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MODIFICATION, DEVELOPMENT AND APPLICATION OF EXTRACTION METHODS FOR PAHs IN SEDIMENTS AND WATER

by

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A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry

Johannesburg, 2012

Declaration

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in Chemistry to the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other university.

……...

(Signature of candidate)

……day of……2012

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are an important class of persistent organic pollutants (POPs) that are commonly found in the environment at low concentrations. POPs are long-lived organic compounds and originate almost entirely from anthropogenic activities such as chemical industries, combustion and agriculture activities. Owing to their potential carcinogenicity, mutagenicity and teratogenicity, PAHs have been determined in several matrices, particularly water, soil and sediment. Hazards associated with these compounds are owing to their hydrophobic character, persistence and bioaccumulation properties of several individual PAHs. Hence the primary goal of this research was to identify and determine PAHs in water and sediment samples, from rivers and dams in greater Johannesburg area, South Africa.

In this work, three extraction techniques have been optimized for the extraction of PAHs in various samples. The extraction techniques based on hollow fiber liquid phase microextraction (HF-LPME) and solid phase extraction (SPE) were optimized for aqueous samples. Microwave assisted extraction technique (MAE) was optimized for solid samples. The optimized methods were applied to real water and sediment samples in and around Johannesburg area. HF-LPME and SPE techniques were compared with each other, while MAE was compared with Soxhlet (SE) in terms of their extraction efficiencies, enrichment factors, detection limits, relative standard deviations and concentrations of PAHs found in real samples.

HF-LPME technique involved extraction of PAHs from a 20 mL sample containing 20% acetonitrile as a modifier. The PAHs were extracted into a 5 cm hollow fibre filled with heptane as organic solvent. At a stirring speed and extraction time of 600 rpm and 30 minutes, respectively, the acceptor solvent was collected to be analysed by GC-MS. The obtained enrichment factors ranged from 40 to 95 and the recoveries ranged from 3-8% depending on individual PAHs. The detection limits ranged from 23.0-95.0 ng L^{-1} while relative standard deviations for the recoveries were less than 5%, (n=3). The concentrations obtained in real water samples ranged from 30.3-213.8 ng L^{-1} and the relative standard deviations were between 0.8-11.9%, (n=3).

SPE technique involved extraction of PAHs from a 100 mL sample containing 10% methanol as a modifier. The PAHs were extracted using C_{18} cartridges with 40% methanol in water as conditioning solvents and 3 mL acetone: THF (1:1) as eluting solvents. After eluting, the analyte is reduced to 1 mL under nitrogen and then analysed using GC-MS. The obtained enrichment factors ranged from 78-135 depending on the individual PAH. The detection limits ranged from 20.0-52.0 ng L^{-1}. The relative standard deviations for the obtained enrichment factors were less than 6%. The obtained concentrations from real water samples ranged from 21.4-615.7 ng L^{-1} and the relative standard deviations were between 1.9-13.0%, (n=3).

In MAE technique, the extraction was carried out with 20 mL of hexane: acetone (1:1, v/v) mixture with 1 g sample at 250 W for 20 minutes. After extraction, the extract was cleaned and reduced to 1 mL under nitrogen and then injected into an HPLC-Fluoresence system. The obtained recoveries ranged from 61-98% depending on the individual PAHs. The detection limits obtained ranged from 0.03-0.5 µg L^{-1} for HPLC-Fluorescence. The relative standard deviations were less than 6% for the obtained enrichment factors. The obtained concentrations in the sediments ranged from 61-45281µg kg^{-1} and the relative standard deviations ranged from 2.1-10.8%, (n=3).

The possible major sources of PAHs pollution in rivers and dams in great Johannesburg area was suspected to be due to poor wastewater and solid waste management in informal settlements and a large oil spill accident that happened. This is supported by the large concentrations of PAHs found in sediments from Jukskei River which passes through one of the largest informal settlements. The pH in this river is also basic with large conductivity suggesting the presence of anions. The recipient dam of this river had also problems of algae and weeds growing in it. The influence of oil spill that happened is supported by decreasing concentrations of PAHs in the sediments with distance away from the accident area in the Blaauwpan dam. Other possible sources of PAHs are vehicle emission since studies have shown that from about 2005, the number of these in high ways in the city have been increasing by about 15-20% per year.

Dedication

This thesis is dedicated to my late mom, who taught me that perseverance is the key to success and that those who never quit are those who win in life. I also dedicate it to my granny who taught me to know who I am, to keep on believing in myself and in all I want my life to be.

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Abbreviations

Ace	Acenaphthene
ACN	Acetonitrile
ATSDR	Agency for Toxic Substances and Disease Registry
BaP	Benzo(a)pyrene
C_{18}	Octadecyl
Chry	Chrysene
CI	Chemical Ionization
CPE	Cloud point extraction
CPI	Positive Chemical Ionization
DLLME	Dispersive liquid-liquid micro extraction
DMC	Dichloromethane
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
ECD	Electron capture detector
EI	Electron Ionization
EMEP	European Monitoring and Evaluation Program
EPA	American Environmental Protection agency
FD	Fluorescence detector
FID	Flame Ionization Detector
Fl	Fluoranthene
FSESI	Florida Spectrum Environmental Services Incorporation
GC	Gas Chromatography
HF-LPME	Hollow fiber-liquid phase micro-extraction
HLLE	Homogeneous liquid-liquid extraction
HSDB	Hazardous Substances Data Bank
LLE	Liquid Liquid Extraction
LOD	Limits of Detection
LPG	Liquified Petroleum Gas
LPME	Liquid Phase Micro Extraction
MAE	Microwave Assisted Extraction

MASE	Microwave Assisted Solvent Extraction
MMLLE	Micro-porous membrane liquid liquid extraction
MS	Mass Spectrometry
MSPD	Matrix solid phase dispersion
NCI	Negative Chemical Ionization
nd	Not detected
NIWR	National I nstitute for Water Research
Np	Naphthalene
NR	Not Reported
OSPAR	Oslo and Paris Convention
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
Ph	Phenanthrene
POPs	Persistent Organic Pollutants
Pry	Pyrene
RSD	Percentage Relative Standard Deviation
SBSE	Stir bar sorptive extraction
SDME	Single drop micro extraction
SE	Soxhlet Extraction
SFE	Supercritical Fluid Extraction
SIM	Selective ion monitoring
SLM	Supported liquid membrane extraction
SPE	Solid Phase Extraction
SPME	Solid - phase micro extraction
THF	Tetrahydrofuran
UE	Ultrasonic extraction
UNEP	United Nations Environmental Programme
VOC’s	Volatile Organic Compounds
WWF	World Wide Fund for nature

Chapter One – Introduction

1.1 Background

The intensive development of civilization together with evolution of industry has caused profound changes in the quality of the environment in which human beings live. Our world is full of synthetic chemicals which pollute air, water, soil and food. These chemicals can be found in places at a very long distance from their place of production or use. Human activities also introduce many kinds of chemical activities into the atmospheric environments of urban areas through manufacturing industries, automobiles, etc (Matsumoto and Hanya, 1980).

Studies in the last few years have showed the presence of more than 600 organic compounds in the environment, the most important of which belong to the following classes: petroleum hydrocarbons, polycyclic aromatic hydrocarbons, ketones, aldehyde and alcohols (Grynkiewicz *et al.*, 2002). Organic pollutants are brought to the atmosphere due to their volatility. They either evaporate from the earth's surface or through emissions from the human activities and are subsequently transported with the masses of air over long distances. Water in clouds become saturated with these substances and precipitation contaminates surface waters and soil, sometimes far away from the emission sources (Grynkiewicz *et al.*, 2002).

Organic pollutants are mainly classified as either volatile organic compounds (VOCs) or semi volatile organic compounds, most of these are also persistent organic pollutants (POPs). The class of VOCs includes species with different physical and chemical behaviors. Pure hydrocarbons containing C and H as the only elements (e.g. alkanes, alkenes, alkynes and aromatics) are an important class of VOCs. POPs on the other hand are chemical substances that persist in the environment, bioaccumulate through the food web and also pose a risk of causing adverse effects to human health and the environment. With the evidence of long-range transport of these substances to regions where they have never been used or produced and the consequent threats they pose to

the whole environment, their continual monitoring is therefore essential to minimize their effect in the environment (UNEP, 2009).

There is a growing concern about persistent organic pollutants (POPs) including PAHs. In May 2001, a global treaty for the regulations of POPS was signed: the 'Stockholm convention' which includes instruments for the total elimination of the 12 POPs on a global scale (Garban *et al.,* 2002). Large-scale programs are conducted on a long-range transboundary atmospheric pollution (European Monitoring and Evaluation Program, EMEP) or their discharge in to the sea (Oslo and Paris Convention OSPAR). PAHs are part of the list of the 12 POPs and benzo(a)pyrene (BaP) is the most toxic of the PAHs. The objective of this convention is to control, reduce or eliminate discharges, emission and losses of POPs into the environment (Garban *et al.,* 2002).

Owing to the different physicochemical properties of organic contaminants, PAHs tend to interact to different extent with water, soil/sediments and biota. Sediment-pore water interaction is one of the most dominant process controlling the distribution and behavior of PAHs in the river (Guo *et al.,* 2009). Dynamics of a river or dam ecosystems are complex with some pollutants adsorbed onto organic matter while some undergo microbial degradation etc. Since POPs are capable of undergoing bioconcentration and biomagnifications, comprehensive risk assessment can only be performed by monitoring the presence and levels of these compounds in common freshwater fish of the region.

The levels and presence of pollutants in fish therefore have a direct bearing on human health risk. The concentration of pollutants in fish also presents the bioavailable fraction of the pollutants in water bodies. Measurements of the levels of PAHs in water, fish and sediments give almost a complete picture of the distribution of the chemical in the aquatic ecosystem. However, chemical analysis of PAHs in fish is more complex than in water and sediments. The complexity of PAHs analysis in fish is due to their rapid metabolism by fish, which then lead to steady state tissue levels of these compounds and thus account for the failure to demonstrate appreciable levels of PAHs in the sample. It is also due to the accumulation and depuration of PAHs in fish which can be

influenced by various factors including route and length of exposure, lipid content of tissues, environmental factors, age, sex and exposure to other xenobiotics (DouAbul *et al.*, 1997). Therefore, often water and sediment samples are assessed for PAHs or other organic pollutants.

The adverse effect of PAHs on human health as a result of inhalation of dust particles have been described as the most detrimental to human health. This is because small particles are associated with the higher pollutants concentrations. Also soil ingestion has been recognized to be as an important exposure route as water and food to human (Mostert, 2008).

PAHs are widespread environmental contaminants that have been studied due to their carcinogenicity, mutagenicity and teratogenicity. Therefore monitoring the level of PAHs in the environment is of paramount importance, especially because of their toxic nature (Marlow and Hurtubise, 2004: Guo *et al.*, 2009).

The field of sample preparation for environmental monitoring has undergone a revolution in the last 30 years. The conventional methodologies are gradually being replaced by modern instrumental techniques (Hussen, 2007). The concern of using hazardous solvents in the laboratories, the cost of solvent disposal and also the demand for sample throughput and the increasing number of substances that need to be analyzed, have pushed the development of instrumental approaches forward. The modern techniques address the need for reduction of solvent use, automation and miniaturization. They are typically designed and more complex, with heaters, valves and pumps to facilitate a level of process tuning that is not attainable with conventional techniques. In general the modern techniques are easier to operate compared to conventional methods but provide optimization challenges (Hussen, 2007).

The available literature as well as the investigations by the authors indicates that although there are excellent techniques for separation and detection of PAHs, e.g. GC and HPLC, a number of problems and difficulties have been encountered in the determination of these compounds (Adkonis *et al.*, 2006). Samples are not introduced

directly into chromatographic systems as a result of numerous limitations, the main ones being low concentration levels of analytes compared to the detection capabilities in chromatographic techniques, the presence of minerals, solid particles, and high-molecular-weight compounds in a soil sample. Consequently, a sample preparation step has to precede the final determination so that the sample introduced onto a chromatographic column yields reliable results and causes a minimum disturbance in the operation of a chromatograph (Adkonis *et al.,* 2006).

In the case of determination of analytes belonging to PAHs (as well as the majority of other organic compounds), sample preparation requires the following steps, sample collection, transport, storage, isolation of analytes, extract cleanup, validation and finally determination of analyte. Chromatographic techniques are well developed and the final determination step yields reliable results. It is therefore essential to realize that if an error is made at one or more of the earlier stages of an analytical process, the use of even the most sophisticated instrumentation will not ensure a reliable final result of the analysis (Adkonis *et al.,* 2006).

The importance of the initial stages of analysis is due to the tendency of organic compounds (especially PAHs) to adsorb both on suspended matter and the walls of containers and equipment used for their determination (Adkonis *et al.,* 2006). This tendency results from their physicochemical properties, such as low solubility in water as a result of their hydrophobic nature or low volatility of the analytes due to their high boiling points. These properties may cause problems and difficulties at the initial stages of the analytical procedure. Despite the available literature, standards and recommendations, there is still little information regarding the problems encountered during the initial steps of sample preparation for the final determination. Moreover, there is no systematic knowledge and quantitative description of these problems (Adkonis *et al.*, 2006).

This thesis therefore covers both traditional (Soxhlet extraction) and modern (microwave assisted extraction, solid phase extraction and hollow fiber liquid phase microextraction) sample preparation techniques. The techniques were first optimized in

order to get best extraction parameters and then applied to real samples for the determination of PAHs in water and sediment samples in rivers and dams around great Johannesburg area in South Africa.

1.2 Problem Statement

PAHs are of special interest because of their carcinogenicity, mutagenicity and teratogenicty. Their significant importance lies on the awareness about their biochemical and toxicological roles in humans and animals (Ogunwokan *et al.,* 2003). Because of their many sources, they are wide spread in the environment. In South Africa, very little information is available on the distribution and levels of PAHs in the environment (Das *et al*., 2008; Nekhavhambe, 2008; Quin *et al.,* 2009; Niewoudt *et al.,* 2011). It is therefore imperative to develop/modify extraction techniques that are most suitable for their monitoring in the environment. In this work SPE, HF-LPME and MAE extraction techniques have been optimized and then applied to real water and sediments samples for the determination of PAHs.

Chapter Two – Literature Review

2.1 Introduction

This chapter discusses the findings of a number of researchers in the literature with regards to PAHs in the environment. Issues that are discussed in this chapter include the history, uses, properties as well as health and environmental effects of PAHs. It also reviews various sample preparation techniques used by other researchers worldwide to determine PAHs in liquid and solid samples. Finally, review includes also the separation and detection techniques for PAHs.

2.2 Polycyclic Aromatic Hydrocarbons (PAHs)

2.2.1 What are PAHs?

Polycyclic aromatic hydrocarbons (PAHs) also known as polynuclear aromatic hydrocarbons are organic compounds consisting of two or more fused benzene rings in a linear or cluster arrangement. They are very stable organic pollutants that are made up of only carbon and hydrogen. Generally they occur as complex mixtures, not as single compounds. PAHs usually occur naturally, but they can also be synthesized as individual compounds for research purposes. As pure chemicals, PAHs generally exist as colourless, white or pale yellow-green solids (ATSDR, 1990). They can have a faint and pleasant odor (Crystal and Foster, 1991).

They are known to have carcinogenic, mutagenic and teratogenic properties. Due to these properties as well as their persistence in the environment, they have been placed on the list of priority pollutants by the United States Environmental Protection Agency (US-EPA) and also the European Environment Agency (Adkonis *et al*., 2006). It is therefore important that their concentrations in the environment be monitored (Charalabaki *et al*., 2005). Furthermore they have high boiling and melting points as well as high molecular weights and are able to survive high temperatures from the

combustion of fuel from automobiles and airplanes engines. Most of them have low water solubility (Nekhavhambe, 2008). More detailed description of some PAHs is given below:

i) Naphthalene

Naphthalene is also referred to as naphthalin, bicyclo[4.4.0]deca-1,3,5,7,9-pentene or antimite. It is a crystalline, white solid with formula $C_{10}H_8$ which is a structure of two fused benzene rings. It is known as the traditional or primary ingredient of mothballs. Naphthalene is volatile, it forms a flammable vapor and it readily sublimes at room temperature thus producing a characteristic odor that is detectable at concentrations as low as 0.08 ppm by mass (Amoore *et al.*, 1983). It had been derived from a kind of naphtha (a broad term encompassing any volatile, flammable liquid hydrocarbon mixture, including coal tar). Exposure to large amounts of naphthalene may damage or destroy red blood cells (Amoore *et al.*, 1983).

ii) Acenaphthene

Acenaphthene is also known as 1,2-dihydroacenaphthylene, which emphasizes that it is a hydrogenated form of acenaphthylene. It is a PAH that is made up of naphthalene with an ethylene bridge that is connecting positions 1 and 8. It is also a constituent of coal tar and has the formula $C_{10}H_{12}$ (ATSDR, 1990).

iii) Phenanthrene

Phenanthrene is a PAH made up of three fused benzene rings and has the formula $C_{14}H_{10}$. The name phenanthrene is a composite of phenyl and anthracene. In its pure form, it is found in cigarette smoke. It is known as an irritant and photosensitizing skin to light. It appears as a white powder with blue fluorescence. It is obtained by fractional distillation of coal-tar oils (Wendland *et al.*, 1963).

iv) Fluoranthene

Fluoranthene is a PAH that is made up of naphthalene and benzene unit that are connected by a four-membered ring. It has a molecular formula $C_{16}H_{10}$ and it is a structural isomer of the alternant PAH pyrene. Nevertheless it is not as thermodynamically stable as pyrene because its electrons cannot resonate throughout the complete structure as the corresponding ones in pyrene. It is found in many combustion products, along with other PAHs. Its presence is an indicator of less efficient or lower-temperature combustion, since non-alternant PAHs are less preferred information than alternant PAHs. Fluoranthene has also been isolated from coal tar pitch (FSESI, 2009).

v) Pyrene

Pyrene is a PAH with a molecular formula $C_{16}H_{10}$ and made up of four fused benzene rings, which results in a flat aromatic system. It is a colourless solid which is the smallest peri-fused PAH (one where the rings are fused through more than one face). It is formed during incomplete combustion of organic compounds. Pyrene was first isolated from coal tar, where it occurs up to 2% by weight (Senkan *et al.*, 2003). As it is a peri-fused PAH, pyrene is much more resonance stabilized than its five-member-ring containing isomer fluoranthene. Therefore it is produced in a wide range of combustion conditions. For example, automobiles produce about 1 μg/km (Senkan *et al.*, 2003). Although it is not as problematic as benzopyrene, animal studies have shown that pyrene is toxic to the kidneys and the liver (Senkan *et al.*, 2003).

vi) Chrysene

Chrysene is a PAH with the molecular formula $C_{18}H_{12}$ and made up of four fused benzene rings. It is a natural constituent of coal tar, from which it was first isolated and characterized. It is also found in creosote, which is a chemical used to preserve wood. Chrysene is formed in small amounts during the burning or distillation of coal, crude oil and plant material (FSESI, 2009). The name chrysene originates from Greek *Χρύσος*

(*chrysos*), which is thought to be the proper color of the compound at the time of its isolation and characterization (FSESI, 2009). However, high purity chrysene is colorless, the yellow colour being due to the traces of its yellow-orange isomer tetracene, which cannot be separated easily (FSESI, 2009).

Table 2.1: Toxic PAHs listed on priority list by United States Agency for Toxic Substances and Disease Registry (US EPA), (Ravindra *et al*., 2008)

Priority PAHs		
Acenaphthene	Fluoranthene	Benzo(k)fluoranthene
Naphthalene	Chrysene	Benzo(a)pyrene
Acenaphthylene	Benzo(a)anthracene	Dibenzo(a,h)anthracene
Anthracene	Benzo(b)fluoranthene	Benzo(ghi)perylene
Phenanthrene	Benzo(j)fluoranthene	Indeno(1,2,3-c,d)pyrene
Pyrene		

2.2.2 Sources of PAHs

The sources of PAHs are both natural and anthropogenic. The natural sources include forest fires, volcanic and bacteria decay of organic materials (Naufal, 2008). The anthropogenic sources may be divided into five categories: industrial, automobile, agricultural, natural and domestic (Bjorseth *et al.,* 1979):

i) Industrial sources

The main industrial sources of PAHs include coke oven, aluminium production, iron and steel foundries, coal gasification and coke production (Ravindra *et al.,* 2008). Also petroleum refining and thermal power plant, as well as occupations where co altars, pitch, asphalt (bitumen), shell-oil and creosotes are used which emits much of the PAHs associated with air pollution (Bjorseth, 1979). To study the extent of air pollution by PAHs size fraction of the airborne particulate matter together with the concentration of PAH associated with each size fraction has been conducted (Bjorseth, 1979). The bulk of the PAH are found in those size fractions that are of a respirable size, i.e. 72% and

98% of the particulate PAHs are found attached to particles below 3 μm and 7 μm, respectively (Bjorseth, 1979). This finding explains the epidemiologically proven dangers of the tarry fumes that can be inhaled inside the coke oven plants. Industrial sources of PAHs emission are believed to be decreasing because of the improved energy management which leads to improved combustion thus resulting in lower emissions (Bjorseth, 1979). Although some industries are associated with other chemical pollution indicators, (e.g. metals, other pollutant etc), it is unlikely that there are industries that can be source specific PAH 'finger prints' (Bjorseth, 1979).

ii) Mobile sources

Modes of transport reliant on a combustion engine are the major sources of PAHs. These include aircrafts, shipping, railways, automobiles and other motor vehicles including off-road vehicles and machinery (Ravindra *et al.*, 2008; Bjorseth, 1979).

Motor Vehicles (automobiles, lorries, motorcycles): Motor vehicle internal combustion engines are generally fuelled by gasoline (petrol) or diesel fuels. One of the major influences on the production of PAHs from gasoline automobiles is the air-to-fuel ratio. It has also been found that the amount of PAHs in engine exhaust decreases with a leaner mixture (Ravindra *et al.*, 2008; Bjorseth, 1979). The use of catalytic converters has also been shown to have a significant effect on the reduction of the PAH concentration in the exhaust gases (Bjorseth, 1979). All internal combustion engines have varying PAH emission characteristics which depends on engine temperature (particularly cold-start), load, fuel quality and speed (Bjorseth, 1979). Diesel fuelled vehicles have higher particulate emissions compared to gasoline fuelled vehicles. The particles consist of combustion-generated soot, a solvent extractable hydrocarbon fraction and a mineral fraction. PAHs are found within the solvent extractable fraction (Bjorseth, 1979). There are very few studies that have been carried out on PAHs emissions from off-road vehicles. Those studies include a wide range of vehicle types, from garden/agricultural engines to military vehicles (Bjorseth, 1979).

Railways: The main source of PAHs emissions in rail transportation is the use of diesel and diesel-electric locomotives (Ravindra *et al.*, 2008). The coal-fired steam locomotives no longer represent a large proportion of the rolling stock in operation in the world. Due to some locomotives that are old and produce large amounts of black smoke, they may be a significant source of PAH but no measurement data are available (Bjorseth, 1979).

Aircraft: Few studies have been carried out on PAH emissions of aircraft and most of them have been military aircraft. The results showed that PAH emissions are dependent on fuel composition (volatility) and on the power setting of the engine and tend to decrease as the power setting increases (Ravindra *et al.*, 2008; Bjorseth, 1979).

iii) Domestic sources

The domestic sources of PAH which can influence ambient air quality are mainly heating and cooking. Domestic heating that is based on the combustion of fuels varies from the use of natural gas or liquefied petroleum gas (LPG) through paraffin and heating oil to wood, coal, peat and brown coal. Domestic sources of PAHs are geographically widespread and the PAH emissions are largely unregulated (Bjoserth, 1979).

The burning of wood, coal and peat in open fireplaces is often for the decorative effect in addition to the heating provided. These systems are often hand fed with a low thermal efficiency and they potentially have high PAH emissions. Benzo(a)pyrene (BaP) from these sources is associated with a range of particle size including the <2.5μm fraction (Ravindra *et al.*, 2008; Bjorseth, 1979).

iv) Agricultural sources

Agricultural sources include the following activities, stubble burning and open burning of brushwood and straw among others. All of these activities involve the burning of

organic materials under sub-optimum combustion conditions, thus it is expected that a significant amount of PAHs are produced (Ravindra *et al.*, 2008).

v) Natural sources

Natural sources of PAHs include the accidental burning of forests, woodland etc. due to lightning strikes etc. Meteorological conditions (such as wind, temperature, humidity) and fuel type (moisture content, green vs. seasoned wood, etc.) may play an important role in the degree of natural PAHs production. No data are available regarding these emissions and their contribution to the overall PAH profile (Ravindra *et al.*, 2008).

It has been estimated that stationary sources account for approximately 90% of total PAHs emission, but this is not true in urban and suburban areas where the mobile sources are prevailing (Caricchia *et al.*, 1999). The highest concentrations of atmospheric PAHs can be found in the urban environment, due to the increasing vehicular traffic and the scarce dispersion of the atmospheric pollutants. The risk associated with human exposure to atmospheric PAHs is highest in the cities, considering the density of population (Caricchia *et al.*, 1999). It is however important to note that the contribution of any PAHs source to the atmosphere will depends on a number of factors including the emission rate of the source, its geographical location and the local climatic conditions (Baek *et al.*, 1991).

There are high concentrations of PAHs that are present in crude oil, coal and oil shale (Harvey, 1997). These petroleum and petrochemical products are extensively used to produce fuels and synthetics (fibers and plastics). The widespread use of petroleum products has therefore led to an increase in the level of PAHs in the environment (Naufal, 2008). Vehicle emissions constitute another major source of PAHs, especially in urban areas which generate approximately 35% of PAHs emissions (Naufal, 2008). The chemical composition and concentration of PAH mixtures vary according to the temperatures at which they were generated. High temperatures in the absence of oxygen usually lead to formation of simple mixtures of unsubstituted PAHs (Naufal, 2008). Intermediate temperatures such as smoldering wood will result in more complex

mixtures including alkyl substituted PAHs (Harvey, 1997). At lower temperatures, the reaction rates are slow and the predominant products are methyl and other alkyl substituted polyarenes (Harvey 1991). As a result of an increase in fossil fuel consumption, the levels of PAHs tend to be higher in cold winter months (Naufal, 2008). Also atmospheric conditions, such as temperature inversions, may increase PAHs concentrations near ground level (Naufal, 2008).

2.2.3 Formation of PAHs

There are two general major mechanisms that result in the formation and transformation of PAHs during the combustion of fossil fuel. These are pyrolysis and pyrosynthesis (Sun, 2004). During pyrolysis, the macromolecular aromatic compounds in coal or heavy oil are broken into different size fragments which fragment and then decompose to form small organic fragments (Sun, 2004). In the process of pyrosynthesis, these small fragments, mainly highly reactive free radicals, undergo cyclization and aromatization reactions to form polycyclic compounds (Sun, 2004). In addition to the cyclization of small units to form PAHs, it has been shown that reactions among the growing aromatic species, (e.g. PAH-PAH radical recombination and addition reactions) also contribute to the formation of larger PAHs (Sun, 2004). Straight chain hydrocarbons such as those from diesel fuel undergoes cyclization to form PAHs (Dobbins *et al*., 2006).

