sediment samples because of its good recoveries (US EPA Method 3540-C; Lau, Gan & Ng, 2010*)*.

Accurately weighed duplicates of surface sediments or soils (10 g) were mixed with approximately 2 g of activated sodium sulfate (dehydration purpose) and 2 g of copper powder (desulfurization and removal of some lipids). The mixture was wrapped in a clean filter paper and inserted into the cellulose extraction thimble (28×80 mm Whatman, Sigma-Aldrich) and covered with clean cotton wool. The thimble was placed into the main soxhlet chamber and fitted to a 250 mL round bottom flask containing 100 mL of an equal mixture of DCM/*n*-hexane (chromatographic grade). A condenser was then attached. The samples were extracted for 16 hours under reflux *(*Saim et al., 1997*)*.

After extraction, the crude extracts were cooled down and concentrated to a volume of nearly 5 mL using the rotary vacuum evaporator. The crude extracts were purified by column chromatography. The same procedure was also applied to spiked samples.

3.1.5.2 Purification of extracts

The extracts were purified on a chromatographic column packed with 10 g of fully activated silica gel and 2 g of sodium sulfate anhydrous (Prycek et al., 2007). The column was first conditioned with 25 mL of *n*-hexane before crude extract application (EPA Method 3630-C (EPA, 1996c)). The extracts were loaded on the column followed

by elution with 25 mL of *n*-hexane collected as the first fraction. The residues from the extraction step were added onto the column and eluted with 2×40 mL of *n*-hexane/DCM 1:1 v/v (Prycek et al., 2007). The purified extracts were concentrated to a volume of approximately 5 mL using a rotary vacuum evaporator. A gentle gas stream of purified nitrogen was passed over the extracts to near dryness. A 2 mL aliquot of DCM was added using a micropipette. The extracts were then filtered using a microfilter membrane (0.45 µm) and the cleaned extracts were kept in the fridge until analysis by GC-MS.

3.1.5.3 Instrumental analysis

GC-MS analysis conditions

The analysis of PAHs was carried out on a Gas Chromatograph (GC) coupled to a Mass Spectrometry Detector (MSD) QP-2010 series (Shimadzu, Japan). The injector port was set at 300 °C. The oven temperature was held initially at 40 °C and then increased at three different rates, namely 25 °C/min to 120 °C; 10 °C/min to 160 °C and finally 5 °C/min to 300 °C.

Purified extracts (2 µL) were injected without splitting the injected gaseous plume. The PAHs were separated on a 30 m (I.D. = 0.25 mm; film thickness = 0.25 µm) 5Sil-MS {1,4-bis(dimethyl siloxy)phenylene dimethyl polysiloxane} capillary column. Helium (99.99%) was used as the carrier gas. The most retained PAHs took 45 min to elute from the column and the scan run was in the range of 40 to 450 atomic mass unit (amu).

PAHs were ionized using a 70 eV beam (electron ionization). The ions were analysed on a single quadrupole and two-dimensional, multi-electron detector. The detector was operated in the selected ion monitoring (SIM) acquisition mode.

Reagents and standards

Eight PAH standards of naphthalene (99.0% purity), acenaphthylene (97% purity), fluorene (98% purity), phenanthrene, anthracene, pyrene, chrysene and deuterated naphthalene-d8 (99.0% purity), dichloromethane (DCM) (≥ 99.8% purity) and *n*-hexane (≥ 97.0% purity) chromatographic grade solvents, sodium sulfate and copper powder (particle size < 63 μm) were purchased from Sigma-Aldrich (Steinthein, USA).

Preparation of calibration solutions

The stock solution (100 μg/mL) of each of the 7 PAH standards was prepared separately in 50.00 mL volumetric flasks. A mass of 5 mg for each PAH was weighed accurately and transferred quantitatively into a 50.00 mL flask and dissolved in DCM. The volume was made to the mark using DCM (chromatographic grade). Calibration mixed standards of concentrations ranging from 0.25 μg/mL to 2 μg/mL were then prepared by diluting the stock solution.

The PAHs were quantified by external calibration incorporating a surrogate standard (deuterated naphthalene-8). The averaged ratio of the peak areas of each PAH to that of the surrogate standard were plotted against the concentrations of the PAHs to obtain the calibration curve. A typical calibration curve of naphthalene is presented in Figure 3.2 for GC-MS instrument.
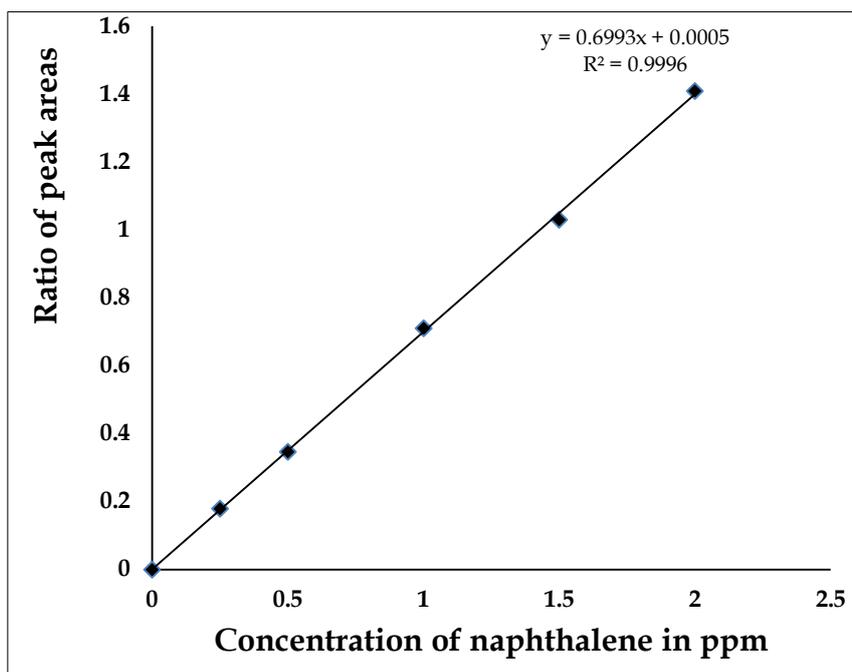
Figure 3.2 Typical calibration curve of naphthalene for GC-MS instrument.

The sample blanks were analysed several times to estimate the detection limit (LOD) of each PAH. The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the calibration slope to be the concentration equivalent to a signal strength equal to three and ten times the standard deviation of the sample blank, respectively. The LOD and LOQ values are presented in Chapter IV, Table 4.2.

Quality assurance

The extraction recoveries of the PAHs from the collected samples were evaluated by analysing the samples together with their spiked aliquots as described in section 3.1.5.1. The calibration curve for each analyte was plotted from the averages for three injections at each concentration as described in section 3.1.5.3

3.1.5.4 Analysis of PAHs by GC-MS

Cleaned extracts (1 mL) were pipetted into clean GC vials. An aliquot of 5 µL of the mixed PAH standards (0.25 µg/mL) was spiked and the solution mixed using vortex. This meant that all injected samples were spiked with each of the 7 PAHs and naphthalene-d8 at a concentration of 1.25×10^{-3} µg/mL. The spiked extracts were sequenced for analysis of PAHs on a GC-MS instrument and the sample was injected in triplicate. The peak area ratio (relative to that of naphthalene-d8) was used to determine the concentration of the spiked samples from the calibration curve. The concentration of each PAH in the unspiked samples was calculated by subtracting 1.25×10^{-3} µg/mL (the spiked concentration) from the concentration deduced from the calibration curve.

The identification of each PAH was done by comparing its retention time in the TIC of the samples to that of the standards. The mass spectra were matched to those of the NIST reference mass spectral data from the onboard library for further verification. The details of the spectral data are summarized in Chapter IV, Table 4.3.

Typical TIC chromatograms, mass spectra and confirmation ions of PAH compounds are presented in Figures 1-9 (see Appendix 3) and in Chapter IV, Figures 4.1 and 4.2.

Figure 3.3 summarizes analytical procedures which were followed for extracting PAHs from water, wastewater, soils and surface sediments from their treatment processes to their analysis with GC-MS instrument (EPA Method 8270 (EPA, 1996d)).

Water and wastewater samples	Soils and surface sediments

A 500 mL of water was subjected to LLE with 100 mL of DCM (HPLC grade) using separator funnel + surrogate standard.

Air-dried (in a dark room) on aluminium foil, ground with a mortar and pestle and sieved.

Dehydration with 20 g of sodium sulfate, filtration, and concentration of the extracts with rotary vacuum evaporator to a volume about 5 mL

A 10 g of the soil or surface sediment + 2 g of activated copper powder + 2 g of the sodium sulfate anhydrous + surrogate standard were soxhlet extracted with 100 mL of DCM: *n*-hexane 1:1 v/v for 16 hours.

Clean up of the extracts with chromatography column filled with 10 g of activated silica gel + 2 g of sodium sulfate anhydrous.

The concentration of the extracts to a volume about 5 mL using rotary vacuum evaporator

Wash the column with 25 mL of *n*-hexane, elute the first fraction with 25 mL of *n*-hexane and elute the second fraction with 2×40 mL of DCM: *n*-hexane 1:1 v/v.

Clean up of the extracts with chromatography column filled with 10 g of activated silica gel + 2 g of sodium sulfate anhydrous.

The concentration of the extracts to a volume about 5 mL using rotary vacuum evaporator and reduction of the solvent under a gentle gas stream of purified nitrogen to near dryness.

Reconstitution of the volume to 2 mL, filter using a microfilter (0.45 µm) + PAH standards and run GC-MS instrument for PAH analysis.

Figure 3.3 Flow diagram of protocol analysis

58

Chapter IV

Results and discussions

4.1 Calculation of Limit of Detection, Limit of Quantification and Quality Control of Analysis

The limits of detection (LOD) and quantification (LOQ) for each of the 7 PAHs were calculated from the calibration slope as a calculated concentration equivalent to 3 and 10 times the standard deviation of the signal strength of the field blank, respectively. Distilled water and a soil sample taken from a relatively assumed unpolluted area were chosen as field control blanks. PAHs were extracted from distilled water and the uncontaminated soil as described in section 3.1.5.1 for the collected samples (river water, soils and surface sediments).

The final extract (1 mL) from the field blank samples were analysed several times on the GC-MS instrument. The signals averaged and the LOD and LOQ values were calculated from the calibration slope of each PAH. The results are summarised in Table 4.1. The calculated LODs and LOQs of the PAHs by the GC-MS instrument for the distilled water and the soil were taken as good estimates of the detection capability of the instrument for river water, effluent water and sediments, respectively. The LODs of the PAHs by GC-MS ranged from 0.27 to 0.77 ng/L for water and from 0.12 to 0.70 ng/g for soil while the LOQs ranged from 1.00 to 1.31 ng/L for water and from 1.01 to 2.40 ng/g for soil (Table 4.1). The calculated percentage recoveries are also summarised in Table 4.1.

Table 4.1 LODs and LOQs (for extracts of distilled water and uncontaminated soil) and % recoveries of 7 PAHs from spiked sediments and water.

PAHs	LOD distilled water (ng/L)	LOQ distilled water (ng/L)	LOD soil blank (ng/g)	LOQ in solid samples (ng/g)	Recovery in spiked sediments (%)	Recovery in spiked river water (%)	R^2
NA	0.50	1.02	0.55	1.01	79.16±0.01	99.66±0.14	0.9996
ACY	0.72	1.07	0.70	2.40	99.10±0.02	95.77±0.32	0.9985
FLUO	0.77	1.01	0.36	1.06	95.19±0.01	105.56±0.01	0.9995
PHEN	0.34	1.00	0.40	1.07	83.79±0.65	80.35±0.05	0.9955
ANTH	0.61	1.05	0.12	2.21	98.59±0.21	92.15±0.01	0.9990
PYR	0.40	1.10	0.38	1.04	95.56±0.03	80.30±0.02	0.9995
CHRY	0.27	1.31	0.30	1.05	101.28±0.02	96.15±0.01	0.9985

R^2 is the coefficient of determination of a linear regression, **LOD** is the limit of detection and **LOQ** is the limit of quantification.

To evaluate the extraction efficiency of the targeted PAHs and their detection by GC-MS, samples of water, soils and surface sediments in duplicates were spiked with known amounts of the 7 PAH standards, and left to equilibrate for 2 days. The unspiked and the equilibrated spiked split portions were extracted as described in section 3.1.5.1 and analysed by GC-MS as described in section 3.1.5.4. Examples of total ion chromatograms (TICs) for extracts of spiked samples are shown in Figures 4.1 and 4.2. The surface sediment was spiked, equilibrated and extracted to have a final volume of 1.00 mL and a concentration of 1.25×10^3 µg/mL of each PAH.
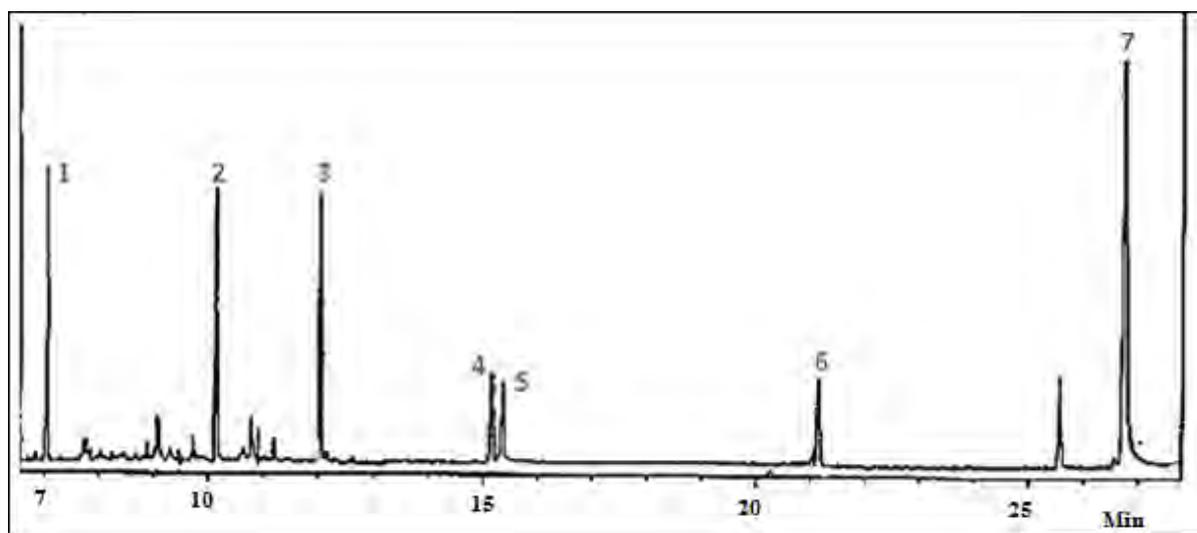
Figure 4.1 A TIC recorded for an extract of a spiked sediment {collected from the agricultural area (AA)} during the autumn of 2014. 1 (Naphthalene), 2 (Acenaphthylene), 3 (Fluorene), 4 (Phenanthrene), 5 (Anthracene), 6 (Pyrene) and 7 (Chrysene).
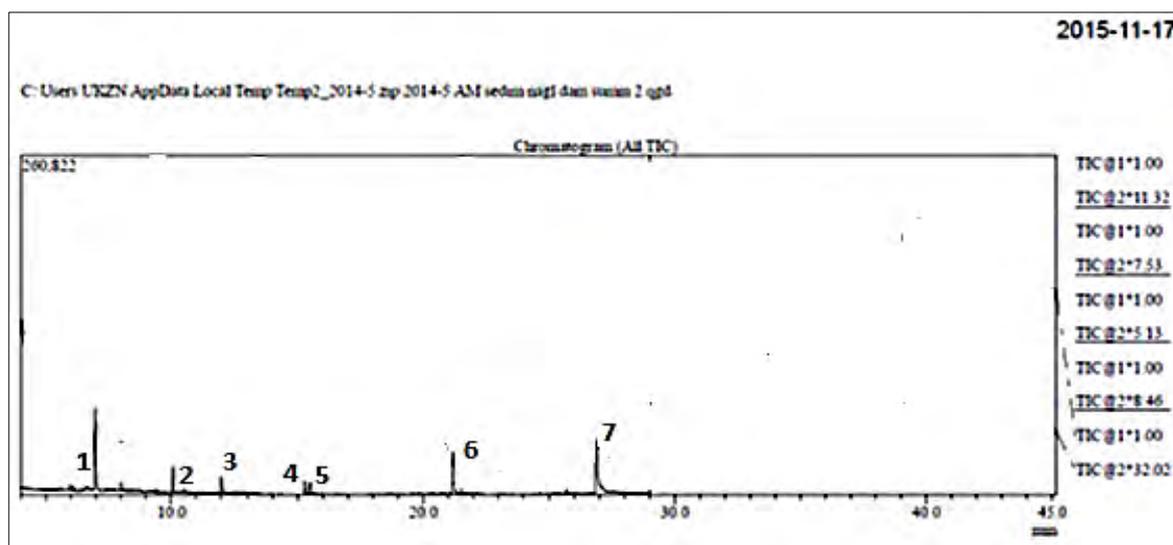
Figure 4.2 *TIC chromatogram of an extract of sediment from the Nagle Dam (NDA) sampling site during the summer season of 2014.* 1 (Naphthalene), 2 (Acenaphthylene), 3 (Fluorene), 4 (Phenanthrene), 5 (Anthracene), 6 (Pyrene) and 7 (Chrysene).

The TIC retention times, molecular weights (g/mole) of each PAH and their *m/z* values used to identify them are presented in Table 4.2. As shown in Figure 4.1 (with the TIC retention times in Table 4.2), the 7 PAHs are well separated from each other, including phenanthrene (4) and anthracene (5), which elute close together at retention times around 15.22 and 15.41 minutes, respectively.

Table 4.2 TIC retention times and confirmation m/z values of analysed PAHs.

PAH*	Retention times (minutes)	M.W. (g/mole)	Confirmation ions (*m/z*)
NA-d8	6.950	136	136
NA	6.960	128	128; 127
ACY	10.01	152	152; 151
FLUO	11.93	166	166; 165
PHEN	15.22	178	178; 176
ANTH	15.41	178	178; 89
PYR	21.08	202	202; 101

CHRY	26.78	228	226; 228

*NA-d8: naphthalene-d8, NA: naphthalene, ACY: acenaphthylene, FLUO: fluorene, PHEN: phenanthrene, ANTH: anthracene, PYR: pyrene and CHRY: chrysene.

As shown in Table 4.2, all of the PAHs were detected at *m/z* values corresponding to their molecular ions, with few fragmentation peaks being observed. This is consistent with the chemical stability of the compounds owing to their aromaticity and delocalisation of electrons within their entire conjugated rings. Thus, the PAH molecules survive bombardment by the 70 eV electron beam directed in their paths. A single electron is removed from the highest molecular orbital of each PAH molecule to form a stable and an odd-electron radical molecular ion. The *m/z* values of each PAH were used to identify (as complementary data to the retention times from the TIC) the 7 PAHs, which were selected for quantitative analysis (see also the figures presented in Appendix 3 for some confirmation ions of PAHs).

To confirm the identity (and complement the retention data from the TIC) of the 7 PAHs and to subsequently quantify them, GC-MS in SIM mode was used to identify and quantify the selected PAHs. In this mode, the percentage recovery of each PAH was calculated by subtracting the amount measured from the unspiked duplicates measured at set molecular ion peaks (Table 4.2).

4.2 Physical parameters and concentrations of PAHs in the Msunduzi River Basin

4.2.1 Conductivity, pH and temperature of the Msunduzi River water

Table 4.3 presents the results of the conductivity, pH and ambient temperatures recorded during the autumn, winter, spring and the summer seasons of 2014 and 2015.

Table 4.3 Physical parameters of the Msunduzi River water.

Seasons	Parameters	Sampling sites								
		HD	CD	DuTV	DWWTP Inlet	DWWTP Outlet	AA	MT	JUM	NDA
Autumn of 2014	pH of water	7.00	6.65	6.31	5.67	6.70	5.86	8.10	5.72	7.71
	Conductivity (µS/cm) at 25 ºC	103.8	187.9	144.1	655.0	698.0	279.0	348.0	30.0	91.8
	Water T (ºC)	16.8	16.6	17.8	23.0	24.0	18.3	18.7	18.3	20.1
	Ambient T (ºC)	28.2	25.3	25.5	29.3	29.2	29.2	21.6	18.3	24.1
Winter of 2014	pH of water	7.99	7.85	7.79	7.34	7.39	8.69	9.02	8.84	7.80
	Conductivity (µS/cm) at 25 ºC	120.4	225.0	169.6	946.0	703.0	387.0	435.0	318.0	90.3
	Water T (ºC)	12.9	16.3	19.9	22.2	16.7	17.8	19.3	19.5	20.3
	Ambient T (ºC)	26.6	31.0	32.5	21.1	21.4	33.8	34.7	33.3	30.4
Summer of 2014	pH of water	7.60	7.42	7.39	7.39	7.77	7.68	7.88	8.10	8.33
	Conductivity (µS/cm) at 25 ºC	83.9	132.9	125.2	865.5	543.0	300.3	329.0	279.5	105.6
	Water T (ºC)	25.0	27.1	23.1	28.7	27.7	27.9	27.8	27.2	28.7
	Ambient T (ºC)	27.5	29.0	31.8	31.4	31.4	32.8	37.7	33.0	37.0
Spring of 2015	pH of water	6.78	7.79	7.67	7.21	7.37	8.64	8.32	6.74	8.54
	Conductivity (µS/cm) at 25 ºC	103.7	384.2	267.2	1165.0	928.2	253.5	231.7	355.7	119.0
	Water T (ºC)	15.3	21.5	16.2	19.7	16.0	25.0	17.9	20.0	26.2
	Ambient T (ºC)	16.3	17.1	18.4	16.1	20.6	25.2	19.5	26.8	28.5

HD (Henley Dam), CD (Camps Drift), DuTV (Du Toit), DWWTP Inlet (Darvill Wastewater Treatment Plant Inlet), DWWTP Outlet (Darvill Wastewater Treatment Plant Outlet), AA (Agriculture Area), MT (Msunduzi Town), JUM (Joining Point of Msunduzi and Umgeni Rivers, NDA (Nagle Dam)

The pH of surface water indicates the acidity and alkalinity of the natural water (Gupta & Sharma, 1996). In cases where there is a minimum external injection of acidic or basic wastes, the pH of the natural water depends strongly on the equilibria involving absorption and the evolution of CO_2 as well the HCO_3^-/CO_3^{2-} buffer system (Hutchinson, 1975). The latter comes from the dissolution of limestone and shale ($Ca/MgCO_3$), which make up the bedrock of most river basins. However, the overall pH of the water is also affected by input loads of organic contaminants (humic and fulvic acids) from surface runoff (Sahu et al., 1998).

During the autumn season, the pH of the river water and wastewater presented the results of normal water as shown in Table 4.3. Normally, a decrease of pH in river water may be due to the dissolution of minerals into the river water while its increase may be due to the depletion of inorganic compounds in water.

During the winter season, the pH of water varied between 7.32 and 9.02. The river water presented a pH within the range of a weak base. A slight decrease was found from the mouth of the river to the DWWTP Outlet sampling site. The water in the lower reaches of the river, such as the JUM and the NDA sampling sites, had higher pH values, which may be attributed to the biochemical processes that occurs in these areas during this season.

The electrical conductivity of water is primarily ascribed to the dissolved ions derived from the putrefied plant material (Singh et al., 2013) and an input of inorganic as well as organic waste (Wright & Hamilton, 1982).

The electrical conductivity (EC) generally increased from the source of the river up to the DWWTP Outlet sampling site and decreased gradually up to the mouth of the river. This can be explained by the variation of dissolved ions and pollutants in the water as the river flows and its presence is also due to the variation in ambient temperature as EC is temperature dependent.

The EC values varied between 90.2 and 946 µS/cm. The higher range of EC observed in winter can be related to the higher than normal ambient temperature values recorded during this season. Research has been reported that the temperature of water

can affect its conductivity by increasing ionic mobility as well as the solubility of many salts and minerals, especially during the day. For example, an increase of 1 °C can cause an increase of the electrical conductivity of water from 2 to 4% (Whipple, 2002). Indeed, the electrical conductivity in the water is normally affected by the compartment of the ionisable organics on which different organic pollutants such as PAHs can adsorb and get stabilized in water (SFM, 2015).

During the spring season, the pH and the EC varied slightly in the surface water from the source to the mouth of the river. The only exception in EC was observed in the wastewater, where the DWWTP both Inlet and Outlet presented high values of EC around 1000 µS/cm. This may be due to the higher concentrations of different types of dissolved ions and pollutants released into the wastewater from the city of Pietermaritzburg during this season. The presence of organic pollutants such as PAHs in the aquatic system depends on physical parameters of water, such as dissolved solids as well as EC and the suspended particulate matter on which they can adsorb.

Winter and spring seasons presented higher averages of the EC, which could be a result of the breakup of industrial wastes in the water. This is because in these two seasons there is generally little rain to wash away these wastes, so they spent a long time in the river water thus increasing its electrical conductivity. Normally, the electrical conductivity increases with an increase in water temperature. The warmer temperatures tend to increase solubility of the ions and solids, resulting in higher dissolved solids which in turn contribute to increased conductivity. Therefore the warmer the water, the higher the electrical conductivity. Conductivity is therefore reported as conductivity at 25 °C (Wetzel, 2001; SFM, 2015).

During the summer season, the pH varied gradually as the river flowed. The pH ranged from 7.39-8.33 while the electrical conductivity of water was very low in the middle reaches as shown in Table 4.3. The pH in the autumn, spring and summer seasons was between 6 and 8, which meant that the Msunduzi River water was in the normal range of stream water (falling in the range of drinking water from 6 to 9). During the winter season it was in the range of weak base or seawater pH. The average electrical conductivity in this study was 281.98±0.07 µS/cm for the autumn,

376.89±0.06 µS/cm for the winter, 422.77±0.05 µS/cm for the spring and 305.75±0.05 µS/cm for the summer seasons.

The average temperature of water was 18.78±0.02 °C for the autumn, 17.87±0.03 °C for the winter, 19.93±0.01 °C for the spring and 27.01±0.05 °C for the summer season, whilst the ambient temperature was 25.87±0.02 °C for the autumn, 28.96±0.05 °C for the winter, 21.02±0.03 °C for the spring and 31.86±0.05 °C for the summer seasons. The abnormal average of ambient temperature observed during the winter season resulted in the fact that samples were taken during a sunny (warm) day.