2.2.4 Molecular structures and Physical Properties of PAHs

The general physical characteristics common to PAHs are high melting and boiling points and very low solubility in water (Table 2.2). PAHs are relatively neutral and stable molecules. They have low solubility in water and low volatilities except small components like naphthalene. The solubility of PAHs in water decreases with increasing molecular weight. Their liphophilicity is high, as measured by water-octanol partition coefficients (Henner *et al.,* 1997). Due to their hydrophobic nature, the concentrations of dissolved PAHs in water are very low. They also show long half lives in geological media. For example, in aerobic sediment, their half lives range from three

weeks for naphthalene up to 300 weeks for benzo (a) pyrene. PAHs are regarded as POPs in the environment and this persistence increases with an increase in the ring number and condensation degree (Henner *et al.*, 1997).

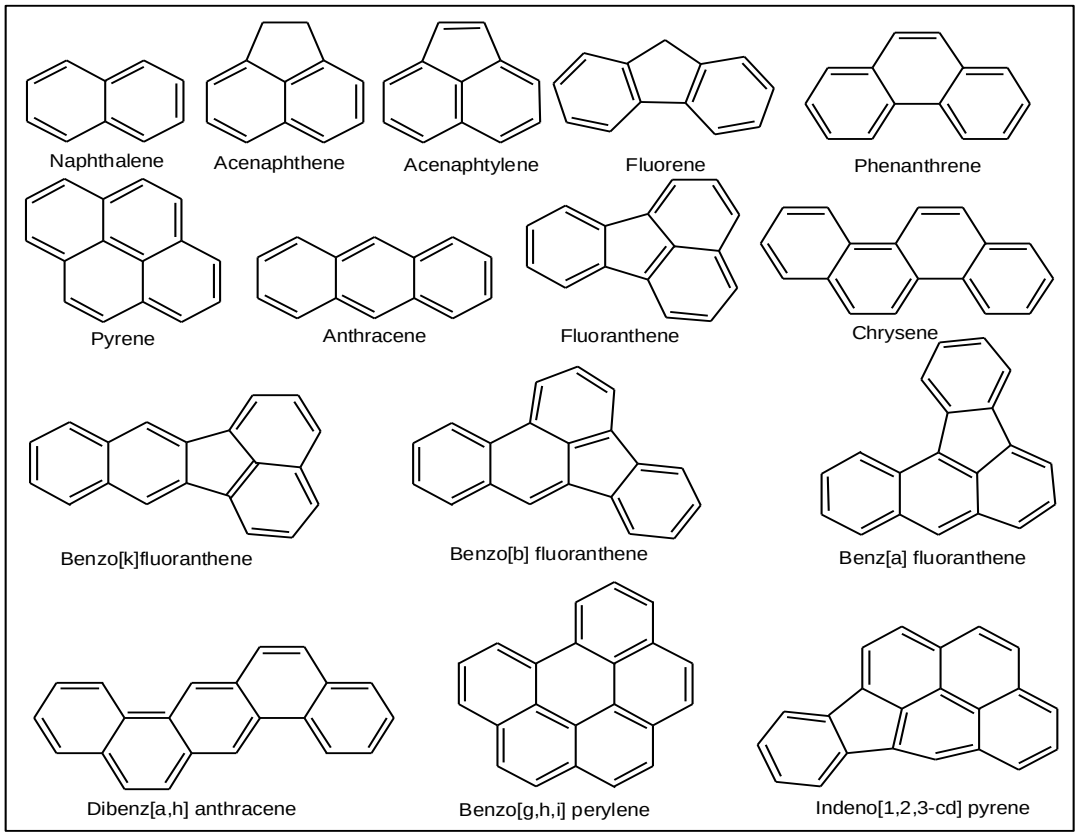


Figure 2.1 Structures of common PAHs (Zuydam, 2007).

Physical properties of some of the PAHs defined as priority pollutants by the American Environmental Protection agency (EPA) are shown in Table 2.2 (Gaga, 2004).

Table 2.2: Physical Properties of PAHs (Manoli and Samara, 1999; Nekhavhambe, 2008; Zuydam, 2007).

PAH	Chemical Formula	Molecular Weight ($gmol^{-1}$)	Water Solubility	Melting Point ^{o}C	Boiling Point ^{o}C	Log K_{ow}	Vapour Pressure kPa
Naphthalene, Np	$C_{10}H_{12}$	128	2.169	81	218	3.37	$1.1x10^{-2}$
Acenaphthene, Ace	$C_{10}H_{12}$	154.2	3.93	93.4	279	3.98	$2.1x10^{-3}$
Phenanthrene, Ph	$C_{14}H_{10}$	178.2	1-1.6	100	340	4.45	$2.3x10^{-5}$
Fluoranthene, Fl	$C_{16}H_{10}$	202.3	0.206	107	384	4.90	$6.5x10^{-7}$
Pyrene, Py	$C_{16}H_{10}$	202.3	0.129	147	404	4.88	$3.1x10^{-6}$
Chrysene, Chry	$C_{18}H_{12}$	228.3	0.0015	254	448	5.61	$5.7x10^{-10}$

2.2.5 Uses of PAHs

PAHs are produced on a small scale for research and commercial purposes. For example anthracene is used as an intermediate in the production of dyes and in the manufacture of synthetic fibres (Hawley, 1987). It is further used as diluents for wood preservatives, and is also useful in the synthesis of the chemotherapeutic agent (Amascrime) (Wardler *et al*., 1986). Acenaphthene is also used as an intermediate in dye production and in the manufacture of pharmaceuticals and plastics, insecticides and fungicides (Windholz, 1983). Fluorene is utilized in the formation of polyradicals for resins and dyestuffs (HSDB, 1994). Phenanthrene is also used in dyestuff, explosives and biological research (Hawley, 1987; HSDB, 1994). Fluoranthrene is used as a lining material to protect the interior of steel and ductile-iron drinking water pipes and storage tanks (NRC, 1983). Pyrene on the other hand is used commercially to make dyes and dye precursors. Chrysene is used in the manufacture of some dyes. Naphthalene is also used in the synthesis of 2-naphthol, a precursor for various dyestuffs, pigments, and rubber

processing chemicals and other miscellaneous chemicals and pharmaceuticals (Collin *et al.*, 2003).

2.2.6 Effects of PAHs

The effects of PAHs are mostly known from animal experiments, but because of the similarity of biological systems in different species, it is likely that all mammals including humans will be affected in a similar way. This is unless they metabolize these substances differently, because in general, it is the metabolic products of the PAHs which give rise to their toxicity (Gaga, 2004).

Several PAHs (including benz(a)anthracene, benzo(a)pyrene (BaP), benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(ah)anthracene, indeno(1,2,3-cd)pyrene) have caused tumours in laboratory animals by inhalation, oral ingestion or by skin contact (Potter, 1994). Experiments with BaP, which is a representative of other cancer-causing PAHs, show that fish seem to be most susceptible to peak exposures of BaP, particularly after earlier exposure to lower levels (Potter, 1994). This has been found to be possibly caused induction of the metabolizing enzymes required to activate the BaP (Potter, 1994; Goulomb *et al.*, 1997).

Also PAHs disrupt the sex hormones and thus potentially possess reproductive and developmental toxicity (Thomas, 1990). There is currently a lack of comprehensive data on the reproductive and developmental effects of many individual PAHs and even for BaP, the data are conflicting. Although animal studies suggest effects on sperm quality on men, females may be at increased risk of reproductive dysfunction because oocyte and follicular destruction can occur as a result of exposure (Thomas, 1990). Since the testes and the ovaries contain rapidly proliferating cells, they are probably particularly susceptible to damage by PAHs (Thomas, 1990).

Studies suggest that PAHs can affect the immunocompetence of wildlife and humans (WWF, 1998). At high doses BaP has been shown to markedly inhibit the immune

system (WWF, 1998). Furthermore, scientists have demonstrated suppression of immune reactions in fish taken from environments highly contaminated with PAHs (WWF, 1998). Also skin exposure to PAHs may result in an increased risk of developing skin cancer, particularly where there is exposure to sunlight (WWF, 1998). Furthermore, other adverse effects of PAHs that have been noted include sebaceous gland destruction, skin ulcerations, and alterations in epidermal cell growth (WWF, 1998).

2.2.7 Standard exposure limits of PAHs in various environmental compartments

The maximum allowable concentrations (MACs) of PAHs in soil and water (ATSDR, 2006) are presented in Table 2.3. No standards exist for the amount of PAHs allowed in the air, but it is recommended that the levels be no higher than 0.004 ppm.

Table 2.3: Maximum allowable concentrations (MACs) of PAHs in soil and water (ATSDR, 2006)

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

2.2.8 Exposure pathways of PAHs to humans

PAHs are present throughout the environment, therefore humans may be exposed to these substances at home, in the fields or at the workplace. Typically, humans will not be exposed to an individual PAH but to a mixture of PAHs. However, the occurrence of PAHs in urban air has caused particular concern because of the continuous nature of the exposure and the size of the population at risk. This is because the urban atmosphere is a very complex and dynamic system consisting of a large variety of interacting chemical species in both the gas and the particulate phases (ATSDR, 2003).

There are four different modes by which human bodies may be exposed to PAHs compounds. These are direct inhalation of polluted air, direct inhalation of tobacco, ingestion of contaminated processed food, water and lastly through dermal contact.

i) Direct inhalation of polluted air

Breathing air contaminated with PAHs in the workplace of coke, coal-tar and asphalt production plants, smokehouses as well as municipal trash incineration facilities are all exposure through air (ATSDR, 2003). Other exposure through air includes breathing air containing PAHs from wood smoke, vehicle exhausts, asphalt roads or agricultural burn smoke. Although environmental air levels are lower than those associated with specific occupational exposures, they are of public health concern when they are spread over large urban populations. The background total levels of seventeen of the ATSDR's toxicological profile priority PAHs in ambient air should be between 0.02–1.2 ng m^{-3} in rural areas and 0.15–19.3 ng m^{-3} in urban areas (Becher and Bjorseth, 1983).

ii) Direct inhalation of tobacco

Cigarette smoking and environmental tobacco smokes are also other sources by which man can be exposed to PAHs (ATSDR, 2003). Smoking one cigarette can yield an intake of 20-40 ng of benzo (a) pyrene. Smoking one pack of unfiltered cigarettes per day yields 0.7 µg per day benzo(a)pyrene exposure, while smoking a pack of filtered cigarettes per day yields 0.4 µg per day (ATSDR, 2009). Environmental tobacco smoke

contains a variety of PAHs, such as benzo(a)pyrene and more than forty are known or suspected as human carcinogens. Side-stream smoke (smoke emitted from a burning cigarette between puffs) contains PAHs and other cytotoxic substances in quantities that are much higher than those found in mainstream smoke (exhaled smoke of smoker) (ATSDR, 2009). Furthermore, more than 70 PAHs compounds have been analyzed in cigarette smoke, hence smokers have eight times more probability of cancer attack than non smokers. For instance, tobacco smoking alone accounts for 30% of total mortality due to cancer every year (Martin, 2008).

iii) Ingestion of contaminated and processed food and water

Swallowing food or drinking water that contain PAHs are other routes for PAHs to enter the body, but absorption is generally slow when PAHs are swallowed. PAHs can enter all the tissues of the body that contain fat. They tend to be stored mostly in the kidneys, liver and fat. Smaller amounts are stored in the spleen, adrenal glands and also ovaries (ATSDR, 2009). PAHs are changed by all tissues in the body into many different substances of which some of them are more harmful and some are less harmful than the original PAHs. Exposure through food is mainly through eating contaminated meat such as eating grilled or charred meats and contaminated cereals, flour, bread, vegetables, fruits, meats as well as processed or pickled foods. PAH concentrations in foodstuffs vary (Adamson and Thorgeirsson, 1995). Charring meat or barbecuing food over a charcoal, wood or other type of fire greatly increases the concentration of PAHs. For example, the PAH level for charred meat can be as high as 10–20 µg/kg. Charbroiled and smoked meats and fish contain more PAHs than do uncooked products, with up to 2.0 µg/kg of benzo(a)pyrene was detected in smoked fish (ATSDR, 2009). Tea, roasted peanuts, coffee, refined vegetable oil, cereals, spinach and many other foodstuffs may also contain PAHs. Some crops such as wheat, rye and lentils may synthesize PAHs or absorb them via water, air, or soil (Buha and Williams, 2011). Another mode of exposure to PAHs is drinking contaminated water or cow's milk. Nursing of infants by mothers who are already exposed to PAHs can also transfer PAHs through their mother's milk. This is because PAHs bioaccumulate in fatty tissue of living organisms which include the milk (ATSDR, 2009).

iv) Dermal contact

Mixtures of carcinogenic PAHs cause skin disorders in humans and animals. However, specific effects in humans of individual PAHs, except for benzo[a]pyrene, have not been reported. Adverse dermal effects have been noted in humans following intermediate-duration dermal exposure to benzo[a]pyrene in patients with the pre-existing dermal conditions of pemphigus vulgaris (acute or chronic disease characterized by occurrence of successive crops of blisters) and xeroderma pigmentosum (a rare disease of the skin marked by disseminated pigment discolorations, ulcers and cutaneous and muscular atrophy) (Cottini and Mazzone, 1939). 1% benzo[a]pyrene solution typically applied to patients with pemphigus resulted in local bilious eruptions characteristic of the disease.

The extent to which humans are exposed to PAHs is a function of several parameters. These include the prevailing atmospheric conditions, concentrations in ambient air, partition between the gas and particle phase and the size distribution of airborne particulates. Risk assessment associated with inhalatory PAHs uptake is often estimated on the basis of the B[a]Py concentration in air. The evaluation of the health risk due to inhalatory exposure to PAHs is based on epidemiological findings. However, it has to be considered that B[a]Py is just one carcinogenic compound in a mixture of carcinogens in the atmosphere (Papageorgopoulou *et al.*, 1999).

Not only does the PAH itself play a major role in the adverse biological outcomes of the exposure, but so does the size of the particle on which it may be adsorbed. Particulates less than 10 mm in diameter are more likely to contain greater amounts (per unit mass) of PAHs due to their large surface area to volume ratio. This is a great concern since it is the smaller diameter particles that are retained by the lung (Duggan, 2001). In the human respiratory system, particles with diameters larger than 10 mm do not reach the thorax, particles ranging from 2.1 to 10 mm are preferentially retained by pharynx, trachea and bronchi while particles below 2.1 mm can reach terminal bronchi and alveoli. Therefore, a physical detrimental action of inhalable particles (i.e., the

development of pulmonary emphysema) is observed along with the chemical impact due to that particle toxicity (Cecinato *et al.,* 1999).

2.2.9 Fate of PAHs in the environment

PAHs enter the air mostly as releases from volcanoes, forest fires, burning coal and automobile exhaust. Once in air they can be attached to dust particles. These can thus travel long distances depending on wind speed. Some PAH particles can readily evaporate into the air from soil or surface waters. Although PAHs can break down by reacting with sunlight and other chemicals in the air, over a period of days to weeks and they are generally known to be persistent. They can also enter surface water through discharges from industrial plants and waste water treatment plants. They can be released to soils at hazardous waste sites if they escape from storage containers. The movement of PAHs in the environment depends on properties such as how easily they dissolve in water (although in general they do not easily dissolve in water), and how easily they evaporate into the air (ATSDR, 1996).

PAHs can be released from their sources either in a gas phase or they can be associated with particles by nucleation and condensation, forming particulate matter. The particulate form of PAHs are initially in the gaseous phase at high combustion temperature, however when the temperature decreases, gaseous phase PAHs adsorb or deposit on fly ash particles (Zuydam, 2007). The smaller the particle size the greater the surface area for the adsorption of PAHs. The ambient temperature is very important for the gas-particle distribution of PAHs. They are present in air as vapours or stuck to the surfaces of small solid particles. They can travel long distances before they return to earth in rainfall or particle settling (Zuydam, 2007).

As mentioned earlier most PAHs do not dissolve easily in water, they therefore adhere to solid particles and settle to the bottoms of lakes or rivers. This is because these are hydrophobic with high octanol-water partition coefficient (Irwin *et al.*, 1997). Microorganisms can also break down PAHs in soil or water after a period of weeks to months. PAHs in soil are most likely to stick tightly to particles. However certain

PAHs can still move through soil to contaminate underground water. The type of soil determines how long the PAHs stick on the soil particle. Clay soil for example will adsorb more PAHs compared to sandy soil. The concentration of PAHs may be higher in plants and animals than in soil or water in which they live and this is due to possible magnification and bioaccumulation of PAHs especially in animals (ODH, 2004).

2.2.10 PAHs in the Atmosphere

Polycyclic aromatic hydrocarbons are emitted into the atmosphere either as vapours or associated with primary aerosol particles. Once they are in the atmosphere, the residence times and ultimate fates of these semi volatile chemicals depend on their distributions among vapour, particle and droplet phases. This partitioning is in turn controlled by the vapour pressures, aqueous solubilities of the compounds and by the concentrations and size distributions of particles and droplets in the atmosphere (Poster and Baker, 1996). As the particle associated and gas phase PAHs are transported through the atmosphere, they may be lost to the vegetation, soil, rivers, lakes, and oceans through the processes of dry and wet deposition. Although neither mechanism is adequately understood since pollutant concentrations can be measured directly in precipitation, wet deposition is better characterized quantitatively than is dry (Gaga, 2004).

2.2.10.1 Gas to Particle Distribution of PAHs in the Atmosphere

The distribution of PAH in the atmosphere between the gas and particulate phases is determined by several factors, which include the vapour pressure of the PAH (as a function of temperature), the amount of fine particles (in terms of available surface area for adsorption of PAHs), the ambient temperature, PAH concentration and the affinity of the individual PAH for the particles organic matrix (Baek *et al.,* 1991). Low molecular weight PAH compounds primarily prefer to be in the gas phase while high molecular weight PAHs are primarily associated with the particulate phase. On the other hand gas phase percentages are generally higher in summer than in winter due to

increasing temperature which increases the vapour pressure of the compounds (Odabası, 1998).

The phase (particulate or vapour) in which the chemical will be found is determined by the vapour pressure of a PAH molecule. The approximation for urban particulate matter is that compounds with vapour pressures above 1 x 10^{-5} kPa should occur almost entirely in the gas phase, whereas compounds with vapour pressures less than $1x10^{-9}$ kPa should exist predominantly in the particulate phase. Any compound with a vapour pressure between these approximate limits would be expected to occur in both the vapour and particle phase (Gaga, 2004).

The effect of ambient temperature on the vapour pressure of the PAH is significant and must also be considered. It has been shown that there is approximately an order of magnitude change in the vapour pressure of compounds such as benzo(a)pyrene and coronene for a temperature change of 20 °C (Murroy *et al.*, 1974). Since many regions can experience summer to winter variations of 50 °C or more, the vapour pressure of the PAHs in the ambient environment can vary over two orders of magnitude. This can cause a shift in the vapour to particle distribution of PAHs. Consequently, one would expect to find more PAHs associated with particulate matter in the winter than in the summer (Gaga, 2004).

2.2.10.2 Air Water Gas Exchange of PAHs

Vapour phase PAHs can transfer from the air to the water and vice-versa. This transfer is governed by Henry's law (Equation 2.1) which states that the concentration in water is proportional to the partial pressure of PAHs in air:

$$p_a = H_a X_w \qquad (2.1)$$

Where:

P_a is the partial pressure in air, H_a is Henry's constant (both in pressure units), and X_w is the mole fraction in water. The lower the Henry's constant, the more likely the gas will partition from air to water (Fisher, 2001).

2.2.10.3 Chemical Transformations of PAHs

The atmospheric chemical and photochemical reactions of the PAHs are important for two reasons:

i) Particular PAHs can be removed from the atmosphere as a result of chemical reactions, and

ii) Decomposition products of PAHs may be more hazardous to human health than the PAH from which they were derived (Gaga, 2004).

A number of experimental studies have demonstrated that many PAHs are susceptible to photochemical and/or chemical oxidation under simulated atmospheric conditions (Pitts *et al.,* 1985a; Nielsen, 1984; Kamens *et al.,* 1988). Although results from the laboratory simulation studies are difficult to extrapolate to the reactivities of PAH under real atmospheric conditions, there is however potential for chemical transformation of PAH by gas-particle interactions in emission plumes, exhaust systems or even during atmospheric transport (Baek *et al.,* 1991).

Generally photochemical transformation have been considered to be the most important mode of atmospheric decomposition of PAHs of both phases and the extent of photochemical decay is strongly dependant on the nature of the substrate on which they are adsorbed. Although photochemistry is the major mechanism for decomposition of PAHs, various PAHs may also degrade by non-photochemical pathways such as evaporative or oxidative reactions with gaseous pollutants (Baek *et al.,* 1991).

2.2.11 Degradation of PAHs

Air

The processes that transform and degrade PAHs in the atmosphere include photolysis and also reaction with NOx, N_2O_5, OH, ozone, sulfur dioxide, and peroxyacetyl nitrate (Baek *et al.,* 1991, NRC 1983). Possible atmospheric reaction products are oxy-, hydroxy-, nitro- and hydroxynitro-PAH derivatives. Photochemical oxidation of a

number of PAHs has been reported with the formation of nitrated PAHs, quinones, phenols, and dihydrodiols (Holloway *et al.*, 1987, Kamens *et al.*, 1986). Some of these breakdown products are mutagenic (Gibson *et al.,* 1978). Some PAHs have been degraded by oxidation reactions that have been measured in the dark to eliminate the possibility of photodegradation.

Water

The most important processes that contribute to the degradation of PAHs in water are photooxidation, chemical oxidation and biodegradation by aquatic microorganisms (Neff, 1979). However hydrolysis is not considered to be an important degradation process for PAHs (Radding *et al.*, 1976). The contribution of the individual processes to the overall fate of a PAH will depend largely on the temperature, depth, pollution status, flow rate and oxygen content of the water. As a result, a process that is a major loss/degradation process for a particular PAH in certain surface water may not be in surface water with different water quality.

The rate and extent of photodegradation vary widely among the PAHs. For example, the rate of aquatic photolysis of naphthalene (containing two benzene rings) is much slower than anthracene which contains three benzene rings (Anderson *et al.,* 1983). The rate of photolysis can also be accelerated by the presence of certain sensitizers (Zepp and Schlotzhauer, 1979) and can conversely be decreased by the presence of certain quenchers in water e.g., certain carbonyl compounds. The importance of photolysis will also decrease with the increase of depth in a body of water, particularly in turbid water, because of light attenuation and scattering (Zepp and Schlotzhauer, 1979).

Sediment and soil

Microbial metabolism is the major process for degradation of PAHs in soil environments. Photolysis, hydrolysis, and oxidation are generally not considered to be important processes for the degradation of PAHs in soils (Sims and Overcash, 1983). However, in a study of PAH losses from four surface soils amended with PAHs in

sewage sludge, losses due to volatilization and photolysis from sterilized soils were considered to be important for PAHs composed of less than four aromatic rings, whereas abiotic losses were insignificant for PAHs containing four or more aromatic rings (Wild and Jones, 1993).

The rate and extent of biodegradation of PAHs in soil is affected by environmental factors such as the organic content, structure and particle size of the soil, characteristics of the microbial population, the presence of contaminants such as metals and cyanides that are toxic to microorganisms, and the physical and chemical properties of the PAHs (Wilson and Jones, 1993). Also environmental factors like temperature, pH, oxygen concentration, PAH concentrations and contamination history of soil, soil type, moisture, nutrients, and other substances that may act as substrate co-metabolites influencing the rate of PAH degradation in soil (Sims and Overcash, 1983). This is mainly because these factors may affect the size and composition of microbial populations. For example, in low-pH soils, fungi are dominant over bacteria, and thereby control microbial degradation in these environments. Sorption of PAHs to organic matter and soil particulates also influences the bioavailability, and hence, biotransformation potential, this may limit the biodegradation of compounds that would otherwise rapidly undergo metabolism (Weissenfels *et al.*, 1992).

2.2.12 Organic Matter

Organic matter content is typically measured as total organic carbon (TOC) and dissolved organic carbon (DOC), which are essential components of the carbon cycle. In water the organic content consists of thousands of components which include macroscopic particles, colloids and dissolved macromolecules. Organic carbon content in river water depends on the size of a water region, climate and also the season at which the sample is collected. The organic carbon determination is used to identify the organic substances that are present in sediments. The organic carbon content depends on geographical location, pollutants entering rivers and also the layer depth of the tested sediments (Niemirycz *et al.*, 2006). Dissolved organic matters which are ubiquitous in aquatic systems are largely composed of humic substances and they tend to bind to

PAHs owing to their high content of organic carbon. It therefore can be considered as both the mobile phase for enhancing the solubility of PAHs and sorbed phase itself on soil particle for retarding their mobility (Moon and Park, 2001).

Soil plays a crucial role in the fate transport and behavior of PAHs. PAHs are affected by the pore size distribution in soils as well as the amount and nature of the organic matter. A number of studies has been done and have found organic to be the most important soil component for PAHs sorption. Therefore the bioavailability and environmental persistence of PAHs are most affected by organic matter. The aspects of the nature of organic matter such as its origin, composition and maturation have impact on the sorption of organic pollutants such as PAHs. Soil contains a wide range of physical and chemical different forms of organic matter such as different humic substances, char materials, black carbon (Ahangar, 2010).

Many of the physical, chemical and biological properties of soils are influenced by the organic matter. Soil structure, soil compressibility and shear strength are some of the properties influenced by organic matter. It also affects the water holding capacity, nutrient contributions, biological activity and water and air infiltration rates (Reddy, 2002).

2.3 Sample Preparation Techniques

Sample preparation is among the most important steps in any analytical process. This is due to the fact that it plays an important role in the removal of macromolecules and other matrix constituents that may adversely interfere with the detection system. In addition to that, sample-preparation methods bring about a possibility of enrichment of the analytes in very dilute samples or where low detection limits are required (Jönsson, 1999). Sample-preparation processes therefore have a direct impact on accuracy, precision and quantification limits and are often a limiting step for many analytical methods (Majors, 2002). The main purpose of extraction is to transfer the analyte(s) from the matrix to a suitable medium for introduction into the analytical instrument for analysis or further clean-up prior to analysis. The PAHs might be present in very low

concentrations therefore a pre-concentration step is required for their detection and quantification. As the samples might have complex and many matrix components, a clean-up step is normally required to separate the analyte from the matrix (Guo *et al.*, 2009).

There is a wide variety of extraction techniques used for extracting organic pollutants from liquid and solid samples. Some of the most common sample preparation techniques reported is given in Table 2.4.

Table 2.4: Sample preparation techniques

Liquid samples	**Solid samples**
Liquid-liquid extraction (LLE)	Soxhlet extraction (SE)
Solid phase extraction (SPE),	Microwave assisted extraction (MAE)
Solid phase micro extraction (SPME),	Pressurized liquid extraction (PLE)
Membrane extractions:	Supercritical fluid extraction (SFE)
Supported liquid membrane (SLM) extraction	Matrix solid phase dispersion (MSPD)
Hollow-fiber liquid phase micro extraction	Ultrasonic extraction (UE)
(HF-LPME)	Ultrasonication
Single drop micro extraction (SDME),	
Homogeneous liquid-liquid extraction (HLLE)	
Dispersive liquid-liquid micro extraction	
(DLLME)	
Stir bar sorptive extraction (SBSE)	
Cloud point extraction (CPE),	

With so many extraction techniques available for both solid and liquid samples, the choice depends on the availability of the technique in the laboratory, easy and cost to run it and its selectivity. Soxhlet and liquid-liquid extractions are the oldest techniques but are also the most time consuming, less selective while they use large amount of organic solvents. SFE is one of the most selective techniques for solid samples but is not easily available. In many laboratories SPE is the most popular technique used for liquid samples. The hollow fiber-liquid phase microextraction is the simplest and cheap technique to perform for liquid samples. It also gives very high enrichment factors. Extraction principles and application of some of these techniques are discussed below.

Source of error in sample preparation

The extraction technique is dictated by the sample to be analysed (liquid, sediments or soil). Analyte extraction step has to precede the final determination so that the sample introduced onto a chromatographic column yields a reliable result and causes a minimum disturbance in the operation of a chromatograph (Adkonis *et al*., 2006). Although analysis can be performed in a few minutes, sample preparation can require hours or days for completion. Typically two thirds of analysis time is spent on sample processing (Figure 2.2). Another significant aspect of chromatographic sample preparation is its contribution to analytical error. The accuracy and the precision of data generated are often more dependent upon the operations performed on the sample before analysis rather than upon the measurement itself. Chromatographic sample processing typically accounts for approximately 30% of the error generated during the entire analytical method. Operator error generally accounts for roughly 19% (Figure 2.3), (Majors, 2002). Therefore, improving and automating sample preparation can decrease error by as much as 50% (Shah, 2002).

Sample preparation for organic analysis usually involves extracting the analyte of interest from the matrix into an appropriate manner that is suitable for the introduction into the analytical technique. The most widely used extraction technique is Soxhlet for solid samples. This method is labor intensive and they involve the use of large quantities of solvents, which often must be evaporated to concentrate the analyte before analysis. The filtration and sample transfer steps which are often mandatory also represent a potential source of error from analyte loss (Shah, 2002). SPE and MAE also require solvent evaporation after extraction because extracts are in relatively large volume (few mL) compared to microlitres for injection.

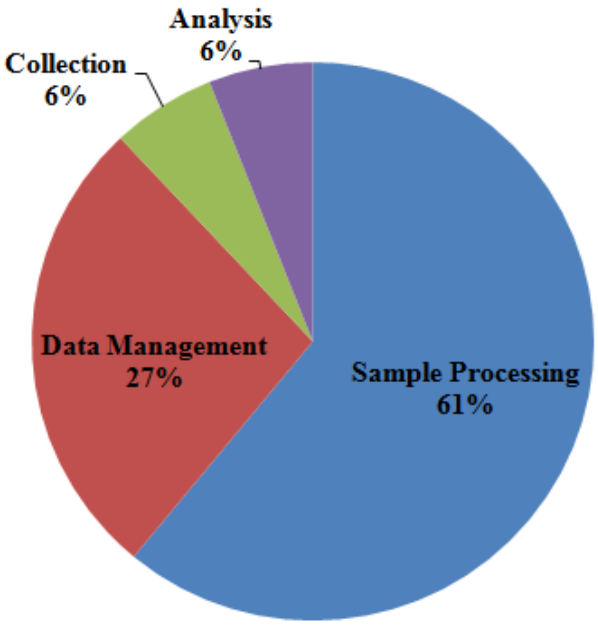


Figure 2.2: Time spent on typical chromatographic analysis (Majors, 2002).

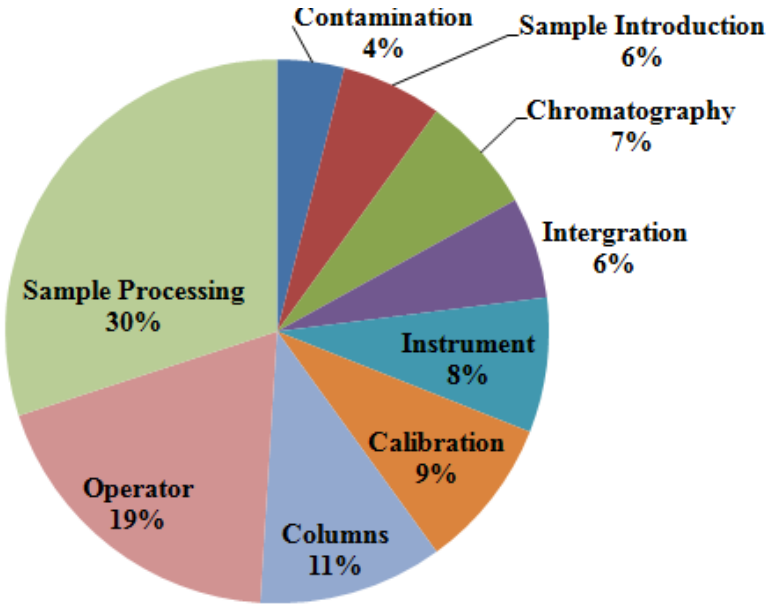


Figure 2.3: Sources of errors generated during chromatographic analysis (Majors, 2002).

Reliable results from the extraction are given by calculating percentage recoveries. This can be done by spiking a sample to be analysed with a known content of the analyte, leaving it to equilibrate and then determine the concentration of the analyte after

extraction. The spike is not always bound in the same way as the naturally occurring compounds, therefore recovery assessments can be overestimated. Certified reference material analysis may be used as a good tool to ascertain accuracy, though it has a limitation as it can only be useful in cases where they contain integral and not spiked species (Nsengimana, 2007). In this work the analytes evaporation was performed at a very slow rate in order to minimise the error due to evaporation. Glass containers were used as appropriate sample containers, since adsorption on to the bottle's surface is likely to occur for PAHs compounds. Brown bottles were used as it reduces photosensitive reactions to a considerable extent (Dryden aqua, 2009). Scrupulous cleanliness of containers was adapted due to the low detection levels. All experiments were done in triplicates. The reference material was also used for the evaluation of extraction methods.

2.3.1 Extraction of PAHs from water samples

2.3.1.1 Hollow fibre micro-extraction technique

The hollow fibre liquid phase micro-extraction (HF-LPME) technique belongs to extraction techniques currently called liquid phase micro-extraction (LPME) technique. These are techniques for extraction of aqueous samples that use small solvent and sample. In this technique, a porous polypropylene hollow fibre strand is used and is filled with a very small volume of acceptor solution (in micro-litre ranges). The filled hollow fibre is then exposed to an organic liquid to impregnate the pores before being placed in an aqueous sample where extraction will take place. Liquid phase micro extraction can be carried out in either a two phase (MMLLE) or a three phase (SLM) depending on the analyte being extracted. MMLLE suites non polar organic compounds like PAHs. In a three-phase system i.e. the supported liquid membrane (SLM), ionisable organic compounds or metal ions are extracted.

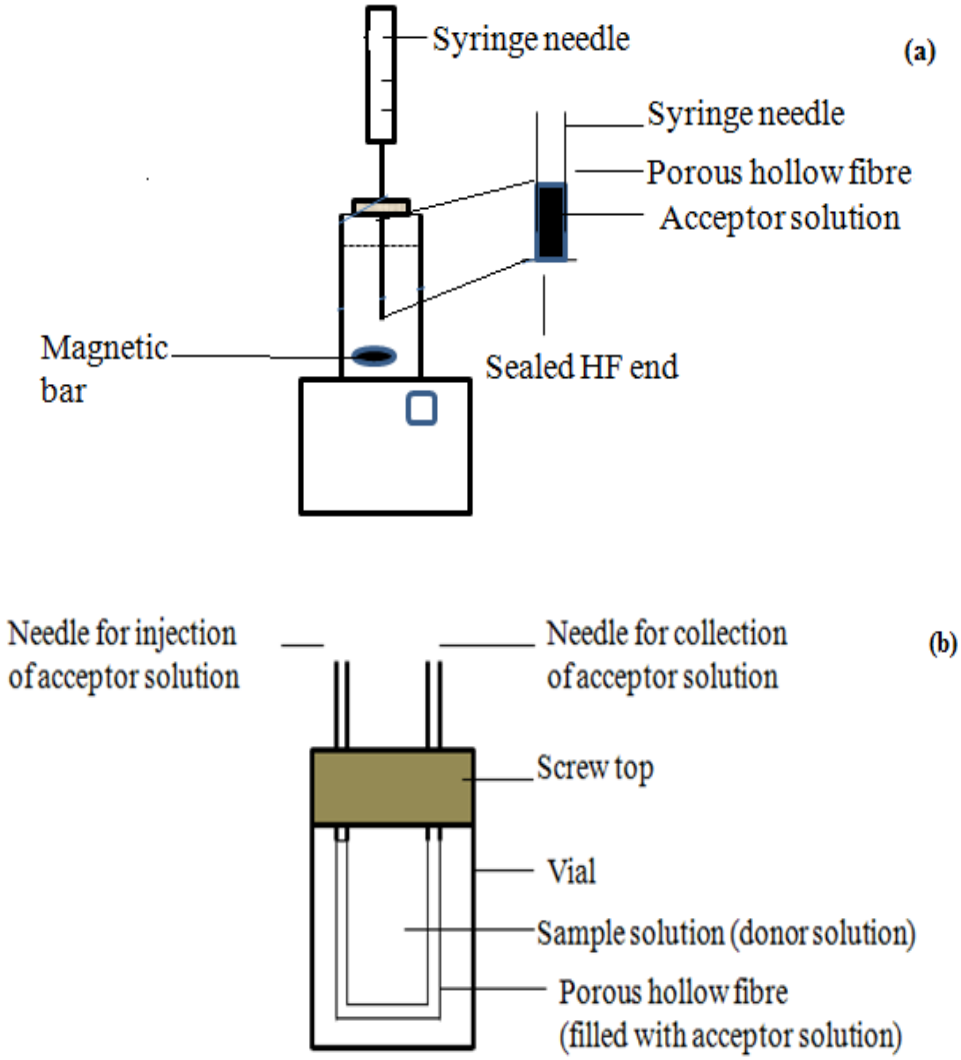


Figure 2.4: Hollow fibre configuration invented by [a) Shen and Lee, 2002, b) Rasmussen *et al.*, 2002].

Theory of HF-LPME two phase system

In two phase HF-LPME, the analytes are extracted from the sample aqueous solution (donor phase) into the organic solvent (acceptor phase) which is present in the pores and also inside the lumen of the hollow fiber (Ho *et al.*, 2002). This process is illustrated by the following equation:

A (donor phase) ← →A (acceptor phase) (2.2)

Where A represents the analyte of interest. The partition coefficient $K_{a/d}$ is:

$$K_{a/d} = \frac{C_{eq,a}}{C_{eq,d}} \quad (2.3)$$

Where $C_{eq,a}$ the concentration of A in the acceptor is phase at equilibrium and $C_{eq,d}$ is the concentration of A in the donor phase at equilibrium. The initial amount of analyte n_i is equal to the sum of the individual amount of analyte present in the two phases during the whole extraction:

$$n_i = n_d + n_a \quad (2.4)$$

where n_d is the amount of analyte present in the donor phase and n_a is the amount of analyte present in the acceptor phase. At equilibrium, Eq (2.4) can also be written as:

$$C_i V_d = C_{eq,d} V_d + C_{eq,a} V_a \quad (2.5)$$

where C_i is the initial analyte concentration in the sample, V_d and V_a are sample volumes donor phase and acceptor phase respectively. At equilibrium, the amount of analyte extracted into the acceptor phase $n_{eq.a}$ of the system can be expressed by:

$$n_{eq,a} = \frac{K_{a/d} V_a C_i V_d}{K_{a/d} V_a + V_d} \quad (2.6)$$

The recovery (R) of the analyte is calculated by the equation:

$$R = \frac{100 n_{eq,a}}{C_i V_d} = \frac{K_{a/d} V_a}{K_{a/d} V_a + V_d} x100 \quad (2.7)$$

The enrichment (E) of the analyte can be calculated by the formula:

$$E = \frac{C_a}{C_i} = \frac{V_d R}{100 V_a} \quad (2.8)$$

C_a is the concentration of A in the acceptor phase at the end of extraction. Eqs. (2.7) and (2.8) may also be used for two phase LLE. For the two phase HF-LPME, the actual recovery is lower than calculated by Eq (2.7) because the fraction of the organic solvent which is immobilized in the pores of the hollow fiber is not available for further analysis. Its only the fraction present in the lumen that may be collected into a micro insert (Ho *et al.*, 2002). In a two phase system (HF-LPME) as described above, the steps for the analyte to be extracted involves: i) Diffusion from the bulk of the sample to the

membrane surface, ii) Partitioning into the organic phase in the pores of the membrane and, iii) Diffusion through the pores into the bulk of the organic acceptor solution.
From the three steps mentioned, since the sample is stirred, diffusion from the bulk of the sample into the membrane surface is not a rate limiting step. Most organic compounds that have been extracted in a two phase system are nonpolar so partitioning into the organic liquid in the pores of the membrane is also not a limiting step. The rate limiting step is often diffusion through the pores into the bulk of the acceptor solution.

The advantages and disadvantages of this technique are summarized below (Li *et al.*, 2008):

Advantages:

- Solvent stability is greatly improved since the organic phase is protected by the hollow fiber.
- Higher stirring rate can be used to reduce equilibrium and extraction time. As a result, better extraction efficiency and sensitivity are achieved.
- The hollow fiber can also prevent the interferences caused by large molecules or suspended solid particles in sample solution.
- Can be very selective.
- Uses little organic solvent, therefore environmentally friendly.
- Faster, simpler and inexpensive.
- High enrichment factors.

Disadvantages:

- Clogging of the pores for samples with high dissolved solids
- Leakage of the phases if organic solvent is partly water soluble or at high pressure.
- Suitable for nonpolar organic compounds.

Application of HF-LPME in water samples

Two phase HF-LPME has been applied for the extraction of PAHs in water samples, some of the extraction solvent, stirring speed, extraction time and hollow fiber length that has been used are reported in Table 2.5. 1-octanol and toluene have been found to be the common solvent for extraction of PAHs. The reason could be their immiscibility with the sample as well as their compatibility with the hollow fiber as it is made with polypropylene which has hydrophobic properties (Frenich *et al*., 2011). Stirring speed of 500-1100 rpms has been reported to be the appropriate speed. The reason for this could be that the solvent is confined in the hollow fiber (which is its holder) which makes the solvent column to be stable and thus not tolerate high stirring speed (Zhao *et al*., 2002). Higher stirring also results in loss of organic solvent due to evaporation during extraction procedure. 10-35 minutes extraction time has been reported. The reason for this could be that the HF-LPME is dependent on the equilibrium rather than exhaustive extraction (Zhao *et al.*, 2002).

Table 2.6 shows some of the detection limits, relative standard deviations, enrichment factors and concentrations that have been obtained in the literature for organic compounds. Two phase HF-LPME has been applied on extraction of organic compounds like PAHs, OCPs ect. This is because they are nonpolar and partitioning into the organic to the pores of the membrane will not be a limiting step. The obtained relative standard deviations ranged from 1.3-13.6% which indicates the reproducibility of the method. The reported detection limits ranged from 0.002-0.74 µg L^{-1}. The enrichment factor ranged from 22.4-167 µg L^{-1}.

Table 2.5: The extraction solvent, stirring speed**,** extraction time and hollow fiber length that has been used in the extraction of PAHs in water samples

Matrix	Extraction solvent Used	Stirring speed (rpm)	HF length (cm)	Extraction Time	Reference
Water	1-octanol	1000	NR	10 minutes	Zhao *et al.*, 2002
Water	1-octanol	1100	6.5	30 minutes	Marlow et al., 2004
Rain water	Toluene	700	1.3	35 minutes	Basheer *et al.*, 2003
Natural water	Toluene	1000	5.1	15 minutes	Charalabaki *et al*., 2005
River water	Toluene	500	NR	15 minutes	Li *et al*., 2008

Table 2.6: Application of HF-LPME in organic compounds

Compound	Analytical Method	LOD ($\mu g\ L^{-1}$)	RSD (%)	Enrichment factors	Concentration ($\mu g\ L^{-1}$)	Reference
PAHs	GC-MS	NR	2.8 – 4.5	42 – 75	-	Zhao *et al.*, 2002
PAHs	Fluorescence spectroscopy	NR	3.5 – 10.6	22.4 – 44.6	6.7 – 44.6	Marlow and Hurtubise, 2004
PAHs	GC-MS	0.002 – 0.74	1.3 – 13.6	46 – 167	0.005 – 0.162	Basheer *et al*., 2003
OCP's	GC-MS	0.013 – 0.059	1.7 – 13.8	63 – 155	0.005 – 0.063	Basheer *et al*., 2003
PAHs	GC-MS	0.005– 0.011	2.7 – 11.3	NR	0.006 – 0.366	Charalabaki *et al*., 2005
PCB's	GC-MS	13 – 41	3.4 – 7.3	718 – 840	0.54 – 2.54	Li *et al.*, 2008
Pesticides	HPLC-MS-MS	0.028 – 0.082	0.2 – 11.8	88 – 96	0.030 – 1.674	Trtić-Petrović *et al*., 2010

2.3.1.2 Solid Phase Extraction (SPE)

SPE is the mostly widely used technique for the preparation and preconcentration of samples. SPE involves a partitioning between the liquid and the solid where the solid material is the extracting/sorbent material (Supelco, 1998). It has been widely used to preconcentrate and remove pollutants from environmental and food samples. Although SPE can be used to prepare liquid samples and extract semi volatile and non-volatile analytes, it can be used with solid samples that are pre extracted into solvents. It is excellent for sample extraction, concentration and cleanup (Supelco, 1998).

Principles of SPE extraction

SPE procedure involves four steps (conditioning the sorbent, sample application, washing and elution of the target analytes). Figure 2.5 shows the steps involved in solid phase extraction. Conditioning is composed of two sub steps. In the first step, the sorbent is passed through the SPE material in order to wet the bonded functional groups and thus prepare the cartridge for reproducible interaction with the sample matrix. The common conditioning solvents are methanol and acetonitrile. The second step equilibrates the sorbent bed. In this case the solvent should be similar (in polarity, pH, etc) to the sample matrix in order to maximize the retention (water or same aqueous solution that the sample is prepared is used). The second conditioning solvent should be weak (low strength) so that it does not act as an eluting solvent. Buffers can be used in order to control ionization of potentially charged compounds (Supelco, 1998). Conditioning is followed by sample application to the conditioned cartridge which then results in the analyte, and perhaps other matrix components being retained on the sorbent surface due to one or more specific chemical interactions. The third step is washing, which is passing solvents through the cartridge with the aim of rinsing away additional interfering compounds while leaving the analyte undisturbed within the sorbent bed. It should remove weakly retained interferences without being strong enough to elute the analyte. Increasing the organic content or decreasing the pH and changing the ionic strength may all help in increasing the clean-up. A common rinse solvent for a non-polar extraction on a C_{18} sorbent would be water. The final step is

elution of the target analyte, which is obtained by passing an appropriate solvent through the cartridge. The eluting solvent is specifically chosen to disrupt the analyte-sorbent interaction, resulting in selective elution of the analyte. The elution step should be able to remove all the target analytes with minimal solvent in order to maximize sensitivity. Sometimes this requires a combination of solvents to break both the primary and secondary interactions (Supelco, 1998). In general, the strength of the solvent is directly related to the target compound. A polar target compound elutes best in polar solvents and vice versa i.e "like dissolve like" principle. Table 2.7 gives a summary of some solvents used in SPE steps for PAHs.

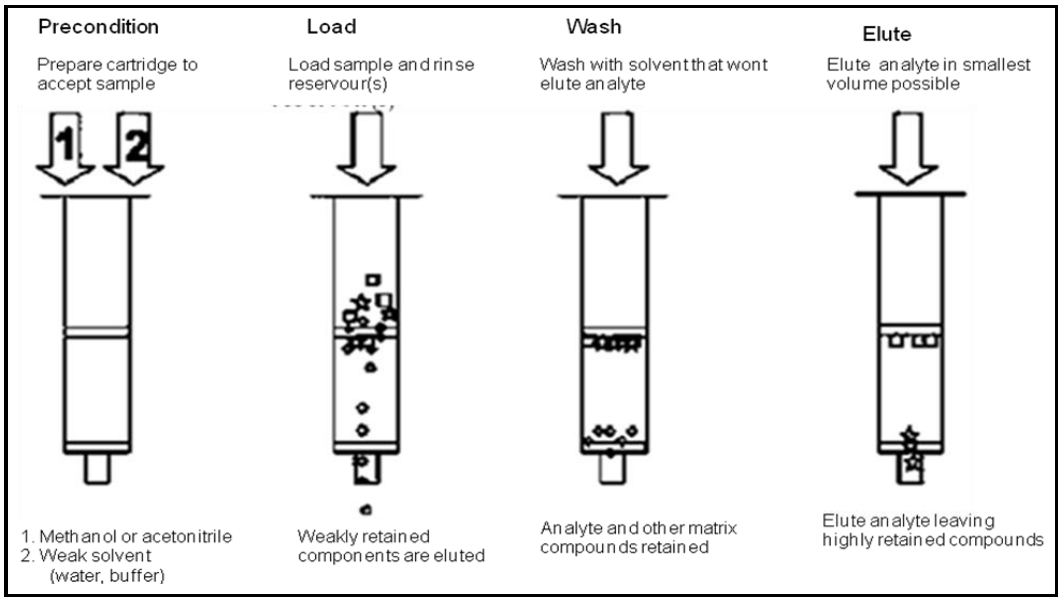


Figure 2.5: Schematic diagram representing the steps involved in solid phase extraction (Supelco, 1998)

Table 2.7: Conditioning, washing and eluting solvents that have been used for PAHs extraction in water

Matrix	Conditioning	Equilibration	Washing	Eluting	Reference
River water	10 mL hexane and methanol	10 mL ultra pure water	NR	15 mL hexane	Ma *et al.*, 2010
River water	10 mL dichloromethane, methanol and acetone	10 mL water	NR	10 mL acetone, methanol and dichloromethane	Zhang *et al.*, 2007
Well water	3 mL methanol and acetone	NR	NR	9 mL dichloromethane	Jungang *et al.*, 2009
Water	5 mL dichloromethane and methanol	10 mL acetone-water	NR	3 mL dichloromethane	Li *et al*., 2007
Water	3 mL acetonitrile/methanol	3 mL water	3 mL water	acetonitrile/methanol	Pinxteren *et al.*, 2009
Water	3 mL methanol	3 mL water	NR	9 mL dichloromethane	Kouzayha *et al*., 2011
Sea water	acetonitrile, ethanol	Water	Deionised water	2 mL benzene	Triantafyllaki *et al.*, 2005
Drinking water	6 mL 2-propanol	6 mL water/2-propanol (9:1, v/v)	2 mL water/2-propanol (9:1, v/v)	2 mL dichloromethane	Kicinski *et al*., 1989

Theory of SPE

The extraction ability of the sorbents in SPE bed depends on the bed capacity, the volume of sample loaded in the bed, the nature and volumes of conditioning solvents and eluents. Other critical parameters include the breakthrough volume, volume of rinsing and eluting solvents which depends on the kinetic properties of the SPE bed, its holdup volume and retention factor (Bielicka-Daszkiewics, 2009). The breakthrough volume (V_B) is the parameter that characterizes the SPE sorbent bed. It is defined as sample volume that can be loaded onto the SPE bed providing a given ratio of outlet to inlet analyte concentration at a given temperature, and it is a maximum sample volume which can be applied with a 100% recovery (Figure 2.6). V_B depends on the concentration of the analytes in the solution that is loaded to the sorbent, the

temperature, the flow-rate and also the number of theoretical plates (Bielicka-Daszkiewicz, 2008).

Breakthrough volume V_B is defined as (Figure 2.6):

$$V_B = V_R - 2\sigma_v \quad (2.9)$$

Where V_R is the retention volume, which can be determined graphically from the diagram. It corresponds to 0.5 of the average value of maximum analyte concentration in the effluent (C_E). It can be expressed analytically by the equation below. σ_V is defined below.

$$V_R = V_M(1-k) \quad (2.10)$$

Where:

V_M is the interparticle volume of the sorbent bed (hold-up volume), k is the retention (capacity) factor of the solute (for the analyte in the sample). σ_V and σ_V^* are the standard deviations of the derivative curve determined graphically from the breakthrough curve. Due to its asymmetry these are given suitably as values corresponding to 0.159 and 0.841 of the average value max concentration of analyte in the effluent (C_E), respectively.

It can be given as follows:

$$\sigma_v = V_M \frac{1+k}{\sqrt{N}} \quad (2.11)$$

N is the plate number for the sorbent bed (Poole *et al*., 2000), calculated by Eq (2.12)

$$N = V_R(V_R - \sigma_V)/\sigma_V^2 \quad (2.13)$$

V_E is the equilibrium volume defined as $V_E = V_R + 2\sigma_v^*$ (2.14)

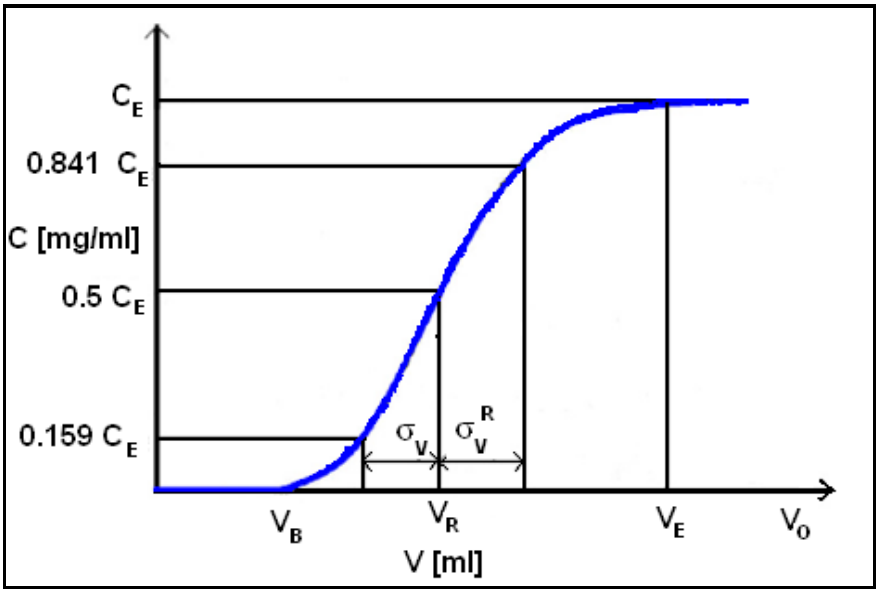


Figure 2.6: Typical breakthrough curve (Bielicka-Daszkiewicz and Voelkel 2009)

Although the solid phase extraction technique is the widely preferred sample preparation technique over classical liquid-liquid extraction, its lack of selectivity is the major drawback. For example when a C_{18} sorbent is used, all non polar analytes compete for retention. This drawback has lead to the development of selective sorbents such as molecular imprinted polymers (Żwir-Ferenc, *et al* 2006). The sorbents typically used in SPE are silica based reversed phase (C_8, C_{18}), normal phase, ion exchange (cation and anion exchange) and adsorption based phases (alumina, graphitised carbon, silica gel) (Oliferova *et. al*., 2005).