The average pH obtained in this work was 6.63 for autumn, 8.07 for winter, 7.67 for spring and 7.72 for summer. The winter season appeared to have a higher average pH, which might be related to the lack of rainfall (normal rainfall has a pH of 5.60, slightly acidic due to CO_2 from the atmosphere) during this season.

4.2.2 Concentration of PAHs in the surface water and the wastewater

The surface water and effluent wastewater samples were analysed without filtering them in order to measure the concentration levels of PAHs that humans, plants, and animals are exposed to. PAHs may enter the environmental system through various routes and are usually found as a mixture containing two or more of these compounds and therefore humans are also exposed to this mixture, and not to one compound. This allows for the summation of their concentrations during their analysis as they form the same class of POPs with the same physical, chemical and toxicological properties (Buha, 2011). The concentrations of the selected 7 PAHs seasonally determined in the Msunduzi River water and wastewater are presented in Tables 4.4-4.7 as well as Figures 4.3-4.6.

Autumn season of 2014

Most of the water samples collected along the Msunduzi River basin during the autumn season of 2014 (March-May) were contaminated with PAHs ranging between 12.47 and 718.93 ng/L, see Table 4.4. The sum of the concentrations of the PAHs in river water was the highest at DuTV (718.93 ng/L) sampling site and the lowest at NDA sampling site (12.47 ng/L). The NDA is located at Umgeni River and was selected as a comparative point during this study. The average concentration of the 7 PAHs for the Msunduzi River basin was calculated to be 367.09±0.25 ng/L.

The total concentrations found at the junction of the Umgeni and Msunduzi Rivers (JUM) (464.61 ng/L) and agricultural area (AA) site (226.89 ng/L) (lower reaches) varied from those measured in the water collected at Camp's Drift (CD) near the city centre of Pietermaritzburg (PMB) (upper reaches).

DuTV is a site located under the N3 Freeway in the city of Pietermaritzburg. Due to the Freeway's large volume of vehicular traffic as well as surface runoff from coal-tar asphalts, PAHs may be introduced at high influx rates to the nearby soils and eventually leach into the Msunduzi River water at this site. Furthermore, this site is close to the city centre and thus, it may also receive surface runoff from the municipal wastes as well as some illegal dumping (Tony, 2012). The summed concentrations of the 7 PAHs at this site has no significant difference ($P < 0.05$) to that calculated for the wastewater from the Darvill wastewater treatment plant (DWWTP) Outlet (695.58 ng/L). An elevated Σ[7-PAHs] at DuTV was expected, given also that the water at this site usually receives wastes and plastics possibly from illegal dumping from the shanty town sprawling along the river around the city of Pietermaritzburg.

The variation pattern of the Σ[7-PAHs] along the Msunduzi River basin, indicates that in general, sites located near the city of PMB (middle reaches of the river) had elevated concentrations of PAHs during the autumn season. This suggests the major input sources of PAHs are from human-related activities. These sites are DuTV and DWWTP. As shown in Table 4.4, the Σ[7-PAHs] generally increased from the Henley

dam (HD) up to the sites located in the city of Pietermaritzburg (CD, DuTV and DWWTP) before they decrease up to its inlet junction with the Umgeni river (JUM).

Sites upstream or downstream of the Msunduzi River recorded lower summed concentrations of the 7 PAHs (< 700 ng/L). Sites upstream of the Msunduzi River presented the background concentration levels of PAHs from natural inputs such as atmospheric fallouts or wet deposition after natural veld fires commonly occurring in KZN during the pre-autumn season. Sites downstream of the Msunduzi River were expected to have higher levels than those of upstream. The comparable lower levels may be due to the dilution effects, even if wastewater with high concentrations of PAHs is returned to the Msunduzi River.

Since five of the seven studied PAHs were LMW-PAHs, their leaching into the river water may come from surface runoff, complete burning of gasoline, diesel fuel, and kerosene. All these occurrences are common in an urban locations like Pietermaritzburg (Sakari, 2012).

Other possible sources of emissions of PAHs into the river are industrial discharges, burning of organic wastes, illegal dumping and urban surface runoff (Tony, 2012). PAHs are commonly emitted from automobiles, domestic and garage discharges, burning of organic materials, plastics and coal (RHP, 2015) and use of creosote during preservation of wooden poles commonly used in the distribution of electricity in KwaZulu-Natal (Thulasaie, 2008).

Another interesting aspect was the prevalence of each PAH at each site along the river basin during this season. Figure 4.3 indicates that a number of PAHs were detected at concentration levels in water greater than 100 ng/L at CD (ACY, FLUO, PHEN, ANTH), DuTV (CHRY) and DWWTP Outlet (PYR).

River water collected from the CD site was contaminated with the following LMW-PAHs (at the measured concentration level): FLUO (118.83 ng/L), ACY (120.11 ng/L), ANTH (140.22 ng/L) and PHEN (156.21 ng/L).

Two HMW-PAHs, namely CHRY and PYR were found at significantly higher concentrations ($P > 0.05$) in the water from the DuTV Bridge (550.08 ng/L) and in the wastewater from the DWWTP Outlet (506.93 ng/L), respectively as shown in Figure 4.3. This is despite the low aqueous solubility of CHRY (log *Kow* value: 5.79) and PYR (log *Kow* value: 4.88). It is likely that CHRY and PYR are stabilized by physico-chemisorptive adsorption on the surface of suspended or dissolved micro particulate organic matter, suspended in the wastewater and river water. The low vapour pressure and biochemical stability of CHRY and PYR also increases their residence time in the water, occurring as adsorbates on the suspended colloidal matter (Miller et al., 1985). HMW-PAHs have been reported to be persistent and resistant in the aquatic systems compared to their lower molecular counterparts (Albers, 1995; van Kemenade et al. 1995; Sayles et al. 1999; Schneider et al. 1996).

The highest concentration of PYR found in the wastewater from the DWWTP Outlet was surprising. Normally, higher concentrations of pollutants are expected to be in the raw and untreated water (inlet point of WWTPs) than in the treated water (outlet point of WWTPs). As shown in the measured values in Table 4.4, this was not the case at DWWTP. Higher concentration levels of other persistent organic pollutants (POPs) such as PCBs, DDT, *etc.* in wastewater collected from the outlet of the WWTP have been reported (Gakuba et al., 2015). In support of this anomaly between the concentration of PAHs at the inlet and outlet of the DWWTP is the high electrical conductivity value of 698 μS/cm recorded in its treated wastewater (see Table 4.1). If the highest electrical conductivity value is due to the ionised dissolved organic matter and fine particles, then the unusually higher PAH concentration values for all the measured PAHs is due to adsorptive stabilization on the dissolved organic matter (Maskaoui, 2002).
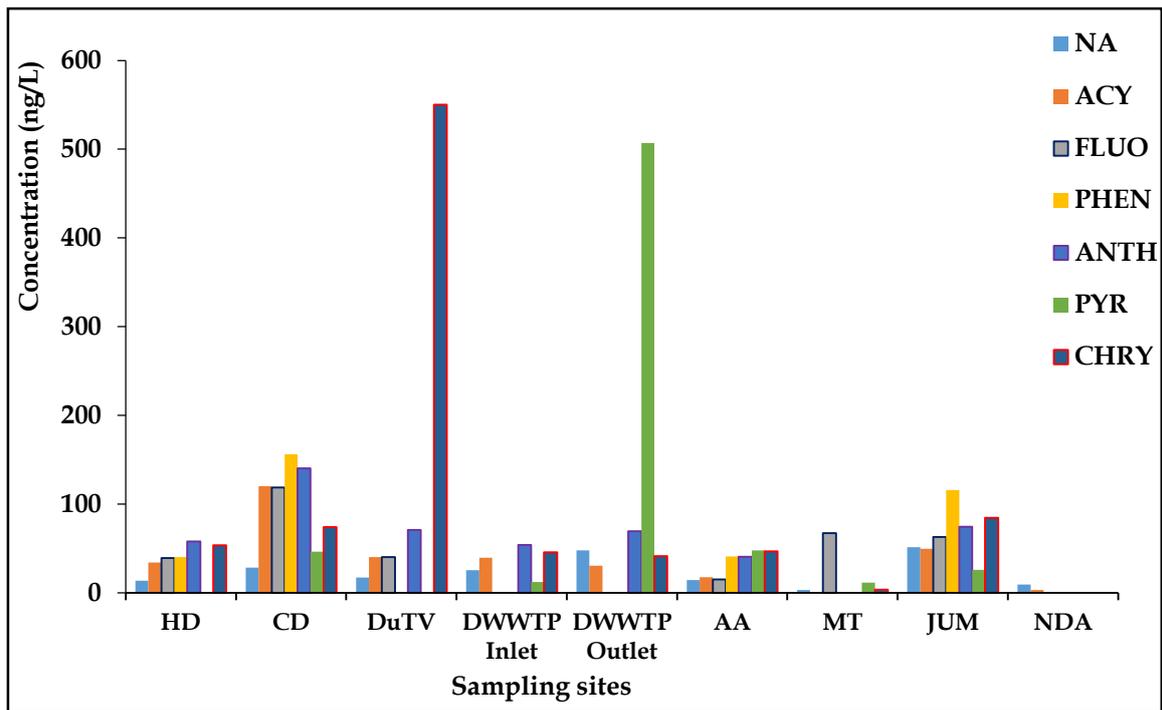
Figure 4.3 *Concentrations (ng/L±SD) of the 7 PAHs in the water and wastewater from sites along the Msunduzi River during the autumn season.*

It may also be due to the accumulation of organic pollutants in treated wastewater and other inputs such as re-dissolution of PAHs from sediment as well as atmospheric fallouts as it travels a long distance (passing through the bird sanctuary area in an open channel where the wastewater flow is slow) before being discharged into the Msunduzi River (Maskaoui, 2002). This can also be related to rehabilitation and extension of this treatment plant, which could have a big impact on the treated wastewater through the aging process and release from the engines. In addition, this season also had low rainfall, which may have resulted in lack of dilution of the discharged wastewater.

Table 4.4 *Concentrations of the 7 PAHs in the water (ng/L±SD) at each site during autumn of 2014.*

Sites	LMW-PAHs					HMW-PAHS		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	13.52±0.01	34.31±0.03	39.34±0.02	40.22±0.03	57.78±0.02	ND	53.55±0.03	238.72±0.21
CD	28.34±0.02	120.11±0.11	118.83±0.01	156.21±0.13	140.22±0.01	46.43±0.02	74.17±0.04	684.31±0.67
DuTV	17.38±0.01	40.44±0.03	40.31±0.03	ND	70.72±0.01	ND	550.08±0.20	718.93±0.31
DWWTP Inlet	25.42±0.01	39.70±0.02	ND	ND	54.07±0.01	12.05±0.01	45.56±0.03	176.80±0.09
DWWTP Outlet	47.67±0.04	30.40±0.01	ND	ND	69.33±0.06	506.93±0.04	41.25±0.03	695.58±0.24
AA	14.52±0.01	17.76±0.01	15.24±0.01	41.10±0.03	40.60±0.02	47.76±0.03	46.91±0.04	226.89±0.30
MT	3.30±0.01	ND	67.17±0.04	ND	ND	11.50±0.01	3.60±0.02	85.57±0.19
JUM	51.38±0.10	49.47±0.05	62.90±0.04	115.91±0.02	74.41±0.01	26.03±0.03	84.51±0.01	464.61±0.26
NDA	9.40±0.01	3.07±0.04	ND	ND	ND	ND	ND	12.47±0.05

ND: not detected, LMW-PAHs: lower molecular weight PAHs, HMW-PAHs: higher molecular weight PAHs and the Σ[7-PAHs]: the sum of concentrations of seven PAHs found at each site. LOD values are shown in Table 4.1.

These results are not surprising given that DWWTP treats different kinds of domestic and industrial wastes from the city of Pietermaritzburg before discharging the treated waste into the Msunduzi River. Failure to completely reduce the concentration of the dissolved solutes and suspended colloids will result in elevated levels of PAHs in water. Possible sources of CHRY and PYR into the DWWTP wastewater and water could be attributed to the incomplete burning of oil, agricultural waste, wood and coal-tar, urban surface runoff containing cigarette ashes and soil runoff, domestic and industrial discharge containing paints and dyes, illegal dumping and atmospheric fallouts from long-range transport. The unusual spike in PYR concentration is also an indication of the high variability in the composition of the wastewater.

The summed concentrations of the 7 PAHs at CD (684.31 ng/L) were also high. This site was also selected as the most contaminated site during this study.

Winter season of 2014

As shown in Table 4.5, only NA and CHRY were detected at all sampling sites while PYR (as a HMW-PAH with low aqueous solubility) was only detected in four sampling sites during the winter season (June-August 2014). The sum of the concentrations of the 7 PAHs in the water or wastewater ranged between 73.35 and 553.62 ng/L with an average concentration of 244.04±3.16 ng/L (Figure 4.4).

Wastewater samples from the DWWTP Inlet and Outlet had the highest concentration levels of PYR at 303.77 and 376.02 ng/L, respectively as shown in Figure 4.4. This is due to the accumulation of different pollutants and total suspended solids from different points of Pietermaritzburg city that make their way into this wastewater treatment plant (built for the Msunduzi local Municipality) as reported in the previous season. This treatment plant normally receives the raw municipal wastewater and industrial wastewater with a higher amount of dissolved ions as reported in section 4.3.1. It also operates through an activated sludge process by removing higher excess of phosphorus and other nutrients to prevent eutrophication in the Msunduzi River (Barnard, 1976).
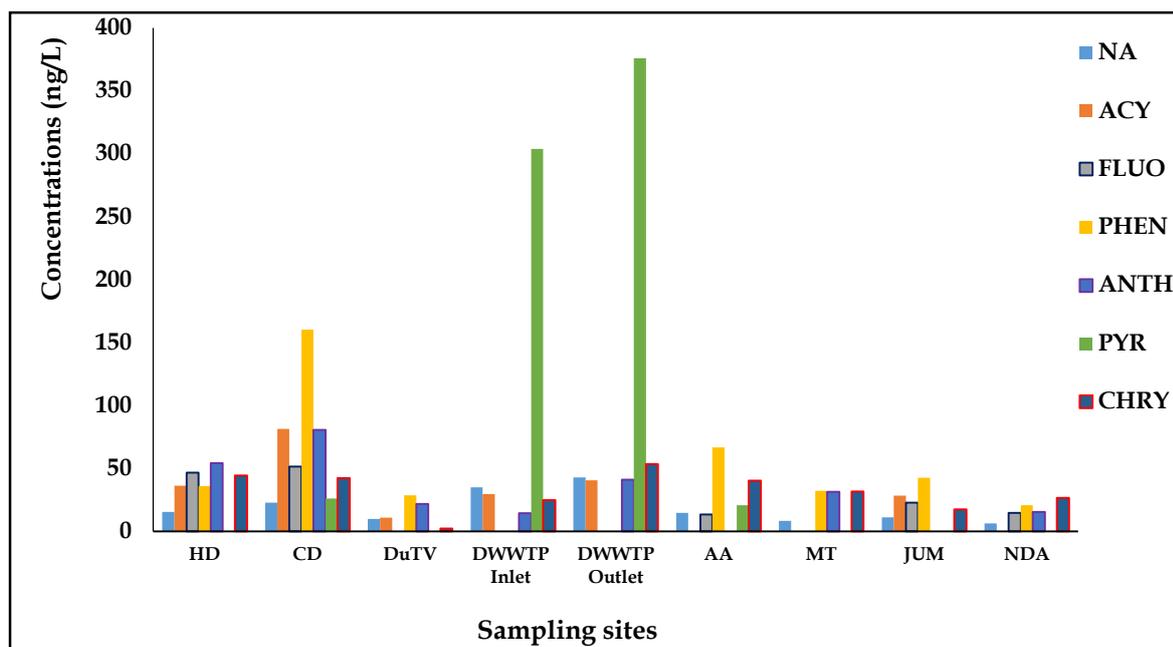
Figure 4.4 Concentrations (ng/L±SD) of the 7 PAHs in the water and wastewater from sites along the Msunduzi River during the winter season.

The occurrence of PYR in higher concentration levels at DWWTP (inlet & outlet) may also be explained by its high resistance and persistence towards biochemical degradation processes in the water or wastewater as reported in section 4.3.1.

Only LMW-PAHs can easily undergo biodegradation processes while most HMW-PAHs remain resistant in the environment due to their low aqueous solubility/bioavailability and their degradation rates are extremely limited by desorption and dissolution (Ukiwe et al., 2013).

Within this season, the higher values of electrical conductivity (EC) were recorded from the DWWTP Outlet (703 µS/cm) and the CD (225.0 µS/cm) sampling sites (Table 4.1, section 4.3.1). The higher summed concentrations of the 7 PAHs in these sampling sites may also be related to these high values of EC recorded during this season. It may also be due to the accumulation of contaminants, aging of the treated wastewater and other inputs from surface runoff as well as atmospheric fallouts. This can be enhanced by the long distance taken by the treated wastewater from the DWWTP

(passing through the bird sanctuary area, where the wastewater flow is too slow) to be discharged into the Msunduzi River as reported in the previous season.

As denoted during the autumn season, the sum of the concentrations of the investigated 7 PAHs was found to be low in the lower reaches of the Msunduzi River, which implies that even in this season higher pollution of water is mainly occurring within and around the city of Pietermaritzburg (upper and middle reaches). This can be highly related to the lack of rainfall in these two seasons, where all discharged waste and pollutants reside for long periods in the upper and middle reaches.

Table 4.5 Concentrations(ng/L±SD) of the 7 PAHs in the water at each site during the winter of 2014.

Sites	LMW-PAHs					HMW-PAHS		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	15.45±0.01	36.23±0.04	46.67±0.03	35.87±0.02	54.32±0.06	ND	44.27±0.04	232.81±0.20
CD	22.70±0.02	81.46±0.11	51.35±0.05	160.16±0.19	80.50±0.04	26.07±0.05	42.21±0.04	464.45±0.82
DuTV	9.92±0.01	10.75±0.03	ND	28.60±0.04	21.78±0.03	ND	2.30±0.02	73.35±0.13
DWWTP Inlet	34.83±0.01	29.66±0.01	ND	ND	14.27±0.01	303.77±0.20	24.85±0.02	407.38±0.45
DWWTP Outlet	42.73±0.04	40.54±0.05	ND	ND	40.90±0.08	376.02±0.29	53.43±0.06	553.62±0.62
AA	14.56±0.01	ND	13.24±0.01	66.64±0.12	ND	20.81±0.04	40.34±0.04	155.60±0.22
MT	8.33±0.02	ND	ND	32.12±0.11	31.43±0.02	ND	31.54±0.09	103.42±0.34
JUM	10.97±0.01	28.42±0.01	22.84±0.01	42.56±0.03	ND	ND	17.34±0.01	122.13±0.07
NDA	6.23±0.01	ND	14.54±0.02	20.75±0.01	15.44±0.01	ND	26.66±0.01	83.62±0.06

Spring season of 2015

As shown in Table 4.6 as well as in Figure 4.5, PYR and CHRY were not detected like in previous seasons, and in some sampling sites were detected at low concentrations due to their low aqueous solubility. Only LMW-PAHs were detected in almost all sampling sites during the spring season (September-October 2015). The detection and solubility in water of 7 PAHs studied decreased as their molecular weights increased.

The summed concentrations of the 7 PAHs ranged between 74.42 and 1799.14 ng/L with an average concentration of 527.80±0.73 ng/L, which was significantly different with 95% confidence level.

The higher sum of the concentrations of the investigated 7 PAHs was found in the water from the JUM sampling site (1799.14 ng/L), which was about two times of that measured in the water from the CD sampling site (967.10 ng/L). This site also had a high conductivity of 355.2 μS/cm which is normally associated with a high total dissolved solids content typical of polluted sites. . The presence of high total dissolved solids can result in some persistent organic pollutants such as PAHs to adsorb strongly on suspended particulate matters and dissolved solids available in the water column itself or in the pore water (Wong et al., 1995).

The lowest summed concentrations of the 7 PAHs was observed in the water collected from the NDA sampling site (74.42 ng/L). The lowest concentration found at this site was expected, as it was selected as a non-contaminated comparative point in this study as reported in previous seasons.

ACY (689.63 ng/L) and FLUO (627.95 ng/L) as LMW-PAHs (characterised by low log *Kow* values), presented higher concentration levels in the water collected from the JUM sampling site. The concentration of ACY was also high in the water collected from the CD sampling site (314.97 ng/L) as shown in Figure 4.5.
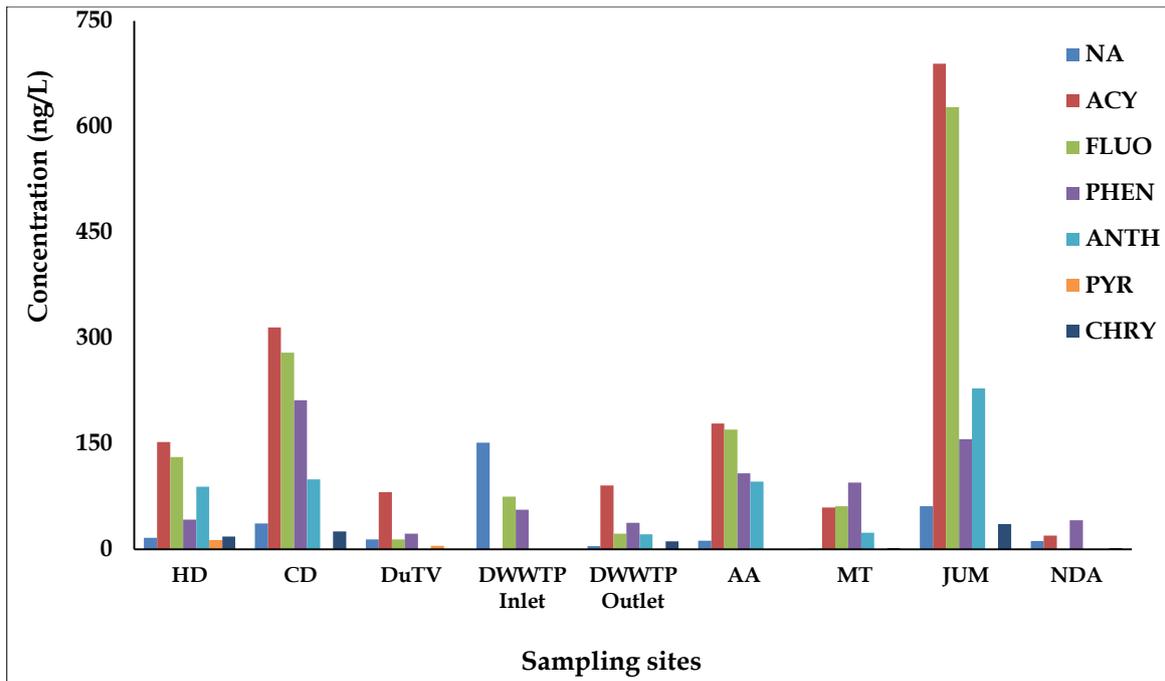
Figure 4.5 Concentrations (ng/L) of the 7 PAHs in the water and wastewater from sites along the Msunduzi River during the spring season.

The CD is a site located in the upper reaches of the Msunduzi River, which is very close to the city of Pietermaritzburg and may receive industrial waste, experience illegal dumping and burning of different kinds of organic materials (Tony, 2012). Pollution from these LMW-PAHs may be attributed to the wet deposition and runoff waters from several petrogenic activities (as spring is a wet season in South Africa) occurring within the Msunduzi town and different types of local inputs (use of coal tar, gasoline, kerosene and creosote). Research carried out in Switzerland and Germany, showed that the highest concentrations of PAHs were identified in runoff waters as well as in rainwater (Lopes & Dionne, 2003; Klimaszewska, Polkowska & Namiesnik, 2007; Sulej et al., 2011).

This season showed an exception in the concentration levels of the 7 PAHs in the water from the source to the mouth of the river. They increased from the upper to the lower reaches (JUM) as shown in Figure 4.5 due to the transportation of loaded and discharged waste by rainfall water from the upper and middle to the lower reaches.

In addition, LMW-PAHs (acutely toxic) was seen to have higher concentrations during this season (wet season) due to their higher solubility in water than the HMW-PAHs (carcinogenic and mutagenic).

The spring season follows the winter season, which is a dry season during which time burning of grasslands occurs in preparation for the planting season of spring. This burning will produce PAHs and together with the high rainfall during the spring season could be a possible reason for the high concentrations of PAHs observed.

Table 4.6 Concentrations (ng/L±SD) of the 7 PAHs in the water at each site during the spring of 2015.

Sites	LMW-PAHs					HMW-PAHS		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	16.28±0.82	152.45±0.07	130.92±0.14	42.03±0.05	88.85±0.08	13.31±0.01	18.24±0.02	462.08±1.19
CD	36.60±0.02	314.97±0.23	279.02±0.21	211.64±0.15	99.25±0.08	ND	25.47±0.02	967.10±0.71
DuTV	14.13±0.01	81.20±0.02	13.97±0.33	22.32±0.02	ND	5.06±0.02	ND	136.68±0.40
DWWTP Inlet	151.50±0.13	ND	74.61±0.01	55.95±0.09	ND	ND	ND	282.06±0.23
DWWTP Outlet	4.67±0.49	90.78±0.90	22.32±0.43	37.70±0.05	21.41±0.05	20.28±0.02	11.26±0.01	208.39±1.89
AA	12.00±0.06	178.55±0.15	169.76±0.17	107.85± 0.16	96.20±0.17	15.17±0.01	ND	579.53±0.72
MT	0.67±0.12	59.40±0.06	60.93±0.06	94.84±0.02	23.35±0.03	ND	1.70±0.04	240.89±0.28
JUM	61.25±0.04	689.63±0.33	627.95±0.55	156.43±0.08	228.30±0.11	ND	35.58±0.02	1799.14±1.12
NDA	11.65±0.01	19.61±0.02	ND	41.36±0.06	ND	ND	1.80±0.01	74.42±0.10

As indicated in Table 4.7 as well as in Figure 4.6, PAHs like FLUO, PHEN, ANTH, and CHRY were not detected in the wastewater collected from DWWTP Inlet, CHRY was not detected in the water from the HD sampling site and ANTH was also not detected in the water from the NDA sampling site during the summer season (November-February 2014).

The sum of the concentrations of the 7 PAHs in the surface water and wastewater samples varied significantly among the sites studied as the values ranged between 283.49 and 1749.05 ng/L with an average concentration of 816.94±0.28 ng/L as shown in Table 4.7 and Figure 4.6.

The highest sum of the concentrations of the 7 PAHs were found in DWWTP Inlet (1 749.05 ng/L) and HD (1380.83 ng/L) sampling sites. Other significantly high sums of concentrations of the 7 PAHs were observed in the water collected from CD (953.95 ng/L), MT (652.59 ng/L) and the JUM (650.55 ng/L) sampling sites. Lower sums of the concentrations of the 7 PAHs were detected in wastewater and surface samples collected from the DWWTP Outlet (584.75 ng/L), DuTV Bridge (570.86 ng/L), NDA (526.46 ng/L) and the AA (283.49 ng/L), respectively.

The concentration level of PYR (HMW-PAH with higher log Kow value), was found to be high in the wastewater collected from the DWWTP Inlet sampling site (1727.21 ng/L). This may be due to the pollution from anthropogenic activities (such as the combustion of fossil fuels) occurring in the city of Pietermaritzburg. The pollutants released accumulate in the water and make their way into this treatment plant as reported in section 4.3.1. This can also be explained by its higher affinity onto organic particulate matter and fine particles suspended in the water coming into this treatment plant.

The other higher concentration level in water of an individual PAH was for PHEN found in the surface water collected from the HD sampling site (1294.73 ng/L). PHEN was the dominant PAH found in almost all sampling sites except at DWWTP Inlet as shown in Figure 4.6. The occurrence of PHEN in the environment is suspected to be

coming from local vegetation fires, long-range transport input as well as wet deposition.
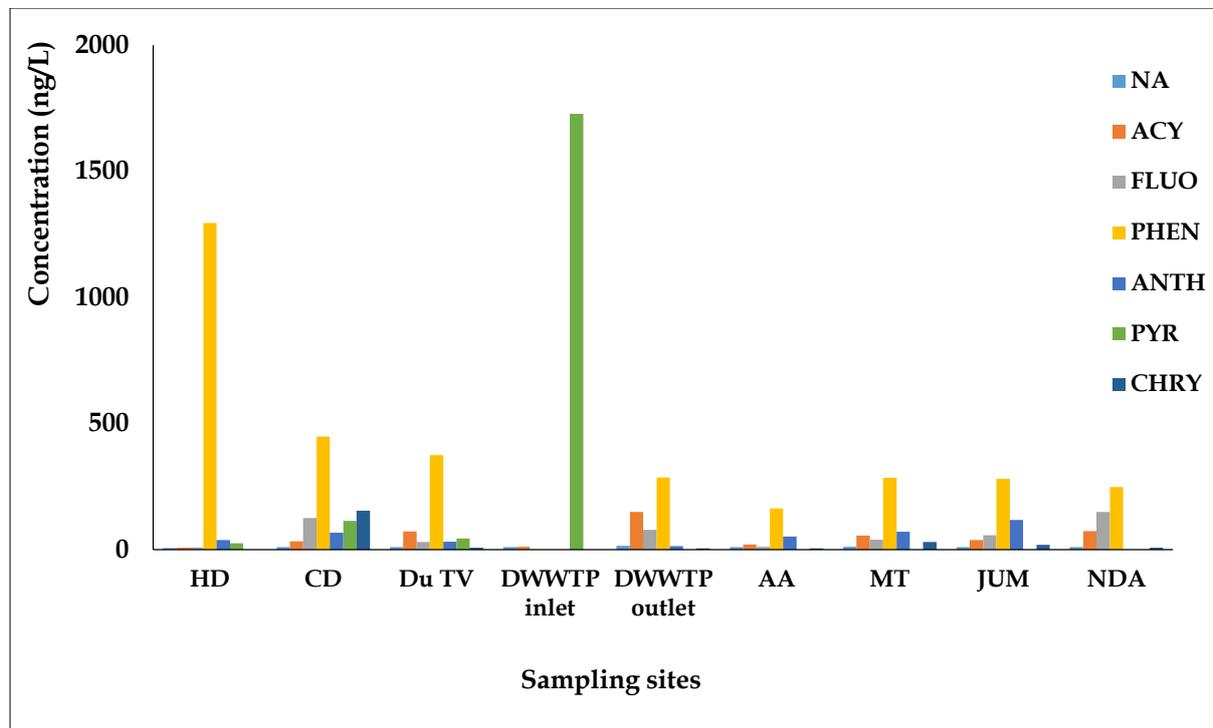
Figure 4.6 Concentrations (ng/L±SD) of the 7 PAHs in the water and wastewater from sites along the Msunduzi River during the spring season.

As shown by the sum of the concentrations of the 7 PAHs in Table 4.7, sums of the concentrations were high in the upper and middle reaches (between HD and DWWTP Inlet sampling sites). This indicated that higher pollution of water during this season occurred within the sites very close to the city of Pietermaritzburg.

Table 4.7 Concentrations (ng/L±SD) of the 7 PAHs in the water at each site during the summer of 2014.

Sites	LMW-PAHs					HMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	6.42±0.01	7.55±0.01	8.45±0.03	1294.73±0.39	38.19±0.02	25.49±0.01	ND	1380.83±0.47
CD	10.52±0.01	33.34±0.02	125.53±0.11	448.20±0.04	67.30±0.01	114.50±0.05	154.56±0.01	953.95±0.25
Du TV	10.46±0.02	71.71±0.04	30.15±0.02	374.28±0.03	31.99±0.01	44.07±0.02	8.20±0.01	570.86±0.14
DWWTP Inlet	10.17±0.01	11.67±0.01	ND	ND	ND	1727.21±0.27	ND	1749.05±0.29
DWWTP Outlet	14.90±0.01	149.36±0.08	78.33±0.02	285.98±0.12	14.12±0.01	36.72±0.01	5.34±0.03	584.75±0.28
AA	9.72±0.03	20.46±0.01	11.25±0.01	162.92±0.02	52.24±0.03	22.20±0.02	4.70±0.01	283.49±0.13
MT	11.04±0.23	56.31±0.04	39.87±0.02	285.07±0.16	70.85±0.02	159.27±0.13	30.18±0.03	652.59±0.63
JUM	9.97±0.01	38.00±0.02	57.46±0.01	281.66±0.03	118.35±0.01	126.38±0.03	18.73±0.01	650.55±0.12
NDA	10.44±0.01	73.50±0.01	149.67±0.07	248.05±0.12	ND	37.12±0.03	7.68±0.01	526.46±0.25

The seasonal variation of PAH concentrations in the water normally depends on the availability of rainfall, which mainly carries and transports all released, emitted, discharged or produced organic pollutants into the aquatic system through wet deposition and surface runoff. It also highly depends on the physical parameters characterising each season. For example, in some warm seasons (with high temperatures), the conductivity of the water may be increased due to higher ionic mobility and dissolution of many salts and minerals on which organic pollutants such as PAHs can rapidly adsorb (Whipple, 2002).

During the wet seasons, all organic pollutants released into the atmosphere through evaporation may return into the aquatic environment through wet deposition, thus leading to higher concentrations observed in water, soils as well as in surface sediments. Indeed, those discharged or dumped into the river system may be dissolved and transported from far sources to other points along the river. In South Africa, spring and summer (as wet seasons) are characterized by heavy rainfall and warm weather, which increases runoff from the surrounding area into the river system. Then, this leads to higher concentrations of many pollutants such as PAHs in aquatic systems.

Overall, there are wide ranges of the summed concentrations of investigated PAHs in water across the river during the spring season (74.42-1799.14 ng/L) followed by the summer season (283.49-1749.05 ng/L) and the autumn season (12.47-718.93 ng/L). The lower concentration range of investigated PAHs was found during the winter season (73.35-553.62 ng/L).

These ranges are globally low compared to the concentration ranges of PAHs found in the water collected from the Tonghui River of Beijing in China (192-2651 ng/L for 16 PAHs) (Zhang, Huang & Hong, 2004), Almendares River in Cuba (836-15811 ng/L for 14 PAHs) (Santana et al., 2015) and the Daliao River watershed in China (46-13448 ng/L for 18 PAHs) (Guo et al., 2007).

Few studies in South Africa have been done on PAH concentrations in the wastewater and water (Nekhavhambe et al., 2009; Tikilili & Nkhalambayausi-Chirwa, 2011; Sibiya

et al., 2012). For example, the concentration levels of PAHs in wastewater collected from the Cape Town Province have been reported. All 16 priority PAHs were detected with the concentration levels fluctuating between 0.001 and 25.1 mg/L (Tikilili & Nkhalambayausi-Chirwa, 2011), which are higher than the results of this study.

PAHs in the surface water collected from Limpopo Province have also been determined (Nekhavhambe et al., 2009). The sum of the concentrations of 6 PAHs obtained ranged between 0.1 and 2500 µg/L for water samples, which are higher than the results of this study.

Five PAHs in the surface water collected from Johannesburg in Gauteng Province have also been determined (Sibiya et al., 2012). Their total concentration levels ranged between 30 and 615 ng/L, which are only higher than the results obtained during the winter season.

4.2.3 Concentration of PAHs in soil collected from the banks of the river

The concentration levels of investigated PAHs in soils collected from the banks of the Msunduzi River and Nagle Dam during the autumn, winter and summer of 2014 and spring of 2015 are presented in Tables 4.8-4.11 as well as in Figures 4.7-4.10.

Autumn season of 2014

As shown in Table 4.8 and Figure 4.7, the sum of the concentrations of the 7 PAHs are very high in the soils collected from the AA (109.40 µg/g), which was approximately twice that observed for the MT (54.99 µg/g) sampling site. The summed concentrations of the 7 PAHs at MT site has no significant difference (P < 0.05) to that calculated for the soils from HD (53.87 µg/g). The sum of the concentrations of the PAHs in the soils ranged from 3.21 to 109.40 µg/g with an average concentration of 38.55±2.33 µg/g dry weight.

Only PHEN, ANTH and PYR were not detected in the soils collected from the NDA sampling site (a comparative point as reported in section 4.3.2) as shown in Figure 4.7.
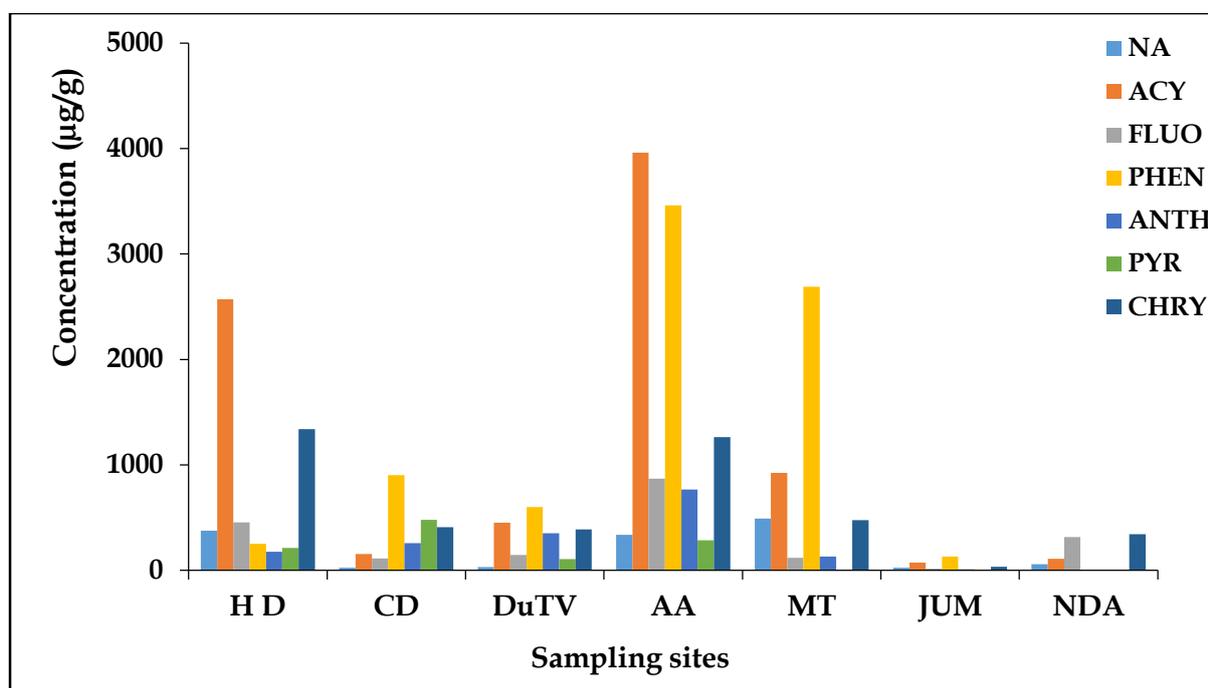
Figure 4.7 Concentrations (µg/g±SD) of the 7 PAHs in the soils from the banks of the Msunduzi River during the autumn season.

The highest concentrations of ACY were found in the soils collected from the AA sampling site (39.59 µg/g dry weight) and the HD area (25.72 µg/g dry weight), located in the upper and middle reaches of the river. The higher sum of concentrations of PAHs found at the AA sampling site is probably referable to the higher nutrients and total dissolved solids from agricultural runoff on which these pollutants can strongly adsorb. It may also be due to the application of sewage sludge and wastewater irrigation as fertilizer to the agricultural area.

PAHs were found to possess a higher affinity with clay-type soil (Hundral et al., 2001), with the soil collected from this sampling site (AA) comprising a higher amount of clay and organic matter. As reported in the study conducted by Hundral et al. (2001), when soils contain a higher portion of organic matter, a large amount of PHEN is highly retained by smectites.

LWMW-PAHs presented higher mean concentrations than HMW-PAHs as shown in Table 4.8. This may be due to their high bioavailability and solubility in the aqueous environment and may also be attributed to the higher petrogenic activities as well as atmospheric fallouts occurring during this season. In addition this may also be a result of the accumulation of different types of pollutants discharged from the DWWTP and sludge sewages. The DWWTP also showed higher concentrations of PAHs in wastewater samples collected from its effluent during the autumn and winter seasons (as reported in section 4.3.2). The AA is the first point receiving this treated effluent wastewater as shown on the map of sampling sites in Chapter III, Figure 3.1. Autumn (as a dry season) is normally characterized by lack of rainfall and also the Msunduzi River, in the AA area, is highly characterized by low water flow rate, which can enhance the accumulation of PAHs in the soils near the banks of the river through leaching.

As shown in Figure 4.7, the upper and middle reaches (close to the city of Pietermaritzburg, from HD to MT) had higher concentrations of PAHs than the lower reaches. This higher pollution level may be due to the leaching of wastes generated by anthropogenic activities and urban surface runoff. The lower sum of the concentration of the 7 PAHs observed from the JUM sampling point may be due to the volatilization and biodegradation processes. The JUM sampling point was a site located in a rural area with very little vehicular traffic as well as no industrial activities which may be the reason for the low concentrations of PAHs observed.

Table 4.8 Concentrations (μg/g ±SD) of the 7 PAHs in the soils at each site during the autumn of 2014.

Sites	LMW-PAHs					HMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	3.77±0.73	25.72±4.02	4.55±0.12	2.52±0.95	1.76±0.09	2.14±1.44	13.39±1.17	53.87±8.52
CD	0.26±0.01	1.56±0.62	1.14±0.08	9.03±1.04	2.57±0.44	4.78±1.06	4.10±0.10	23.45±3.35
DuTV	0.29±0.03	0.45±0.02	1.45±0.09	5.97±0.54	3.52±0.93	1.06±0.27	3.89±1.12	16.65±3.00
AA	3.37±0.28	39.59±6.61	8.67±1.02	34.59±5.01	7.67±0.17	2.86±0.44	12.64±2.43	109.40±15.96
MT	4.88±1.43	9.23±2.34	1.18±0.07	26.89±4.18	1.30±0.03	6.66±2.32	4.77±1.67	54.99±12.04
JUM	0.25±0.02	0.75±0.02	0.17±0.05	1.30±0.20	0.09±0.02	0.30±0.08	0.35±0.08	3.21±0.47
NDA	0.58±0.04	1.10±0.02	3.17±0.13	ND	ND	ND	3.43±0.16	8.28±0.35

Winter season of 2014

As shown in Table 4.9, the sum of the concentrations of the 7 PAHs in the soils ranged from 2.67 (HD sampling site) to 77.34 µg/g (AA sampling site) with an average concentration of 18.33±1.45 µg/g dry weight. The higher concentrations of PAHs in the soils collected from the AA sampling site during the winter season of 2014 may be related to the composition and texture of the soil at the sampling sites as explained during the autumn season of 2014.

The high sum of concentrations of the 7 PAHs observed the from DuTV sampling site may be ascribed to the incomplete combustion of gasoline, fuel oil, and diesel fuel while those observed from AA and NDA areas can be the results of leaching from agricultural runoff and atmospheric fallouts (Sakari, 2012). In addition, people living in the AA area use fires (burning grasslands) as a means of controlling the growth of the grasses and preparing the ground for the incoming planting season (spring). These processes can likewise contribute to the high amount of PAHs (especially PHEN) found in the rural areas through flying ash and land washing (Grova et al., 2002).

Furthermore, people from these areas tend to burn wood and fuel during the colder months as a source of heat for homes, which would further lead to the high levels of PAHs in these areas. The other suspected sources of PAHs at the MT sampling site could be the vehicular activity releasing fumes and oil leakage, which may contain some organic pollutants including PAHs.

As shown in Figure 4.8, high concentrations of the investigated PAHs in this season have been found at the AA site but lower than compared to the previous season. This is due to the continuous leaching of PAHs from the top soil to the ground soil, biological degradation or volatilization processes especially the LMW-PAHs.

Table 4.9 Concentrations (µg/g±SD) of the 7 PAHs in the soils at each site during the winter of 2014.

Sites	LMW-PAHs					HMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	0.25±0.05	0.41±0.09	0.13±0.01	1.25±0.04	0.23±0.09	ND	0.39±0.03	2.67±0.31
CD	0.11±0.09	ND	ND	2.69±0.10	0.62±0.27	1.81±0.46	2.00±0.54	7.26±1.46
DuTV	0.12±0.10	0.51±0.08	0.87±0.05	6.74±2.78	1.21±0.12	2.76±0.08	1.90±0.36	14.15±3.57
AA	2.87±0.53	28.97±4.46	4.10±1.05	27.35±5.82	3.35±1.82	0.90±0.73	9.77±2.32	77.34±16.73
MT	0.06±0.06	1.06±0.65	0.34±0.20	1.43±0.81	ND	0.34±0.34	1.95±1.09	5.20±3.15
JUM	0.24±0.03	ND	ND	5.41±1.99	1.04±0.77	0.91±0.76	1.02±0.72	8.63±4.27
NDA	0.15±0.10	0.26±0.04	0.03±0.01	10.93±3.62	0.24±0.16	0.57±0.08	0.82±0.02	13.03±4.03
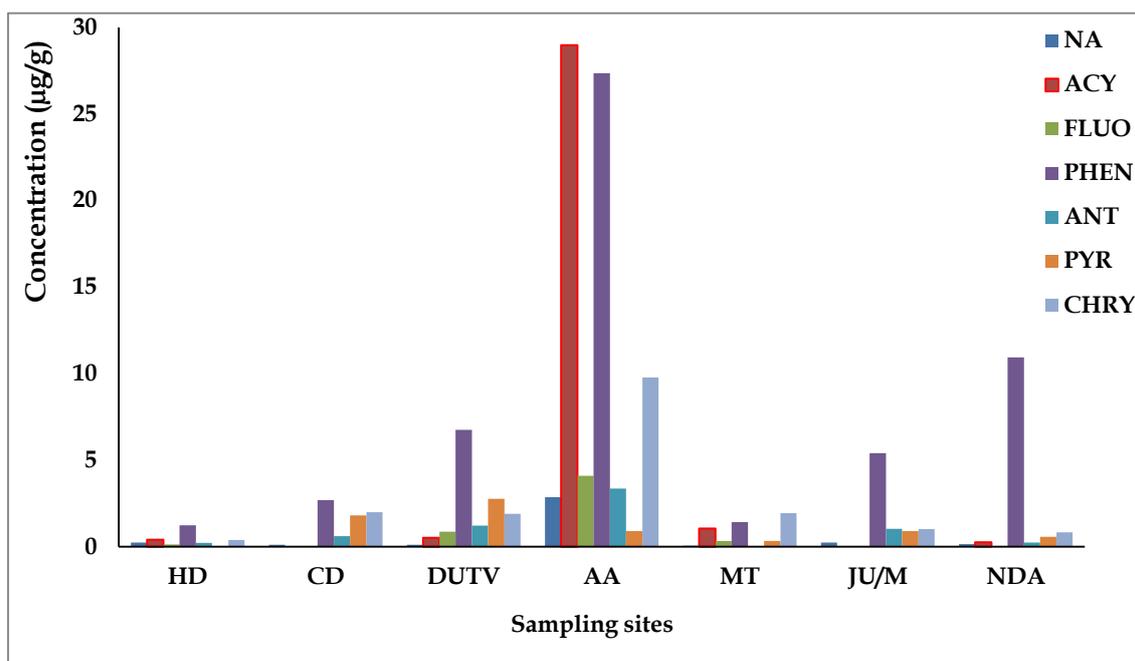
Figure 4.8 Concentrations (µg/g±SD) of the 7 PAHs in the soils collected from the source to the mouth of the river during the winter season.

Spring season of 2015

As shown in Table 4.10, only ANTH was not detected in the soils collected from the JUM and AA sampling sites. The higher sum of the concentrations of the 7 PAHs was found in the soils collected from the DuTV Bridge sampling site (256.75 µg/g) followed by that found in the soils collected from the CD sampling site (150.89 µg/g). The sum of the concentrations of the 7 PAHs varied considerably in the other sites as shown in Figure 4.9 with higher concentrations in the upper reaches of the river. Their values fluctuated between 12.44 and 256.75 µg/g with an average concentration of 89.04±13.27 µg/g dry weight.
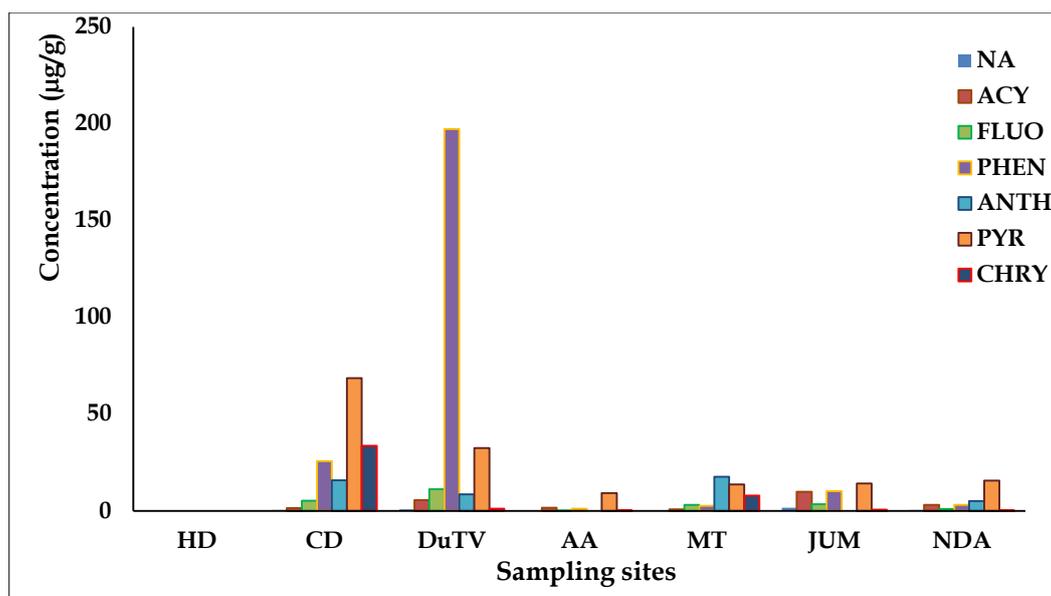
Figure 4.9 Concentrations (µg/g±SD) of the 7 PAHs in the soils from the banks of the Msunduzi River during the spring season.

DuTV Bridge is very close to an N3 Freeway and experiences large volumes of traffic, which may introduce these high levels of PAHs due to exhaust fumes from vehicular activity and runoff from coal tar. It may also experience some illegal dumping, which can contaminate the soil through leaching (Tony, 2012).

The higher concentrations of PAHs observed at the DuTV and CD were expected and these sites were selected as the most contaminated areas due to their location in the urban area of the Pietermaritzburg city. Thus, the possible sources of PAHs in the soils of these sites may be attributed to leaching from petrogenic and pyrogenic processes like incomplete combustion of organic textiles, agricultural waste and the release of petroleum products or deposition from long-range transport through atmospheric fallouts (Wilcke et al., 2005).

PHEN (LMW-PAH) was also found in higher concentration levels in the soils collected at this site (197.10 µg/g) which was approximately three times the order of magnitude to that of PYR (HMW-PAH) found in the soils collected from the CD sampling site.

Table 4.10 Concentrations (µg/g±SD) of the 7 PAHs in the soils at each site during the spring of 2015.

Sites	LMW-PAHs					HMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	-	-	-	-	-	-	-	-
CD	0.52±0.11	1.49±0.20	5.26±3.66	25.66±3.47	15.75±3.02	68.55±8.94	150.89±9.66	150.85±29.06
DuTV	0.72±0.09	5.58±0.96	11.22±2.18	197.10±20.75	8.51±1.21	32.46±1.92	1.16±0.30	256.75±27.41
AA	0.37±0.01	1.56±0.32	0.03±0.01	1.08±0.72	ND	9.16±0.15	0.24±0.02	12.44±1.21
MT	0.11±0.04	0.78±0.47	3.03±0.21	2.64±0.94	17.68±4.85	13.60±3.35	7.84±2.98	45.68±12.84
JUM	1.57±0.18	9.90±2.07	3.41±0.36	10.21±0.57	ND	14.14±0.92	0.70±0.62	39.93±4.72
NDA	0.45±0.01	3.12±1.07	0.91±0.60	3.09±2.25	5.08±0.14	15.58±0.13	0.38±0.17	28.61±4.37

- No soil samples were taken from the HD site during the spring season of 2015. The site was inaccessible.

Summer season of 2014

All PAHs were detected in the soils collected from all sampling sites by presenting significant amounts compared to the previous seasons. This may be due to the higher temperature and heavy rainfall normally present during this season, which would transport PAHs from higher up soil banks closer to the river banks (due to runoff) where the soil was sampled.