Type of SPE formats

The modified silica sorbents are the most commonly used cartridges for extraction of organic compounds. The sorbent is packed as precolumn for online extraction and as cartridges or discs for offline modes. The disc format allows using high flow rates thus reducing the extraction time for large volume of samples. The cartridges are disposable. The precolumn allows online injection of the whole extract onto the separation technique. It thus gives low detection limits.

In SPE the stationery phase comes in a form of a packed syringe-shaped cartridge, a 96 well plate, a 47 or 90 mm flat disk each which can be mounted on its specific type of extraction manifold. The manifold allows multiple samples to be processed by holding several SPE media in place and allowing a number of samples to pass through them simultaneously. Typically cartridge SPE manifold can accommodate up to 24 cartridges while a disk SPE manifold can accommodate 6 disks (Supelco guide to Solid phase, 1998). In this work cartridges have been used (figure 2.7).

Cartridges

Examples of cartridge format

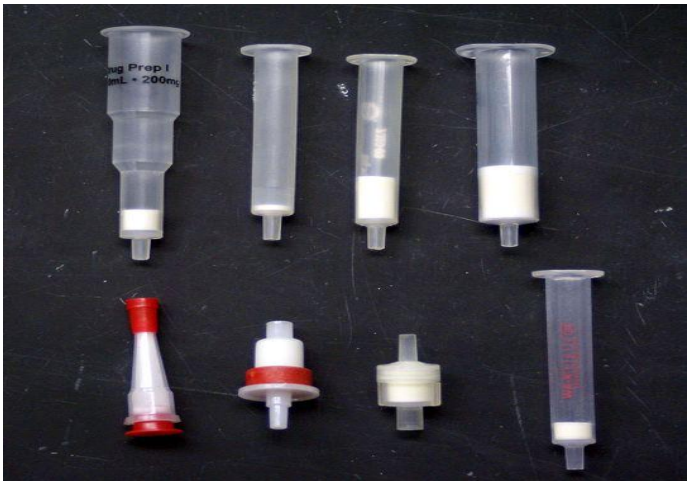


Figure 2.7: Types of SPE cartridges (Supelco, 1998

Type of SPE phases

Solid phase extraction cartridges and disks are available with a variety of stationary phases, each of which can separate analytes according to different chemical properties. Most stationary phases are based on silica that has been bonded to a specific functional group. SPE phases include reversed phase, normal phase, ion exchange and adsorption. In this versed phase was used.

Reversed phase (bonded silica)

This is the most popular SPE sorbent used in sample preparation of organic analytes. The most popular bonded silica is octadecyl (C_{18}). C_8 has also been used in some cases. Reverse-phase SPE sorbents are used to extract non-polar and moderately polar compounds such as pesticides, herbicides, hydrocarbons, steroids and also fat soluble vitamins from polar samples such as water. Retention of an analyte is primarily due to the non polar-non polar interactions and van der Waals or dispersion forces. The retention of compounds on the bonded silica correlates to the octanol-water partition coefficient ($logK_{ow}$). Organic compounds with $logK_{ow}$ above 3 normally give 95% recovery. To obtain a better extraction efficiency and a totally apolar material, the trends are to minimise the number of residual silanol groups of the original silica. For this purpose, a trifunctional silane is used for bonding the n-alkyl chains. After bonding the n-alkyl chains the end endcapping is carried out with trimethylsilane (Hennion, 1999).

Applications of SPE Technique

Table 2.8 gives some results reported in the literature on application of SPE technique on organic compounds. GC-MS has been reported to be mostly employed for analysis of organic compounds. The reason could be it high sensitivity for quantification in selected ion monitoring mode (Ma *et al*., 2010). C_{18} sorbent has also been universally used than other sorbent which could be due to it high recovery and enrichment factors (Ma *et al.,* 2010). The LODs obtained ranged from 1-200 ng L^{-1}. The recoveries ranged from 23-125% with RSDs of 1-29.3%. The reported obtained concentrations ranged from 0.7-35000 ng L^{-1}.

Table 2.8 Application of SPE technique to organic compounds

Compound	Analytical Method	Sorbent	LOD (ng L^{-1})	RSD (%)	Recoveries (%)	Concentration (found) (ng L^{-1})	Reference
PAHs	GC-MS	carbon nano tubes	2 – 8	1.2 - 12.1	75.8 - 125.3	spiked 0.2	Ma *et al.*, 2010
PAHs	GC-MS	C_{18}	NR	1.0 - 11.3	44 - 82.5	0.7 - 38.2	Zhang *et al.*, 2007
PCBs	GC-MS	C_{18}	NR	3.9 - 18.3	56.6 - 120.8	1.6 - 128.2	Zhang *et al.*, 2007
PPCPs	GC-MS	C_{18}	NR	1.2 - 25.2	72.5 - 102.5	4.7 - 113.7	Zhang *et al.*, 2007
PAHs	GC-MS	NR	1	5 – 10	70 – 93	1 – 35000	Cao *et al.*, 2005
Biphenyls	HPLC-UV	Fluorocarbon polymer	5	0.03 – 0.6	NR	40 – 5200	Olifevora *et al*., 2005
PAHs	LC-UV	C_{18}	0.02 – 1.1	4.2 – 17.6	90 – 108	0.2 – 4	Sun *et al*., 1998
PAHs	GC-MS	C_{18}	0.17 – 4.7	1.4 – 17.2	71.8 – 123.6	0.9 – 60.9	Jungang *et al*., 2009
PAHs	GC-MS	C_{30}	7 – 21	0.8 – 9.2	61 – 115	0.27 – 6.39	Li *et al*., 2007
Pesticides	HPLC MS-MS	C_{18}	0.5 – 5.5	8 – 12	Close to 100	1 – 1410	Pinxteren *et al*., 2009
PAHs	GC-MS	C_{18}	NR	1 – 14	70 – 85	spiked 50	Kouzayha *et al*., 2011
PAHs	HPLC-Fluorescence	C_{18}	0.8 – 39	NR	NR	0.9 – 142.2	Triantafyllaki *et al*., 2005
PAHs	GC-MS	C_{18}	0.4 – 200	2.0 – 24.7	23 – 78	spiked 100	Azevedo *et al*., 2004
Pesticides	GC-MS	C_{18}	0.6 – 20	2.1 – 29.3	82 – 119	spiked 100	Azevedo *et al*., 2004

2.3.2 Extraction of PAHs from solid samples

2.3.2.1 Soxhlet extraction (SE)

Soxhlet extraction is recommended by the US Environmental Agency (EPA) for extracting semi-volatile and non-volatile organics from solid matrices (Banjoo *et al.*,

2005). It is a standard method and is preferred because it is easily standardized, with high recoveries when it is compared to matrix dependent techniques like microwave extraction, pressurized liquid extraction and supercritical fluid extraction (Banjoo *et al.*, 2005). A solid material containing some of the desired compound is placed inside a thimble, which is loaded into the main chamber of the Soxhlet extractor (Harwood *et al.*, 1999). The Soxhlet extractor is placed onto a flask containing the extraction solvent. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the material slowly fills the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by siphon side arm, with the solvent running back down to the distillation flask. This cycle repeats many times, over hours or days. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded (Jensen, 2007).

The advantages of SE are summarized below:

Advantages:

- Filtration of the final extract is not required.
- Very inexpensive technique.
- Unattended operation.
- Instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.
- The sample phase is repeatedly brought into contact with fresh portions of the solvent, thereby enhancing the displacement of the analyte from the matrix

Disadvantages:

- Large amount of solvent close to 200 mL.

- Slow extraction close to 24 hours.
- Mandatory evaporation of extract.

Guo *et al.*, (2009) measured the total PAHs concentration by Soxhlet extraction in sediments samples. The concentrations varied between 6.3 and 46.4 µg L^{-1}). The extraction solvent used was dichloromethane/hexane (250 mL of 1:1, v/v) for 24 h in a water bath maintained at 60 °C. The extract of the sediment sample was passed to a 1:2 alumina: silica glass column (1.00 g) anhydrous sodium sulphate overlying the silica gel for clean-up and fractionation. The eluent containing PAHs was collected by eluting 70 mL of hexane/ dichloromethane (7:3, v/v), and was concentrated to 0.5 mL under a gentle purified nitrogen stream. The analytical instrument used was GC-MS.

2.3.2.2 Microwave extraction (MAE)

MAE method is a procedure for extracting water insoluble or slightly water soluble organic compounds from soils, clays, sediments, sludges or just solid wastes. It uses microwave energy to produce elevated temperature and pressure conditions in a closed vessel containing the sample and organic solvent(s) to achieve analyte recoveries equivalent to those from Soxhlet extraction (Ganzler *et al.*, 1986).

The principle is that a sample and an appropriate solvent (or solvent mixtures) are put in a vessel, which is then pressurized and heated by microwaves. After typically 5-20 minutes the extraction is complete, and the vessels are allowed to cool down before removing the sample/solvent mixture. The solvent must be filtered to remove sample particles prior to analysis of the extracted components. A schematic representation of the MAE apparatus is shown in Figure 2.8.

Microwaves heat up the molecules by dual mechanism of ionic conduction and dipole rotation (Smith and Carpentier, 1995). There are two types of oscillating perpendicular fields that generate microwaves, and those are the electric field and magnetic field (Bethe, 1947). When the microwaves interact with polar solvents, heating of the substance is caused which is due to the ionic conduction or dipole rotation, individually

or simultaneously. The electrophoretic migration of ions under the influence of the changing electric field is called ionic conduction (Anastassiades *et al.*, 1992). If the solution offers a resistance to this migration of ions, a friction is generated and the solution is heated. The realignment of the dipoles of the molecule with the rapidly changing electric field is called dipole rotation (Burfoot, 1967). There is a generation of heat through frictional force when the solvent molecules try to align themselves with the changing electric field, but the molecules fail to realign themselves (Burkert *et al.*, 1993). No heating occurs when the frequency is greater than 2450 MHz and the electrical component changes at a much higher speed (Carroll, 1970; Collier, 1985). No heating occurs when the frequency is less than 2450 MHz and the electrical component changes at a much lower speed. The inference from the above mentioned mechanisms is that only dielectric material or solvents with permanent dipoles get heated up under microwave (Corney, 1988). The value of dissipation factor (tan δ), is a measure of the efficiency with which different solvents heat up under microwave.

The dissipation factor is given by the equation:

$$\tan\sigma = \varepsilon'' / \varepsilon \qquad (2.15)$$

where, ε" indicates the efficiency of converting microwave energy to heat i.e., the dielectric loss. ε is the measure of the ability to absorb microwave energy, i.e., the dielectric constant (Feyman *et al.*, 1966).

Microwave systems for extraction and laboratory use are available in two forms (Kristenson, 2006):

- Closed extraction vessels/Multi-mode microwave ovens (used in this work) and
- Focused microwave ovens

The microwave extraction assembly comprises of four major components:

a) Microwave generator, which is responsible for generation of microwaves
b) Wave guide, which is used to direct the propagation of microwave from the source to the microwave cavity.

c) Applicator, where the sample holder along with the sample is placed.

d) Circulator, which regulates the movement of microwaves only in the forward direction.

In case of multi-mode systems the applicator is a closed cavity inside which a random dispersion of microwaves is brought about. Beam reflectors or turntables help in bringing about a uniform distribution of microwave energy inside the cavity, irrespective of the position of placement of sample. In case of focused microwave systems, the microwave waveguide acts as the applicator and the extraction vessel is placed directly in the cavity (Kristenson, 2006).

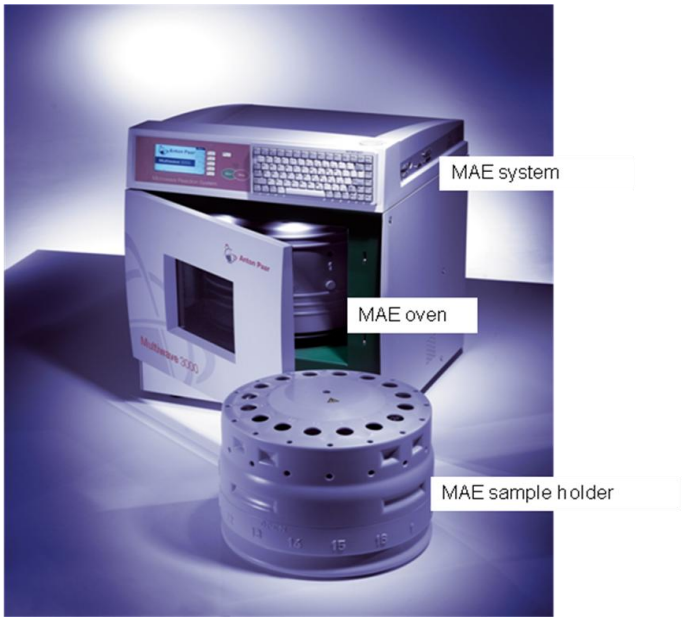


Figure 2.8: Schematic representation of MAE instrument

The advantages and disadvantages of MAE are given below:

Advantages of the closed system:

- It can reach higher temperatures than open system because of the increased pressure inside the vessel that raises the boiling point of the solvents used. The higher temperatures in turn decreases the time needed for the microwave treatment.

- The loss of volatile substances during microwave irradiation is virtually completely avoided.
- Less solvent is required. This is because there is no evaporation, hence there is no need to continually add solvent to maintain the volume. Also the risk of contamination is avoided since there is little or no risk of airborne contamination.

Disadvantages of the closed system:

- The high pressure used pose safety (explosion) risks.
- The amount of sample that can be processed is limited.
- The usual constituent material of the vessel, PTFE does not allow high solution temperatures.
- The single-step used excludes the addition of reagents or solvents during operation.
- The vessels must be cooled down before it can be opened after the treatment to avoid loss of volatile constituents.
- The extraction solvent must be able to absorb microwaves.
- Analyte clean-up is required.

Applications of MAE technique

Table 2.9 gives some of the reported extraction solvents, extraction time and power that has been used in the extraction of PAHs by MAE. Dichloromethane has been reported to be one of the common PAHs extraction solvents. The extraction time ranged between 10-30 minutes. Table 2.10 gives LODs, %RSD, extraction efficiency and concentrations that have been reported in the literature on the analysis of organic compounds by MAE. The LODs ranged from 0.0016-7.78 ng m^{-3}. Extraction efficiency ranged from with 49-117.7 with RSDs of 1.2-16.2%. Concentrations ranged from 0.04-2250 ng g^{-1}.

Table 2.9: The extraction solvent, extraction time and power used in MAE for the extraction of PAHs

Matrix	Extraction solvent Used	Extraction power	Extraction Time	Temperature	Extraction Power	Reference
Sediments	30 mL dichloromethane	30 W	10 minutes	NR	NR	Bundzinski *et al.*, 1999
Sediments	30 mL Acetonitrile	NR	20 minutes	120 ^{0}C	NR	Pensado *et. al*, 2000
Sediments	Dichloromethane and ethyl acetate	NR	30 minutes	150 ^{0}C	1MPA	Itoh *et al.*, 2008

Table 2.10: Application of MAE in organic compounds (sediment and soil samples)

Compounds	Analytical Method	LOD (ng m^{-3})	RSD (%)	Extraction efficiency	Concentration (ng g^{-1})	Reference
PAHs	GC-MS	NR	2.6 – 16.2	85 – 106	135 - 1300	Budzinski *et al.*, 1999
PAHs	LC-Fluorescence	0.0016 – 0.027	1.2 – 19	56.7 – 112	0.04 – 27.9	Castro *et al.*, 2009
PAHs	GC-FID	0.78 – 7.48	1.2 – 4.6	87 – 99	NR	Pensado *et al.*, 2000
PAHs	GC-MS	NR	1.2 – 16.1	49 – 117.7	1.51 – 24.7	Itoh *et al.*, 2008
PAHs	HPLC-UV	NR	NR	NR	26 – 2255	Villar *et al.*, 2006
PCBs	GC-MS	NR	7 - 11	NR	spiked 50	Criado *et al.*, 2004

2.4 Separation Techniques

The analysis of environmental samples for PAHs and for other organic pollutants has been developed to high standards. However, the complexity of the samples and also the low concentration levels of organic contaminants within the sample matrix continue to actively encourage research interest directed towards achieving more convenient and cost effective analytical methods (Manoli and Samara, 1999).

Chromatographic separations of mixtures of various organic compounds are based on their distribution between a stationary and a mobile phase, which are present in a chromatographic column. There are several types of chromatographic separation techniques and these include high performance liquid chromatography (HPLC) and gas chromatography (GC) (Harwood, 1989).

The compounds that are present in the final extract need to be individually separated and then detected by selective separation techniques so that they can be identified and quantified correctly. This ensures satisfactory baseline separation, high obtainable recoveries, and low detection limits. GC with different types of stationary phases has been developed. GC is used for the separation of organic constituents present in environmental samples. The sensitivity of the GC permits the analysis of samples containing μg L^{-1} or even less of the target analytes. GCs equipped with different detection techniques such as flame ionization detection (FID) (Magdic & Pawliszyn, 1996) and mass spectrometry (MS) (Aguilar *et al*., 1998), have been used. HPLC with Fluorescence detector has also been used for the detection of PAHs from environmental samples (Mao and Turker, 2002).

2.4.1 Gas Chromatography

Gas chromatography (GC) is an analytical technique that separates compounds based primarily on their volatilities. Gas chromatography provides both qualitative and quantitative information for individual compounds present in the sample (Bailon *et al*., 2000).

2.4.1.1 Principle

The compounds in gas chromatography move through the column as gases, either because these compounds are normally gases or they have been vapourised into a gaseous state. The compounds partition between the stationery phase, which can either be solid or liquid and a mobile phase (gas). The differential partitioning into the stationery phase allows the separation of compounds in time and space. GC includes

carrier gas, injector, column, oven detector and data recorder (Pavia *et al*., 2006) (Figure 2.9).

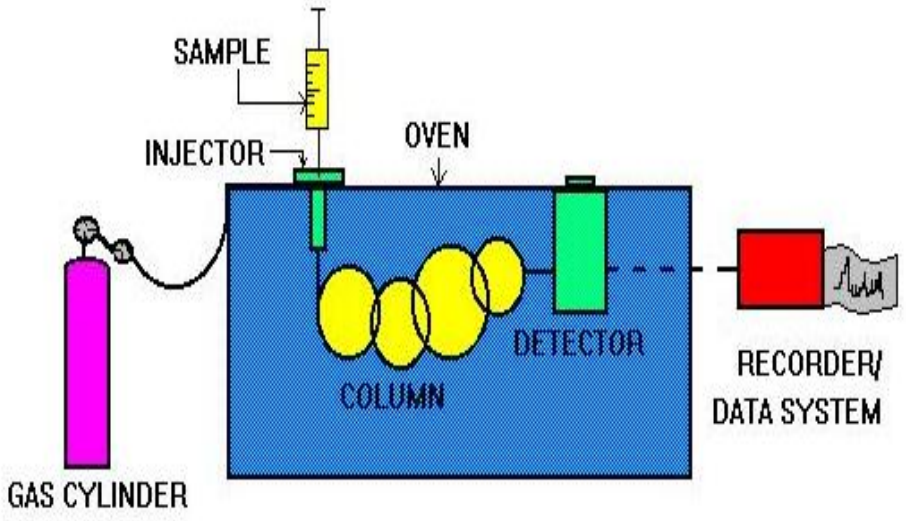


Figure 2.9 Schematic representation of Gas Chromatography (Bailon *et al*., 2000).

The GC column is the heart of the GC system. The column is coated with a stationary phase which greatly influences the separation of the compounds. The structure of the stationary phase affects the amount of time that the compounds take to move through the column. Typical stationary phases are large molecular weight polysiloxane, polyethylene glycol or polyester polymers of 0.1 to 2.5 micrometer film thickness. Columns are available in many stationary phase sizes. A typical capillary column is 15 to 60 meters in length and 0.25 to 0.32 mm in diameter, while a typical packed column is 1.8 to 3.6 meters long and 2.2 mm diameter. In gas chromatography, gaseous analytes are transported through the column by a gaseous mobile phase, which is called the carrier gas. The stationary phase in this type of chromatography is usually a non-volatile liquid bonded onto a solid but it can sometimes be only a solid (Harries, 1987).

There are two types of columns which are encountered in gas chromatography, i.e., the packed and the capillary columns. Chromatographic columns vary in length (from less than 2 m to 50 m or more) and they are usually made of stainless steel, glass, fused silica or teflon. Capillary columns have two basic types: wall coated open tubular (WCOT) and support-coated open tubular (SCOT). Wall coated columns are simply

capillary tubes coated with a thin layer of the stationary phase. SCOT column have the inner surface of the capillary lined with a thin film (~30 μm) of a support material. This type of column holds several times as much stationary phase as the wall coated column and hence has greater sample capacity. Although the efficiency of SCOT column is generally less than that of WCOT it is significantly greater than of packed columns (Skoog and Leary, 1992).

In this study a 5% (phenyl) methylpolysiloxane (HP 5-MS) WCOT column with a nonpolar stationary phase was used. Many capillary GC stationary phases that are designed for optimally separating complex PAH mixtures are commercially available from different suppliers. In general, nonpolar stationary phases, such as methyl polysiloxane or phenyl methyl polysiloxane are the most suitable for the separation of these compounds (Santos and Galceran, 2002).

The weakness of a gas chromatography is that it requires volatile compounds for analysis. However, its major problem is that it lacks definitive proof of the nature of the detected compounds as they are separated. This requires having a detector at the end just like in any other separation technique (McMaster, 2008).

2.4.2 Detectors in Gas Chromatography

A number of detectors are used in gas chromatography. Flame Ionisation Detector (FID) is the easiest and most common detector, but it is not sensitive for PAHs. Coupling GC with MS affords greater (Santos and Galceran, 2002).

2.4.2.1 Flame Ionization detector (FID)

FID (Figure 2.10) is the most useful and available GC detector and also by far the most commonly used in GC analyses. It has a very wide dynamic range, a high sensitivity and it can detect all carbon containing substances (with exception of a few low molecular weight compounds) (Scott, 1957). In the FID, hydrogen is mixed with the column eluent and burned at a small jet. The flame is surrounded by a cylindrical

electrode as well as a relatively high voltage which is applied between the jet and the electrode to collect the ions that are formed in the flame.

The resulting current is amplified by a high impedance amplifier and the output fed to a data acquisition system or a potentiometric recorder. The detector usually requires three separate gas supplies together with their precision flow regulators. The gases normally used are hydrogen for combustion, helium or nitrogen for the carrier gas and oxygen or air as the combustion agent. The detector is normally thermostated in a separate oven; this is not because the response of the FID is particularly temperature sensitive but to ensure that no solutes condense in the connecting tubes (Scott, 1957).

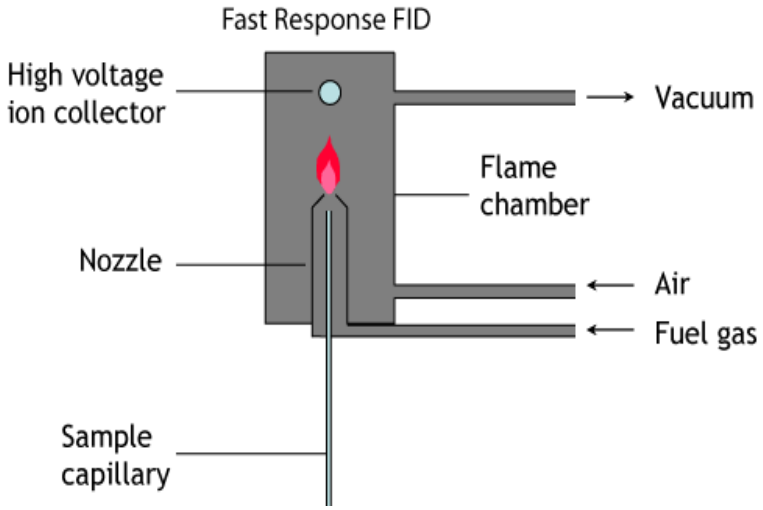


Figure 2.10: Flame Ionization Detector (Harwood *et al*., 1989)

2.4.2.2 Mass Spectrometry Detector

Mass spectrometry (MS) is an analytical technique that measures the mass to charge ratio (m/z) of the charged particles. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule and also for elucidating the chemical structures of molecules, such as peptides and other chemical compounds. The principle of the MS consists of ionizing chemical compounds to generate charged molecules or molecular fragments and the measurement of their mass to charge ratios (Gohlke and McLafferty, 1993).

In MS, a sample is loaded onto the MS instrument where it undergoes vaporisation. The components of the sample are ionized by one of a variety of methods (e.g., by impacting them with an electron beam), which results in the formation of charged particles (ions). The ions are then separated according to their mass to charge ratio in an analyzer by an electromagnetic field. The ions are usually detected by a quantitative method and ion signal is processed into mass spectra. A typical GC/MS instrument is capable of performing both functions either individually or unitedly, depending on the setup of that particular instrument (Gohlke and McLafferty, 1993).

Full scan MS

A target range of ions is determined and put into the instrument's method when collecting data in the full scan mode. An example of a typical broad range of ions to monitor would be between m/z 50 to m/z 400. The scan range to be used is largely determined by what one expects to be the content of the sample while being sensible of the solvent and other possible interferences. If one is to use a large scan range, then the sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of ions (McLafferty *et al*., 1974).

The full scan is useful when determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in the full scan mode in order to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method (McLafferty *et al*., 1974).

Selected ion monitoring (SIM)

In selected ion monitoring certain ions are entered into the instrument method and only those ions are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three ions) during each scan and more scans can take place at a time.

Since only a few ions of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios are comparable to a known reference standard (McLafferty *et al*., 1974).

2.4.3 Types of Ionization

As the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they can be ionized by various methods with typically only one method being used at any given time. Once the sample is ionized it will then be detected, usually by an electron multiplier, which essentially turns the ion current into an electrical signal that is then detected. The ionization technique chosen is independent of the scan mode, i.e. either a Full Scan or SIM (Gohlke and McLafferty, 1993).