As shown in Table 4.11, the summed concentration values ranged from 12.16 to 72.72 µg/g with an average concentration of 41.32±3.21 µg/g dry weight. The highest sum of the concentrations of the 7 PAHs was recorded in the soil collected from the AA sampling site followed by that found in the soils from MT, JUM and CD sampling sites. A lower sum of the concentrations of the 7 PAHs was observed in the soil samples collected from the DuTV Bridge and NDA sampling sites. The concentration level of PHEN (42.17 µg/g) was found to be higher in the soils collected from the AA sampling site, and this was followed by that of PYR (42.17 µg/g) found in the soils collected from the MT sampling site as shown in Figure 4.10.
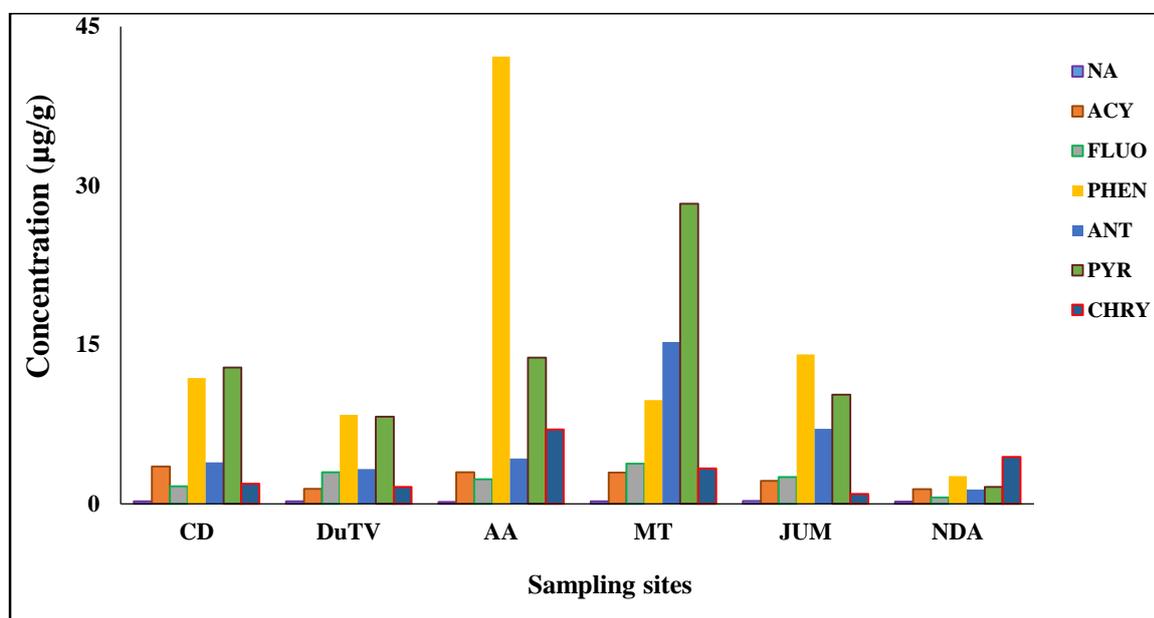
Figure 4.10 Concentrations (µg/g±SD) of the 7 PAHs from the soils from the banks of the Msunduzi River during the summer season.

These results suggested that PAHs accumulated or leached into the Msunduzi River soils may come from different sources such as industrial discharges, urban surface runoff, and incomplete combustion of grasses or forest during the cultivation and fuel combustion emissions.

Sometimes, the presence of PHEN in soils can be as a result of biological activities, local vegetation fires, or imported by long-range transportation. Many studies also showed that PHEN occurs in plants, which may synthesize it biologically and during putrefaction of plant materials, which can then reach the soil through the leaching process (Laflamme & Hites, 1978; Wickstrom & Tolonen, 1987).

During this season, the higher sum of the concentrations of investigated PAHs was observed within the middle and lower reaches as the river flows from the source to the mouth of the river. This is due to the higher amount of rain, which is normally available in this season, transporting all loaded materials into the river from one place to another, which finally accumulates in the lower reaches.

Table 4.11 Concentrations (µg/g±SD) of the 7 PAHs in the soils at each site during the summer of 2014.

Sites	LMW-PAHs					HMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	-	-	-	-	-	-	-	-
CD	0.25±0.01	3.52±0.21	1.66±0.12	11.86±1.86	3.91±0.55	12.84±1.42	1.89±0.04	35.95±4.21
DuTV	0.23±0.03	1.42±0.33	2.97±0.03	8.40±0.04	3.27±0.06	8.23±0.08	1.58±0.66	26.11±1.23
AA	0.18±0.05	2.98±0.84	2.30±0.11	42.17±5.04	4.28±0.37	13.78±1.48	7.02±1.18	72.72±9.07
MT	0.22±0.09	2.94±0.65	3.78±1.63	9.79±1.23	15.25±2.97	28.27±1.64	3.33±1.42	63.70±9.63
JUM	0.25±0.01	2.16±0.03	2.52±0.17	14.08±3.77	7.07±0.03	10.27±2.09	0.91±0.60	37.29±6.70
NDA	0.21±0.05	1.38±0.31	0.59±0.40	2.62±0.86	1.34±0.89	1.58±0.41	4.42±0.24	12.16±3.16

- No soil samples were taken from the HD site during the summer season of 2014. The site was inaccessible.

During this work, the wider range of the summed concentrations of the 7 PAHs was found in the soils collected during the spring season (12.44-256.75 µg/g) followed by that of the autumn season (3.21-109.40 µg/g) and the winter season (2.67-77.34 µg/g). The lower range was observed during the summer season (12.16-72.72 µg/g).

The concentration of PAHs in soil may depend on the composition of the soil (availability of clay), texture, location and seasonal behaviour. For example, during the warmer season different pollutants can undergo volatilization or evaporation and decomposition while during the rainy seasons, many pollutants leach from the surface due to the higher permeability of the soils.

Spring is the first season following two consecutive dry seasons (autumn and winter, characterized by lack of a rainfall and cold weather) in which temperature starts to increase and also the rain starts falling. Thus, the moderate rainfall available during the spring season makes the soils softer by increasing its moisture facilitating the leaching of many contaminants from different compartments through water runoff (Shabalala et al., 2013).

In comparison with global contamination of PAHs in soils, the results of this work were lower than the concentrations detected from the urban area of Beijing in China (0.36-278.25 µg/g for 16 PAHs) (Tang et al., 2005) and those found from different contaminated soil areas (297.4-458.0 µg/g for 16 PAHs) in the study conducted by Dean et al., 1995.

This range of concentrations of PAHs found in the spring season was higher than the range found in the urban park soils collected from Hong Kong in China (not detected-19.50 µg/g for 16 PAHs) (Chung et al., 2007), in soils from Delhi in India (0.83-3.88 µg/g for 16 PAHs) (Agarwal et al., 2009), from the Seine River basin in France (0.45-5.65 µg/g for 14 PAHs) Motelay-Massei et al., 2004) and from Shanghai in China (0.44-19.70 µg/g for 16 PAHs) (Jiang et al., 2009). It was also higher than the range found in the analysed soils collected from the Weihe River in the Northwest China (0.36-15.66 µg/g for 16 PAHs) (Chen, Jia & Yang, 2015).

Few studies, in South Africa, have been done on soils (Nieuwoudt et al, 2011; Okedeyi et al., 2013; Vogt, 2014). For example, the study on PAHs was conducted on the soils around three power plants in this country, namely the Rooiwal located in Gauteng, the Lethabo located in Free states and the Matla located in Mpumalanga Provinces. The results of total concentration levels of 15 PAHs obtained in the soils ranged between 9.73 and 61.24 µg/g dry weight (Okedeyi et al., 2013), which are very low compared to the range obtained during the spring season, but closer than that obtained during the summer and winter seasons.

Sixteen PAHs in the soils sampled from central South Africa (residential, agricultural and industrial areas), and the Vaal Triangle River in the Gauteng Province were found to be in the range of 0.04 and 39 µg/g dry weight (Nieuwoudt et al, 2011), which are also very much lower than the results of this study.

The concentration levels of 23 PAHs in the KwaZulu-Natal soils (eThekwini Municipality) have been found to be in the range of 0.006 and 3.23 µg/g dry weight (Vogt, 2014), which were low compared to the results of the present study.

4.2.4 Concentrations of PAHs in the surface sediments from the Msunduzi River

The concentration levels of the 7 investigated PAHs in the surface sediments collected from the Msunduzi River basin and Nagle Dam during the autumn, winter and summer of 2014 and spring of 2015 are presented in Tables 4.12-4.15 as well as Figures 4.11-4.14.

Autumn season of 2014

As shown in Table 4.12, the sum of concentrations of the 7 PAHs along the Msunduzi River ranged from 1.09 µg/g to 146.16 µg/g with an average concentration of 23.43±2.78 µg/g dry weight. The sum of the concentration of the 7 PAHs was higher

in the surface sediments collected from the AA sampling site (146.16 µg/g dry weight) with PHEN (42.92 µg/g) and ACY (50.21 µg/g) as the dominant PAHs as shown in Figure 4.11.
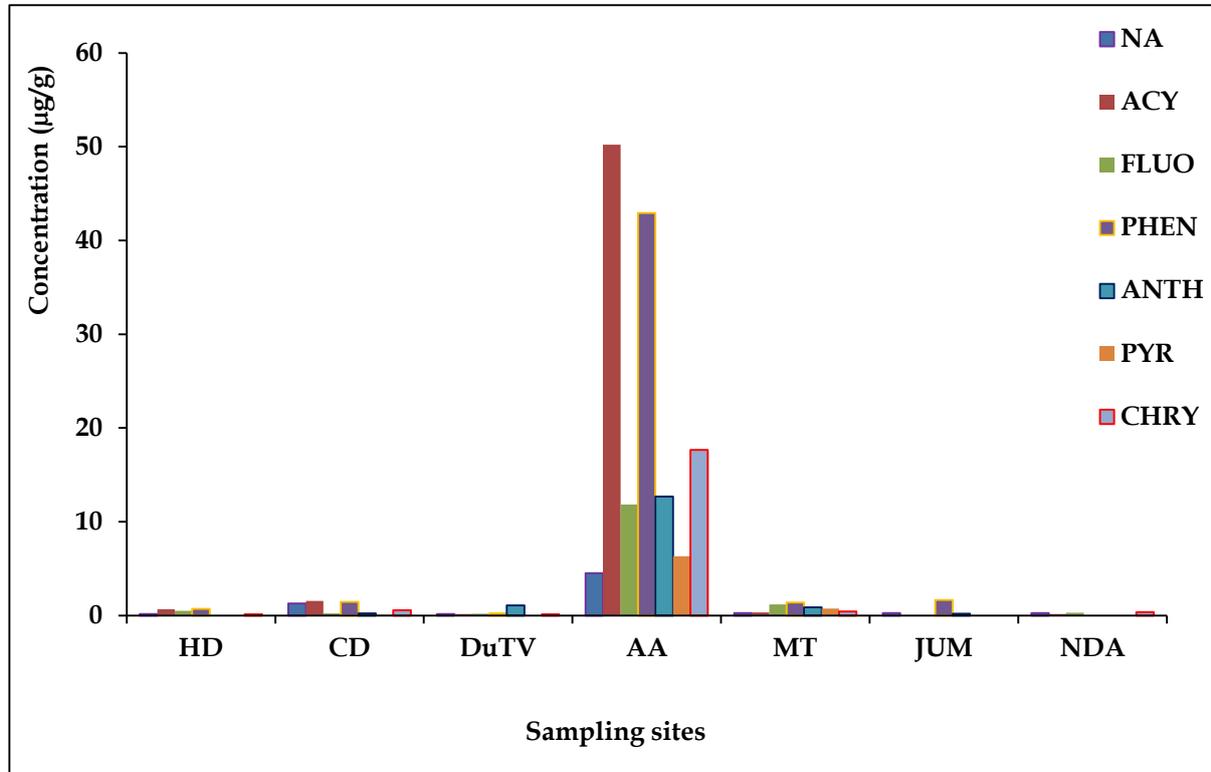
Figure 4.11 Concentrations (µg/g±SD) of the 7 PAHs from the surface sediments from the Msunduzi River during the autumn season.

Specifically, the distribution and the fate of PAHs in the environment depend on the capacity of organic matter in the sediment, the octanol-water partition coefficients of the PAHs and the particle size distribution of the sediments. The octanol-water partition coefficients (log *Kow*) of the PAHs normally increases as the number of benzene rings increase (Arias et al., 2009).

In the aquatic systems, the PAHs are strongly sorbed to the clay particles because of their high log *Kow* and as a consequence, they resist biochemical conversion to other products or to water washing due to their low water solubility (Douglas et al., 1996).

The surface sediments collected from the AA sampling site were found to incorporate a large amount of the clay-type sediment and this could be the major contributing factor to this high concentration level and persistence of the PAHs in this area.

This increased pollution could be induced by the surface runoff containing burnt residues of grass (in preparation for the planting season), and the combustion of fuel oils, gasoline, and the diesel as a source of the heat for homes in this area during the winter and the autumn seasons, which may reach surface sediment by leaching.

Table 4.12 Concentrations (µg/g±SD) of the 7 PAHs in the surface sediments at each site during the autumn of 2014.

Site	LMW-PAHs					HMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	0.16±0.14	0.67±0.50	0.50±0.04	0.69±0.24	ND	ND	0.11±0.05	2.15±0.97
CD	1.27±0.36	1.56±0.62	0.22±0.20	1.45±0.13	0.24± 0.08	0.07±0.03	0.57±0.54	5.39±1.96
DuTV	0.16±0.01	0.16±0.06	0.20±0.10	0.24±0.09	1.06± 0.53	ND	0.13±0.02	1.96±0.81
AA	4.51±1.84	50.21±11.66	11.85±3.86	42.92±11.06	12.70±8.52	6.31±1.44	17.65±5.03	146.16±43.41
MT	0.25±0.20	0.32±0.05	1.18±0.29	1.37±1.21	0.87±0.24	0.73±0.08	0.41±0.02	5.15±2.09
JUM	0.25±0.21	ND	ND	1.64±1.02	0.19±0.02	ND	ND	2.09±1.25
NDA	0.24±0.10	0.17±0.02	0.30±0.14	ND	ND	ND	0.36± 0.25	1.09±0.51

Winter season of 2014

The winter season showed a considerable reduction in the PAH concentration levels in surface sediments compared to the previous season as shown in Table 4.13 and Figure 4.12 where the values of the sum of concentrations ranged between 3.73 and 13.50 µg/g with an average concentration of 8.76±0.80 µg/g dry weight.

During this season, many people burn coal, wood, and fuel to warm up their houses which emit smoke and generate residue materials. This may introduce PAHs or other organic pollutants into the atmosphere. However, there is no rainfall to return these released pollutants to the environment due to this season being dry and generally has strong cold winds by which these pollutants can be transported to other places or remote areas. This could be the cause of lower concentrations obtained during this season. The HMW-PAHs are governed by precipitation and the particle size of sediment while the LMW-PAHs depend on the air temperature (He & Balasubramanian, 2011). In contrast, the autumn season is characterized by some slight rainfall as well as mild climate and the medium temperatures, getting colder as it progresses to the winter season. These conditions govern the deposition of both LMW- and HMW-PAHs, and hence may be the reason for the higher concentration in the autumn than in the winter season.

In addition, biodegradation of LMW-PAHs (slightly soluble in water), may also be the reason some PAHs were not detected at some sampling sites. The NDA sampling site (located at the Umgeni River) showed a significant amount of the sum of concentrations of the 7 PAHs even if it was selected as a comparative site to the Msunduzi River in this study.

Table 4.13 Concentrations (µg/g± SD) of the 7 PAHs in the surface sediments at each site during the winter of 2014.

Sites	LMW-PAHs					LMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	0.02±0.01	0.09±0.25	1.68±0.63	1.10±0.57	ND	0.98±0.08	0.43±0.26	4.32±1.80
CD	0.09±0.04	0.18±0.02	0.63±0.08	7.39±3.90	1.59±0.13	0.93±0.04	1.67±0.98	12.52±5.19
DuTV	0.12±0.06	ND	ND	3.15±0.19	4.44±0.45	1.60±0.66	1.10±0.11	10.42±1.47
AA	0.18±0.05	0.40±0.28	2.15±0.70	2.49±0.32	1.04±0.38	0.11±0.05	7.17±2.18	13.58±3.96
MT	0.02±0.01	0.02±0.02	0.30±0.69	1.65±0.67	0.19±0.03	0.91±0.35	0.62±0.33	3.73±2.10
JUM	0.04±0.01	ND	0.35±0.11	2.83±0.84	0.92±0.24	1.43±0.57	2.55±0.63	8.65±2.40
NDA	0.25±0.20	0.02±0.01	0.165±0.09	6.26±1.68	0.83±0.70	ND	0.53±0.41	8.07±3.09
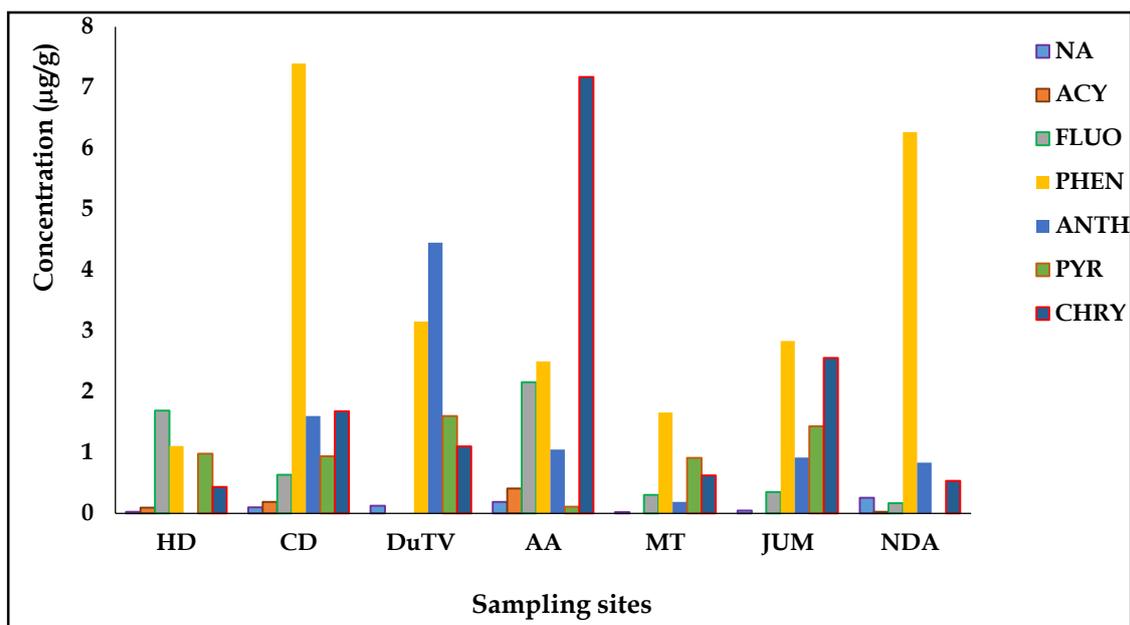
Figure 4.12 Concentrations (μg/g±SD) of the 7 PAHs from the surface sediments from the Msunduzi River during the winter season.

PHEN in the surface sediment samples collected from the CD (7.39 μg/g) and NDA (6.26 μg/g) sampling sites and CHRY from AA (7.17 μg/g) gave higher concentrations (Table 4.13 and Figure 4.12). As a LMW-PAH, the presence of PHEN in the environment may be ascribed to the petrogenic processes and the surface runoff from the garages and burning of vegetation waste. CHRY (HMW-PAH) is suspected to come from the incomplete combustion of organic matter and biomass. All of these activities may occur in these sites as CD is very close to the city of Pietermaritzburg while the AA is located in the rural area.

In South Africa, the agricultural wastes and the grasslands are generally burnt before and after cultivation in order to prepare the environment for the new rainy seasons. Generally, PAHs and other organic pollutants have a strong affinity to adsorb onto the organic particles present in the sediments as well as the pore water, hence, higher concentration levels were expected to occur in the surface sediments than in the surface water (Gan et al., 2012).

Spring season of 2015

During this season, only PYR was not found in the surface sediments collected from the MT sampling site (Table 4.14 and Figure 4.13). The sum of the concentrations of the 7 PAHs ranged between 13.50 and 160.05 µg/g with an average concentration of 88.30±15.57 µg/g dry weight.

The higher sum of the concentration levels of PAHs was found in the surface sediments collected from the middle reaches such as the AA sampling site (160.05 µg/g dry weight) and the DuTV Bridge sampling site (130.97 µg/g dry weight).

The surface sediments collected from the DuTV Bridge sampling site showed a higher concentration level of PYR (69.07 µg/g dry weight), which may be attributed to inputs from anthropogenic activities as described in section 4.3.1. This was followed by that of ACY found in the surface sediments collected from the AA sampling site (67.33 µg/g dry weight). Other higher concentrations of individual PAHs were for FLUO found in the surface sediments collected from the JUM sampling site (41.72 µg/g).
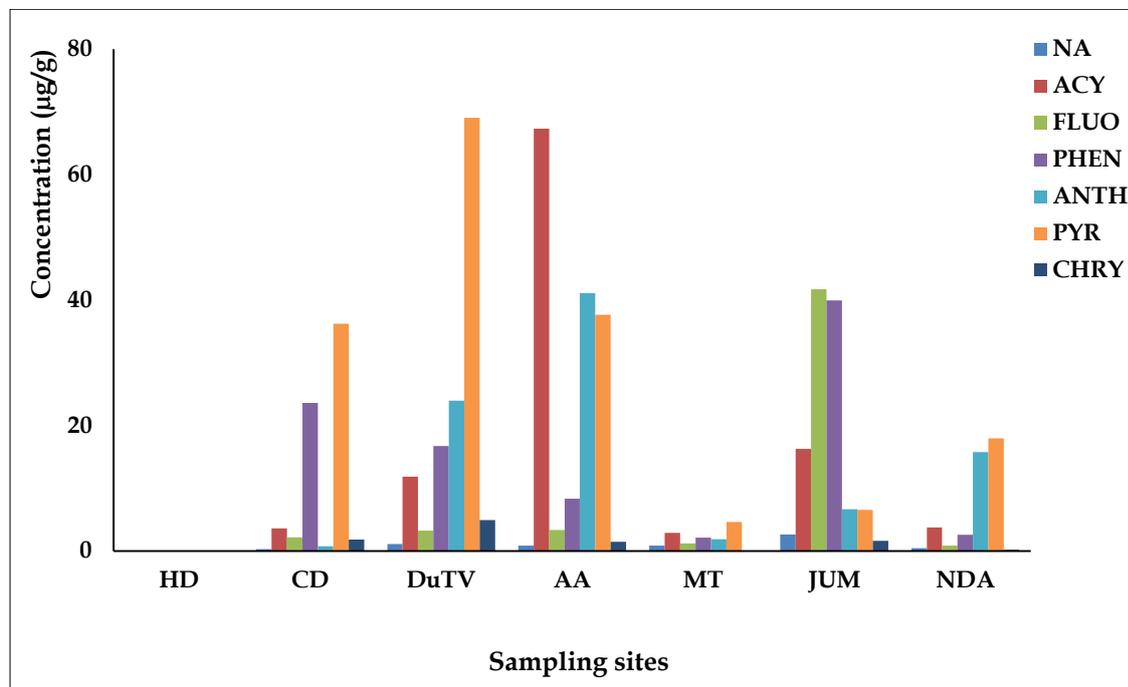
Figure 4.13 Concentrations (µg/g±SD) of the 7 PAHs in the surface sediments from the Msunduzi River during the spring season.

The surface sediments collected from the JUM and NDA (along the Umgeni River) sampling sites were higher than the sum of the concentrations of PAHs found in the surface sediment collected from the MT sampling site. The NDA sampling site (as a comparative point) also had significant concentrations of PAHs, which may reveal that the sediment from the Umgeni River may also be contaminated by PAHs.

Table 4.14 Concentrations (µg/g ±SD) of the 7 PAHs in the surface sediments at each site during the spring of 2015.

Sites	LMW-PAHs					LMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	-	-	-	-	-	-	-	-
CD	0.27±0.15	3.60±2.05	2.18±1.27	23.60±2.57	0.72±0.08	36.23±5.92	1.82±0.73	68.42±12.77
DuTV	1.12±0.33	11.86±2.46	3.26±1.84	16.71±9.95	23.98±5.25	69.07±14.26	4.92±0.59	130.92±34.68
AA	0.86±0.07	67.33±8.17	3.34±0.97	8.31±0.18	41.13±0.62	37.65±0.20	1.43±0.72	160.05±10.93
MT	0.85±0.06	2.86±0.34	1.18±0.29	2.12±1.19	1.87±0.29	4.62±0.41	ND	13.50±2.58
JUM	2.65±0.39	16.28±3.03	41.72±8.94	39.92±5.78	6.67±0.76	6.57±1.98	1.60±0.02	115.41±20.90
NDA	0.46±0.06	3.77±0.02	0.83±0.76	2.59±0.33	15.75±0.14	17.94±9.93	0.18±0.04	41.52±11.28

- No surface sediment samples were taken from the HD site during the spring season of 2015. The site was inaccessible.

Summer season of 2014

As shown in Table 4.15, the higher summed concentrations of PAHs in the sediments were found in the lower reaches than in the upper ones. Their values ranged from 9.46 to 55.71 µg/g with an average concentration of 30.37±3.76 µg/g dry weight. The highest sum of concentrations of the 7 PAHs was recorded in the surface sediments collected from the JUM sampling site followed by that in surface sediments collected from the NDA, AA, and the CD sampling sites, respectively. The lower summed concentration levels were found in the surface sediment samples collected from the MT and the DuTV Bridge sampling sites.

Due to the locations of the above sampling sites, possible sources of PAHs may be the products of waste from anthropogenic activities such as dumping, burning of plastics and grass, as well as surface runoff and wet deposition. The high PAH concentrations at JUM may also be a result of increasing pollution load from both the Msunduzi River and the upper reaches of the Umgeni River. NDA had a fairly low flowrate and this would have encouraged settling of PAHs into the sediment at this site.

The PYR's concentration level was found to be high in the surface sediments collected from the JUM sampling site followed by that of PHEN found in the surface sediments collected from the NDA site. FLUO, PHEN, and PYR were the dominant individual PAHs in the surface sediments during the summer season as shown in Figure 4.14.