2.4.3.1 Electron Ionization

The electron ionization (EI) is by far the most common and also standard form of ionization. In EI the molecules enter into the MS where they are bombarded with free electrons emitted from a filament, not much unlike the filament one would find in a standard light bulb. The molecules are bombarded by a high-energy electron beam. An electron which strikes a molecule may impart enough energy to remove another electron from that molecule i.e $M + e^- \rightarrow M^{+\cdot} + 2\ e^-$. This hard ionization technique results in the creation of more ions of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. The molecular fragmentation pattern depends upon the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with National Institute of Standard (NIST-USA) library of spectra applying algorithmic matching programs and the use of methods of analysis written by many method standardization agencies (Stein and Scott, 1994).

2.4.3.2 Chemical Ionization

In chemical ionization a reagent gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and the analyte and cause a soft ionization of the molecule of interest. In positive chemical ionization (PCI) the reagent gas interacts with the target molecule, usually with a proton exchange. This then produces the ions in relatively high amounts. In negative chemical ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply. One of the main benefits of using chemical ionization is that an ion closely corresponding to the molecular weight of the analyte of interest is produced (Gohlke *et al*., 1993).

2.4.4 Applications of GC in PAHs extraction

PAHs have been measured in solid samples using ultrasonic extraction with GC-MS (Sun, 1998) and obtained the levels ranging from 0.7 to 9.7 μg g^{-1} in soil. The extraction solvent used was acetone and the samples were sonicated for 30 minutes in a sonication bath. The extracts were concentrated in RP-SFE and eluted with a solution of acetone: THF (1:1).

ASE with GC-MS has been used for the extraction of PAHs in dried compost (Brandli *et al*., 2006). The limits of detection obtained were between 1.1 and 37.2 mg kg^{-1}.

PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene) have been determined in aquatic species using SPME sampling followed by GC-MS analysis (Guillén, 2002). The obtained concentration levels ranged from 0.5 to 5 ng g^{-1}.

Soxhlet extraction has been used to measure the total amount of PAHs in sediments samples (Guo *et al.*, 2009). The obtained concentrations varied between 6.3 and 46.4 μg L^{-1}. The extraction solvent used was 250 mL of dichloromethane/hexane (1:1, v/v) for

24 h in a water bath maintained at 60 °C. The sediment extract was then passed through an alumina: silica (1:2) glass column with 1 g anhydrous sodium sulphate overlying the silica gel for clean-up and fractionation. The eluent containing PAHs was collected by eluting with 70 mL of hexane/ DMC (7:3, v/v), and was concentrated to 0.5 mL under a gentle purified nitrogen stream and analysed with GC-MS.

LLE for extraction of PAHs in liquid samples has been used (Jong *et al.*, 2006). The detection limits obtained using GC-MS were between 0.01 and 0.065 μg L^{-1}. Three sets of 500 mL of water sample was liquid/liquid extracted with 3 × 30 mL dichloromethane. The extracts were then combined. The water extracts were concentrated, solvent-exchanged to hexane, and purified by passing through a 1:2 alumina:silica column with anhydrous sodium sulfate. The first fraction containing aliphatic hydrocarbons was eluted with 20 mL of hexane and the second fraction containing PAHs was eluted with 70 mL of dichloromethane/hexane (3:7, v/v). The PAHs fraction was concentrated to 0.5 mL under a gentle stream of nitrogen, and ready for instrumental analysis. The PAHs concentrations were then determined with a Finnigan Trace 2000 GC Ultra gas chromatograph, equipped with a Finnigan PolarisQ mass spectrometer in the selected ion monitoring (SIM) mode.

DLLME has been used for the extraction of PAHs in surface water (Rezaee *et al.*, 2006). The recovery obtained ranged from 60.3 to 111.3%. For most of the analytes, the limit of detection was between 0.007-0.030 μg L^{-1} for most of analytes. The relative standard deviations (RSDs) for 2 μg L^{-1} of PAHs in surface water was obtained by using an internal standard and was found to be in the range of 1.4-10.2%. The analytical instrument used is GC-FID.

Pre concentration and determination of PAHs by HLLE method in wastewater samples using gas chromatography–flame ionization detection (GC–FID) was investigated (Tavakoli *et al.*, 2008). Dynamic linear range of 0.1-400 μg $L^{-1}$was obtained. The limits of detection of 0.02-0.18μg L^{-1} were obtained. The extracting solvent used was chloroform.

Solvent bar micro extraction has been applied for the analysis of polyaromatic hydrocarbons in water samples using GC-ECD (Foo, 1997). Obtained concentration ranged from 0.015-0.044 μg L^{-1}.

2.4.5 High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography or high-pressure liquid chromatography, (HPLC) is a chromatographic technique that can separate a mixture of compounds and it is used in biochemistry and analytical chemistry for identification, quantification and purify the individual components of a mixture (Hung *et al*., 1998). HPLC typically uses different types of stationary phases, a pump that moves the mobile phase and analyte through the column, and a detector that provides a characteristic retention time for the analyte. The retention time of the analyte varies depending on the strength of its interactions with the stationary phase, the ratio or composition of the solvent(s) used, and the flow rate of the mobile phase. With HPLC, a pump that provides a higher pressure is required to propel the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes that are packed within the column. This allows for a better separation on columns of shorter length when compared to ordinary column chromatography (Xiang *et al*., 2006).

2.4.5.1 Principle of HPLC

In HPLC the sample to be analyzed is introduced in small volumes to the stream of the mobile phase. The solution is slowly moved through the column by specific chemical or physical interactions with the stationary phase present within the column. The velocity of the solution moves depending on the nature of the sample and on the compositions of the stationary (column) phase. The time at which a specific sample elutes is called the retention time and under particular conditions it is considered an identifying characteristic of the given sample. The use of smaller particle size column packing increases the linear velocity thus giving the components less time to diffuse within the column and hence improving the chromatogram resolution. Common solvents that are used include any miscible combination of water or various organic liquids (the most

common are methanol and acetonitrile). Water may contain buffers or salts to assist in the separation of the sample components, or compounds such as trifluoroacetic acid which acts as an ion pairing agent (Xiang *et al.*, 2006).

Another HPLC is to vary the mobile phase composition during the analysis; gradient elution. A normal gradient for reversed phase chromatography might start at 5% methanol and progress linearly to 50% methanol over 25 minutes and this is dependent on the hydrophobicity of the sample. The gradient separates the sample mixtures as a function of the affinity. This partitioning process is similar to that which occurs during a liquid-liquid extraction except that it is continuous and not in a step-wise manner. The choice of solvents, additives and gradient depend on the nature of the column and the sample to be analysed. Often a series of tests are performed on the sample together with a number of trial runs in order to find the HPLC method which gives the best peak separation (Xiang *et al.*, 2006).

2.4.5.2 Types of Liquid Chromatography

There are three types of liquid chromatography i.e normal phase, reversed phase and ion exchange chromatography. In this work reversed phase was used.

Reversed-phase chromatography (RPC)

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, less polar molecules have a longer retention time while polar molecules elute more readily. The retention time can be increased by addition of more water to the mobile phase, thus making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RP-HPLC is so commonly used that it is often incorrectly referred

to as ‘HPLC’ without further specification. The pharmaceutical industry regularly employs RH-HPLC to qualify drugs before their release (Horvath *et al.,* 1967).

RP-HPLC operates on the principle of hydrophobic forces, which originate from the high symmetry in the dipolar water structure and it plays the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. Upon association with the ligand, the binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C_{18}-chain versus the complex of both. The energy released in this process is proportional to the surface tention of the eluent (water: 7.3×10^{-6} J cm^{-2}, methanol: 2.2×10^{-6} J cm^{-2}) and to the hydrophobic surface of the analyte and the ligand respectively. The retention time can thus be decreased by adding a less polar solvent (methanol, acetonitrile) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the analysis (Xiang *et al*., 2006).

The structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, -NH_2, COO^- or -NH_3^+ reduce retention as they are well integrated into water. Very large molecules, however, can result in an incomplete interaction between the large analyte surface and the ligand's alkyl chains thus can have problems entering the pores of the stationary phase. The retention time also increases with hydrophobic (non-polar) surface area and branched chain compounds elute more rapidly than their corresponding linear isomers because overall the surface area is decreased. Similarly organic compounds with single C-C-bonds elute later than those with a C=C or C-C-triple bond, as the double or triple bond is shorter than a single C-C-bond.

Other than the mobile phase surface tension, other mobile phase modifiers can affect analyte retention in a column. For example, the addition of inorganic salts causes a moderate linear increase in the surface tension of aqueous solutions which is about 1.5×10^{-7} J cm^{-2} per Mol for NaCl and 2.5×10^{-7} J cm^{-2} per Mol for $(NH_4)_2SO_4$). This is because the entropy of the analyte-solvent interface is controlled by surface tension hence the addition of salts tends to increase the retention time. This technique is useful for mild separation and recovery of proteins as well as protection of their biological activity during protein analysis (Snyder *et al*., 2009).

Another important component is the influence of the pH since it can influence the hydrophobicity of the analyte. For this reason most methods use a buffering agent, such as sodium phosphate, to control the pH. The buffers serve multiple purposes. They control pH, neutralize the charge on any residual exposed silica on the stationary phase and also act as ion pairing agents to neutralize charge on the analyte (Hung *et al*., 1988)

In comparison, reversed phase columns are quite difficult to damage than normal silica columns. However, many reversed phase columns consist of alkyl derivatized silica particles and should never be used with aqueous bases because these will destroy the underlying silica particles. They can be used with aqueous acid, but the column should not be exposed to the acid for too long because it can corrode the metal parts of the HPLC equipment. RP-HPLC columns should be flushed with a clean solvent after use so that residual acids or buffers are removed. These columns should therefore be stored under appropriate composition of solvent (Xiang *et al*., 2006).

2.4.6 Detectors in HPLC

There are different types of detectors that have been used with HPLC for the detection of PAHs, i.e. fluorescence and UV detectors. A fluorescence detector has been used in this work.

2.4.6.1 Fluorescence Detector (FD)

Fluorescence detectors are probably the most sensitive among the existing modern LC detectors. With this type of detectors, it is possible to detect even the presence of a single analyte molecule in the flow cell. Typically, fluorescence sensitivity is 10 -1000 times higher than that of the UV detector for strong UV absorbing materials. Among other optical detectors, fluorescence detectors are the most very specific and selective detectors. This is normally used as an advantage in the measurement of specific fluorescent species in samples. During fluorescence compounds with specific functional groups are excited by shorter wavelength energy and they emit higher at wavelength radiation which is called fluorescence. Usually, the emission is measured at right angles to the excitation (Scott, 2003).

Roughly about 15% of all compounds have a natural fluorescence. The presence of conjugated pi-electrons especially in the aromatic components gives the most intense fluorescent activity. Also, aliphatic and alicyclic compounds with carbonyl groups and compounds with highly conjugated double bonds fluoresce, but usually to a lesser degree. The degree of fluorescence of most unsubstituted aromatic hydrocarbons increases with the increase in the number of rings, their degree of condensation and their structural rigidity. Fluorescence intensity depends on both the excitation and emission wavelength, and this allows for selective detection of some components while suppressing the emission of others. The detection of any component significantly depends on the chosen wavelength (Scott, 2003).

Fluorescence detectors can either be simple or complex. The simplest consists of a single wavelength excitation source and a sensor that monitors fluorescent light of all wavelengths. For certain samples, this form of fluorescence detector can be very sensitive and relatively inexpensive. However, employing excitation light of a single wavelength and only a broad emission wavelength is not very versatile. Conversely, the fluorescence spectrometer fitted with a small sensor cell is far more complex but it becomes more versatile with both selectable excitation wavelengths and emission wavelengths. In addition, the excitation and emission spectra can be obtained as

required (LCGC, 2004). The two types of fluorescence detectors are the single wavelength excitation fluorescence detector and the multi wavelength fluorescence detector. Single wavelength was used in this work.

The Single Wavelength Excitation Fluorescence Detector

The single wavelength excitation fluorescence detector (Figure 2.11) is probably the most sensitive LC detector that is available. However it is achieved by forfeiting versatility. The excitation light is normally provided by a low pressure mercury lamp which is comparatively inexpensive and provides relatively high intensity UV light at 253.7 nm. Many substances that fluoresce will be excited by light of this wavelength (Scott, 2003).

The excitation light is focused by a quartz lens through the cell. A second lens, set normal to the incident light, focuses the fluorescent light onto a photo cell. A fixed wavelength fluorescence detector will have a sensitivity (minimum detectable concentration at an excitation wavelength of 254 nm) of about 1×10^{-9} g mL^{-1} and a linear dynamic range of about 500 with a response index of $0.96 < r < 1.04$ (Scott, 2003).

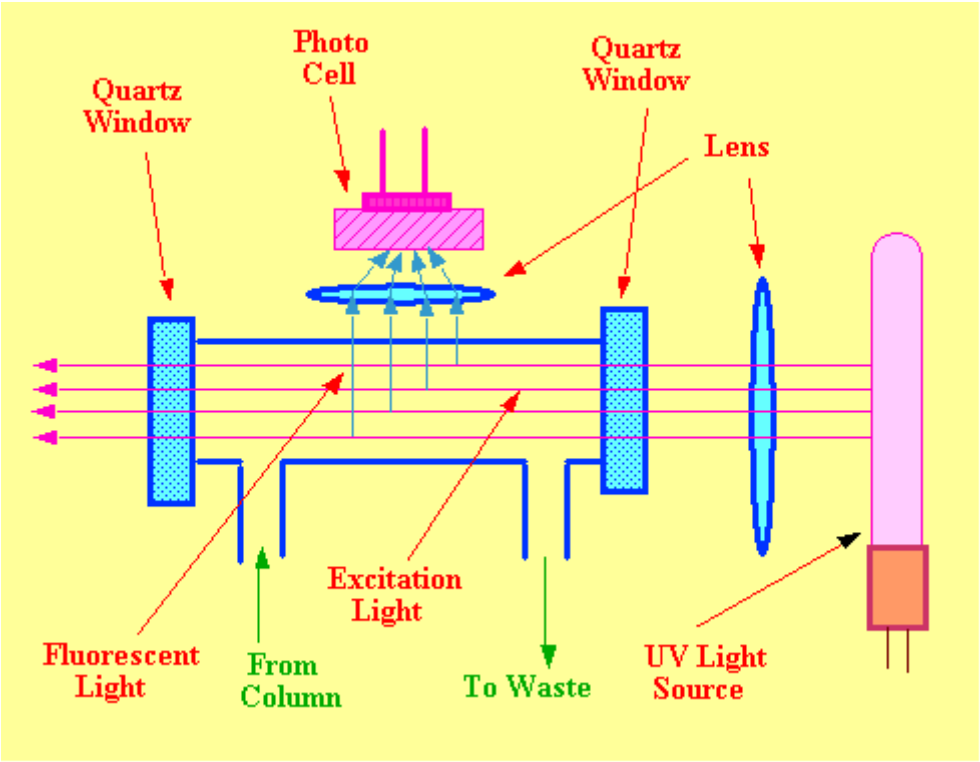


Figure 2.11: The Single Wavelength Excitation Fluorescent Detector (Scott, 2003)

PAHs have a high natural fluorescence so they are easy to discriminate against matrix components with a fluorescence detector. Fluorescence detectors can be monochromator based allowing for wavelength programming to achieve optimum sensitivity (Furata and Otsuki, 1983).

2.4.7 PAHs determination using HPLC

There have been a number of applications of these detection techniques in recent years and examples of these are given in the subsequent paragraphs. MSPD with HPLC-fluorescence detection has been proporsosed for the first time for the isolation PAHs from soil samples (Pena *et al*., 2007). The obtained quantification limits were between 0.01 and 0.6 ng g^{-1} dry mass. The sorbent used was C_{18} and the extraction solvents were hexane-dichloromethane (1:1, v/v).

Polyvinylidenefluoride (fluorocarbon polymer sorbent) with surface area 8.4 m^2g^{-1}, non-porous, particle size 15–30 µm) for on-line SPE–HPLC have been used for the

determination of naphthalene, biphenyl, acenaphthene, anthracene and pyrene in natural water (Oliferova *et al.,* 2005). The sorbent was conditioned by 5% (v/v) acetonitrile-water mixture. Detection limits of SPE-HPLC method were as followed: 5 ng L^{-1} for biphenyl, 7 ng L^{-1} for anthracene, 8 ng L^{-1} for acenaphthene, 30 ng L^{-1} for pyrene and 40 ng L^{-1} for naphthalene. These were obtained using UV spectrophotometric detection.

LPME has been used for extraction of PAHs in aqueous samples (Raich-Montiu 2008). Obtained concentrations were between 0.1 and 50 µg L^{-1}. A polypropylene hollow fibre with a 200 µm wall thickness, a 600 µm inner diameter and 0.2 µm pore size was used. These were obtained using undecane as the organic solvent and the analytical instrument used was LC-MS/MS.

MAE has been used to extract PAHs in fish samples (Purcaro *et al.*, 2009). The obtained concentrations ranged from 2 to 19 µg kg^{-1}. The extraction solvent used was hexane, the extraction time was 15 minutes and the separation technique used was HPLC.

PAHs in river samples have been measured using cloud point extraction with HPLC-FID (Vassilakis *et al.*, 1998). The btained recoveries ranged from 35 to 103%. The levels of PAHs ranged from 26.8 mg L^{-1} for FLU to 1.6 ng L^{-1} for BaP. The surfactant used was Triton X-114 nonionic surfactant.

SBSE and HPLC- UV were also used for the extraction of PAHs in lake water and soil samples (Yu *et al.*, 2008). The obtained limits of detection ranged from 0.007 to 0.103 µg L^{-1} and the relative standard deviations were in the range of 6.3-12.9%.

The identification and quantification of six PAHs on EPA's priority pollutant list: anthracene, pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, and benzo[g,h,i]perylene using the HPLC-fluorescence detector technique has been reported (Obana *et al*., 1981). The levels measured in human tissue ranged from 0.006 to 0.460 ng g^{-1}.

PAH levels in the ng L^{-1} range have also been successfully determined in water using flotation enrichment and HPLC/fluorescence detection (Xu and Fang, 1988). Good recoveries 86-107% were achieved, and the RSD was in the range of 2.7-13.6% RSD.

Errors during separation procedure

The analysis of unknown complex real samples such as environmental samples plays an important role in environmental chemistry and other fields. It has thus far become one of the hot spots and difficult point in modern analytical chemistry. The hyphenated chromatographic techniques are generally recommended to attach these problems due to their advantages in chromatographic separation and spectral identification. However, the complete separation of PAHs especially in real complex samples is not an easy task at all. Incompletely separated chromatographic peaks are commonly observed in practice, which then bring about difficulty in the qualitative and quantitative analysis of the unknown complex real samples (Hailin *et al*., 1998). Gas chromatography, using capillary or packed columns and liquid chromatography are the main separation methods used so far. GC has become a powerful tool in the determination of traces of organic compounds. Whereas for most environmental applications packed columns are abandoned for the determination of traces of organic compounds because of poor separation and time-consuming procedures. In gas chromatography the separation is done by changing temperature, injection, controlling oven and detector temperatures and precautions should be taken to preserve the compound integrity in the column (Nsengimana, 2007). The quantification of the analyte is generally based on peak area or the peak height, therefore the peaks have to be well resolved. In environmental samples many unwanted compounds can be co-extracted and may interfere with the analyte, thus well resolved peaks are needed (Nsengimana, 2007).

Direct analysis of PAHs in complex mixtures using RP-HPLC is complicated due to the fact that most complex organic materials contain compounds that are not usually miscible in acetonitrile, the solvent of choice for reverse phase separation of PAHs (McKinney *et al*., 2000). In this work the problem of solvents that are not miscible with acetonitrile (such as THF, dichloromethane) was encountered, thus solvent exchange

was then done. This problem was solved by evaporating the extract to 1 mL, thereafter 1 mL of methanol was added to the extract and then evaporated to 1mL. This procedure involved more evaporation steps, which could lead to the loss of analyte being extracted.

Errors during detection

Ultra high-performance chromatographic separations are increasingly accepted as a key approach to getting more analytic information in less time, even for complex sample mixtures. Nevertheless, there are some challenges not only that the chromatography system have to handle the higher pressures (600 bar or more), the detection systems have to keep pace with the increasing speed of the analysis. Though UV detectors can generally cope with this, fluorescence detectors are more difficult to optimize. This is especially true with wavelength switching. This technique is used to gain the maximum in sensitivity with fluorescence detection by switching the excitation and emission wavelengths to best match the spectral properties of the separated analytes. In practice, it implies a certain delay during data acquisition due to the mechanical inactivity of the equipment (Martin, 2008). As a consequence, marginally resolved and narrow analyte bands with different wavelength setting requirements could not be detected by fluorescence in the past. The total switching time was a summation of both wavelength switching times and the delay caused by the detector response time (Martin, 2008).

In this work the difficulty of optimising the florescence detector was experienced. To find one compromise wavelength suitable for all the PAH compounds worked with was never possible. The optimum wavelength (225-460 nm) was good for naphthalene, acenaphthene and fluoranthene. Phenanthrene was not well separated with acenaphthene even after changing the mobile phase composition from 80:20 to 70:30 (acn:water, v/v). The mobile phase could not be changed further as it led to peak broadening for the later eluting compounds (fluoranthene) and also increased retention times. Phenanthrene was then excluded as part of the compounds to work with. Pyrene was not detected at a wavelength of 225-460 nm, it was then analysed at 333-390 nm which was the best wavelength for it. Therefore two different wavelengths were used per sample in this

work, which then led to more time spent per analysis. This is because the fluorescence detector used could not allow wavelength switching during analysis.

One of the major problems associated with the analysis of PAHs is that many organic compounds can coextract with PAHs and thus interfere with their separation, identification and quantification Most of them are structurally similar which also makes their separation and identification difficult (Chiu *et al*., 1997).

From the literature overview in this chapter, the sources, effects of exposure and distribution of PAHs has been highlighted. PAHs bioaccumulate and are therefore persistent in the environment. Due to their ubiquitous occurrence and carcinogenic activity, PAHs have gathered significant environmental concern hence evaluating and monitoring their levels from different environment matrices is important. It is therefore necessary to develop and/or modify suitable extraction methods for the determination of PAHs in solid and water samples. MAE, SE, SPE and HF-LPME were the techniques that were used to extract the PAHs that were present in the samples. These techniques were chosen because of their great selectivity towards PAHs, high enrichment factors, the ease to use and also because of their availabity.

Chapter Three – Research Objectives

3.1 General and Specific Objectives

3.1.1 General Objectives

The following objectives have been formulated in an attempt to answer the hypothesis below.

- To develop and compare extraction techniques that is most suitable for the extraction of PAHs in river water and sediment samples.
- To evaluate the extent of PAHs distribution in the water and sediment environmental compartments in and around the greater Johannesburg area.

3.1.2 Specific Objectives

The specific objectives of this research were as follows:

- To develop and optimize extraction methods based on solid phase extraction (SPE) and hollow fiber liquid phase microeaxtraction (HF-LPME) techniques for PAHs in water samples.
- To apply and compare the optimized SPE and HF-LPME techniques for determining PAHs in river water samples in and around great Johannesburg.
- To develop and optimize Microwave assisted extraction (MAE) method for determining PAHs in sediment in Johannesburg rivers.
- To compare MAE and Sohxlet (SE) methods in the determination of PAHs in river sediments in and around Johannesburg.
- To identify the PAHs that may be present in river water and sediments in the area under investigation.
- To determine the total concentration of PAHs in river water and sediments in the area under investigation.
- To have an idea of the risk posed by PAHs in the river water and sediments in the area under investigation.

- To evaluate the hot spots and possible sources of PAHs in river water and sediments in the area under investigation.

3.2 Hypothesis and Research Questions

3.2.1 Hypothesis

- The area in and around Johannesburg has a lot of PAHs in its environment.
- The existing extraction methods for PAHs in water can be further optimized.

3.2.2 Research Questions

The main aim of this research is therefore to answer the following questions:

- Are PAHs present in the samples to be analyzed?
- If they are present, in which concentrations and what are the main sources?
- Are there any relationships between the concentrations of PAHs in the field water samples and their physicochemical properties?
- Is there any relationship between PAHs in the river water and the sediments in the study area?
- In which way can the extraction methods be improved for PAHs in sediments and river water?
- What are the factors that can be optimized in order to achieve better quantification and identification of the low concentrations of PAHs in the environment?

3.3 Justification of the research

The growing extent of the pollution of the environment as a result of industrial and human activities has initiated a wide complex of legislative measures. Reliable and relevant data on concentrations of pollutants in the environment is necessary for policy implementation and formulation and environmental protection. The largest problems encountered are in the case of organic micro pollutants, where the analyst has to cope

with many different compounds occurring at trace concentrations. Thus the need for reliable data on occurrence of organic micro pollutants in the environment is an important driving force in initiating the development of modern analytical techniques and procedures. Two major target areas of interest can be distinguished in the process of development of environmental organic trace analysis (Liśka, 2000). The first area that was given major attention in the past was analytical separation and detection. In this field, remarkable progress has been achieved during the past several decades. The second field, sample preparation has always been in the shadow of the first one, often being considered as a boring but inevitable part of the whole analytical method. Only after the highly sensitive analytical systems had become a common standard for environmental analysts, it was realized that the preparation of samples was an important braking factor in general progress in environmental analysis (Liśka, 2000).

Reliable environmental analysis of organic compounds like PAHs is a prerequisite for their risk assessment. The analytical methods used for the determination of organic compounds in complex aqueous and solid samples require sample extraction, clean up and pre concentration steps because these compounds exist in low concentrations. Traditional sample preparation techniques such as liquid-liquid extraction and Soxhlet extraction are laborious and time consuming and also consume large organic solvent volume. Extraction techniques such as microwave extraction, solid phase extraction, hollow fiber liquid membrane extraction etc have been developed to overcome the drawbacks of the traditional extraction techniques. This is because they are fast and use little solvent volumes which make them environmentally friendly. In this work environmentally friendly techniques were modified/developed for the extraction of PAHs in water and sediments samples. These modified techniques were then applied to real South African samples.

Generally, the levels of environmental pollutants in South Africa are not well known, despite the fact that the country is the largest economy in Africa (Nieuwoudt *et al.*, 2011). Although some research has been done on selected POPs especially pesticides such as DDT, there is very little known about the levels of PAHs in South Africa (Nieuwoudt *et al.*, 2011). The PAHs that have been selected as priority pollutants by the

US EPA are mainly of petrogenic or pyrogenic origin. That means that they are generally part of the crude 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 inefficient combustion of organic materials, domestic heating, power generation, incineration, vehicle exhaust emissions, or natural fires (Masih and Taneja, 2006). All of these sources occur in South Africa, either concentrated in industrial parks or distributed in residential and rural landscapes. Exposure of humans to PAHs may lead to elevated levels of DNA mutation, reproductive defects and an increased risk of cancer and other adverse health effects. It is therefore very important to determine the levels of PAHs in the South African environment, especially in areas where human health may be negatively affected by the presence of these compounds (Nieuwoudt *et al.*, 2011).

A few studies on the presence of PAHs in South Africa have been done (Cele, 2005; Tikilili and Chirwa, 2004; Das *et al.*, 2008; Nekhavhambe, 2008: Nieuwoudt *et al.*, 2011). PAHs in South African sludge samples have been analysed (Cele, 2005). The average concentrations obtained were 0.55 mg kg^{-1} for benzo(a)pyrene in KwaZulu Natal province, 0.55 mg kg^{-1} in Gauteng province with only one sample exceeding the regulatory limit, which is 2.55 mg kg^{-1} and 0.75 mg kg^{-1} in North West and Western cape province. However, the South African guideline" does not cater for other PAHs that might be harmful to the environment (Cele, 2005).

PAHs in South African wastewater samples have been analysed in Cape Town (Tikilili and Chirwa, 2004). All 16 priority PAHs were detected in the sample with concentrations ranging from 0.001mg L^{-1} to 25.1 mg L^{-1}. The results obtained showed that there are high levels of PAHs in the wastewater exceeding the World Health Organisation (WHO) recommended maximum values for safety, which is 0.05 µg L^{-1}.

PAHs in sediments and surface water in Cape Town have also been analysed (Das *et al.*, 2008). The obtained concentrations were 36.9 ng mg^{-1}in sediments and 0.01-13.9 ng L^{-1} in surface water.

PAHs in rivers, surface runoff and sediments in Thohoyandou, Limpopo Province have been determined (Nekhavhambe, 2008). Concentrations obtained were between 0.0001-2.5 µg L^{-1} in water samples and 0.41-34.4 mg kg^{-1} in sediment samples.

Since there is a growing extent of environmental pollution, it is necessary to establish a data source on the occurrence of PAHs around Johannesburg. Also, the modification and development of modern analytical techniques and procedures is necessary to easily monitor the concentrations of different types of PAHs. The objectives that have been set up were therefore used to attain the main aim of the study. This is necessary to ensure that data that will help to avert the adverse health effects associated with exposure to PAHs is readily available.

Chapter Four – Research Methodology

4.1 Introduction

This chapter presents the methodology and experimental procedures used in this work. It also describes the sampling procedure as well as the data collection procedure. The methodology for the determination of PAHs in the environmental samples involved a preliminary survey to identify the sampling sites. This was followed by a three steps procedure: sampling, extraction by SPE and HF-LPME (for liquid samples), MAE and SE (for solid samples), and then analysis by GC-FID, GC-MS and HPLC-Fluorescence. Quality assurance procedure is also addressed.

4.2 Standards and reagents

Heptane (99.9%) and methanol (99.9%) were purchased from Fischer Scientific (Loughborough, UK), acetone (99.8%) and acetonitrile (99.9%) from Lab Scan Analytical Scientific (Dublin, Ireland), toluene (99.9%) from Sigma-Aldrich (Steinhein, USA), isooctane (99.5%), hexane (99.5%) and dichloromethane (99.5%) were from Fluka (Steinheim, USA), tetrahydrofuran (99.5%) from Merck (Darmastadt, Germany), phenanthrene and naphthalene (97.9 and 98% purity respectively) from Supelco (Bellefonte, PA, USA), acenaphthene , pyrene, fluoranthene and chrysene with 99% purity from Sigma-Aldrich (Steinhein, USA), sodium sulphate anhydrous granulated (99.0%) from Scharlau Chemie, S.A (Barcelona, Spain). All reagents were used without any further purification. A PP Q3/2 Accural capillary hollow fiber membrane from Membrana GmbH was from (Wuppertal, Germany). SPE cartridges, bond elute LRC C_{18} (500 mg, 6mL) were from Varian (Torrance, CA, USA). Certified reference materials (QCO-259 for water samples and SQC017 for sediment samples) were obtained from Industrial Analytica (Pty) LTD (Johannesburg, South Africa).

4.3 Equipment

The 460 Elma Transsonic Ultrasonic bath from (Elma, Singen, Germany) was used. The ultrasonic bath was employed for dissolution of standards and for the elimination of bubbles from freshly prepared HPLC mobile phase. It was also used for the cleaning of the hollow fiber membrane. A Buchi Rotapor R11, from Labotec (Flawil, Switzerland) was used. Evaporator was employed for solvent reduction of Soxhlet extracts. A Fritsch pulverisette 6 (Fritsch GmbH, Idar-Oberstein, Germany) was used to grind sediment samples prior to extraction. Crushing was achieved at 400 rpm for 20 minutes. Anton Paar Multiwave 3000 solv (Swisslab, Johannesburg, South Africa) was used for the extraction of sediment samples. Soxhlet extraction was assembled from existing components within the laboratory. SPE unit with a C_{18} column (Supelco, Park Bellefonte, USA) packed with 500 mg of the sorbent in a 6 mL polypropylene syringe barrel was used. SPE was used for extraction of water samples. It was also used for clean-up of MAE and SE sediment extracts.

Eutech instrument Cyberscan pH 510 meter (Leeds, UK) was used for measuring pH of the samples. Winlab Dataline conductivity meter (Clausthal-Zellerfed, Germany) was used for measuring conductivity of the samples. Metler Toledo PB 303 balance (METLER TOLEDO, Zurich, Switzerland) and Precisa 180A balance (Delta Laboratory Services, London, United Kingdom) were used for mass measurements.

Agilent 7890A gas chromatography system (Agilent technologies, California, USA) equipped with flame ionization detector and chemstation software (Agilent technologies, Santa Clara, USA) was used for preliminary optimization of HF-LPME, SPE and MAE. A Varian gas chromatography 3800 without an auto sampler (Varian Chromatography Systems, Walnut Creek, CA), connected to mass spectrometer Varian Saturn GC-MS 2000 and WorkStation v5.4 software was used for optimization of HF-LPME and SPE because this was more sensitive then GC-FID. For application to real water, two GC-MS instruments were used. A Finnigan TraceGC ultra (Thermo Electron S.p.A, Rodano, Milan, Italy) without an auto sampler, connected to pyramid triple Quad Mass spectrometer (Mass Spec corporation, South Africa, Cape Town) and peak simple

software was used. A quadrupole QP2010 gas chromatograph connected to mass spectrometry GC-MS system (Shimadzu, Kyoto, Japan) with an auto sampler was also used for detection and quantitation of the analytes. Analytes were separated using a Zebron-1ms column with dimensions 30 m x 0.25 µm (Phenomenex, Torrance, California, USA) and peak simple software. A Supelco 5 µL manual syringe SGE (SUPELCO Analytical, Pennsylvania, USA) was used for injection of 1 µL of sample into the GC. A HPLC-fluorescence consisting of Waters pump (Milford, Massachusetts) and RF-10AxL Shimadzu fluorescence detector (Kyoto, Japan) and clarity software (Podohradska, Prague, Czech) was used for acquiring chromatograms.

4.4 Preparation of solutions

1000 mg L^{-1} stock solution of each of the six PAHs (naphthalene, acenaphthene, phenanthrene, fluoranthene, pyrene and chrysene) was prepared in a 50 mL volumetric flask separately. 50 mg of each PAH was weighed and transferred quantitatively into a 50 mL volumetric flask and dissolved in methanol. The volume was made to the mark using methanol for GC-FID and GC-MS. Another 1000 mg L^{-1} stock solution of PAHs was prepared by dissolving 50 mg of PAHs in acetonitrile. The volume was diluted to the 50 mL mark with acetonitrile for HPLC-Fluorescence. From the 1000 mg L^{-1}, a 100 mg L^{-1} stock solution of the mixture of PAHs was prepared which was then used for spiking low concentrations levels. Standard solutions ranging from 0.25 to 2.0 mg L^{-1} for GC-FID, 0.2 to 1.0 mg L^{-1} for GC-MS and 0.1 to 1.0 mg L^{-1} for HPLC-Fluorescence were prepared and used for the calibration curve. All stock solutions and standards solutions were stored at $4^{o}C$ and new solutions were prepared every month.

The composition of the mobile phase was 80% acetonitrile and 20% water for HPLC. The prepared mobile phase was filtered through hydrophilic membrane of 0.45 µm pore size using a vacuum pump and then sonicated for 20 minutes in an ultrasonic bath to get rid of the air bubbles.

4.5 Instrumental and analysis conditions

In GC, most of the optimization experiments were performed using a Varian gas chromatography 3800 without an auto sampler, connected to an ion trap mass spectrometer Varian Saturn GC-MS 2000. Analytes were separated using WCOT fused silica capillary column (30×0.25mm ID, 0.25 µm film thickness). Helium was used as a carrier gas. The injector was set at 280 ^{o}C in splitless mode. Injection volume was 1 µL. The temperature programme was: 40 ^{o}C, held for 5 min, rate 10 ^{o}C/min to 179 ^{o}C, held for 2 min, rate 9 ^{o}C/min to a final temperature of 300 ^{o}C, held for 10 min. The mass spectrometer was operated in the elecron electron ionization (EI) mode. Samples were analysed in the selected ion monitoring (SIM) mode. Scan runs were made in the range from 40-650 amu. Some of the earlier optimization was performed using an Agilent gas chromatography system equipped with flame ionization and electron capture detectors. Flame ionization detection was used in this case. Other conditions used were similar to the GC-MS system. Manual injection was also performed with 1µL. Application to real samples for SPE was performed using Finnigan TraceGC ultra (Thermo Electron S.p.A, Rodano, Milan, Italy) without an auto sampler, connected to pyramid triple Quad Mass spectrometer (Mass Spec corporation, South Africa, Cape Town) and peak simple software. Other parameters were similar as described above. A quadrupole QP2010 gas chromatograph connected to mass spectrometry GC-MS system (Shimadzu, Kyoto, Japan) with an auto sampler was used for HF-LPME samples. Analytes were separated using a Zebron-1ms column with dimensions 30 m x 0.25 µm (Phenomenex, Torrance, California, USA) and peak simple software. Other conditions were similar to other instruments used.

A Waters pump (LA, California, USA) HPLC system with a Fluorescence detector and a clarity chromatographic software was used sediment samples for MAE. A Discovery HS C_{18} column with dimensions 5 µm x 4.6 mm x 25 cm was used (Supelco, Bellefonte, PA, USA).The injection volume was 20 µL.

4.6 Sampling

The sampling areas were chosen with the aim of representing the whole of Johannesburg in order to have overall overview on PAHs contamination in the city of Johannesburg area. The east area of Johannesburg which is the East rand was represented by Blaauwpan Dam, Homestead Lake, Middle Lake and Kleinfontein Lake, which flows to each other (Figure 4.1). The Centurion area which is North of Central Johannesburg was represented by Centurion River and Centurion Lake. Germiston area which is also part of the east side of the city was represented by Natalspruit River. The Alexandra/Thembisa area was represented by Jukskei River. The west area was represented by Hartbeespoort Dam. The other reason for the selection of East rand (Benoni) dams was the assumption that they might be polluted with PAHs. This assumption was due to the jet fuel spill that occurred in the area, since fuel is one of the sources of PAHs. The incident took place near Johannesburg International airport when a mechanical failure on one of the refueling valves caused a jet fuel spillage (News24, 26 Sept 2006 at 4:56 pm). Even though the dam was cleaned, PAHs could still be found in that area because of their persistence in the environment. Figure 4.1 shows a schematic diagram for the sampling areas used in this work.

The samples were collected between December 2010 and October 2011. Water samples were sampled in two sets. One set which was the top water was collected in the surface and the other set which was the bottom was collected 15 cm depth below the surface. They were stored in pre-cleaned 1 L brown glass bottles and kept at $4^{o}C$ until analysis. Sediment samples were sampled in three sets. One set which was the top layer was collected between 0-9 cm and 0-12cm from the surface. The second set which was the middle layer was collected below the surface at depths of 9-18 cm and 12-24 cm. The third set which was the bottom layer was collected below the surface at depths 18-27 cm and 24-36 cm. The distance between the sampling spots was between 100 m to 10 km per sampling area.

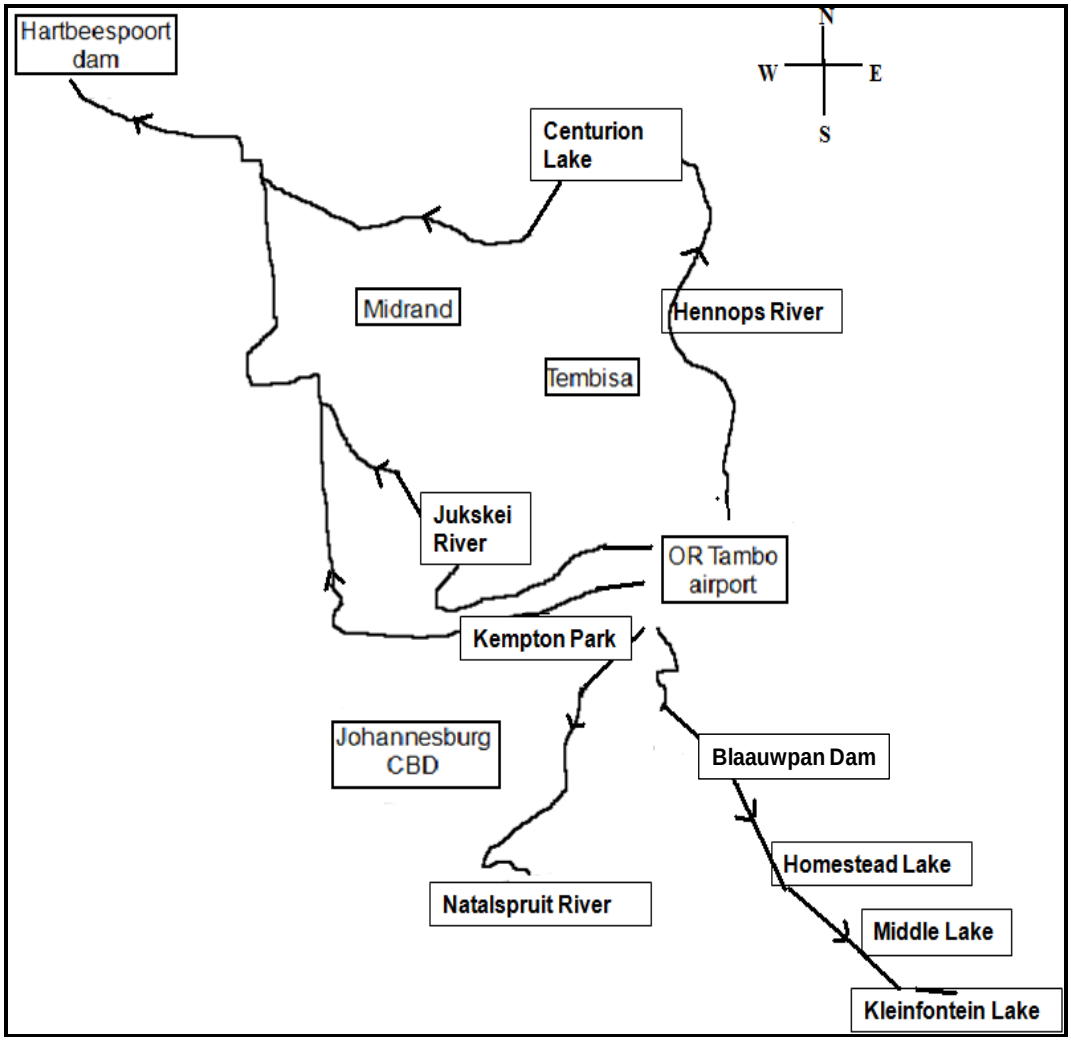


Figure 4.1: Location of sampling areas around Johannesburg

4.6.1 Sampling areas details

Potentially toxic substances enter the aquatic environment from either a direct source point or non-point source. A direct source point is where discharges enter a water source at a single point, e.g. discharges of domestic sewages and industrial effluents. Non-point source is where a toxic substance enters surface and underground water through runoff from urban and industrial areas, leachates from domestic and solid waste disposal sites and mining operations (Roux 1994, Sutton and Oliveira 1987). The aquatic environment is affected by different types of chemicals that are toxic. These chemicals originate from both natural (e.g. heavy metals and various PAHs) and anthropogenic sources (e.g.

PAHs, polychlorinated biphenyls (PCBs), pesticides and heavy metals) (Visser, 2008). In order to monitor the effect of these contaminants, analysis from different environmental matrices has to be done.

Hartbeespoort dam

The Hartbeespoort dam is situated on the confluence of the Crocodile River, about 16 km southwest of the town of Brits and 37 km due west of Pretoria (SANCOLD, 1978) and in the Highveld region of northern South Africa, which is 259 km south of the Capricorn (Figure 4.2) (Hely-Hutchinson and Schuman, 1997). It is a large recreational attraction for many locals and tourists. It has five catchment basins which are from west to east, the Magalies/Skeerpoort, the Crocodile, the Jukskei, the Hennops and the Swartspruit basin (Van Reit, 1987). The Crocodile River is one of the most intensive irrigation system used river in South Africa with numerous points and diffuse sources of domestic and industrial pollution (Heath and Classen, 1999).

The Hartbeespoort dam was built in 1923 on the confluence of the Crocodile River and Magalies River, and was raised in the 1971 with 2.12 m. It has a total storage capacity of 185.49 $x10^6$ m^3 and a catchment area of 4 112 km^2 (Rossouw, 1992). The land usage in the Hartbeespoort dam can be divided into two categories, namely rural and urban. The commercial, residential and industrial that are associated with the northern suburbs of Johannesburg and also other smaller towns of the Witwatersrand make up the land use. On the other hand the rest of the area is used for natural reserves and agriculture (NIWR, 1985).

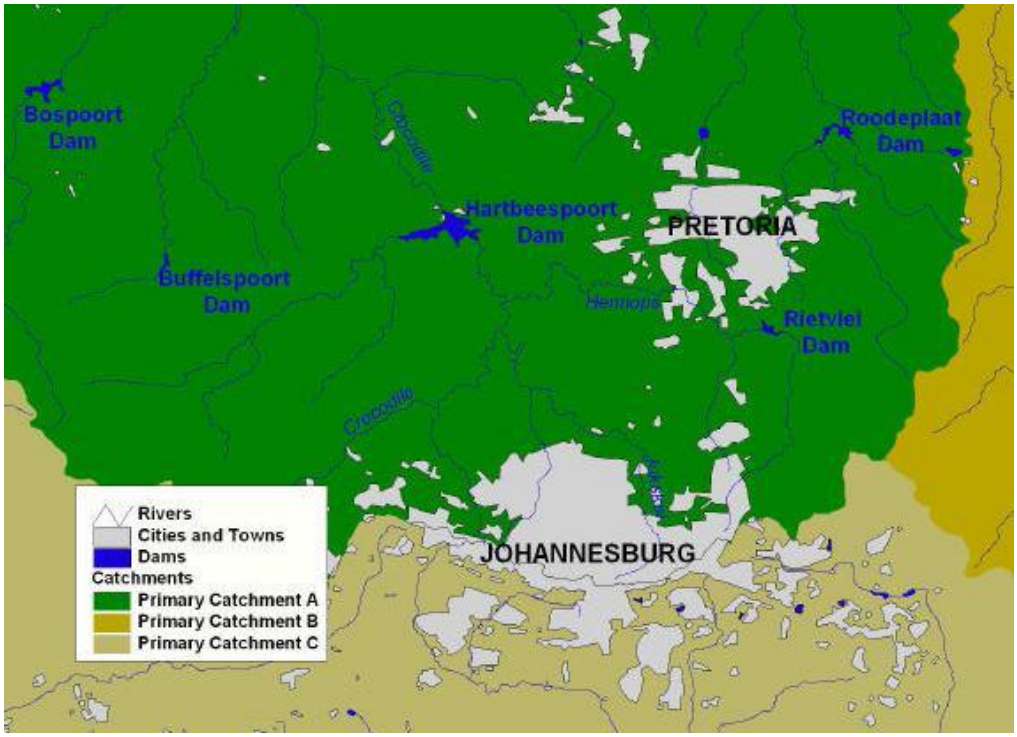


Figure 4.2: A map showing the catchment areas, rivers and urban/ industrial areas of Johannesburg

The rivers that flow into the Hartbeespoort dam are carrying an ever increasing volume of the wastewater from a rapidly growing industrial and urban complex (Aucamp *et al.*, 1987). It has been stated that the water of the Hartbeespoort dam is becoming unsuitable for agriculture, development and recreation (Van Reit 1987). The upper reaches of the Crocodile River drains the Johannesburg Northern suburbs and its Hennops tributary drains the Kempton Park, Tembisa, Midrand and Centurion. The Magalies River drains the town of Magaliesburg and Swartspruit drains the town of Hartbeespoort (Sutton and Oliveira, 1987). Other catchment areas include towns like Clayville, Olifantsfontein, Alexandria and a part of Atteridgeville and Saulsville (Rossouw, 1992).

Hartbeespoort dam has the potential to decrease water quality of the natural resources due to the dumping of the effluents and solid-waste, mines, industrial activities. Also sewage treatment plants of Johannesburg, Midrand, Kempton Park, Centurion, Olifantsfontein, Krugersdorp and Roodepoort area are there. Industrial sites include AEK (Pelindaba and Valindaba), AECI-Modderfontein as well as Kelvin power station.

There is also a potential contamination of storm water run-off from industrial areas including Clayville, Isando and Eastleigh as well as residential areas like Tembisa, Alexandria and Atteridgeville. The biggest influence on water quality in the Hartbeespoort Dam is the Moordefontein stream which forms the confluence with the Jukskei River and the Crocodile River (Rossouw, 1992).

Hartbeespoort Dam was built for irrigation purposes. It then became a water source for primary consumption and also an attractive recreational destination for many water sports enthusiasts, anglers and local and international tourists (DWA, 2009). The volumes of water and the loads of plant nutrients such phosphates and nitrogen that reach the dam have increased. This was due to rapid urban development as well as industrial growth in the Hartbeespoort Dam catchment area. As the urban areas in the catchment are expected to grow steadily, this trend may continue in the future. These nutrients are commonly found in fertilizers, industrial wastewater, sewage effluent and products such as soap and washing powders. Even though they are present in very low concentrations in the dam water and sediment, these plant nutrients cause excessive growth of algae and waterweeds such as hyacinth (Figure 4.3) (DWAF 1996a). The growth of alga causes problems in the environment (DWA, 2009).



Figure 4.3: Excessive growth of alga due to plant nutrients (DWA, 2009)

The contamination of Hartbeespoort Dam resulted to the following problems:

- Agriculture -Algal produced toxicity of water which in non portability for animals.
- Domestic - Non-potability (algal toxins), high purification costs for purification and bad taste after purification.
- Inhabitants - Seasonally strong unpleasant odours as well as excessive algal growth. Dam is not pleasant for recreation
- Anglers/recreation users - Algal mats and scums, reduced diversity of fishery, bad taste and odours in flesh of fish and health risks (toxic algae) and reduced visibility of water.
- Tourism and lodging operators - Reduced influx of tourists, loss of business and job opportunities. The dam lacks the attraction of a clean and safe water body.
- Proprietors - Reduced property values, (DWA, 2009).

Samples were collected in different areas around the Hartbeespoort dam. Figure 4.4 and Figure 4.5 shows the exact spots where the samples were taken.

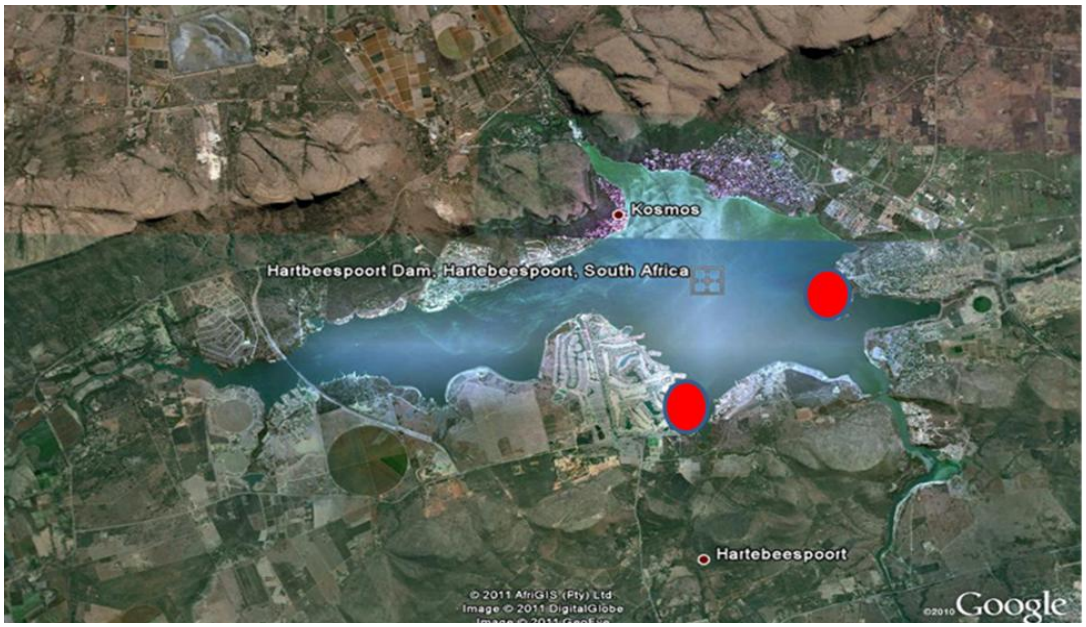


Figure 4.4: Sampling spots (red dots) in Hartbeespoort Dam



Figure 4.5: Sampling spots (red dots) in Hartbeespoort Dam

Centurion/Hennops River

Hennops River is situated on the southern part of Pretoria, it is synonymous with the river flowing to the countryside, golf estate, residential areas, recreational areas, industrial areas and business areas. Hennops River flows in the north-west direction through these areas including Centurion Lake. This enhances the aesthetic value of the surroundings as a result of a variety of trees, lush vegetation and birdlife that are present along its banks. A hiking trail along this part of the Hennops River was also created during the early nineties. The Centurion Lake is totally surrounded by businesses, hotels, shops and restaurants which have positive impact on the areas economy and job creation opportunities (Jan-Marten, 2004).

The progressive degradation of Hennops River owing to factors such as urban development, industrial activities, agricultural activities and informal settlements is being experienced. This degradation of the Hennops River and consequently Centurion has reduced the benefits of having river flowing through the centre of the Centurion CBD and intrinsic value it offers over the past three decades. An increase in hydrological and water quality problems such as riverbed erosion and microbiological

pollution has become issue of concern. More noticeable consequence of the Hennops River's degradation is the silting up of the Centurion Lake and the fact that the river's water has become unfit for recreational use. Recreational activities that were associated with the Centurion Lake include occasional swimming, windsurfing, angling (Freeman *et al*., 2000).