During this season, a slight increase in the sum of concentrations of the PAHs was observed as the river flowed from the upper to the lower reaches due the increased rainfall which led to the transportation and deposition of particulate matter in these areas as shown in Figure 4.14.
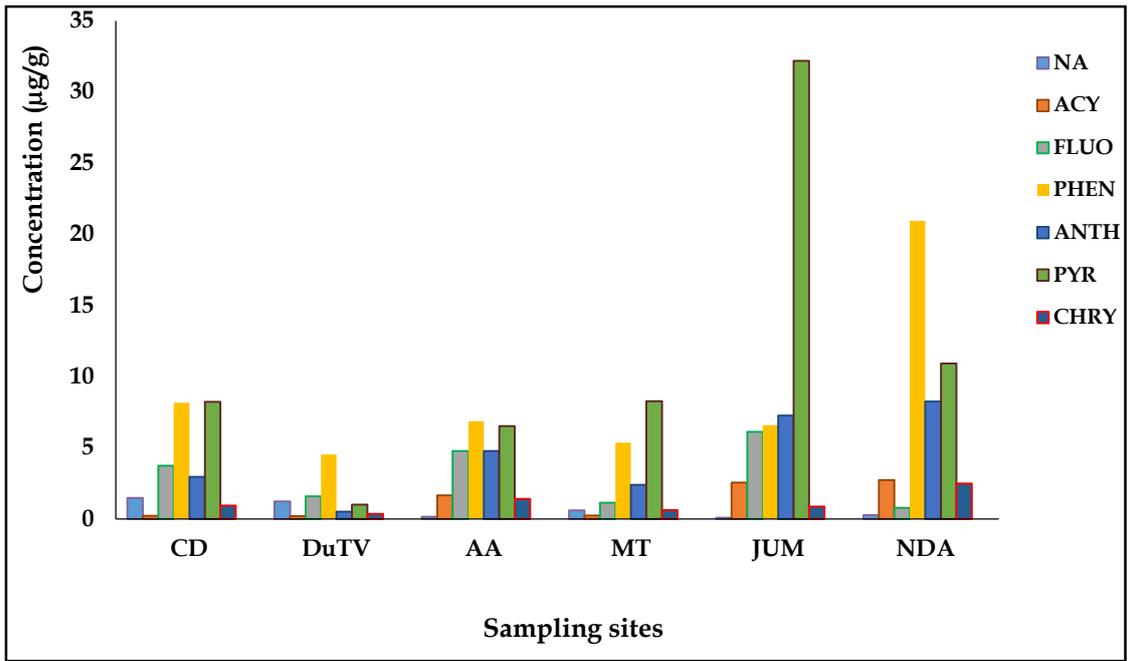
Figure 4.14 *Concentrations (µg/g±SD) of the 7 PAHs from the surface sediments of the Msunduzi River during the summer season.*

Table 4.15 Concentrations (µg/g±SD) of the 7 PAHs in the surface sediments at each site during the summer of 2014.

Sites	LMW-PAHs					HMW-PAHs		Σ7-PAHs
	NA	ACY	FLUO	PHEN	ANTH	PYR	CHRY	
HD	-	-	-	-	-	-	-	-
CD	1.48±0.02	0.23±0.50	3.74±0.32	8.17±1.88	2.96±0.88	8.22±3.69	0.93±0.47	25.70±7.76
DuTV	1.24±0.08	0.19±0.06	1.59±0.82	4.53±1.11	0.51±0.33	1.01±0.77	0.36±0.02	9.46±3.19
AA	0.16±0.09	1.67±0.49	4.76±2.37	6.86±0.39	4.77±1.96	6.53±2.61	1.42±0.80	26.20±8.71
MT	0.61±0.01	0.25±0.46	1.15±0.07	5.35±0.56	2.40±1.53	8.27±1.88	0.63±0.43	18.68±4.94
JUM	0.09±0.03	2.55±0.33	6.12±0.65	6.59±0.48	7.27±1.45	32.19±6.69	0.88±0.37	55.71±10.00
NDA	0.27±0.01	2.74±0.02	0.78±0.11	20.95±0.04	8.26±0.01	10.91±1.47	2.49±0.01	46.43±1.67

- No surface sediment samples were collected from the HD site during the summer season of 2014. This site was inaccessible.

In surface sediment, the wider range of summed concentrations of the 7 PAHs was also observed during the spring season of 2015 (13.50-160.05 μg/g) followed by the autumn season (1.09-146.16 μg/g) and the summer season (9.46-55.71 μg/g) while the lower one was observed during the winter season (3.73-13.50 μg/g) of 2014.

Autumn and spring seasons are normally characterized by mild weather and a medium precipitation. All these factors allow these seasons to have high concentrations of organic pollutants due to wet deposition and accumulation processes. In addition, the surface sediment showed to have the higher range of the sum of concentrations of PAHs than in the soils, which confirms the strong affinity of PAHs to organic matter in sediments, which is their main sink than in the soils.

A comparison of the global contamination of PAHs in sediments found in the literature with the results of this study has been made. It showed that our results were lower than those found in the surface sediments collected from the Santander Bay in Northern Spain (0.02-344.60 μg/g) (Viguri, Verde & Irabien, 2002) and from the Tianjin River in China (0.79-194.30 μg/g PAHs) for 16 PAHs (Shi et al., 2005). They were higher than that observed in the surface sediments collected from the Meiliang Bay (Taihu Lake) in China (1.20-4.75 μg/g) for 16 PAHs (Qiao et al., 2006).

Few studies on the presence of PAHs have been done on the surface sediments in South Africa *(Das et al., 2008; Nekhavhambe et al., 2009; Nieuwoudt et al., 2011; Sibiya et al., 2013)*.

PAHs in the surface sediments collected from Limpopo and Cape Provinces have been determined. The sum of the concentrations of 6 PAHs studied ranged between 0.11 and 344 μg/g (Nekhavhambe et al., 2009*)* and the sum of the concentrations of 23 PAHs was 369 μg/g (Das et al., 2008*)* , which are higher than the results of this study.

PAHs in the surface sediments sampled from central South Africa (residential, agricultural and industrial areas), and the Vaal Triangle River in Gauteng Province were determined and found to be in the range of 0.044 and 39 μg/g dry weight (Nieuwoudt et al, 2011), which are lower than the results of this study.

The concentration levels of PAHs in the KwaZulu-Natal surface sediments have been found to be in the range of 0.006 and 3.24 µg/g dry weight (Vogt, 2014), which were low compared to the results of the present study.

4.3 Seasonal Variations of PAH Concentration and Physical Parameters of the Water

The seasonal behaviour on the origin of the PAH emissions is closely associated with rainfall, climate, temperature, wind velocity, automobile trafficking, heating systems, soil volatilization, industrial waste, and natural and anthropogenic activities. The distribution of the sum of the concentration levels ($\sum$[7-PAHs]) in the water, soils and the surface sediments of the Msunduzi River during the four seasons were shown in the above tables (Chapter IV).

The range of the sum of the concentrations of the 7 PAHs in the water for all seasons decreased in the order: $\sum$[7-PAH] $_{spring}$ > $\sum$[7-PAH] $_{summer}$ > $\sum$[7-PAH] $_{autumn}$ > $\sum$[7-PAH] $_{winter}$ while in the surface sediments was in the order: $\sum$[7-PAH] $_{spring}$ > $\sum$[7-PAH] $_{autumn}$ > $\sum$ [7-PAH] $_{summer}$ > $\sum$[7-PAH] $_{winter}$ and in the soils were in the order: $\sum$[7-PAH] $_{spring}$ > $\sum$[7-PAH] $_{autumn}$ > $\sum$[7-PAH] $_{winter}$ > $\sum$[7-PAH] $_{summer.}$ Normally, the seasonal variation of the concentrations of PAHs in soils and surface sediments should follow the same order. This variation in the results is acceptable and expected because of the many different sources identified in this study. The sum of the concentrations of PAHs in the surface sediments during the summer season was high but lower than in soils in winter. This anomaly may be due to the heavy rainfall occurring during the summer season, which can wash all pollutants such as PAHs from surface soils and accumulate them in the marine environment resulting in higher concentrations of PAHs in surface sediments than in soils. It may also be due to the higher loss of pollutants through evaporation and volatilization processes occurring in summer (higher temperatures) than in winter, resulting also in lower concentrations of contaminants in soils than in sediments.

The concentration of PAHs was found to be relatively higher in the soils and surface sediments than in the water samples. This is because soil and sediment contain more organic matter to which PAHs prefer to partition to, thus reducing their concentrations in the water column.

The occurrence of PAHs in the environment varies seasonally because each season has its own physical and chemical parameters. For example, during the rainy period in some countries, PAHs attach to the particulate materials in the atmosphere and are brought back down to the land environment, where they form dry or wet deposits in the environmental systems. This can make them more available in these compartments than in the atmosphere. When the temperature increases, PAHs can undergo different chemical reactions and volatilization from the aquatic system into the atmosphere. Thus, higher concentration levels may be found moving into the atmosphere with a high probability of polluting remote and pristine areas (Slaski et al., 2000).

In South Africa, both of these phenomena normally happen during the summer and spring seasons, where the temperature is high (Table 4.1), hence the significant concentration levels of PAHs in the atmosphere. By precipitation or wet deposition due to the availability of rain in these seasons, all the released PAHs are brought back down, thus increasing the occurrence of these pollutants in the water, soils, and surface sediments.

The results of average pH shown in section 4.3.1 indicated that the water of the Msunduzi River during this work was in the range of drinking water, normally ranging from 6 to 9. The higher value of average pH observed during the winter season might be attributed to presence of carbonates in water, which can increase its alkalinity. The average temperature of water was ranging between 17 and 28 $^{\circ}$C, whilst the ambient temperature fluctuated between 21 and 32 $^{\circ}$C during all seasons. The average electrical conductivity in this study ranged between 281.98 and 422.77μS/cm in all seasons as shown in section 4.3.1. The electrical conductivity of water can be affected by salinity, pH, rainfall and temperature. It can also be affected by the geology of the region through which the river flows. For example, river that runs through areas

with granite bedrock tend to have lower conductivity, as granite comprises more inert materials that do not dissolve into ionic forms, when eroded into the water. Studies reported that the concentrations of different organic pollutants in water depend on its conductivity, which indicates the level of suspended matter and dissolved solids on which these pollutants like PAHs can easily adsorb (Furukawa & Wolanski, 1996).

4.4 Source Apportionment of PAHs in this Study

The major sources of PAHs in the environment can be classified as either petrogenic (petroleum) or pyrogenic (combustion). In general, petrogenic sources emit organic particulates characterized by a high mole fraction of LMW-PAHs (those of 2 or 3 fused benzene rings). Through calculations of source-apportioning concentration ratios, reliable pointers (> 80% probability) of the origins of anthropogenic emitters of PAHs in a given matrix can be made. The ratios frequently used to test concentration data of PAHs in the aquatic environment are the following: [Anth]/[Phen] or [Anth]/([Anth]+[Phen]) for LMW-PAHs or [Flua]/([Flua]+[Pyr]) for HMW-PAHs, respectively *(Neff, 1979; Budzinski et al., 1997; Soclo et al., 2000)*.

In this study, the concentration ratios of {[Anth]/([Anth]+[Phen]} *(Soclo et al., 2000; Dong et al., 2012)* was used to make apportionment of the origins of PAHs along the Msunduzi River basin. The results are summarized in Table 4.16.

Table 4.16 *Calculated concentration ratio {[Anth]/([Anth]+[Phen]} for sediments and probable source of PAHs in the Msunduzi River.*

Calculated concentration ratio	Sites	Autumn 2014	Winter 2014	Spring 2015	Summer 2014
ANTH/(ANTH+PHEN)	HD	-	-	-	-
	CD	0.15	0.20	0.02	0.25
	DuTV	-	0.60	0.70	0.10
	AA	-	0.30	0.80	0.41
	MT	0.39	0.31	0.45	0.30
	JUM	0.10	0.20	0.15	0.50
	NDA	-	0.10	0.85	0.30

Petrogenic source: ANTH/(ANTH+PHEN) < 0.40 and pyrogenic source: ANTH/(ANTH+PHEN) > 0.40

In this work, the main sources of PAHs were only identified from the surface sediment samples collected during the autumn, winter and summer seasons of 2014 and spring season of 2015. The surface sediments are normally considered as the main sink of PAHs. During the autumn season, all calculated values were < 0.40, implying petrogenic process. During the winter season, only one calculated value was > 0.40, implying pyrogenic source while the spring season of 2015 showed higher percentage of pyrogenic sources. During the summer season, only two ratios in six calculated were > 0.40, these also implied pyrogenic inputs. By counting the calculated ratios in all seasons, 70.0% showed that the studied PAHs during this study were mostly coming from petrogenic sources.

Normally, pyrolytic or pyrogenic PAHs (pyrene and chrysene in this study) generally comprise more fused benzene rings than petrogenic PAHs. The most common sources of pyrogenic PAHs are the forest fires, incomplete combustion of the organic matter, coal, tobacco smoke and industrial waste. Petrogenic PAHs are mostly formed during the release of the engine oils, use of fuels, kerosene, crude and the refined petroleum (Adrian et al., 1999). So, as shown by the above results from Table 4.16, a large amount of the PAH pollution in this study is coming from petrogenic activities as the five in seven studied PAHs were LMW-PAHs.

PAHs can be found in the environmental system due to different mechanisms like post-depositional early diagenesis of biogenic precursors like perylene, higher temperature pyrogenic processes or incomplete combustion of vegetation and fossil fuels, lower temperature diagenesis or petrogenesis for long periods of geologic time (oil products) (Meyers & Ishiwatari, 1993). Higher molecular weight PAHs are produced by the pyrogenic process, whereas lower molecular PAHs result from petrogenic processes (Fernández et al., 2002). The distribution of PAHs in the water, soil, and sediment for autumn, winter, spring and summer seasons, 2014-2015 of the Msunduzi River are shown in the above tables. The PAH concentration levels were relatively high in the soil samples collected from the agricultural area for autumn, winter and summer seasons while in the spring season were higher in the samples collected from the Du Toit Viljoen Bridge sampling site.

In the surface sediment samples, higher concentration levels of PAHs were found in the samples collected from the agricultural area for autumn, winter and spring seasons while in the summer were found in the samples collected from the Junction of the Msunduzi and Umgeni Rivers and the Nagle Dam sites, respectively.

In this study, the ranges of the summed concentrations of the 7 PAHs are high in the soils, water, and surface sediments during the spring season. The lower molecular weight PAHs are predominant in soils during the autumn season. The predominance of low and medium molecular weight PAHs in the surface sediments, soils, and water of this study area reflects the presence of significant combustion products from low-temperature pyrolytic processes and/or petrogenic sources.

Table 4.17 shows the maximum concentrations of PAHs in soils allowed by the Catalan legislation depending on the soil uses (Busquet, 1997). The maximum PAH concentrations allowed by the Canadian legislation are also shown (CCME, 2001). Almost all the concentrations of PAHs in soils from this study are below these limits, except that of PHEN (197.10 µg/g) at DuTV during the spring season.

Table 4.17 Maximum concentrations (ng/g) of PAHs in soils allowed by the Catalan and Canadian legislations depending on the soil uses

PAHs	Catalonia		Canada			
	Non-industrial	Industrial	Agriculture	Residential	Commercial	Industrial
NA	5000	1500	100	600	22000	22000
ACY	-	-	-	-	-	-
FLUO	-	-	-	-	-	-
PHEN	5000	10000	100	5000	50000	50000
ANTH	100000	1300000	-	-	-	-
PYR	-	100	10000	100000	100000	100000
CHRY	-	-	-	-	-	-

(Cfr Nadal, Schumacher & Domingo, 2004).

Chapter V

Conclusion and recommendations

5.1 Conclusion

This study investigated the seasonal distribution of seven polycyclic aromatic hydrocarbons in the water, soil and the surface sediment samples. The samples were collected from the Henley Dam, Camp's Drift, Du Toit Viljoen Bridge, Darvill wastewater treatment plant Inlet and Outlet after chlorination, Agricultural area, Msunduzi town, the junction of the Msunduzi and Umgeni Rivers and the Nagle Dam (as a comparative point) along the Msunduzi River.

The liquid-liquid and the soxhlet extraction techniques and the GC-MS method for the determination of these pollutants using standard addition provided comparatively good recoveries (Table 4.2).

Some PAHs were not detected in water or wastewater samples due to their low aqueous solubility as shown in the above tables of results as shown in Chapter IV. In this study, a wide range of the sum of the concentrations of the 7 PAHs (LMW+HMW) was observed in the water, soil, and the surface sediments analyzed during the spring season.

The higher sum of the concentrations of PAHs in soils and surface sediments was found at the agricultural area (AA) in almost all seasons. The reason could be that, this site is the first point to receive all treated wastewater discharged from the DWWTP effluent (low water flow rate of the Msunduzi River in this area) and its soils and sediments may be contaminated with PAHs. This could be due to the accumulation of fine particles and long-term irrigation. Other possible sources of pollution in this area were suspected to be the incomplete combustion of forest areas, grass and other organic materials before cultivation. Water samples from DuTV (autumn of 2014) and JUM (spring of 2015), wastewater from the DWWTP Outlet (winter of 2014) and

DWWTP Inlet (summer of 2014), soils from CD and DuTV (spring of 2015) also presented a higher sums of the concentrations of the studied PAHs in this work. Pyrene (PYR), phenanthrene (PHEN) and chrysene (CHRY) were the dominant individual PAHs in almost all seasons.

Suggested possible sources of PAHs during this study were incomplete combustion process of organic compounds, the exhaust from the motor vehicles, deposition from sewages, oil or gasoline spills, urban runoff, atmospheric fallouts, wood and coal-burning fires, use of creosote, kerosene, gasoline and petroleum products.

By using PAH ratios, the calculated values in all seasons showed that about 70% of the studied PAHs originate from petrogenic sources.

DWWTP showed a high sum of the concentrations of PAHs in wastewater during the autumn and winter seasons due to the fact that this treatment plant was under rehabilitation and extension as well as the lack of rain during these seasons.

According to the water quality information from the World Health Organization (WHO), the concentration levels of PAHs in the water should normally fluctuate between 1000 and 11000 ng/L *(Lewis, 2012)*. The results obtained in the water during all seasons are low compared to this range. Almost all the current concentrations of PAHs in soils along the Msunduzi River are below the Catalan and Candadian allowable limits (see table 4.17), except that of PHEN (197.10 µg/g) found at the DuTV Bridge sampling site during the spring season.

In general, agricultural soils and surface sediments, especially those collected from the sites located around and next to the urban district (Pietermaritzburg city) are the most contaminated sites with PAHs.

5.2 Recommendations

✓ This research was conducted on 7 PAHs selected from 16 PAHs classified by US EPA as priority pollutants to be monitored in different environmental

matrices. Three of them contain 2 benzene rings (naphthalene, acenaphthylene, and fluorene), two contain 3 benzene rings (anthracene ant phenanthrene) and two contain 4 benzene rings (pyrene and chrysene) and no PAH-containing 5 or 6 benzene rings were analyzed. It is recommended that the 5 and 6 rings be analyzed to obtain a full study of PAHs on the Msunduzi River in order to easily compare its pollution level with other rivers in the country and around the world.

✓ A study on benzo[a]pyrene is recommended, which is the only PAH with sufficient toxicological data, in order to estimate the carcinogenicity potency factor of other PAHs in this work.

✓ Continuous assessment should be conducted in order to investigate the long-term effects of PAHs, to determine if their concentration levels remain the same as they are known to be persistent organic pollutants.

✓ Studies on photo-oxidation at the surface and microbial enzymatic production at the bottom of the river, on PAHs, would assist in determining the fate of PAHs in the environment.

✓ More studies need to be conducted on aquatic organisms such as fish, since levels and the presence of organic pollutants in fish have a direct effect on human health. The concentrations of POPs in fish also present the bioavailable fraction in the water bodies. Since PAHs can undergo bioconcentration and biomagnification processes, comprehensive risk monitoring can only be performed by assessing their concentration levels in common freshwater fish of this region.

✓ Bioremediation methods such as phytoremediation, biodegradation, bio-stimulation, land farming and phytoextraction need to be implemented in

order to reduce the concentration levels of these PAH pollutants in this region (Msunduzi River).

- ✓ Studies need to be done on the sediment-pore water as its interaction is one of the most dominant processes controlling the distribution and behavior of PAHs in any river.

- ✓ Continual studies need to be conducted on the water, soil and the surface sediment samples from other rivers flowing through the KwaZulu-Natal in order to obtain a full study of PAHs in the whole province.

Bibliography

Adrian, D. C., Bollag J. M., Frankenberger, W. T. Jr. & Sims, R. C. (1999): Bioremediation of contaminated soils. *American Society of Agronomy, Madison, USA, No. 37*, 67-70.

Agarwal, T., Khillare, P. S., Shridhar, V. & Ray, S. (2009). Pattern, sources and toxic potential of PAHs in the agricultural soils of Delhi, India. *Journal of Hazardous Materials*, Volume *163* (2), 1033-1039.

Aguilar, C., Penalver, S., Pocurull, E., Borrul, F. & Marce, R. M. (1998). Solid-phase microextraction and gas chromatography with mass spectrometry detection for the determination of pesticides in aqueous samples. *Journal of Chromatography A.*, 795, 105-115.

Agunbiade, F. O. & Moodley, B. (2014). Pharmaceuticals as emerging organic contaminants in the Umgeni River water system, KwaZulu-Natal, South Africa. *Environmental Monitoring and Assessment, 186* (11), 7273-7291.

Ahangar, A. G. (2010). Sorption of PAHs in the soil environment with emphasis on the role of soil organic matter: A review. *World Applied Sciences Journal, 11* (7), 759-765.

Albers, P. H. (1995). Petroleum and individual polycyclic aromatic hydrocarbons. *Handbook of ecotoxicology*, *2*.

Albers, P. H. (2002). Sources, fate, and effects of PAHs in shallow water environments: A review with special reference to small watercraft. In: M. J. Kennish, editor. Impacts of motorized watercraft on shallow estuarine and coastal marine environments. *Journal of Coastal Research (special issue)*, 37:143–150.

Allen, J. S. (1947). An improved electron multiplier particle counter. *Review of Scientific Instruments*, *18* (10), 739-749.

Alloway, B. & Ayres, D. C. (1997). Chemical principles of environmental pollution: CRC Press.

Al-Rashdan, A., Helaleh, M. I., Nisar, A., Ibtisam, A. & Al-Ballam, Z. (2010). Determination of the levels of polycyclic aromatic hydrocarbons in the toasted bread using gas chromatography-mass spectrometry. *International Journal of Analytical Chemistry. Hindawi Publishing Corporation, Volume 2010, 1-8.*

Altman, M., Van der Heijden, T., Mayer, M. & Lewis, G. (2002). Employment-oriented Industry Studies.

Arias, A. H., Spetter, C. V., Freije, R. H. & Marcovecchio, J. E. (2009). Polycyclic aromatic hydrocarbons in water, mussels (*Brachidontes sp., Tagelus sp.*) and fish (*Odontesthes sp.*) from Bahía Blanca Estuary, Argentina. *Estuarine, Coastal and Shelf Science*, *85* (1), 67-81.

Armarego, W. L. & Chai, C. L. (2003). Purification of laboratory chemicals. Butterworth–Heinemann, London.

Azhari, A. (2012). Polycyclic aromatic hydrocarbons (PAHs) in air and vegetation: A case study of three selected toll stations along north, south expressway in Johor, Malaysia (Doctoral dissertation, Universiti Tun Hussein Onn Malaysia).

Baek, S. O., Goldstone, M. E., Kirk, P. W. W., Lester, J. N. & Perry, R. (1992). Concentrations of particulate and gaseous polycyclic aromatic hydrocarbons in London air following a reduction in the lead content of petrol in the United Kingdom. *Science of the Total Environment, 111* (2), 169-199.

Bailon, P., Ehrlich, G.K., Fung W. J. & Berthold, W. (2000). An Overview of Affinity Chromatography, Humana Press.

Banjoo, D. R. & Nelson, P. K. (2005). Improved ultrasonic extraction procedure for the determination of polycyclic aromatic hydrocarbons in sediments. *Journal of Chromatography A, 1066* (1), 9-18.

Barnard, J. L. (1976). A review of biological phosphorus removal in the activated sludge process. *Water South Africa*, *2* (3), 136-144.

Batterman, S., Chernyak, S., Gouden, Y., Hayes, J., Robins, T. & Chetty, S. (2009). PCBs in air, soil and milk in industrialized and urban areas of KwaZulu-Natal, South Africa. *Environmental Pollution, 157* (2), 654-663.

Baumard, P., Budzinski, H., Michon, Q., Garrigues, P., Burgeot, T. & Bellocq, J. (1998). Origin and bioavailability of PAHs in the Mediterranean Sea from mussel and sediment records. *Estuarine, Coastal and Shelf Science*, *47* (1), 77-90.

Bendicho, C., De La Calle, I., Pena, F., Costas, M., Cabaleiro, N. & Lavilla, I. (2012). Ultrasound-assisted pre-treatment of solid samples in the context of green analytical chemistry. *Trends in Analytical Chemistry*, *31*, 50-60.

Bente, M., Sklorz, M., Streibel, T. & Zimmermann, R. (2009). Thermal Desorption−Multiphoton Ionization Time-of-Flight Mass Spectrometry of Individual Aerosol Particles: A Simplified Approach for Online Single-Particle Analysis of Polycyclic Aromatic Hydrocarbons and Their Derivatives. *Analytical Chemistry, 81* (7), 2525-2536.

Bercaru, O., Ulberth, F., Emons, H. & Vandecasteele, C. (2006). Accurate quantification of PAHs in the water in the presence of dissolved humic acids using isotope dilution mass spectrometry. *Analytical Bioanalytical Chemistry,* 384:1207–1213

Berset, J. D., Ejem, M., Holzer, R. & Lischer, P. (1999). Comparison of different drying, extraction and detection techniques for the determination of priority polycyclic aromatic hydrocarbons in background contaminated soil samples. *Analytica Chimica Acta, 383* (3), 263-275.

Bevis, M., Businger, S., Herring, T. A., Rocken, C., Anthes, R. A. & Ware, R. H. (1992). GPS meteorology: Remote sensing of atmospheric water vapor using the Global Positioning System. *Journal of Geophysical Research: Atmospheres (1984–2012), 97* (D14), 15787-15801.

Blanco, E., Mahía, P., Lorenzo, S., Rodríguez, D. & Fernández, E. (2000). Optimization of microwave-assisted extraction in the marine sediments: comparison with Soxhlet extraction method. *Journal of Analytical Chemistry, 366,* 283-288.

Bocca, B., Di Mattia, M., Cartoni, C., Fiori, M., Felli, M., Neri, B. & Brambilla, G. (2003). Extraction, cleanup and gas chromatography–mass spectrometry characterization of zilpaterol as a feed additive in fattening cattle. *Journal of Chromatography B*, *783* (1), 141-149.

Bodzek, D., Janoszka, B., Dobosz, C., Warzecha, L. & Bodzek, M. (1997). Determination of polycyclic aromatic compounds and heavy metals in sludge from biological sewage treatment plants. *Journal of Chromatography A*, *774* (1), 177-192.

Boxall, A., Rudd, M. A., Brooks, B. W., Caldwell, D. J., Choi, K., Hickmann, S. & Van Der Kraak, G. (2012). Pharmaceuticals and personal care products in the environment: what are the big questions? *Environmental Health Perspectives, 120* (9), 1221-1229.

Bruckner, G., Vosloo, W., Plessis, B., Kloeck, P., Connoway, L., Ekron, M. & Marais, T. (2002). Foot and mouth disease: the experience of South Africa. *Revue Scientifique et Technique-Office International des Épizooties, 21* (3), 751-761.

Budzinski, Jones, I., Bellocq, J., Piérard, C. & Garrigues, P. (1997). Evaluation of sediment contamination by polycyclic aromatic hydrocarbons in the Gironde estuary. *Marine Chemistry, 58*, 85–97.

Buha, A. (2011). Polycyclic aromatic hydrocarbons. [Online]. Available from: *http://www.toxipedia.org/display/toxipedia/Polycyclic+Aromatic+Hydrocarbons.* [Accessed on August 6th 2015].

Burgess, R. M., Ahrens, M. J. & Hickey, C. W. (2003). Aquatic Environments: Source, Persistence and Distribution. *PAHs: An Ecotoxicological Perspective, 35-45.*

Busquet, E. (1997). Elaboració dels criteris de qualitat del sòl a Catalunya. *Generalitat de Catalunya, Departament de Medi Ambient, Junta de Residus, Barcelona, Spain.*

Camel, V. (2001). Recent extraction techniques for solid matrices using supercritical fluid extraction, pressurized fluid extraction and microwave-assisted extraction: their potential and pitfalls. *Analyst, 126* (7), 1182-1193.

Carro, N., Garcia, I., Ignacio, M. & Mouteira, A. (2012). Optimization of Soxtec Extraction for the Determination of Polychlorinated Biphenyls (PCBs) in Mussel and Comparison with Soxhlet Extraction, Accelerated Solvent Extraction, and Microwave Assisted Extraction. *Analytical Letters, 45* (15), 2161-2175.

CCME. (2001). CCME (Canadian Council of Ministers of the Environment) Canadian soil quality guidelines for the protection of environmental and human health *http://www.ccme.ca/assets/pdf/e8_003.pdf* (2001). [Available online]. Accessed 27th March, 2016.

CDC, U. ATSDR (1995). Toxicological profile for polycyclic aromatic hydrocarbons.

Cele, I. S. (2010). Determination of inorganic and poly-nuclear aromatic hydrocarbon contents in South African sewage sludge. MSc Thesis, School of Chemistry, University of KwaZulu-Natal, South Africa.

Cerniglia. C. E. (1984). Microbial metabolism of polycyclic aromatic hydrocarbons. Advanced in Applied Microbiology. Volume 30, Academic Press.

Cerniglia. C. E. (1993). Biodegradation of polycyclic aromatic hydrocarbons. Microorganisms to Combat Pollution, Springer Netherlands, 227-244.

Chee, K. K., Wong, M. K. & Lee, H. K. (1996). Optimization of microwave-assisted solvent extraction of polycyclic aromatic hydrocarbons in marine sediments using a microwave extraction system with high-performance liquid chromatography-fluorescence detection and gas chromatography-mass spectrometry. *Journal of Chromatography A, 723* (2), 259-271.

Chen, B., Xuan, X., Zhu, L., Wang, J., Gao, Y., Yang, K. & Lou, B. (2004). Distributions of polycyclic aromatic hydrocarbons in the surface waters, sediments and soils of Hangzhou City, China. *Water Research, 38* (16), 3558-3568.

Chen, C. W., Chen, C. F., Dong, C. D. & Tu, Y. T. (2012). Composition and source apportionment of PAHs in sediments at river mouths and channel in Kaohsiung Harbor, Taiwan. *Journal of Environmental Monitoring, 14* (1), 105-115.

Chhabra, P. S., Lambe, A. T., Canagaratna, M. R., Stark, H., Jayne, J. T., Onasch, T. B. & Worsnop, D. R. (2015). Application of high-resolution time-of-flight chemical ionization mass spectrometry measurements to estimate volatility distributions of α-pinene and naphthalene oxidation products. *Atmospheric Measurement Techniques*, *8* (1), 1-18.

Chung, M. K., Hu, R., Cheung, K. C. & Wong, M. H. (2007). Pollutants in Hong Kong soils: polycyclic aromatic hydrocarbons. *Chemosphere*, *67* (3), 464-473.

Clément, B., Cauzzi, N., Godde, M., Crozet, K. & Chevron, N. (2005). Pyrene toxicity to aquatic pelagic and benthic organisms in single-species and microcosm tests. *Polycyclic Aromatic Compounds, 25* (3), 271-298.

Clements, W. H. (1992). Bioaccumulation and Food Chain Transfer of Polycyclic Aromatic Hydrocarbons and Heavy Metals: A Laboratory and Field Investigation: DTIC Document.

Commission of Water Resources (WRC). (2002). State of Rivers Report: Umgeni River and neighboring rivers and streams. WRC Report No. TT, 200 (02).

Das, S. K., Routh, J. & Roychoudhury, A. N. (2008). Sources and historic changes in polycyclic aromatic hydrocarbon input in a shallow lake, Zeekoevlei, South Africa. *Organic Geochemistry*, *39* (8), 1109-1112.

de Hoffmann, Edmond; Vincent Stroobant (2003). Mass Spectrometry: Principles and Applications (Second Ed.). Toronto: John Wiley & Sons.

Dean, J. R., Barnabas, I. J. & Fowlis, I. A. (1995). Extraction of polycyclic aromatic hydrocarbons from highly contaminated soils: a comparison between Soxhlet, microwave and supercritical fluid extraction techniques. Royal Society of Chemistry. In *Analytical Proceedings Including Analytical Communications*, 32 (8), 305-308.

de Saint Laumer, J. Y., Leocata, S., Tissot, E., Baroux, L., Kampf, D. M., Merle, P. & Chaintreau, A. (2015). Prediction of response factors for gas chromatography with flame ionization detection: Algorithm improvement, extension to silylated

compounds, and application to the quantification of metabolites. *Journal of Separation Science*, *38* (18), 3209-3217.

Domínguez, C., Sarkar, S. K., Bhattacharya, A., Chatterjee, M., Bhattacharya, B. D., Jover, E. & Satpathy, K. K. (2010). Quantification and source identification of polycyclic aromatic hydrocarbons in core sediments from Sundarban Mangrove Wetland, India. A*rchives of Environmental Contamination and Toxicology, 59* (1), 49-61.

Dong, C. -D., Chen, C. F. & Chen, C. -W. (2012). Determination of polycyclic aromatic hydrocarbons in industrial harbor sediments by GC-MS. *International Journal of Environmental Research and Public Health, 9* (6), 2175-2188.

Douglas, G. S., Bence, A. E., Prince, R. C., McMillen, S. J. & Butler, E. L. (1996). Environmental stability of selected petroleum hydrocarbon source and weathering ratios. *Environmental Science & Technology, 30* (7), 2332-2339.

DRAFT, C. & HSRC, P. O. (2010). Know your HIV prevention, response (KYR) analysis report for component 3: KwaZulu-Natal Provincial Report.

DUCT (Duzi-Umgeni Conservation Trust). (2006). The DUCT news. [Online]. Available from: *http://www.duct.org.za/.* [Accessed on 30th September 2015].

Dzyuba, S. V. & Bartsch, R. A. (2003). Recent Advances in Applications of Room-Temperature Ionic Liquid/Supercritical CO_2 Systems. *Angewandte Chemie International Edition, 42* (2), 148-150.

El-Nemr, A., Said, T. O., Khaled, A., El-Sikaily, A. & Abd-Allah, A. M. (2007). The distribution and sources of polycyclic aromatic hydrocarbons in surface sediments along the Egyptian Mediterranean coast. *Environmental Monitoring and Assessment, 124* (1-3), 343-359.

Essays, UK. (2013). Threat of Water Pollution in the Msunduzi River. Environmental Sciences Essay. Retrieved from: *http://www.ukessays.com/essays/environmentalsciences/threat-of-water-pollution-on-*

Msunduzi-River-environmental-sciences-essay.php?cref=1. [Accessed on 30th September 2015].

Evenset, A., Christensen, G. N., Carroll, J., Zaborska, A., Berger, U., Herzke, D. & Gregor, D. (2007). Historical trends in persistent organic pollutants and metals recorded in sediment from Lake Ellasjøen, Bjørnøya, and Norwegian Arctic. *Environmental Pollution, 146* (1), 196-205.

Farmer, J. G. (1991). The perturbation of historical pollution records in aquatic sediments. *Environmental Geochemistry and Health, 13* (2), 76-83.

Fernández, P., Vilanova, R. M., Martínez, C., Appleby, P. & Grimalt, J. O. (2000). The historical record of atmospheric pyrolytic pollution over Europe registered in the sedimentary PAH from remote mountain lakes. *Environmental Science & Technology, 34* (10), 1906-1913.

Filser, J. G., Csanady, G. A., Dietz, W., Kessler, W., Kreuzer, P. E., Richter, M. & Stömer, A. (1996). Comparative estimation of the neurotoxic risks of n-hexane and n-heptane in rats and humans based on the formation of the metabolites 2, 5-hexanedione and 2, 5-heptanedione. In *Biological Reactive Intermediates V*. Springer United States, 411-427.

Fountain, S. T, Lee, H. & Lubman, D. M. (1994). Ion fragmentation activated by matrix-assisted laser desorption/ionization in an ion-trap/reflectron time-of-flight device. *Rapid Communications in Mass Spectrometry, 8* (5): 407–16.

Furukawa, K. & Wolanski, E. (1996). Sedimentation in mangrove forests. *Mangroves and salt marshes*, Kluwer Academic Publishers, *1* (1), 3-10.

Gakuba, E., Moodley, B., Ndungu, P. & Birungi, G. (2015). Occurrence and significance of polychlorinated biphenyls in water, sediment pore water and surface sediments of Umgeni River, KwaZulu-Natal, South Africa. *Environmental Monitoring and Assessment, 187* (9), 1-14.

Gan, S. & Ng, H. K. (2012). Current status and prospects of Fenton oxidation for the decontamination of persistent organic pollutants (POPs) in soils. *Chemical Engineering Journal*, *213*, 295-317.

Gaspari, L., Chang, S. S., Santella, R. M., Garte, S., Pedotti, P. & Taioli, E. (2003). Polycyclic aromatic hydrocarbon-DNA adducts in human sperm as a marker of DNA damage and infertility. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 535* (2), 155-16.

Gemmell, M. E. & Schmidt, S. (2013). Is the microbiological quality of the Msunduzi River (KwaZulu-Natal, South Africa) suitable for domestic, recreational, and agricultural purposes? *Environmental Science and Pollution Research, 20* (9), 6551-6562.

Gerlach, R. W., Dobb, D. E., Raab, G. A. & Nocerino, J. M. (2002). Gy sampling theory in environmental studies. 1. Assessing soil splitting protocols. *Journal of Chemometrics*, *16* (7), 321-328.

Girard, J. E. (2013). Principles of Environmental Chemistry: Jones & Bartlett Publishers.

Goates, S. R., Zabriskie, N. A., Simons, J. K. & Khoobehi, B. (1987). Detection of aerosol formation in the effluent of a supercritical fluid chromatography. *Analytical Chemistry*, *59* (24), 2927-2930.

Grobler, D. F., Badenhorst, J. E. & Kempster, P. L. (1996). PCBs, chlorinated hydrocarbon pesticides and chlorophenols in the Isipingo Estuary, Natal, Republic of South Africa. *Marine Pollution Bulletin, 32* (7), 572-575.

Grova, N., Feidt, C., Laurent, C. & Rychen, G. (2002). [14C] Milk, urine and feces excretion kinetics in lactating goats after an oral administration of [14C] polycyclic aromatic hydrocarbons. *International Dairy Journal, 12* (12), 1025-1031.

Guerin, T. (1999). The extraction of aged polycyclic aromatic hydrocarbon (PAH) residues from a clay soil using sonication and a Soxhlet procedure: a comparative study. *Journal of Environmental Monitoring*, *1* (1), 63-67.

Guillén, M. D. & Errecalde, M. C. (2002). Volatile components of raw and smoked black bream (*Brama raii*) and rainbow trout (*Oncorhynchus mykiss*) studied by means of solid phase microextraction and gas chromatography/mass spectrometry. *Journal of the Science of Food and Agriculture, 82* (9), 945-952.

Guo, W., He, M., Yang, Z., Lin, C., Quan, X. & Wang, H. (2007). Distribution of polycyclic aromatic hydrocarbons in water, suspended particulate matter and sediment from Daliao River watershed, China. *Chemosphere, 68* (1), 93-104.

Guo, W., He, M., Yang, Z., Lin, C., Quan, X. & Men, B. (2009) Distribution, partitioning and sources of polycyclic aromatic hydrocarbons in the Daliao river water system in dry season, China. *Journal of Hazardous Materials, 164,* 1379-1385.

Gupta, S. K. & Sharma, R. (1996). Biological oxidation of high strength nitrogenous wastewater. *Water Research*, *30* (3), 593-600.

Gurjar, B. R., Molina, L. T. & Ojha, C. S. P. (2010). Air pollution: Health and Environmental Impacts. CRC Press.

Habaki, H., Hu, H. & Egashira, R. (2015). Liquid-liquid equilibrium extraction of ethanol with mixed solvent for bioethanol concentration. *Chinese Journal of Chemical Engineering*, 1-22.

Harvey, R. G. (1982). Polycyclic Hydrocarbons and Cancer: Certain common environmental pollutants are converted by mammalian cells into metabolites that can induce cancer through their reactions with cellular nucleic acids. *American Scientist, 4* (70), 386-393.

Harvey, R. G. (1991). Polycyclic aromatic hydrocarbons: Chemistry and carcinogenicity: CUP Archive.

Harvey, R. G. (1998). The Handbook of Environmental Chemistry of PAHs. *In PAHs and related compounds: Chemistry.* Springer Berlin, Heidelberg, 1-54.

Harvey, R. G. (2011). Historical overview of chemical carcinogenesis. *In Chemical Carcinogenesis*. Humana Press, 1-26.

He, J. & Balasubramanian, R. (2010). Semi-volatile organic compounds (SVOCs) in ambient air and rainwater in a tropical environment: concentrations and temporal and seasonal trends. *Chemosphere*, *78* (6), 742-751.

Heroux, L. & Hinteregger, H. E. (1960). Resistance strip magnetic photomultiplier for the extreme ultraviolet. *Review of Scientific Instruments*, *31* (3), 280-286.

Hübschmann, H. J. (2015). Handbook of GC-MS: Fundamentals and Applications. John Wiley & Sons.

Hundal, L. S., Thompson, M. L., Laird, D. A. & Carmo, A. M. (2001). Sorption of phenanthrene by reference smectites. *Environmental Science & Technology*, *35* (17), 3456-3461.

Hutchinson, G. E. (1978). A treatise on limnology. Wiley.

Jazestani, J. (2011). Bioavailability and Biodegradation of Organic Xenobiotic Recalcitrant Polycyclic Aromatic Hydrocarbons (PAHs) in Different Soil Environments. McGill University.

Jenkins, R. A., Tomkins, B. & Guerin, M. R. (2000). The chemistry of environmental tobacco smoke: Composition and Measurement: CRC Press.

Jensen, W. B. (2007). The origin of the Soxhlet extractor. *Journal of Chemical Education, 84* (12), 1913.

Jerina, D. M., Sayer, J. M., Thakker, D. R., Yagi, H., Levin, W., Wood, A. W. & Conney, A. H. (1980). Carcinogenicity of polycyclic aromatic hydrocarbons: the bay-region theory. In Carcinogenesis: Fundamental Mechanisms and Environmental Effects. Springer Netherlands, 1-12.

Jiang, Y. F., Wang, X. T., Wang, F., Jia, Y., Wu, M. H., Sheng, G. Y. & Fu, J. M. (2009). Levels, composition profiles and sources of polycyclic aromatic hydrocarbons in urban soil of Shanghai, China. *Chemosphere*, *75* (8), 1112-1118.

JiJi, R. D., Cooper, G. A. & Booksh, K. S. (1999). Excitation-emission matrix fluorescence-based determination of carbamate pesticides and polycyclic aromatic hydrocarbons. *Analytica Chimica Acta*, *397* (1), 61-72.

Johnson, L. L., Collier, T. K. & Stein, J. E. (2002). An analysis in support of sediment quality thresholds for polycyclic aromatic hydrocarbons (PAHs) to protect estuarine fish. Aquatic Conservation: *Marine and Freshwater Ecosystems, 12* (5), 517-538.

Juhani, K., Kettunen, J., Hartonen, K. & Lissa, R. (2004). Pressurised hot water extraction of n-Alkanes and PAHs in soil and sediment from the oil shale industry district in Estonia. *Journal of Soils Sediments, Iran*. 1-8.

Kanchanamayoon, W. & Tatrahun, N. (2008). Determination of polycyclic aromatic hydrocarbons in water samples by solid-phase extraction and gas chromatography. *World Journal of Chemistry*, *3* (2), 51-54.

Kaplinsky, R. & Morris, M. (1999). Trade policy reform and the competitive response in KwaZulu-Natal Province, South Africa. *World Development, 27* (4), 717-737.

Kawamura, K., Suzuki, I., Fuji, Y. & Watanabe, O. (1994). Ice core record of polycyclic aromatic hydrocarbons over the past 400 years. *Naturwissenschaften, 81* (11), 502-505.

Keyte, I. J., Harrison, R. M. & Lammel, G. (2013). Chemical reactivity and long-range transport potential of polycyclic aromatic hydrocarbons–a review. *Chemical Society Reviews*, *42* (24), 9333-9391.

Khan, Z., Troquet, J. & Vachelard, C. (2005). Sample preparation and analytical techniques for determination of polyaromatic hydrocarbons in soils. *International Journal of Environmental Science & Technology*, *2* (3), 275-286.

Kishi, N., Hara, H., Watanabe, T. & Suzuki, K. (2015). *U.S. Patent No. 8,939,012*. Washington, DC: U.S. Patent and Trademark Office.

Klimaszewska, K., Pol*kow*ska, Z. & Namiesnik, J. (2007). Influence of mobile sources on pollution of runoff waters from roads with high traffic intensity. *Polish Journal of Environmental Studies*, *16* (6), 889.

Koltsakidou, A., Zacharis, C. K. & Fytianos, K. (2015). A validated liquid chromatographic method for the determination of polycyclic aromatic hydrocarbons in honey after homogeneous liquid–liquid extraction using hydrophilic acetonitrile and sodium chloride as mass separating agent. *Journal of Chromatography A*, *1377*, 46-54.

Kwazulu-Natal Top Business. (2015). [Online]. Available from: *http://kzntopbusiness.co.za/site/manufacturing*. [Accessed on 24th November 2015].

Laflamme, R. E. & Hites, R. A. (1978). The global distribution of polycyclic aromatic hydrocarbons in recent sediments. *Geochimica et Cosmochimica Acta*, *42* (3), 289-303.

Lau, E. V., Gan, S. & Ng, H. K. (2010). Extraction techniques for polycyclic aromatic hydrocarbons in soils. *International Journal of Analytical Chemistry*, *Volume 2010*, 1-9.

Lee, D. S., Kim, D. W. & Kim, S. K. (2009). Relationship of pyrogenic polycyclic aromatic hydrocarbon contamination among environmental solid media. *Journal of Environmental Monitoring, 11* (6), 1244-1252.

Lewis, B. & Von Elbe, G. (2012). Combustion, flames and explosions of gases. Elsevier. Academic Press Inc. (London) Ltd.

Lewu, F. & Mavengahama, S. (2011). Utilization of wild vegetables in four districts of the northern KwaZulu-Natal province, South Africa. *African Journal of Agricultural Research, 6* (17), 4159-4165.

Likens, G. E., Keene, W. C., Miller, J. M. & Galloway, J. N. (1987). Chemistry of precipitation from a remote, terrestrial site in Australia. *Journal of Geophysical Research: Atmospheres (1984–2012), 92* (D11), 13299-13314.

Loganathan, B. G. & Lam, P. K. S. (Eds.). (2011). Global contamination trends of persistent organic chemicals. CRC Press.

Lohmann, R., Gioia, R., Jones, K. C., Nizzetto, L., Temme, C., Xie, Z. & Jantunen, L. (2009). Organochlorine pesticides and PAHs in the surface water and atmosphere of the North Atlantic and the Arctic Ocean. *Environmental Science & Technology, 43* (15), 5633-5639.

Lopez-Avila, V., Young, R. & Beckert, W. F. (1994). Microwave-assisted extraction of organic compounds from standard reference soils and sediments. *Analytical Chemistry*, *66* (7), 1097-1106.

Lopez-Avila, V., Benedicto, J. & Bauer, K. M. (1998). Stability of organochlorine and organophosphorus pesticides when extracted from solid matrixes with microwave energy. *Journal of AOAC International, 81* (6), 1224-1232.

Lopes, T. J. & Dionne, S. G. (2003). A review of semi-volatile and volatile organic compounds in highway runoff and urban storm water. *National highway runoff water-quality data and methodology synthesis*, *1*, 235-303.

Lough, W. J. & Wainer, I. W. (1995). High-performance liquid chromatography: Fundamental Principles and Practice: CRC Press.

Luque de Castro, M. & Garcıa-Ayuso, L. (1998). Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. *Analytica Chimica Acta, 369* (1), 1-10.

Mabaso, M. L. H., Appleton, C. C., Hughes, J. C. & Gouws, E. (2003). The effect of soil type and climate on hookworm (*Necator americanus*) distribution in KwaZulu-Natal, South Africa. *Tropical Medicine & International Health*, *8* (8), 722-727.

Magdic, S. & Pawliszyn, J. B. (1996). Analysis of organochlorine pesticides using solid phase microextraction, *Journal of Chromatography A,* 723, 111-122.

Majors, R. E. (2013). Sample Preparation Fundamentals for Chromatography. Agilent Technologies, Mississauga, ON, Canada.

Mandigo, A. C., DiScenza, D. J., Keimowitz, A. R. & Fitzgerald, N. (2015). Chemical contamination of soils in the New York City area following Hurricane Sandy. *Environmental Geochemistry and Health*, 1-10.

Manoli, E. & Samara, C. (1999). Polycyclic aromatic hydrocarbons in natural waters: sources, occurrence and analysis. *Trends in Analytical Chemistry, 18* (6), 417-428.

Manoli, E., Kouras, A. & Samara, C. (2004). Profile analysis of ambient and source emitted particle-bound polycyclic aromatic hydrocarbons from three sites in northern Greece. *Chemosphere*, *56*(9), 867-878.

Masih, A. & Taneja, A. (2006). Polycyclic aromatic hydrocarbons (PAHs) concentrations and related carcinogenic potencies in soil at a semi-arid region of India. *Chemosphere, 65* (3), 449-456.

Maskaoui, K., Zhou, J. L., Hong, H. S. & Zhang, Z. L. (2002). Contamination by polycyclic aromatic hydrocarbons in the Jiulong River estuary and Western Xiamen Sea, China. *Environmental Pollution*, *118* (1), 109-122.

Mbatha, B. (2012). A user perspective on the web usage in selected South African public Universities.

McLafferty, F.W., Hertel, R.H. & Villwock, R.D. (1974). Probability based matching of mass spectra. Rapid identification of specific compounds in mixtures. *Organic Mass Spectrometry,* (9), 690-702.

McMurry, J. E. & Simanek, E. E. (2007). Organic Chemistry, Cengage Learning. *Inc., USA*.

Meyers, P. A. & Ishiwatari, R. (1993). Lacustrine organic geochemistry—an overview of indicators of organic matter sources and diagenesis in lake sediments. *Organic Geochemistry*, *20* (7), 867-900.

Miller, M. M., Wasik, S. P., Huang, G. L., Shiu, W. Y. & Mackay, D. (1985). Relationships between octanol-water partition coefficient and aqueous solubility. *Environmental Science & Technology*, *19* (6), 522-529.

Mohrig, J. R., Alberg, D., Holifmeister, G., Schatz, P. F. & Hammond, C. N. (2014). *Laboratory techniques in organic chemistry*. WH Freeman & Co Ltd.

Morrison, Robert, T. & Robert, N. B. (1992). 6th ed. Englewood Cliffs, NJ: Prentice Hall.

Motelay-Massei, A., Ollivon, D., Garban, B., Teil, M. J., Blanchard, M. & Chevreuil, M. (2004). Distribution and spatial trends of PAHs and PCBs in soils in the Seine River basin, France. *Chemosphere*, *55* (4), 555-565.

Müller, L., Fattore, E. & Benfenati, E. (1997). Determination of aromatic amines by solid-phase microextraction and gas chromatography–mass spectrometry in water samples. *Journal of Chromatography A*, *791* (1), 221-230.

Muñoz, B. & Albores, A. (2011). DNA damage caused by polycyclic aromatic hydrocarbons: Mechanisms and Markers. INTECH Open Access, Publisher.

Munson, M. S. & Field, F. H. (1966). Chemical ionization mass spectrometry. I. General introduction. *Journal of the American Chemical Society*, *88* (12), 2621-2630.

Nadal, M., Schuhmacher, M. & Domingo, J. L. (2004). Levels of PAHs in soil and vegetation samples from Tarragona County, Spain. *Environmental Pollution*, *132* (1), 1-11.

Nam, J. J., Sweetman, A. J. & Jones, K. C. (2009). Poly-nuclear aromatic hydrocarbons (PAHs) in global background soils. *Journal of Environmental Monitoring, 11* (1), 45-48.