The main source of Hennops River is found approximately 10 km south of the Centurion Lake, which is in the form of the Kaalspruit that originates in the industrial area of Lethabong and residential area of Birch Acres in Kempton Park, Johannesburg. The Kaalspruit flows through the high density of informal settlements of Tembisa and Ivory Park northerly direction where tributaries such as Olifantspruit converge with the principal stream downstream from here before entering the Centurion Lake as the Hennops River. The main tributaries downstream from Centurion Lake are the Rietspruit and Swartbooispruit before the Hennops River ultimately converges with the Crocodile River to feed the Hartbeespoort Dam which is approximately 35 km north-west of Centurion Lake (Jan-Marten, 2004).

Due to the variety of land uses within the Hennops River catchment that exists are causing difficulty in adequately managing and controlling the water quality and hydrological status of its rivers and streams. This is due to the sources of pollution and hydrological degradation that are varied, cover a large area and also to be addressed in different ways. Land uses include formal and informal housing, commercial, industrial and business development as well as agricultural activities. The different land uses and activities occurring within the catchment influence various types of pollutants which enter the Hennops River and its principal tributaries (Jan-Marten 2004).

Natalspruit River

The Natalspruit (Figure 4.6) is situated in the middle of one of the most highly industrialized areas of the Witwatersrand.



Figure 4.6: Picture of Natalspruit River (Sibiya, 2011)

Natalspruit River extends approximately 25 kilometers from its source to the confluence with the Rietspruit. The Elsburgspruit is approximately 12 kilometers from its source to its confluence with the Natalspruit. The catchment area of these rivers covers most of Germiston, Boksburg, Alberton and the Eastern portion of Southern Johannesburg and is 225 km^2 in extent. There are approximately 1800 major and minor industries ranging from large chemical factories, steel works to minor engineering works in the area (Bodenstein *et al*., 2005). Industrial effluent contributes approximately 60 percent of the flow to the various purification works. Because of this factor illegal toxic discharges (accident or deliberate) often have a pronounced detrimental effect on the purification works over long periods and often lead to discharge of sub-standard effluent. This is due to the industrial nature of the area. A legacy of large slimes, sand and rock dumps as well as abandoned mines were left in the past by extensive gold mining. Seepage and run-off from the dumps create a substantial pollution load to the aquatic environment (Bodenstein *et al*., 2005).

Homestead Lake

Homestead Lake (Figure 4.7) is situated in Farrar mere which is part of Benoni. Guest houses are situated about a few metres from the lake. Wanderers Stadium, CR Swart Dam/Rynfield Dam, Apex Industrial Area and Jet Park are other nearby attractions (Heimann, 2003). Homestead Lake provides public recreation in addition to flood control. The 37-surface-acre reservoir is open to the public for fishing, picnicking, no-wake boating and other activities. The lake was completed in spring 2001. There are two main areas that are open for recreation. The one on the west of the dam, includes a boat ramp and a fishing dock. The other at the far northwest corner of the lake, includes a picnic shelter, additional picnic areas with fire rings and tables and primitive restrooms. Parking is also available at both areas. Portions of the Homestead Lake recreation area were opened for upland game hunting in fall 2003 (Heimann, 2003). Homestead Dam flows into Benoni Lake into Middle Lake (Figure 4.8) and Kleinfontein Lake (Figure 4.9). There is increasingly an amount of hyacinth in Benoni dams but so far only Middle Lake and Kleinfontein Lake has been affected. Figure 4.10 below shows the exact points where the samples were taken in Homestead Lake, Middle Lake and Kleinfontein Lake including Blauuwpan Dam. Three quarters of Kleinfontein is covered with the hyacinth (Heimann, 2003).



Figure 4.7: Picture of Homestead Lake (Sibiya, 2011)



Figure 4.8: Picture of Middle Lake (Sibiya, 2011)



Figure 4.9: Picture of Kleinfontein Lake (Sibiya, 2011).



Figure 4.10: Sampling spots (red dots) in Blaauwpan Dam, Homestead Lake, Middle Lake and Kleinfontein Lake near the OR Tambo airport

Jukskei River

The Jukskei River is in the east of the Alexandra west bank. As a result of rapid urbanization due to the development of a squatter camp on the west bank of the river in Alexandra Township, Jukskei River has been an example of an urban catchment in which problems have arisen. The development in the Alexandra area began in 1905 (Kisembo, 2004). Between the years 1945 and 1948 there was a large influx of people to this area which put a great strain on the environment as no formal sanitation services were yet available. In order to try and limit the number of people flocking to and staying in this area, a number of resettling policies have been implemented over the years (De Jager, 1990). Accommodation shortage in Alexandra Township rose. This led to the establishment of informal settlements on any open land, including Jukskei River banks. The closely built dwellings among several other factors have made refuse removal difficult and sanitation facilities inadequate, hence waste including human excreta is discharged on open lands or into the Jukskei. These wastes affect the water quality of the Jukskei River. Other land uses include: businesses such as spaza shops and shebeens

and community facilities such as schools, clinics and churches. There is a shortage of open space primarily because of the encroachment of informal housing onto public spaces such as pavements, schools and the natural open space system being along the tributaries of the Jukskei River (Matowanyika, 2010). Figure 4.11 to 4.14 shows the sampling areas in Jukskei River.



Figure 4.11: Jukskei River sampling spot1 (red dot) (Sibiya, 2011)



Figure 4.12: Jukskei River sampling spot2 (red dot)

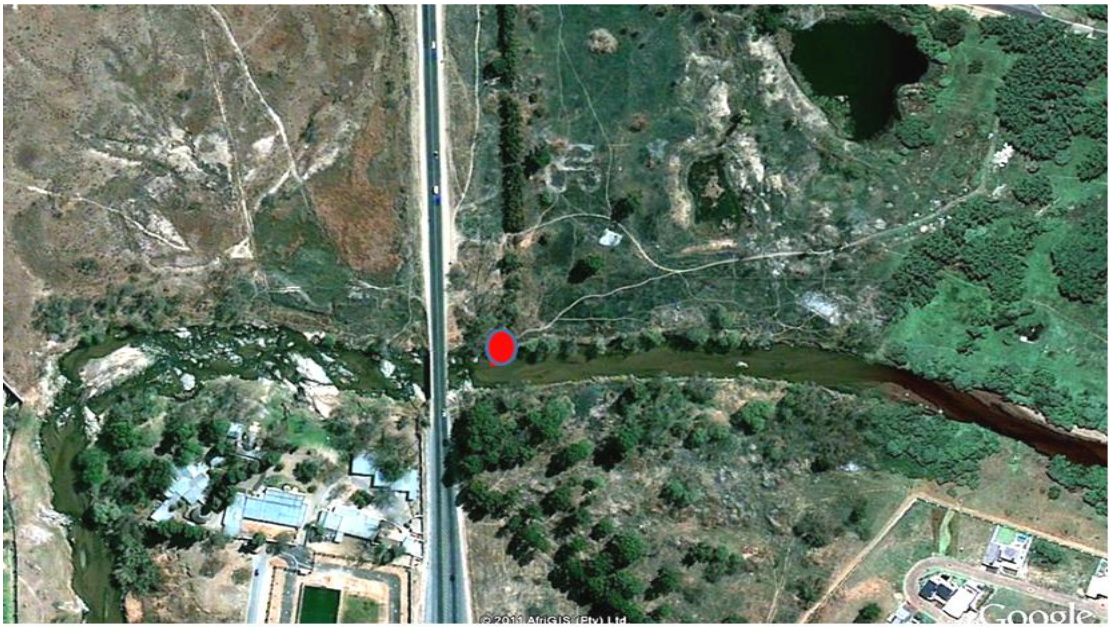


Figure 4.13: Jukskei River sampling spot3 (red dot)



Figure 4.14: Jukskei River sampling spot4 (red dot)

4.7 Sample preparation

The main aim of sample preparation is to transfer the analyte from the matrix to a suitable medium for introduction into the analytical instrument for analysis. Different sample preparation techniques were optimized and then applied to real samples in this work. For water samples HF-LPME and SPE were optimized, for solid samples MAE was optimized and SE was used as a reference method.

4.7.1 HF-LPME procedure

Prior to extraction, the fiber was cut into 5 cm length. For each extraction, a new fiber was used. The fiber was ultrasonically cleaned in acetone for 10 minutes and air dried before used. After drying, it was heat sealed on one end using a hot surface. It was then impregnated in heptane for 10 seconds to open the membrane pores. Heptane was then withdrawn into the syringe. The syringe needle was tightly fitted into the 5 cm length of the hollow fiber. The syringe plunger was pressed so that the fiber was completely filled with the heptane. The fiber was inserted in 20 mL sample solution without removing the syringe. It was then stirred for 30 minutes at 600 rpm (Figure 4.15). After stirring, the extract was collected by pushing heptanes through the fiber into a small vial and 17µl was collected. 1 µl was then injected into the GC-MS and/or GC-FID. Each experiment performed was repeated 3 times. The samples were prepared by spiking 20 mL of water sample with 140 µL of 1 mg L^{-1} stock solution of PAHs to make the final concentration of 7 µg L^{-1} in the solution.

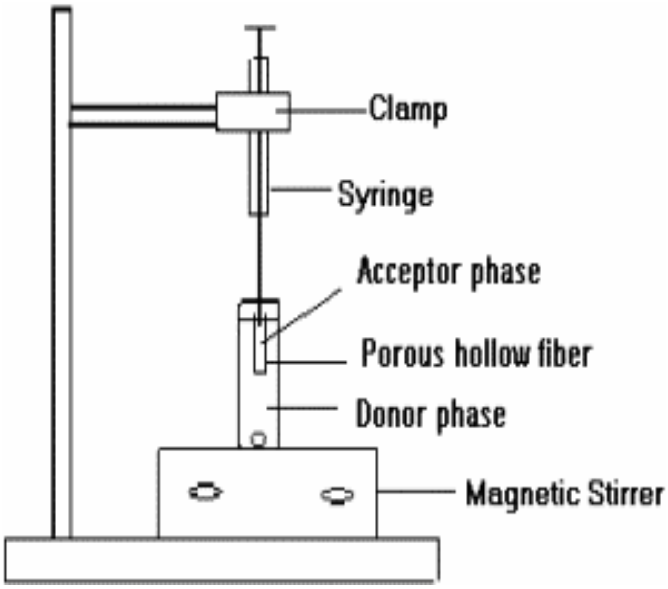


Figure 4.15: Schematic representation of HF-LPME technique (Basheer *et al.*, 2003)

4.7.1.1 Optimization of the critical parameters affecting HF-LPME extraction technique

Hollow fiber liquid phase micro extraction is based on an equilibrium distribution process. The amount of analyte extracted at a certain time depends on the mass transfer of the analyte from the aqueous sample to the organic solvent in the hollow fiber. There are several parameters such as type of organic solvent, stirring speed, extraction time, addition of salt etc that can enhance this distribution process (Fontanals *et al.*, 2006, Basheer *et al.*, 2003). Thus, all of the above mentioned factors affecting the extraction efficiency were optimized. These factors were investigated using deionized water samples spiked with known concentrations of PAHs. The goal was to optimize hollow fiber extraction procedures so as to obtain high analyte recovery and enrichment factors.

Selection of organic solvent

The selection of the type of organic solvent to be used as acceptor phase was performed by varying the acceptor solvents while keeping other parameters constant. For this purpose isooctane, heptane, toluene, hexane and dichloromethane were examined as possible acceptor solvents. The extraction time was kept constant at 30 minutes, the

stirring speed was set at 600 rpm and a concentration of 7 µg L^{-1} mixture of PAHs spiked in deionized water was used.

Stirring speed

The effect of stirring speed was investigated by stirring PAHs solution between 400 and 1000 rpm. Other parameters were kept constant. The extraction time was kept constant at 30 minutes and a concentration of 7 µg L^{-1} mixtures of PAHs spiked in deionized water was used. The acceptor solvent was iso-octane.

Extraction time

To determine the influence of extraction time, the spiked standard deionized water solutions with a concentration of 7 µg L^{-1} mixture of PAHs were extracted for different extraction times ranging from 15 to 60 min. The acceptor solvent was iso-octane and a stirring speed of 600 rpm.

Addition of salt

The possible salting-out effect was also examined in this study by varying the amount of sodium chloride added (0 –100 mg mL^{-1} NaCl) to the aqueous solution containing the target analytes. The extraction time was kept constant at 30 minutes and a concentration of 7 µg L^{-1} mixtures of PAHs spiked in deionized water was used. The acceptor solvent was iso-octane and a stirring speed of 600 rpm.

Addition of acetonitrile (ACN)

PAHs are characterized as hydrophobic compounds with very low water solubility, which is a problem as it leads to adsorption problems during sampling and storage. To increase their solubility an organic solvent or a surfactant is usually added to the sample. The concentration of the organic solvent is a critical parameter, because if it is too high, PAHs will apparently be too soluble in the donor phase to be efficiently extracted (Barri *et al.,* 2004). In this work acetonitrile was investigated as the organic

modifier because it increases the solubility and selectivity of PAHs (Belin *et al.*, 2005). The influence of organic solvent content in water samples was investigated by adding 0-40% acetonitrile in the sample. The extraction time was kept constant at 30 minutes and a concentration of 7 µg L^{-1} mixtures of PAHs spiked in deionized water was used. The acceptor solvent was heptane and a stirring speed of 600 rpm.

Spiked concentration and detection limit

PAHs are generally found at trace levels in water bodies because of their poor solubility. The developed extraction method should allow concentrating and increasing such low levels to values able to be detected by the analytical instrument. PAHs low solubility in water may also affect the recovery especially at different concentrations (Kiss *et al.*, 1996). The effect of sample concentration was investigated by extracting spiked concentrations at 3 and 7 µg L^{-1} mixtures of PAHs. The detection limit was determined as a concentration that gives three times the peak height to that of the background noise. GC-MS was used for analysis.

Application to real samples

Real river water samples from Hennops River, Centurion Lake, Hartbeespoort dam and Jukskei River were collected for analysis. The samples were extracted without any filtration or pretreatment. 20 mL of each sample with 20% ACN was extracted in triplicate using the optimized HF-LPME procedure, heptanes as extraction solvent, 30 minutes extraction time at 600 rpm stirring speed. No salt was added.

4.7.2 SPE procedure

C_{18} spe cartridges were used in this work. The cartridges were conditioned with 5 mL methanol, then 5 mL methanol-water (40:60). 7 µg L^{-1} spiked 100 mL sample with 10% methanol as organic modifier was sucked through each cartridge (Figure 4.16) at a flow rate of 1 mL min^{-1}. Sample loading was followed by 5 mL milli-Q water for washing. The analytes were the eluted by 3 mL acetone: THF (1:1) at a flow rate of 0.5 mL min^{-1}.

The volume was then reduced to 1 mL under nitrogen flow. Finally the concentrated eluate was then injected in GC-MS. Each experiment was repeated three times. The samples were prepared by spiking 100 mL of water sample with 500 µL of 1 mg L^{-1} stock solution of PAHs to make the final concentration of 5 µg L^{-1} in the solution.

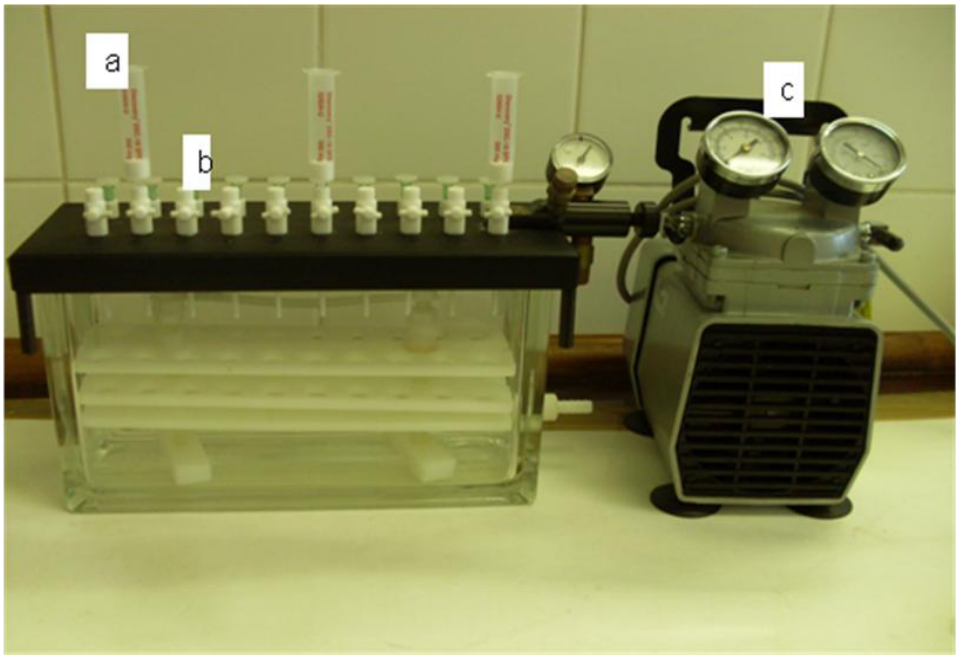


Figure 4.16: Representation of SPE technique. Where, a is the cartridge with sorbent, b is the cartridge holder and the flow rate can be adjusted, c is the pressure vacuum pump

4.7.2.1 Optimization of the critical parameters affecting solid phase extraction technique

There are several parameters such as addition of organic modifier, spiked concentration, sample loaded volume, conditioning solvent, washing solvent and eluting solvent that need to be optimized in order to enhance the extraction efficiency. Thus all of the above mentioned factors affecting the extraction efficiency were optimized. These factors were investigated using Milli-Q water samples spiked with known concentrations of PAHs. The goal was to optimize the solid phase extraction procedure so as to obtain high analyte recovery and enrichment factors.

Volume of sample loaded

The effect on sample volume was investigated by varying the volume between 100-200 mL of spiked deionised water. The extraction was conducted with 20% ACN as the organic modifier, 5 mL of acetone: water (40:60, v/v) as the conditioning solvent, 5 mL of deionized water as the washing solvent, 3 mL of acetone: THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in deionized water was used.

Selection of organic modifier

In order to increase their solubility, an organic solvent or a surfactant is usually added to the sample. In order to investigate the influence of adding the organic modifier methanol, 2-propanol and acetonitrile were investigated at 10%. Other parameters were kept constant, 5 mL of acetone:water (40:60, v/v) as the conditioning solvent, 5 mL of deionized water as the washing solvent, 3 mL of acetone:THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water was used.

Varying methanol amount

The effect of methanol content in the sample was examined by varying methanol amount between 0-20% in the sample. Other parameters were kept constant, 5 mL of acetone: water (40:60, v/v) as conditioning solvent, 5 mL of deionized water as washing solvent, 3 mL of acetone: THF (1:1) as eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water was used.

Selection of conditioning solvent

The effect of conditioning solvent was evaluated at 40% acetone, methanol and 2-propanol in water. Other parameters were kept constant; 5 mL of deionized water as the washing solvent, 3 mL of acetone: THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water and 10% methanol was used as the organic modifier.

Amount of the conditioning solvent

In order to investigate the effect of the amount of methanol content as the conditioning solvent, it was varied between 20-40% in the sample. Other parameters were kept constant; 5 mL of deionized water as the washing solvent, 3 mL of acetone: THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water and 10% methanol was used as the organic modifier.

Selection of eluting solvent

A suitable strength of the eluting solvent was determined by examining 3 mL acetone:tetrahydrofuran (1:1), 3 mL methanol:tetrahydrofuran (1:1) and 3 mL acetonitrile in tetrahydrofuran (1:1). Other parameters were kept constant; 5 mL of acetone: water (40:60, v/v) as the conditioning solvent, 5 mL of deionized water as washing solvent, a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water, 10% methanol as the organic modifier and 40% methanol in water as the conditioning solvent.

Varying spiked concentration

PAHs are characterized by their low solubility in water. Due to their low solubility they are found at trace levels in water bodies, which then require the developed method to be able to allow such low levels to be detected by the analytical instrument. In order to

investigate that effect spiked water concentration was varied between 3 and 7 µg L^{-1} mixture of PAHs.

Validation of extraction method

SPE method was validated by using certified reference material adopted by National Institute for Standards and Technology (NIST). The concentrations in the reference material were 6.67 mg L^{-1} for naphthalene, 3.47 mg L^{-1} for acenaphthene, 1.15 mg L^{-1} for phenanthrene, 0.570 mg L^{-1} for fluoranthene and 1.48 mg L^{-1} for pyrene. The sample was prepared by transferring 1 mL of the concentrated reference material standard containing different concentrations PAHs compounds to a 1 L of water. The final concentrations in 1 L of the sipked water were 6.67 µg L^{-1} for naphthalene, 3.47 µg L^{-1} for acenaphthene, 1.15 µg L^{-1} for phenanthrene, 0.570 µg L^{-1} for fluoranthene and 1.48 µg L^{-1} for pyrene. 100 mL of the prepared sample was extracted in triplicates using the above described SPE procedure. The concentrations of the compounds in the certified material were back calculated from the calibration curve and the enrichment factors of the optimized SPE method.

Application to real samples

The analysed real river water samples were collected from Blaauwpan Dam, Homestead Lake, Middle Lake, Kleinfontein Lake, Hennops River, Centurion Lake, Hartbeespoort Dam and Jukskei River. The samples were extracted without any filtration or pretreatment. 100 mL of each sample was extracted in triplicate using the above described SPE procedure.

4.7.3 MAE procedure

MAE (multiwave 3000) was used for this work. 250 W was used as extraction power. Sediment sample was weighed into reaction vessels (Figure 4.17) followed by the addition of hexane-acetone mixture. MAE was carried out using 1g sample, 20 mL hexane:acetone mixture as extraction volume and extraction solvents and 20 minutes

extraction time. After extraction, the extract was filtered and dried through anhydrous sodium sulphate to remove water. For the cleanup, a column was plugged with glass wool. It was then filled with approximately 7 g activated silica gel and topped with 2 g of anhydrous sodium sulphate. 10 mL of hexane was used to pre-wash the column and allowed to drain to bed level. The raw extract was transferred quantitatively to the column. The column was eluted with 5 mL hexane. After clean-up step, the extract was reduced to 1 mL under nitrogen and then injected into a GC-FID. The samples were prepared by spiking 1 g of sediment sample with 625 µL of 8 mg L^{-1} stock solution of PAHs to make the final concentration of 5.216 mg kg^{-1}. This was thoroughly mixed. Finally, 20 mL of extraction solvent was then added. Blank real sample was also measured since the sample already contained some PAHs.

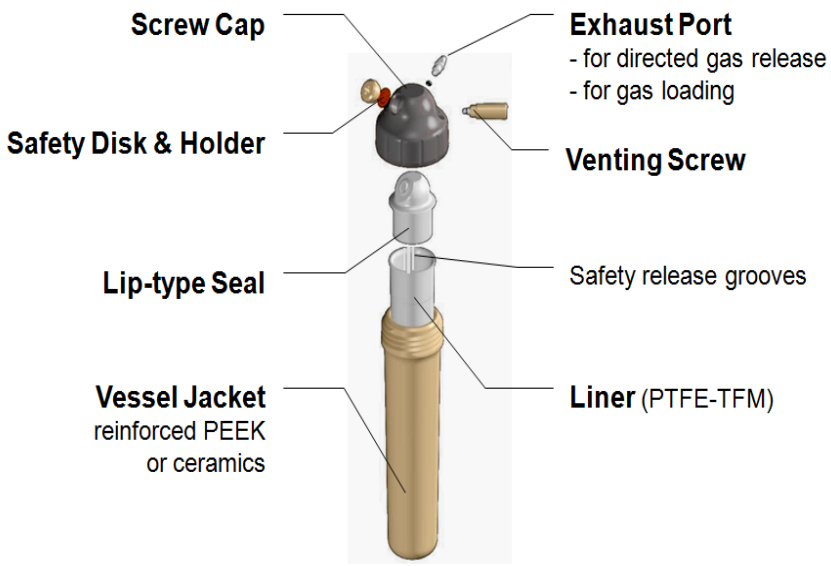


Figure 4.17: Pressure-vessel design of a microwave digestion liner (Anton Paar, 2009)

4.7.3.1 Optimization of the critical parameters affecting microwave-assisted extraction technique

MAE is influenced by many factors such as extraction time, solvent type, solvent volume, sample amount and extraction power. Thus we optimized all of the above mentioned factors affecting the extraction efficiency. These factors were investigated

using sediment samples spiked with known concentrations of PAHs. The goal was to optimize the microwave-assisted extraction procedure so as to obtain high analyte recovery and enrichment factors.

Varying extraction power

The power must be chosen correctly to avoid excessive temperature which could lead to solute degradation and overpressure inside the vessel. In order to investigate the influence of extraction power, it was varied between 200 and 300 W. The extraction was carried out using 20 mL hexane:acetone (1:1) as extraction volume and solvents, 10 minutes as extraction time and 1g as sample mass.

Selection of extraction solvent

The basic factor which affects an extraction process is the choice of appropriate solvent. To select the appropriate solvent for the process, hexane:water (1:1), hexane:acetone (1:1) and hexane were investigated. The other parameters were kept constant, 20 mL as extraction volume, 20 minutes as extraction time, 1g as sample mass and 250 W as extraction power.

Volume of extraction solvent

The solvent volume used should be sufficient enough to immerse the matrix completely in the solvent throughout the entire irradiation process. The effect on volume of extraction solvent was investigated by varying the volume between 20-40 mL. The other parameters were kept constant, hexane: acetone as extraction solvents, 10 minutes as extraction time, 1g as sample mass and 250 W as extraction power.

Varying extraction time

Time is another parameter whose influence needs to be taken into account. To determine the time needed to obtain high recovery, extractions were performed at

different times between 10-30 minutes. The other parameters were kept constant, 20 mL hexane:acetone as extraction volume and solvents, 1g as sample mass and 250 W as extraction power.

Varying the amount of sample

Optimisation of the amount of sample is important in order to assess the homogeneity of the material (Pensado *et al.*, 2000). To assess the sample amount influence, the sample mass was varied between 0.5 and 2 g. The other parameters were kept constant, 20 mL hexane:acetone as extraction volume and solvent, 20 minutes as extraction time and 250W as extraction power.

Validation of extraction procedure

The MAE procedure was validated by using certified reference material adopted National Environmental Laboratory Accreditation Conference (NELAC). Certified material was extracted using the above described MAE procedure. The concentrations in certified reference material had 906 µg kg^{-1} for naphthalene, 533 µg kg^{-1} for acenaphthene, 845 µg kg^{-1} for phenanthrene, 613 µg kg^{-1} for fluoranthene and 341 µg kg^{-1} for pyrene. The concentrations of the compounds in the certified material were back calculated from the calibration curve and the enrichment factors of the optimized MAE method.

Application to real sediment samples

Real river water samples from Blaauwpan Dam, Homestead Lake, Middle Lake, Kleinfontein Lake, Hennops River, Centurion Lake, Hartbeespoort Dam, Natalspruit River (PIT) and Jukskei River were collected for analysis. The samples were air dried for 48 hours, they were then grounded using pulviriser at 400 rpm for 10 minutes. 1 g of each sample was extracted using the MAE procedure described above.