National Research Council (NRC US). (1985). Steering Committee for the Petroleum in the Marine Environment Update. Oil in the sea: inputs, fates and effects (Vol. 1). National Academies.

Neff, J. M. (1978). Polycyclic aromatic hydrocarbons in the aquatic environment and cancer risk to aquatic organisms and man. In: Symposium: Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment. US Environmental Protection Agency Rep, Volume 600, 385–409.

Neff, J. M. (1979). Polycyclic aromatic hydrocarbons in the aquatic environment: sources, fates and biological effects. In Polycyclic aromatic hydrocarbons in the aquatic environment: sources, fates and biological effects. Applied Science.

Neff, J. M., Stout, S. A. & Gunster, D. G. (2005). Ecological risk assessment of polycyclic aromatic hydrocarbons in sediments: identifying sources and ecological hazard. *Integrated Environmental Assessment Management 1* (1), 22-33.

Nekhavhambe, T. J., Van Ree, T. & Fatoki, O. S. (2014). Determination and distribution of polycyclic aromatic hydrocarbons in rivers, surface runoff, and sediments in and around Thohoyandou, Limpopo Province, South Africa. *Water South Africa, 40* (3), 415-424.

Ngcobo, H., Dladla, B. & Worth, S. (2002). Provincial report on education and training for agricultural and rural development in Kwa-Zulu Natal (KZN). KZN Department of Agriculture, Pietermaritzburg.

Nicolas (2014). Column and thin layer chromatography. [Online]. Available from: *http://www.odinity.com/thin-layer-column-chromatography/*. [Accessed on 13rd November 2015].

Nieuwoudt, C., Pieters, R., Quinn, L. P., Kylin, H., Borgen, A. R. & Bouwman, H. (2011). Polycyclic aromatic hydrocarbons (PAHs) in soil and sediment from industrial, residential, and agricultural areas in Central South Africa: An initial assessment. *Soil and Sediment Contamination, 20* (2), 188-204.

Nkansah, M. A. (2012). Environmental Remediation: Removal of polycyclic aromatic hydrocarbons Dissertation for the degree of Philosophiae Doctor (Ph.D.). Department of Chemistry, University of Bergen, Norway.

Notar, M., Leskovšek, H. & Faganeli, J. (2001). Composition, distribution and sources of polycyclic aromatic hydrocarbons in sediments of the Gulf of Trieste, Northern Adriatic Sea. *Marine Pollution Bulletin, 42* (1), 36-44.

Odabasi, M., Vardar, N., Sofuoglu, A., Tasdemir, Y. & Holsen, T. M. (1999). Polycyclic aromatic hydrocarbons (PAHs) in Chicago air. *Science of the Total Environment, 227* (1), 57-67.

Okedeyi, O. O. (2012). Assessment of polycyclic aromatic hydrocarbon (PAHs) and heavy metals in the vicinity of coal power plants in South Africa. (Doctoral dissertation, University of South Africa).

Oluseyi, T., Olayinka, K., Alo, B. & Smith, R. M. (2011). Comparison of extraction and cleanup techniques for the determination of polycyclic aromatic hydrocarbons in contaminated soil samples. African *Journal of Environmental Science and Technology, 5* (7), 482-493.

Ouyang, Y. (2006). Phenanthrene pathway map. [Online]. Available from: *http://umbbd.msi.umn.edu/PHA/PHA_map.html.* [Accessed on 24th July 2015].

Palmqvist, A., Rasmussen, L. J. & Forbes, V. E. (2006). Influence of biotransformation on trophic transfer of the PAH, fluoranthene. *Aquatic Toxicology, 80* (3), 309-319.

Pang, G. F., Fan, C. L., Cao, Y. Z., Yan, F., Li, Y., Kang, J. & Chang, Q. Y. (2015). High Throughput Analytical Techniques for the Determination and Confirmation of Residues of 653 Multiclass Pesticides and Chemical Pollutants in Tea by GC/MS, GC/MS/MS, and LC/MS/MS: Collaborative Study, First Action 2014.09. *Journal of AOAC International*, *98* (5), 1428-1454.

Pavia, D. L. L., Kriz, G. M., Pavia, G. S. D. L., Lampman, G. M. & Kriz, G. S. (1988). Introduction to organic laboratory techniques: a contemporary approach (No. Sirsi) i9780030148132), Agricultural Information and Documentation Service of the Americas (SIDALC).

Pavia, D. L., Lampman, G. M., Kritz, G. S. & Engel, R. G. (2006). Introduction to Organic Laboratory Techniques, 4th Ed., Thomson Brooks/Cole, 797-817.

Pena, M. T., Casais, M. C., Mejuto, M. C. & Cela, R. (2009). Development of an ionic liquid based dispersive liquid–liquid microextraction method for the analysis

of polycyclic aromatic hydrocarbons in water samples. *Journal of Chromatography A, 1216* (36), 6356-6364.

Petersen, D. G., Sundbäck, K., Larson, F. & Dahllöf, I. (2009). Pyrene toxicity is affected by the nutrient status of a marine sediment community: Implications for risk assessment. *Aquatic Toxicology, 95* (1), 37-43.

Petrick, G., Schulz-Bull, D. E. & Martens, V. (1997). An in-situ filtration/extraction system for the recovery of trace organics in solution and on particles tested in deep ocean water. *Oceanographic Literature Review*, *3* (44), 191.

Pies, C., Hoffmann, B., Petrowsky, J., Yang, Y., Ternes, T. A. & Hofmann, T. (2008). Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in river bank soils. *Chemosphere, 72* (10), 1594-1601.

Prycek, J., Ciganek, M. & Simek, Z. (2007). Cleanup of extracts for nitrated derivatives of polycyclic aromatic hydrocarbon analyses prior to their gas chromatography determination. *Journal of the Brazilian Chemical Society, 18* (6), 1125-1131.

Qiao, M., Wang, C., Huang, S., Wang, D. & Wang, Z. (2006). Composition, sources, and potential toxicological significance of PAHs in the surface sediments of the Meiliang Bay, Taihu Lake, China. *Environment International*, *32* (1), 28-33.

Quiroz, R., Grimalt, J. O. & Fernández, P. (2010). Toxicity assessment of polycyclic aromatic hydrocarbons in sediments from European high mountain lakes. *Ecotoxicology and Environmental Safety, 73* (4), 559-564.

Ramos, L., Kristenson, E. M. & Brinkman, U. T. (2002). Current use of pressurized liquid extraction and subcritical water extraction in environmental analysis. *Journal of Chromatography A, 975* (1), 3-29.

Randall, L. G. & Wahrhaftig, A. L. (1978). Dense gas chromatograph/mass spectrometer interface. *Analytical Chemistry*, *50* (12), 1703-1705.

Ravindra, K., Mittal, A. K. & Van Grieken, R. (2001). Health risk assessment of urban suspended particulate matter with special reference to polycyclic aromatic hydrocarbons: A review. *Reviews on Environmental Health, 16* (3), 169-189.

Ravindra, K., Sokhi, R. & Van Grieken, R. (2008a). Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. *Atmospheric Environment, 42* (13), 2895-2921.

Ravindra, K., Wauters, E. & Van Grieken, R. (2008b). Variation in particulate PAHs levels and their relation to the transboundary movement of the air masses. *Science of the Total Environment, 396* (2), 100-110.

Reimer, G. & Suarez, A. (1995). Comparison of supercritical fluid extraction and Soxhlet extraction for the analysis of native polycyclic aromatic hydrocarbons in the soils. *Journal of Chromatography A, 699* (1), 253-263.

Richard, Y. & Poccard, I. (1998). A statistical study of NDVI sensitivity to seasonal and interannual rainfall variations in Southern Africa. *International Journal of Remote Sensing*, *19* (15), 2907-2920.

Rick, L. (2015). How do small particle size columns increase sample throughput? [Online]. Available from: *http://www.restek.com/Technical-Resources/Technical-Library/Pharmaceutical/pharm_A016*. [Accessed on 13rd November 2015].

Ringuet, J., Albinet, A., Leoz-Garziandia, E., Budzinski, H. & Villenave, E. (2012). Reactivity of polycyclic aromatic compounds (PAHs, NPAHs and OPAHs) adsorbed on natural aerosol particles exposed to atmospheric oxidants. *Atmospheric Environment,* 61, 15-22.

Rissato, S. R., Galhiane, M. S., Ximenes, V. F., De Andrade, R. M., Talamoni, J. L., Libânio, M. & Cavalari, A. A. (2006). Organochlorine pesticides and polychlorinated biphenyls in soil and water samples in the North-eastern part of São Paulo State, Brazil. *Chemosphere*, *65* (11), 1949-1958.

Roos, C. (2010). Characterizing the scale and significance of persistent organic pollutants in South African sediments. North-West University.

Rose, N. L., Rose, C. L., Boyle, J. F. & Appleby, P. G. (2004). Lake-sediment evidence for local and remote sources of atmospherically deposited pollutants on Svalbard. *Journal of Paleolimnology, 31* (4), 499-513.

Rossberg, M., Lendle, W., Pfleiderer, G., Tögel, A., Dreher, E. L., Langer, E. & Mann, T. (2006). Chlorinated hydrocarbons. *Ullmann's encyclopaedia of industrial chemistry*.

Rubailo, A. I. & Oberenko, A. V. (2008). Polycyclic Aromatic Hydrocarbons as Priority Pollutants. *Journal of Siberian Federal University*. *Chemistry 2008 1* (4).

Rubio, R. & Ure, A. M. (1993). Approaches to sampling and sample pretreatments for metal speciation in soils and sediments. *International Journal of Environmental Analytical Chemistry*, *51* (1-4), 205-217.

Sahu, K. C., Panda, U. C. & Mohapatra, D. M. (1998). Geochemistry and mineralogy of sediments in Rushikulya estuary, east coast of India. *Chemical Environmental Research*, *7* (1/2), 77-92.

Saim, N. A., Dean, J. R., Abdullah, M. P. & Zakaria, Z. (1997). Extraction of polycyclic aromatic hydrocarbons from contaminated soil using Soxhlet extraction, pressurized and atmospheric microwave-assisted extraction, and supercritical fluid extraction and accelerated solvent extraction. *Journal of Chromatography A, 791* (1), 361-366.

Sakari, M. (2012). Depositional history of Polycyclic Aromatic Hydrocarbons: Reconstruction of petroleum pollution record in Peninsular Malaysia. INTECH Open Access, Publisher.

Santana, J. L., Massone, C. G., Valdés, M., Vazquez, R., Lima, L. A. & Olivares-Rieumont, S. (2015). Occurrence and Source Appraisal of Polycyclic Aromatic Hydrocarbons (PAHs) in Surface Waters of the Almendares River, Cuba. *Archives of environmental contamination and toxicology*, 1-10.

Santos, F. J. & Galceran, M. T. (2002). The application of gas chromatography to environmental analysis. *Trends in Analytical Chemistry,* (21), 672-685.

Saponaro, S., Bonomo, L., Petruzzelli, G., Romele, L. & Barbafieri, M. (2002). Polycyclic aromatic hydrocarbons (PAHs) slurry phase bioremediation of a

manufacturing gas plant (MGP) site aged soil. *Water, Air, and Soil Pollution*, *135* (1-4), 219-236.

Sayles, G. D., Acheson, C. M., Kupferle, M. J., Shan, Y., Zhou, Q., Meier, J. R. & Brenner, R. C. (1999). Land treatment of PAH-contaminated soil: performance measured by chemical and toxicity assays. *Environmental Science & Technology*, *33* (23), 4310-4317.

Schneider, J., Grosser, R., Jayasimhulu, K., Xue, W. & Warshawsky, D. (1996). Degradation of pyrene, benzo[a]anthracene, and benzo[a]pyrene by Mycobacterium sp. strain RJGII-135, isolated from a former coal gasification site. *Applied and Environmental Microbiology*, *62* (1), 13-19.

Shabalala, A. N., Combrinck, L. & McCrindle, R. (2013). Effect of farming activities on seasonal variation of water quality of Bonsma Dam, KwaZulu-Natal. *South African Journal of Science*, *109* (7-8), 01-07.

Shah, S., Ashraf-Khorassani, M. & Taylor, L. T. (1989). Normal-phase liquid and supercritical-fluid chromatographic separations of steroids with Fourier-transform infrared spectrometric detection. *Chromatographia*, *27* (9-10), 441-448.

Shen, J. X., Motyka, R. J., Roach, J. P. & Hayes, R. N. (2005). Minimization of ion suppression in LC–MS/MS analysis through the application of strong cation exchange solid-phase extraction (SCX-SPE). *Journal of Pharmaceutical and Biomedical Analysis, 37* (2), 359-367.

Shi, Z., Tao, S., Pan, B., Fan, W., He, X. C., Zuo, Q. & Wong, P. K. (2005). Contamination of rivers in Tianjin, China by polycyclic aromatic hydrocarbons. *Environmental Pollution*, *134* (1), 97-111.

Sibiya, P. N. (2012). Modification, development and application of extraction methods for PAHs in the water and sediments. (Doctoral dissertation, Faculty of Science, University of the Witwatersrand, Johannesburg).

Sicre, M. A., Marty, J. C., Saliot, A., Aparicio, X., Grimalt, J. & Albaiges, J. (1987). Aliphatic and aromatic hydrocarbons in different sized aerosols over the

Mediterranean Sea: Occurrence and origin. *Atmospheric Environment (1967), 21* (10), 2247-2259.

Sikhakhane, S. S. (2001). Cost-benefit Analysis of the Environmental Impacts of Darvill Wastewater Works, Pietermaritzburg, KwaZulu-Natal (Doctoral dissertation, University of Natal).

Silva, T. F. D., Azevedo, D. D. A. & Aquino Neto, F. R. D. (2007). Distribution of polycyclic aromatic hydrocarbons in surface sediments and waters of Guanabara Bay, Rio de Janeiro, Brazil. *Journal of the Brazilian Chemical Society, 18* (3), 628-637.

Simpson, D. & Pillay, M. (2000). Quantifying externalities: Study of the impact of nutrient loading in the Msunduzi River on downstream users. Presented at the WISA 2000 Biennial Conference, Sun City, South Africa, 2-4.

Sims, R. C. & Overcash, M. R. (1983). Fate of poly-nuclear aromatic compounds (PNAs) in soil-plant systems. In Residue reviews. Springer New York, 1-68.

Singh, T. A., Meetei, N. S. & Meitei, L. B. (2013). Seasonal variation of some physicochemical characteristics of three major rivers in Imphal, Manipur: A comparative evaluation. *Current World Environment*, *8* (1), 93-102.

Slaski, J. J., Archambault, D. J. & Li, X. (2000). Evaluation of polycyclic aromatic hydrocarbon (PAH) accumulation in plants. The potential use of PAH accumulation as a marker of exposure to air emissions from oil and gas flares. Report prepared for the Air Research Users Group, Alberta Environment: Edmonton, Alberta.

Sloan, C. A., Brown, D. W., Pearce, R. W., Boyer, R. H., Bolton, J. L., Burrows, D. G. & Krahn, M. M. (2004). Extraction, Clean up and Gas Chromatography/Mass Spectrometry Analysis of Sediments and Tissues for Organic Contaminants. NOAA Technical Memorandum NMFS-NWFSC-59.

Smart-fertilizer management (SFM). (2015). Electrical conductivity of water. [Online]. Available from: *http://www.smart-fertilizer.com/articles/electrical-conductivity*. [Accessed on 26th August 2015].

Smith, R. D., Udseth, H. R. & Kalinoski, H. T. (1984). Capillary supercritical fluid chromatography/mass spectrometry with electron impact ionization. *Analytical Chemistry*, *56* (14), 2971-2973.

Soclo, H., Garrigues, P. & Ewald, M. (2000). Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: Case studies in Cotonou2 (Benin) and Aquitaine (France) areas. *Marine Pollution Bulletin, 40* (5), 387-396.

South African River Health Program (RHP). (2015a). State of the Rivers Report uMngeni River and Neighbouring Rivers and Streams. [Online]. Available from: *http://www.csir.co.za/rhp/state_of_rivers/state_of_umngeni_02/umngeni.html*. [Accessed on 20th September 2015].

Stashenko, E. & Martínez, J. R. (2014). Gas Chromatography-Mass Spectrometry. Advances in Gas Chromatography, Dr Xinghua Guo (Ed.), ISBN: 978-953-51-1227-3, InTech, DOI: 10.5772/57492. [Online]. Available from: *http://www.intechopen.com/books/advances-in-gas-chromatography/gas-chromatography-mass-spectrometry*. [Accessed on 14th November 2015].

Statistics South Africa (SSA). (2014). Mid-year population estimates. [Online]. Available from: *http://www.statssa.gov.za/publications/P0302/P03022014.pdf*. [Accessed on 3rd November 2015].

Steingass, C. B., Langen, J., Carle, R. & Schmarr, H. G. (2015). Authentication of pineapple (*Ananas comosus [L.] Merr.)* Fruit maturity stages by quantitative analysis of γ-and δ-lactones using headspace solid-phase microextraction and chirospecific gas chromatography–selected ion monitoring mass spectrometry (HS-SPME–GC–SIM-MS). *Food Chemistry*, *168*, 496-503.

Straif, K., Baan, R., Grosse, Y., Secretan, B., El Ghissassi, F., Cogliano, V. & WHO International Agency for Research on Cancer Monograph Working Group.

(2006). Carcinogenicity of household solid fuel combustion and high-temperature frying. *The Lancet Oncology, 7* (12), 977-978.

Suchan, P., Pulkrabová, J., Hajšlová, J. & Kocourek, V. (2004). Pressurized liquid extraction in the determination of polychlorinated biphenyls and organochlorine pesticides in fish samples. *Analytica Chimica Acta, 520* (1), 193-200.

Suess, M. J. (1976). The environmental load and cycle of polycyclic aromatic hydrocarbons. *Science of the Total Environment, 6* (3), 239-250.

Sulej, A. M., Polkowska, Ż. & Namieśnik, J. (2011). Contamination of Runoff Water at Gdańsk Airport (Poland) by Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs). *Sensors, 11* (12), 11901-11920.

Sun, F., Littlejohn, D. & Gibson, M. D. (1998). Ultra-sonication extraction and solid phase extraction cleanup for determination of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons in soils by reversed-phase liquid chromatography with ultraviolet absorption detection. *Analytica Chimica and Acta, 364* (1), 1-11.

Sutton, C. (2008). Urban open space: a case study of the Msunduzi local municipality, South Africa.

Tang, L., Tang, X. Y., Zhu, Y. G., Zheng, M. H. & Miao, Q. L. (2005). Contamination of polycyclic aromatic hydrocarbons (PAHs) in urban soils in Beijing, China. *Environment International, 31* (6), 822-828.

Tao, S., Jiao, X., Chen, S., Liu, W., Coveney, R., Zhu, L. & Luo, Y. (2006). Accumulation and distribution of polycyclic aromatic hydrocarbons in rice (*Oryza sativa*). *Environmental Pollution, 140* (3), 406-415.

Thwala, D.W. (2010). Community participation is a necessity for project success: a case study of rural water supply projects in Jeppes Reefs, South Africa. *African Journal of Agricultural Research, 5*, 970–979.

Tikilili, P. V. & Nkhalambayausi-Chirwa, E. M. (2011). Characterization and biodegradation of polycyclic aromatic hydrocarbons in radioactive wastewater. *Journal of Hazardous Materials*, *192* (3), 1589-1596.

Titato, G. M. & Lanças, F. M. (2005). Comparison between Different Extraction (LLE and SPE) and Determination (HPLC and Capillary-LC) Techniques in the Analysis of Selected PAHs in Water Samples. *Journal of Liquid Chromatography & Related Technologies, 28* (19), 3045-3056.

Tony, C (2012). Durban-Umgeni Water will spend R450 million to upgrade the main Pietermaritzburg wastewater treatment works to reduce sewage contamination levels in Durban's main water storage dam. [Online]. Available from: *http://www.iol.co.za/news/south-africa/kwazulu-natal/r450m-for-wastewater-treatment-site-1.1411648#.VhIVMuyqqko*. [Accessed on 20th September 2015].

Thulasaie, S. (2008). *An environmental risk assessment of the creosote bleedings at the Howick Pole Yard* (Doctoral dissertation, University of Kwa-Zulu Natal).

Ünlü, S. & Alpar, B. (2006). Distribution and sources of hydrocarbons in surface sediments of Gemlik Bay (Marmara Sea, Turkey). *Chemosphere*, *64* (5), 764-777.

US-Environmental Protection Agency (US EPA). (1996a). Method 3630, Revision C. Washington DC, USA.

US-Environmental Protection Agency (US EPA). (1996b). Separator Funnel Liquid–liquid Extraction. EPA Method 3510-C, 40 CFR 136, Appendix A. Third Revision.

US-Environmental Protection Agency (US EPA). (1996c). Method 3540C: Soxhlet Extraction. US Environmental Protection Agency, Washington DC, USA.

US-Environmental Protection Agency (US EPA). (1996d). Semi-volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). EPA Method 8270, 40 CFR 136, Appendix A, Third Revision.

US-Environmental Protection Agency (US EPA). (1994). Method 1613: Tetra- through Octa-chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.

Revision B. Office of Water: Engineering and Analysis Division, Washington, DC. EPA-821-B-94–005.

Van Deemter, J. J., Zuiderweg, F. J. & Klinkenberg, A. V. (1956). Longitudinal diffusion and resistance to mass transfer as causes of non-ideality in chromatography. *Chemical Engineering and Science, 5* (6), 271-289.

Van Kemenade, I., Anderson, W. A., Scharer, J. M. & Moo-Young, M. (1995). Bioremediation enhancement of phenanthrene contaminated soils by chemical pre-oxidation. *Hazardous Waste and Hazardous Materials*, 12 (4), 345-355.

Van Zuydam, C. S. (2009). Determination of Polycyclic Aromatic Hydrocarbons (PAHS) resulting from wood storage and wood treatment facilities for electricity transmission in Swaziland. MSc Thesis, University of KwaZulu-Natal.

Vasconcellos, P. C., Zacarias, D., Pires, M. A., Pool, C. S. & Carvalho, L. R. (2003). Measurements of polycyclic aromatic hydrocarbons in airborne particles from the metropolitan area of Sao Paulo City, Brazil. *Atmospheric Environment, 37* (21), 3009-3018.

Viguri, J., Verde, J. & Irabien, A. (2002). Environmental assessment of polycyclic aromatic hydrocarbons (PAHs) in surface sediments of the Santander Bay, Northern Spain. *Chemosphere, 48* (2), 157-165.

Vogt, N. L. (2014). Quantification of PAHs and PCBs in the eThekwini aquatic systems, using chemical and biological analysis (Doctoral dissertation). North-West University, Potchefstroom Campus.

Vu, V. T., Lee, B. K., Ny, M T. & Kim, T. J. (2010). A study of characteristics of organic carbon and polycyclic aromatic hydrocarbons in PM 10 at the residential and industrial areas in Ulsan in Korea. In *International Forum on Strategic Technology* (IFOST), IEEE, 2000, 263-2566.

Wade Jr, L. G. (2003). Infrared spectroscopy and mass spectrometry. Organic Chemistry. 5th Ed. Pearson Education: Upper Saddle River, NJ, 490-538.

Wang, W., Meng, B., Lu, X., Liu, Y. & Tao, S. (2007). Extraction of polycyclic aromatic hydrocarbons and organochlorine pesticides from soils: a comparison between Soxhlet extraction, microwave-assisted extraction and accelerated solvent extraction techniques. *Analytica Chimica and Acta, 602* (2), 211-222.

Wang, L. & Weller, C. L. (2006). Recent advances in extraction of Nutraceuticals from plants. *Trends in Food Science & Technology, 17* (6), 300-312.

Wang, X. T., Miao, Y., Zhang, Y., Li, Y. C., Wu, M. H. & Yu, G. (2013). Polycyclic aromatic hydrocarbons (PAHs) in urban soils of the megacity Shanghai: occurrence, source apportionment and potential human health risk. *Science of the Total Environment, 447*, 80-89.

Weather online. (2015). [Online]. Available from: *http://www.worldweatheronline.com/Durban-weather-averages/Kwazulu-Natal/ZA.aspx*. [Accessed on 25th September 2015].

Walker, C. H. (2008). Organic pollutants: An Ecotoxicological Perspective. CRC Press.

Wenzl, T., Simon, R., Anklam, E. & Kleiner, J. (2006). Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. *Trends in Analytical Chemistry, 25* (7), 716-725.

Wetzel, R. G. (2001). Limnology: Lake and River Ecosystems (3rd Ed.). San Diego, CA: Academic Press.

Whipple, K. (2002). Factors Influencing Hydraulic Roughness. In Surface Processes and Landforms. [Online]. Available from: *http://ocw.mit.edu/courses/earth-atmospheric-and-planetary-sciences/12-163-surface-processes-and-landscape-evolution-fall-2004/labs/roughnes_handout.pdf*. [Accessed on 26th August 2015].

White, K. L., Lysy, H. H. & Holsapple, M. P. (1985). Immunosuppression by polycyclic aromatic hydrocarbons: a structure-activity relationship in B6C3F1 and DBA/2 mice. *Immunopharmacology, 9* (3), 155-164.

WHO (World Health Organization). (2003). The World Health Report. [Online]. Available from: *http://www.who.int/whr/2003/en/.* [Accessed on 27th July 2015].

Wickstrom, K. & Tolonen, K. (1987). The history of airborne polycyclic aromatic hydrocarbons (PAH) and perylene as recorded in dated lake sediments. *Water, Air, and Soil Pollution*, 32 (1-2), 155-175.

Wischmann, H., Steinhart, H., Hupe, K., Montresori, G. & Stegmann, R. (1996). Degradation of selected PAHs in soil/composts and identification of intermediates. *International Journal of Environmental Analytical Chemistry, 64* (4), 247-255.

Wise, S. A., Schantz, M. M., Benner, B. A. J., Hays, M. J. & Schiller, S. B. (1995). Certification of polycyclic aromatic hydrocarbons in a marine sediment standard reference material. *Analytical Chemistry, 67* (7), 1171-1178.

Witt, G. (1995). Polycyclic aromatic hydrocarbons in water and sediment of the Baltic Sea. *Marine Pollution Bulletin, 31* (4), 237-248.

Wong, C. S., Sanders, G., Engstrom, D. R., Long, D. T., Swackhamer, D. L. & Eisenreich, S. J. (1995). Accumulation, inventory, and diagenesis of chlorinated hydrocarbons in Lake Ontario sediments. *Environmental Science & Technology, 29* (10), 2661-2672.

Wright, D. R. & Hamilton, R. D. (1982). Release of methyl mercury from sediments: effects of mercury concentration, low temperature, and nutrient addition. *Canadian Journal of Fisheries and Aquatic Sciences*, 39 (11), 1459-1466.

Wu, Y., Xia, L., Chen, R. & Hu, B. (2008). Headspace single drop microextraction combined with HPLC for the determination of trace polycyclic aromatic hydrocarbons in environmental samples. *Talanta, 74* (4), 470-477.

Yang, M., Xi, X., Wu, X., Lu, R., Zhou, W., Zhang, S. & Gao, H. (2015). Vortex-assisted magnetic β-cyclodextrin/attapulgite-linked ionic liquid dispersive liquid–liquid microextraction coupled with high-performance liquid chromatography

for the fast determination of four fungicides in water samples. *Journal of Chromatography A*, *1381*, 37-47.

Yu, H. (2002). Environmental carcinogenic polycyclic aromatic hydrocarbons: photochemistry and photo-toxicity. *Journal of Environmental Science and Health, Part C, 20* (2), 149-183.

Yu, M. H., Tsunoda, H. & Tsunoda, M. (2011). Environmental toxicology: Biological and Health Effects of Pollutants. CRC Press, Third Ed., 188-191.

Yunker, M. B., Macdonald, R. W., Vingarzan, R., Mitchell, R. H., Goyette, D. & Sylvestre, S. (2002). PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry, 33* (4), 489-515.

Zdráhal, Z., Karásek, P., Lojková, L., Bucková, M., Večeřa, Z. & Vejrosta, J. (2000). Pressurised liquid extraction of ketones of polycyclic aromatic hydrocarbons from soil. *Journal of Chromatography A*, *893* (1), 201-206.

Zhang, Z., Huang, J., Yu, G. & Hong, H. (2004). Occurrence of PAHs, PCBs and organochlorine pesticides in the Tonghui River of Beijing, China. *Environmental Pollution*, *130* (2), 249-261.

Zhang, L., Dong, L., Ren, L., Shi, S., Zhou, L., Zhang, T. & Huang, Y. (2012). Concentration and source identification of polycyclic aromatic hydrocarbons and phthalic acid esters in the surface water of the Yangtze River Delta, China. *Journal of Environmental Sciences, 24* (2), 335-342.

Zhao, Z. & Barron, A. (2012). Dynamic Headspace Gas Chromatography Analysis. *Journal of Connections: Houston, TX.*

Zhong, Y., Carmella, S. G., Upadhyaya, P., Hochalter, J. B., Rauch, D., Oliver, A. & Zimmerman, C. (2010). Immediate consequences of cigarette smoking: rapid formation of polycyclic aromatic hydrocarbon diol epoxides. *Chemical Research in Toxicology, 24* (2), 246-252.

Zondi, M. (2004). Pietermaritzburg to get R1 billion waterfront, Daily News (South Africa). [Accessed on 30th September 2015].

Appendices

Appendix 1: Sixteen PAHs prioritised for regular monitoring by US EPA, their abbreviations and their number of benzene rings**.**

Table 1: Those 7 PAHs with * are those studied in this study from 16 PAHs classified by **USEPA** as priority pollutants to be monitored in the environment.

No	PAHs	Abbreviations	Number of rings
1	**Naphthalene***	**NA**	**2**
2	**Acenaphthylene***	**ACY**	**2**
3	Acenaphthene	ACE	2
4	**Fluoene***	**FLUO**	**2**
5	**Anthracene***	**ANTH**	**3**
6	**Phenanthrene***	**PHEN**	**3**
7	Fluoranthene	FLA	4
8	**Pyrene***	**PYR**	**4**
9	Benzo[a]anthracene	BaA	4
10	**Chrysene***	**CHRY**	**4**
11	Benzo[b]fluoanthene	BbF	4
12	Benzo[k]fluoanthene	BkF	4
13	Benzo[a] pyrene	BaP	5
14	Dibenzo[a, h] anthracene	DahA	5
15	Indeno[1,2,3-c, d]pyrene	IcdP	5
16	Benzo[g,h,i]perylene	BghiP	6

Appendix 2: Preparation of standard solutions

1. Exactly 5 mg of each PAH standard (naphthalene, acenaphthylene, fluorene, anthracene, phenanthrene, pyrene and chrysene) and 5 mg of naphthalene-d8 as an internal standard were dissolved in 50 mL of DCM to make 100 ppm stock solution.

2. DCM (chromatographic grade, 50 mL) was used to prepare a stock solution of 100 ppm for each PAH.

Serial dilution

1. The solution of 0.25 ppm was prepared by diluting a volume 125 µL of the stock solution into 50 mL of DCM.

2. The solution of 0.5 ppm was prepared by taking a volume 250 µL from the stock solution using micropipette and this was diluted with 50 mL of DCM.

3. To get 1 ppm, the volume of 500 µL was taken from the stock solution and we was diluted with 50 mL of DCM.

4. To obtain 1.5 and 2 ppm, the volumes 750 and 1000 µL were taken from the stock solution and diluted with 50 mL of DCM, respectively.

Preparation of calibration curves

1. The above concentrations were used by running each three times on GC-MS instrument.

2. The calibration curves were plotted using the ratios of peak areas of each PAH analyte dividing by those of naphthalene-d8 verses their concentrations.

3. LODs and LOQs were calculated as three and ten times the signal to noise ratio using three calibration intercepts divided by the slope of the calibration curve, respectively.

Appendix 3: Library search results of naphthalene and pyrene and chromatogram of the 7 PAH standards.
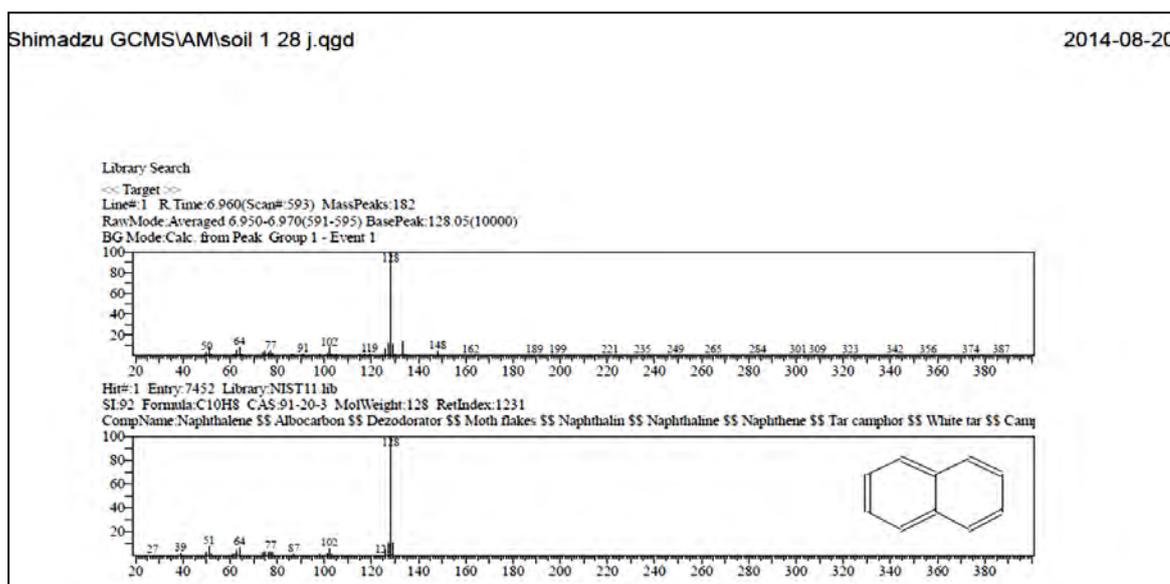
Figure 1: Library search result of naphthalene analysed in the soils collected from Henley Dam sampling site.
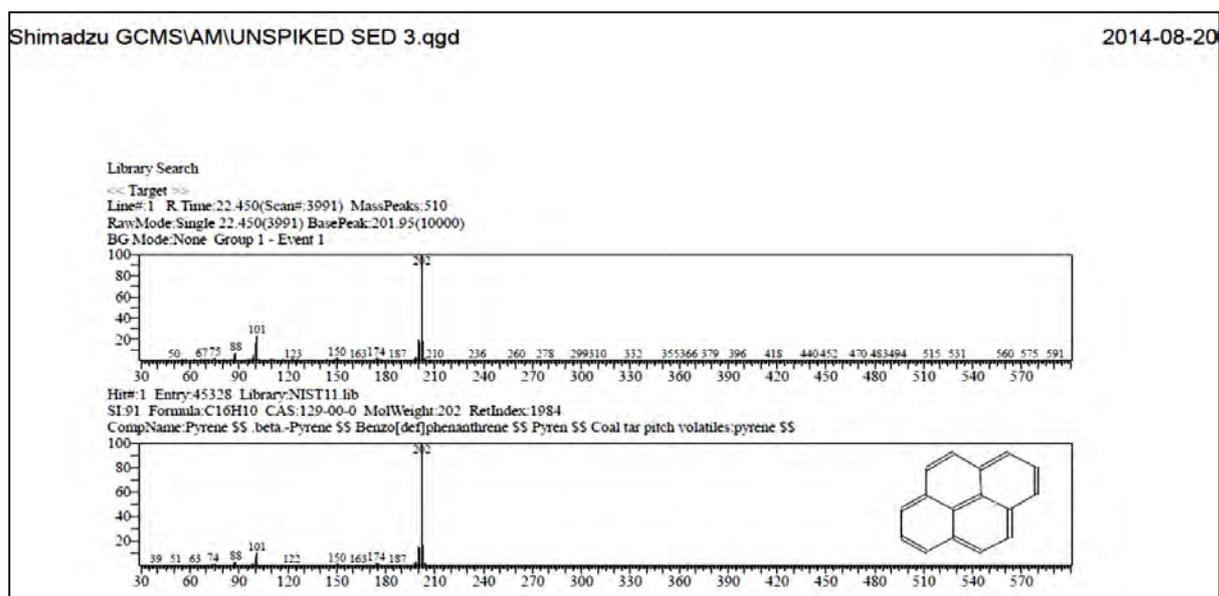

Figure 2: A mass spectrum showing the result from library search for pyrene (202.0 g/mole) in the unspiked surface sediment collected from the Msunduzi Town sampling site.
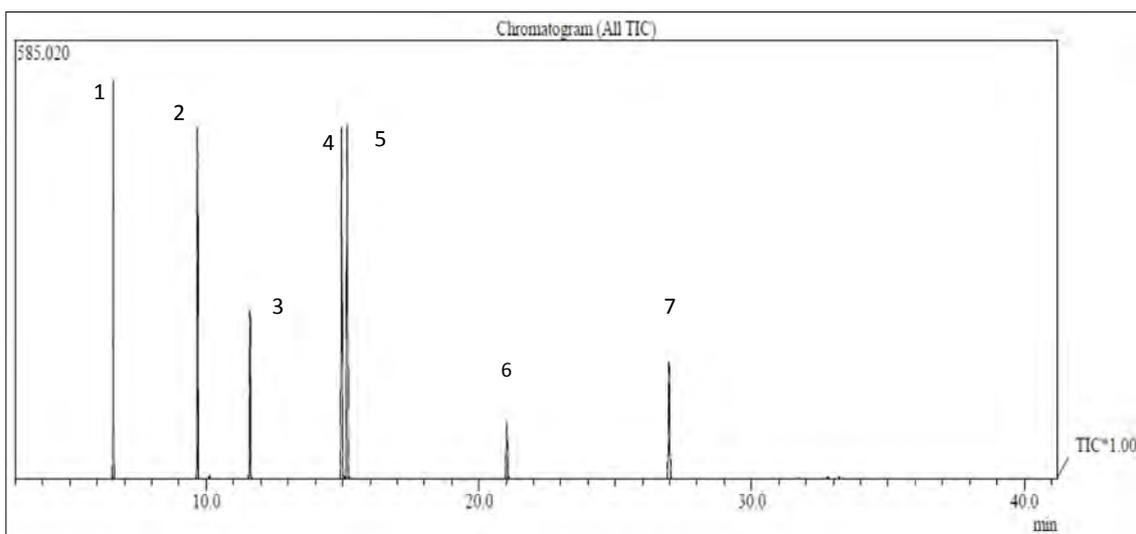
Figure 3: Chromatogram of seven PAH standards in SIM mode. 1 (Naphthalene), 2 (Acenaphthylene), 3 (Fluorene), 4 (Phenanthrene), 5 (Anthracene), 6 (Pyrene) and 7 (Chrysene).

As shown in Figure 3, SIM mode was used to identify and confirm the retention times as well as the molecular weights of the selected 7 PAHs during this study.
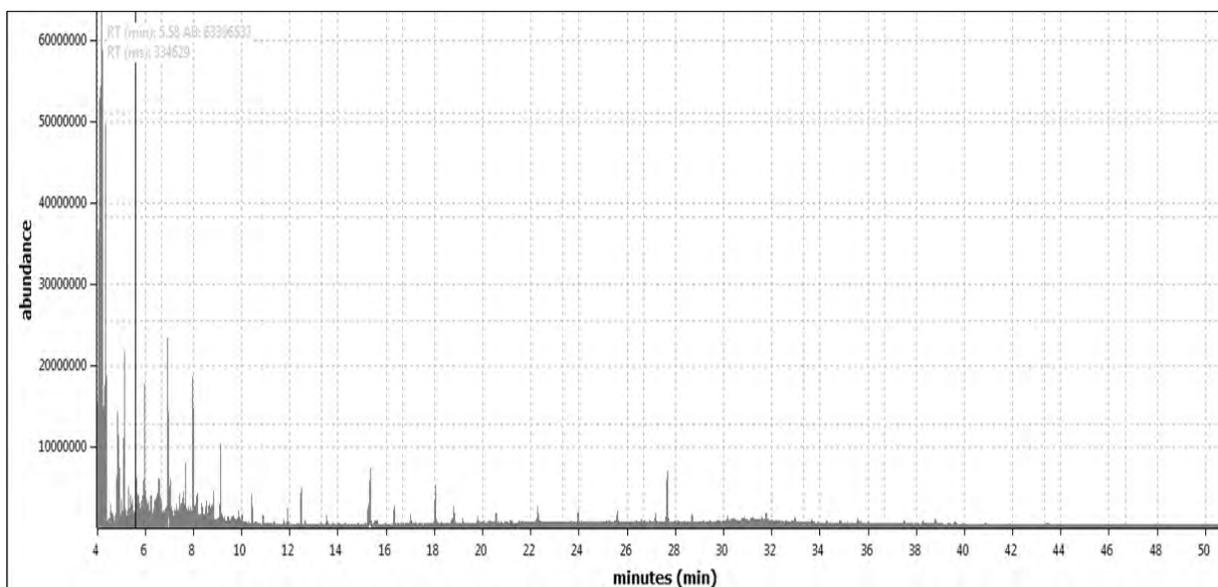

Figure 4: Chromatogram of spiked soils with naphthalene-d8 and 7 PAH standards collected from the Msunduzi Town sampling site.

156
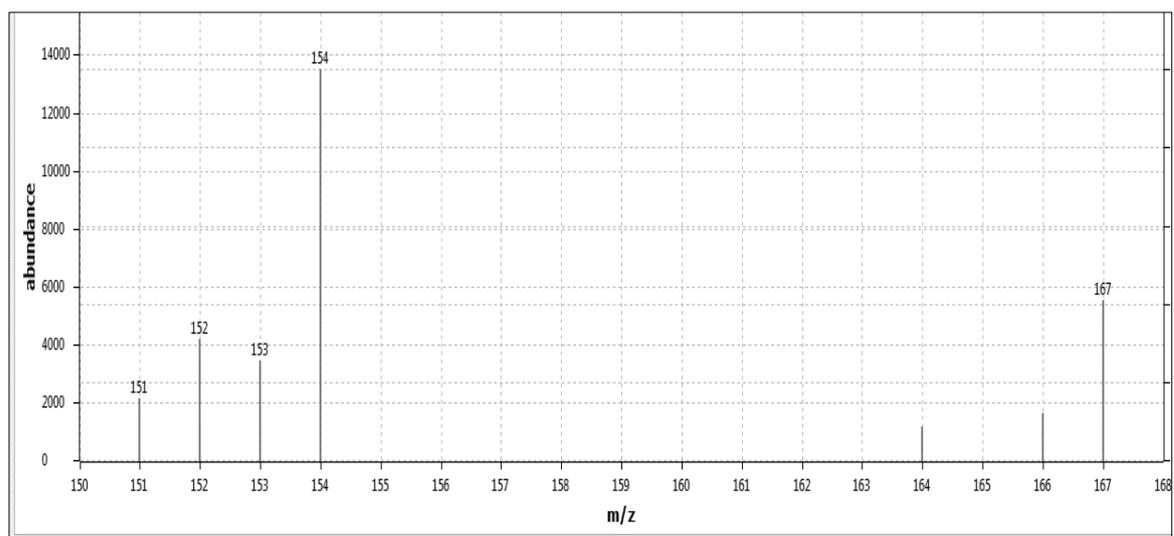
Figure 5: Spectrum showing the fragment ions or *m/z* (151, 152, 153 and 154) of acenaphthylene detected in soil collected from the Msunduzi Town sampling site.
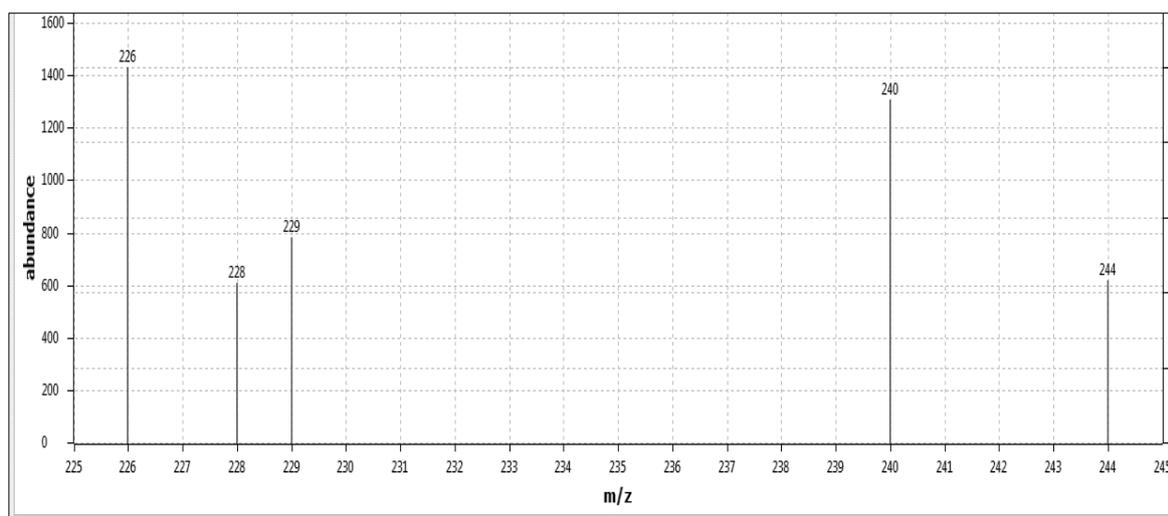

Figure 6: Spectrum showing the fragment ions or *m/z* (226, 228 and 229) of chrysene detected in soil samples collected from the Msunduzi Town sampling site.
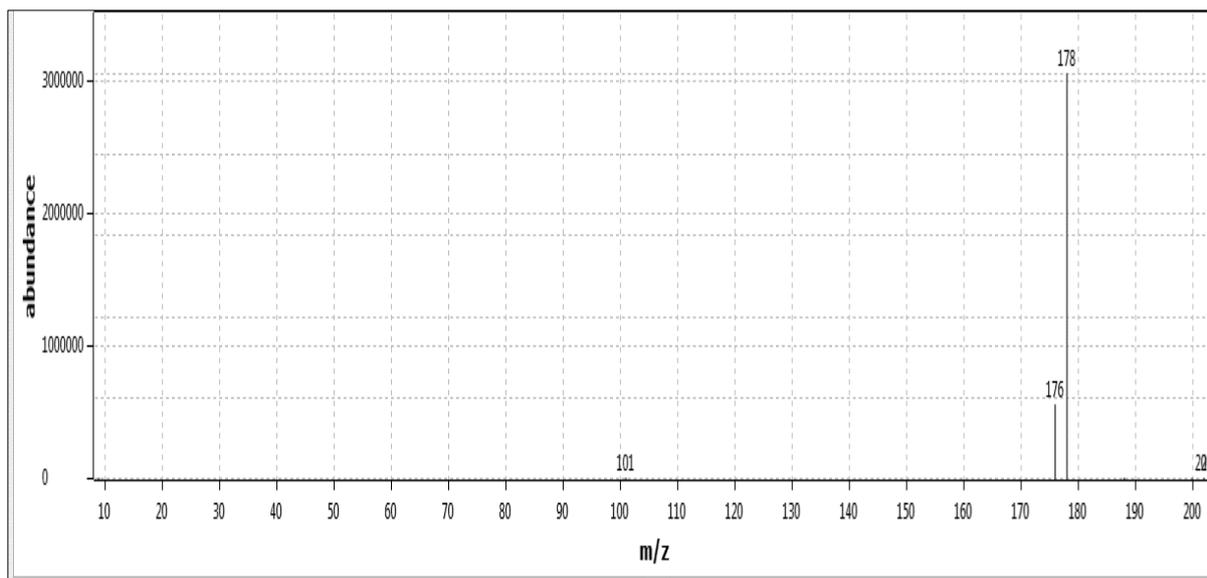
Figure 7: Spectrum showing the fragment ions or *m/z* (176 and 178) of phenanthrene detected in soil samples collected from the Msunduzi Town sampling site.
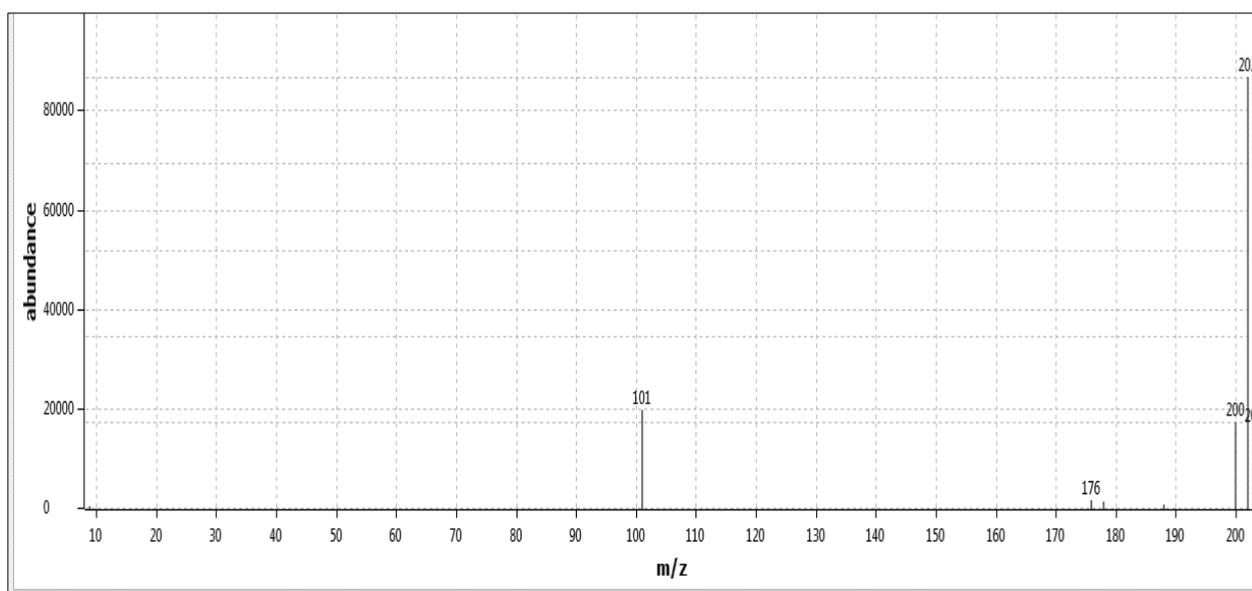
Figure 8: Spectrum showing the fragment ions or *m/z* (101, 200, 202 and 203) of pyrene detected in soil samples collected from the Msunduzi Town sampling site.
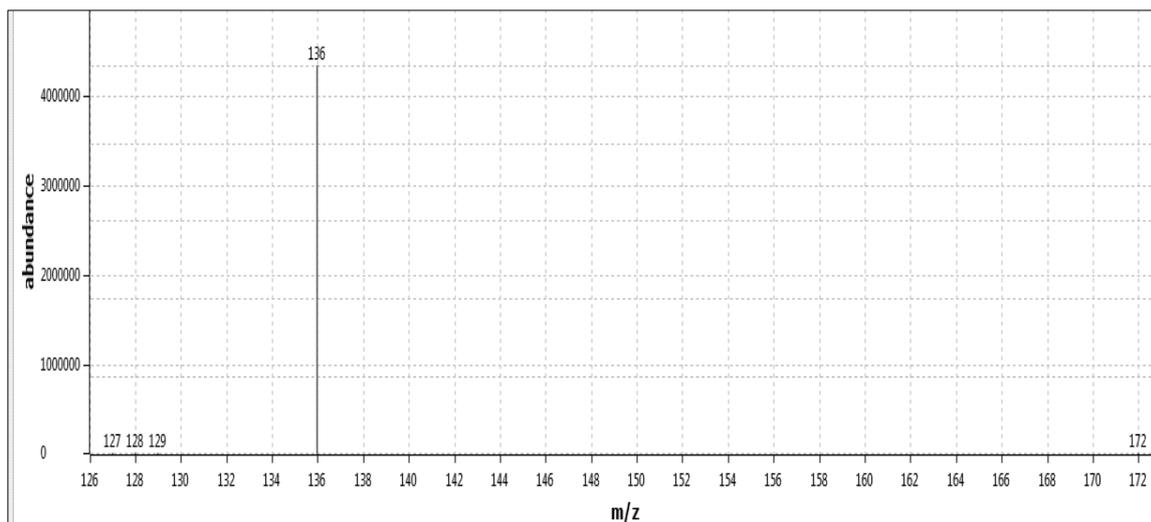
Figure 9: Spectrum showing the *m/z* (136) of naphthalene-d8 (isotope of naphthalene) in soil samples collected from the Msunduzi Town sampling site.