4.7.4 Soxhlet procedure

Soxhlet extraction was used as the reference method for solid, and the procedure was taken from (Guo *et al.,* 2009). The Soxhlet was carried out with 250 mL of dichloromethane: hexane mixture in 15 g sample for 24 hours in a water bath maintained at 60 ^{o}C. The extract was then passed through a 1:2 alumina: silica gel glass column with 1 g anhydrous sodium sulphate overlying the silica gel for clean up and fractionation. The eluents were collected by eluting with 70 mL of dichloromethane: hexane (7:3, v/v). After cleaning the extract was reduced to 0.5 mL under nitrogen and then injected into a GC-FID and/or GC-MS.

4.8 Determination of organic content

The organic content is the ratio of the mass of organic matter in a given mass of soil to the mass of the dry soil. It is expressed as a percentage (Reddy, 2002).

The organic matter in this work was determined by burning in a furnace 2 g of air dried sediment at 425 ^{o}C for 24 hours. The sediment was reweighed after burning to get mass of burnt sediment (M_A). The organic matter was determined by a difference in mass of burnt sediment from the mass of dried sediment

$$M_O = M_D - M_A \tag{4.1}$$

Where $M_{O\ is}$ the organic matter, M_D is the mass of dried sediment; M_A is the mass of burnt sediment.

The organic content (OM) was than calculated using equation (5.2) for all the sediment samples analysed in this work.

$$OM = \frac{M_O}{M_D} X100 \tag{4.2}$$

4.9 Quantification and quality assurance

A number of activities were taken into consideration in order to ensure quality of the final results obtained in the experiments. This included making calibration curves and repeating experiments. Certified reference standards were also used as part of quality assurance.

Calibration

Quantification of the extracts for PAHs compounds was performed by external calibration curves that were linear in concentration range of 0.25 to 2.0 mg L^{-1} for GC-FID and 0.2 to 1.0 mg L^{-1} for GC-MS and 0.1 to 1.0 mg L^{-1} for HPLC-Fluorescence. The calibrations gave good level of linearity with correlation coefficients (r^2) between 0.997-0.999 for GC-FID, 0.983-0.999 for GC-MS and 0.999 for HPLC-Fluorescence. Typical calibration curves (Figure 4.18-4.20) and standard chromatograms for PAHs compounds are shown in Figure 4.21-4.23 below, for GC-FID, GC-MS and HPLC-Fluorescence respectively.

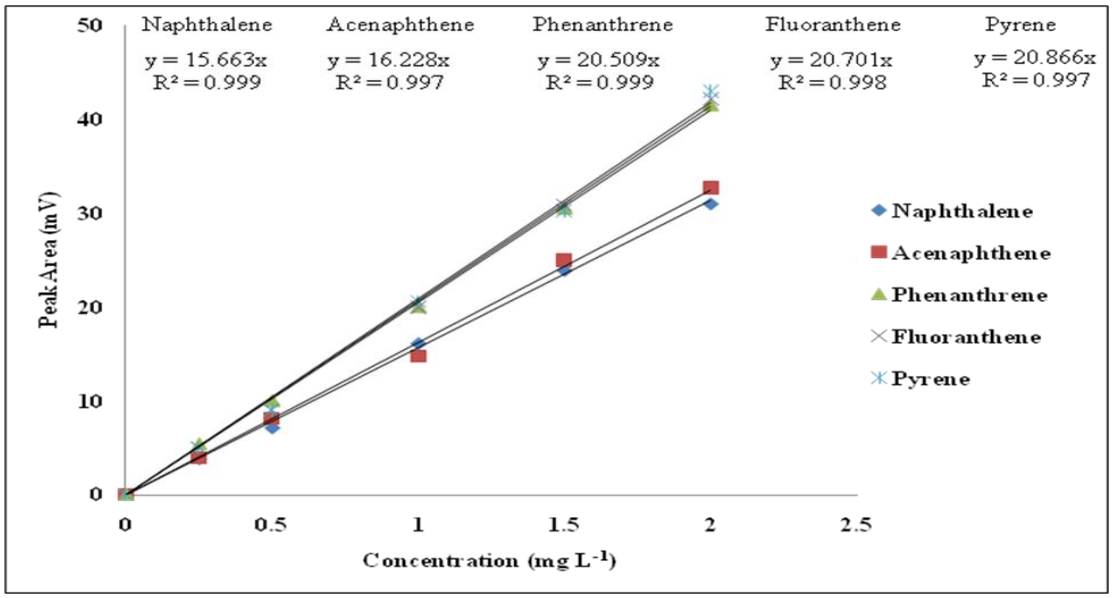


Figure 4.18: Typical calibration curves for GC-FID

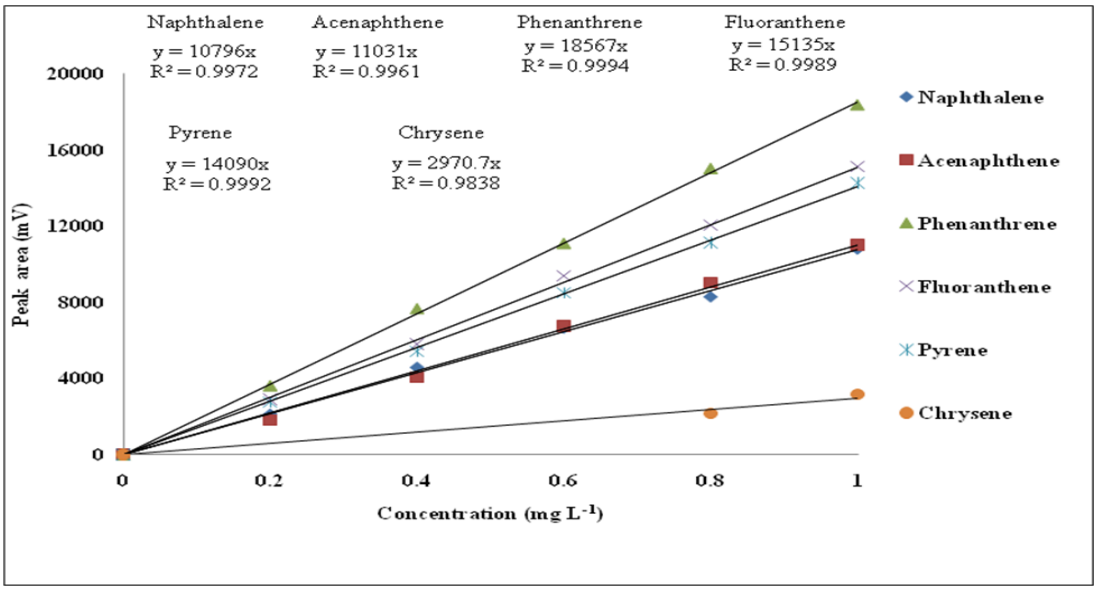


Figure 4.19: Typical calibration curves for GC-MS

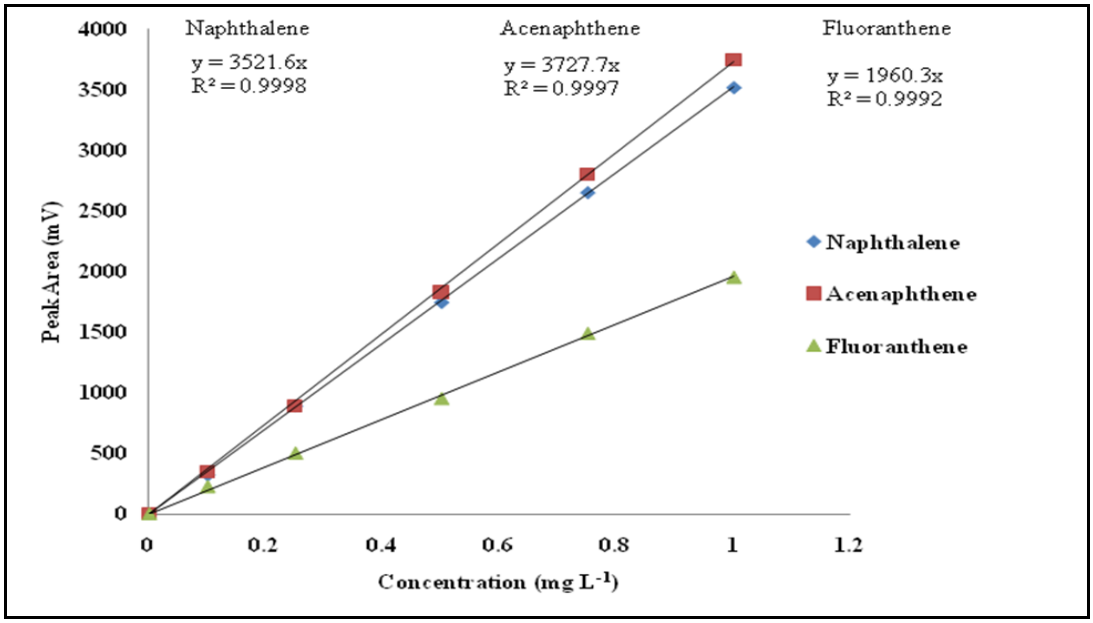


Figure 4.20: Typical calibration curve for HPLC-Fluorescence

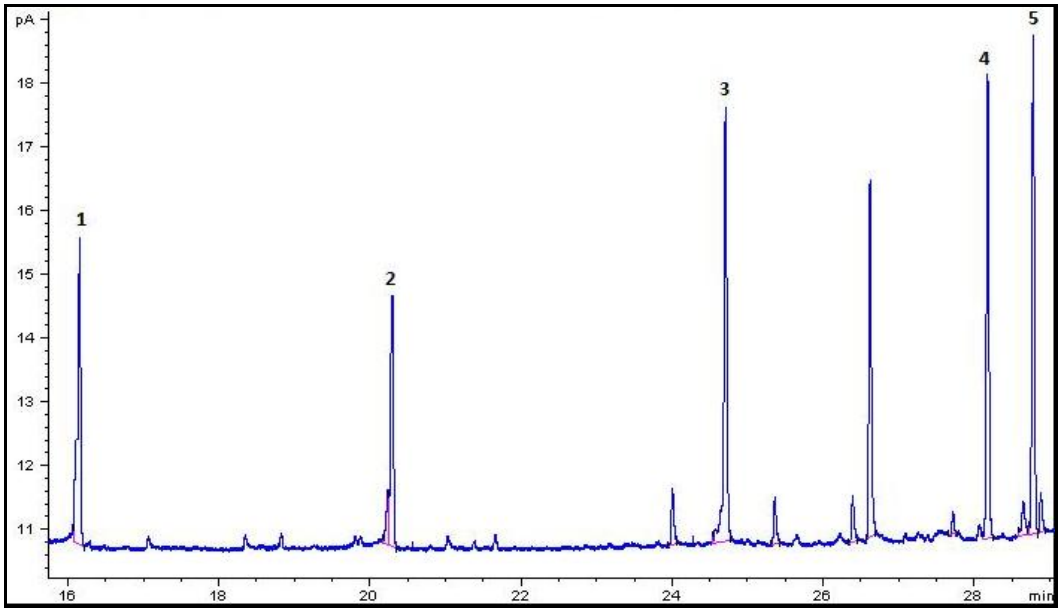


Figure 4.21: A typical chromatogram of 1.0 mg L^{-1} of PAHs standard injection where (1) Naphthalene, (2) Acenaphthene, (3) Phenanthrene, (4) Fluoranthene, (5) Pyrene, using GC-FID.

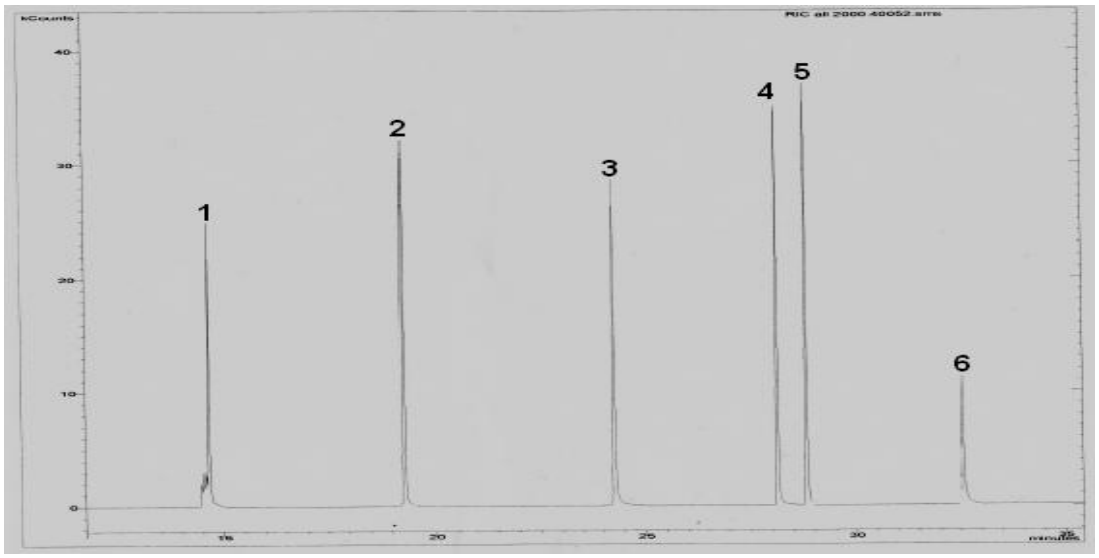


Figure 4.22: A typical chromatogram of 1.0 mg L^{-1} of PAHs standard injection where (1) Naphthalene, (2) Acenaphthene, (3) Phenanthrene, (4) Fluoranthene, (5) Pyrene, (6) Chrysene, using GC-MS.

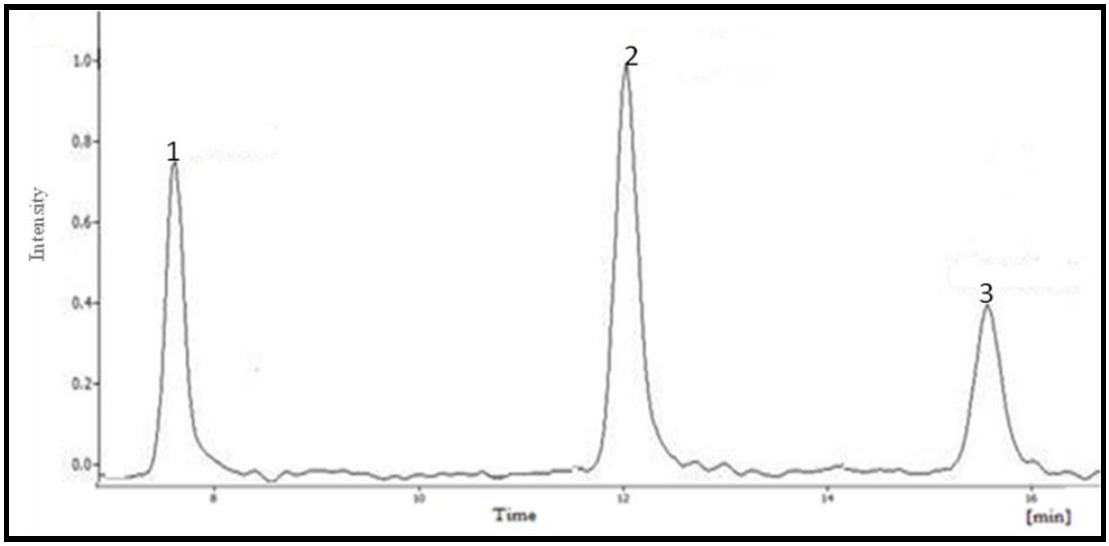


Figure 4.23: A typical chromatogram of 1.0 mgL^{-1} of PAHs standard injection where (1) Naphthalene, (2) Acenaphthene, (3) Fluoranthene, using HPLC-Fluorescence.

Repeatability

The repeatability of the analysis was performed by injecting same standard three times in order to measure the precision of the results, and the reproducibility was performed by repeating each experiment three times. The results showed to be repeatable and also reproducible as the RSD's values obtained were less than 10%.

Spiking of the samples was done in order to determine the extraction efficiency and enrichment factors. This was done by spiking samples with 7 µg L^{-1} of the standards solution. The limits of detection were calculated as three times signal to noise ratio (3xS/N) in order to determine the lowest concentration that can be detected. The blank samples were also analysed in order to check for any possible contamination.

Certified Reference materials

Certified reference materials for both the liquid and solid samples were analysed in order to check the validity of the methods. For solids samples, the certified materials were extracted using MAE optimized method described in 5.8.3 above. For liquid

samples, the reference materials were extracted using SPE optimized method described in 5.8.2 above. The extractions were performed three times for both liquid and solids. The concentrations for all compounds in the original certified reference materials were calculated from the optimized extraction efficiencies and determined concentrations in the extracts.

Other steps taken to ensure quality of the results

The processes of sample collection, transport and storage should be performed in such a way as to ensure that the samples remain unchanged until they are subjected to final analysis. The main processes that damage sample integrity are i) Physical processes, mainly analyte adsorption onto the walls of samplers and vessels, as well as evaporation of volatile components. ii) Chemical reactions including photolysis, oxidation, reduction, complexing. iii) Biological reactions such as biodegradation. To minimize these negative effects, the analysis should be performed as rapidly as possible thus minimizing the time between sampling and final analysis. Also appropriate methods of sample collection, conservation, transport and storage should be applied (Adkonis *et al.*, 2006). Papers dealing with PAHs determinations suggest that drops in analyte concentration could be caused by sorption onto components of the sample preparation system. It was also confirmed that adsorption onto glass may be responsible for a 10–25% drop in the PAH content in a water sample. A study of the literature over many years suggests that our knowledge of the degree of loss of PAHs during sampling, transport and storage is incomplete in spite of the fact that scientists are aware of the potential for loss (Adkonis *et al*, 2006). In this work certain steps were taken in order to account for these errors mentioned and thus control the quality of the results. In order to minimise the error due to adsorption in the containers, acetonitrile and methanol were used as organic modifiers in the samples. Also all glassware was scrupulously cleaned using hot water and soap and rinsed with deionised water. They were then rinsed with methanol and then dried in oven for 24 hours prior any usage.

Chapter Five – Results and Discussion

5.1 Introduction

SPE, MAE and HF-LPME extraction techniques were optimized in this work. The optimized conditions were then applied to real samples. This chapter presents the results obtained in the optimization of various sample preparation techniques as well as their applications on real samples obtained from Hartbeespoort Dam, Centurion Lake, Hennops River, Jukskei River, Natalspruit Dam, Blaauwpan Dam near OR Tambo International Airport, Homestead Lake, Middle Lake and Kleinfontein Lake. It also presents the comparison of SE and MAE in the extraction of sediment samples and comparison of SPE and HF-LPME techniques for water samples. The comparison was based on concentrations obtained, enrichment factors, extraction efficiencies and detection limits.

5.2 Optimization of the HF-LPME extraction technique

5.2.1 Stirring speed

The stirring speed used in the extraction procedure was investigated since agitation of the sample reduces the time to reach thermodynamic equilibrium, especially for the analytes with higher molecular mass (Fontanals *et al.,* 2006). The results are shown in Figure 5.1. Stirring speed enhanced the extraction up to 600 rpm. Above 600 rpm the amount extracted started to decrease, which decreased more with the increase in stirring speed. The observed trend is expected. Initially, the amount extracted increases with stirring speed as this enhances the movement of the PAHs in the bulk of the sample to the hollow fibre surface. However, at too high stirring speed, the amount of analytes extracted decreases due to the generation of air bubbles on the surface of the hollow fibre and due to the loss of the organic solvent and thus analyte loss. 600 rpm was therefore selected as the optimum stirring speed. Varying the stirring speed in the screening of PAHs in soil using hollow fiber membrane solvent micro extraction has been reported (King *et al*., 2002). Stirring speed was varied from 0-1225 rpm. The

amount extracted increased with stirring speed but above 800 rpm setting, the stirring became too violent leading to loss of extraction solvent. Varying the stirring speed from 0-1250 rpm in analysis of PAHs in wastewater treatment plant effluents using hollow fibre liquid phase micro extraction has also been reported (Charalabaki *et al.,* 2005). The amount extracted increased with stirring speed until after 600 rpm where it started decreasing. This decrease was attributed to solvent loss during extraction at higher stirring rate. The obtained optimum stirring speed generally depends on the set-up especially size of the sample container, viscosity of solution and type of magnetic stirrer. This explaines why the optimum obtained in this work is different from that of King *et al*., (2002). However, regardless of these variables the obtained trend should be similar as verified in Figure 5.1.

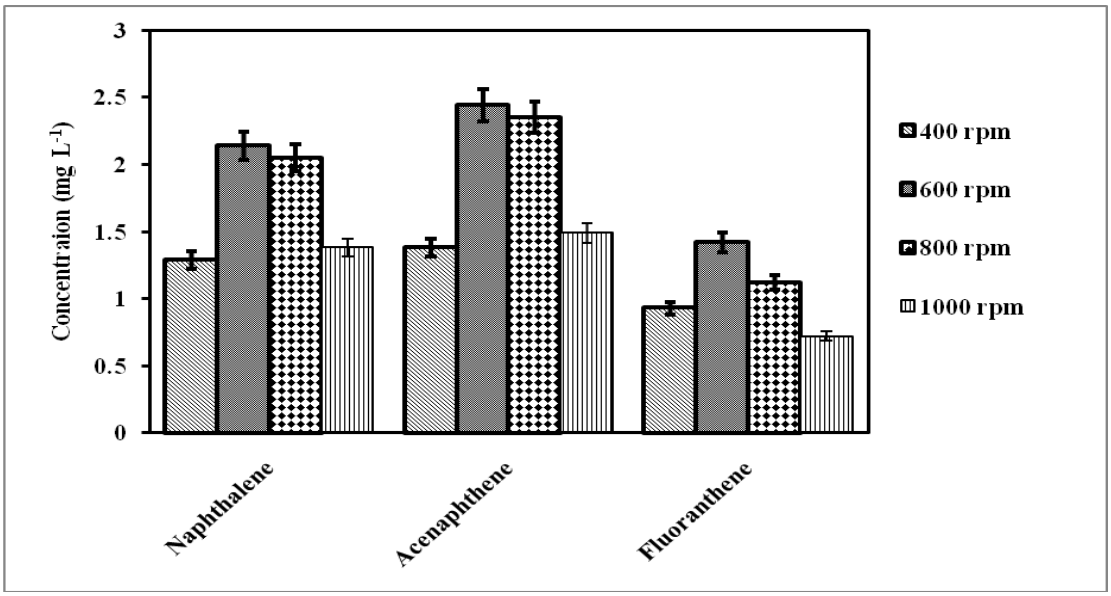


Figure 5.1: Effect of sample stirring speed on the amount extracted. Other parameters kept constant were 30 minutes extraction time, iso octane as the acceptor solvent and a of 7 µg L^{-1} mixtures of PAHs spiked in deionized water was used with no salt added in the sample.

5.2.2 Extraction time

Hollow fiber extraction involves a dynamic partitioning of the target compounds between the hollow fiber and the sample solution and diffusion of the compounds through the impregnated solvent to the bulk of the acceptor solvent. The extraction efficiency therefore depends on both the transfer of the PAHs from sample solution to the hollow fibre surface and diffusion through the organic liquid. The mass transfer is therefore a time-dependent process (Basheer *et al*., 2003). The effect of extraction time was thus examined from 15-60 minutes (Figure 5.2). The extraction profile of the analyte showed an increase with an increase in extraction time up to 30 minutes. Between 30-45 minutes it started to decrease steadily, it then decreased further between 45-60 minutes. This suggests that the equilibrium was reached at 30 minutes. Long extraction time increases solvent loss and thus the decrease in the amount of analyte extracted. On the basis of the results obtained, an extraction time of 30 minutes was selected as the optimum time. Varying the extraction time from 5-25 minutes in similar set-up and similar compounds has been reported (Charalabaki *et al*., 2005). The amount extracted increased with increasing extraction time as observed in this study. Another similar study found out that the amount extracted increased with extraction time up to 30 minutes (Basheer *et al.*, 2003). Between 30 and 40 minutes of extraction time, a plateau was reached. The findings of this study are in consistency with those of (Charalabaki *et al*., 2005; Basheer *et al*., 2003) are also consistency with this study. Even though 30 minutes has been reported as the optimum extraction time for PAHs, but further optimization needed to be done. The reason for this is that even when applying an existing method, the reported parameters might not be applicable due to factors such as differences in analytical separation systems, detection etc.

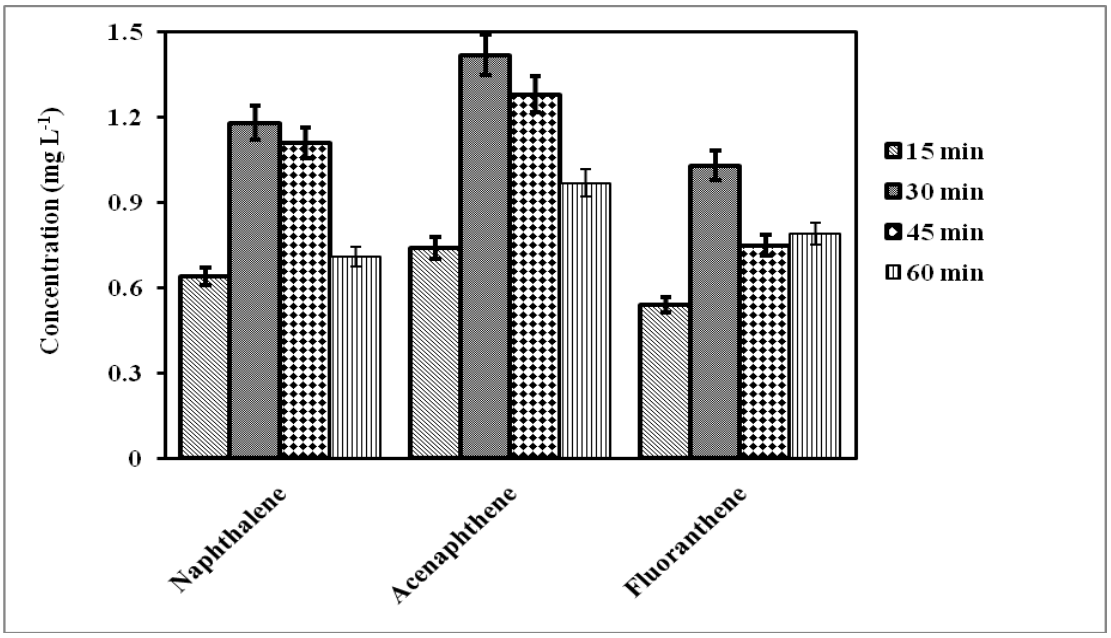


Figure 5.2: Effect of extraction time on the amount extracted. Other parameters kept constant were iso octane as the acceptor solvent, 600 rpm as the stirring speed with deionized water spiked with 7 µg L^{-1} mixture of PAHs as sample. No salt was added in the deionized water.

5.2.3 Addition of salt

NaCl is often added to the water sample in order to increase ionic strength and enhance the analyte extraction through the increased partitioning into the organic phase in the membrane. However, salt addition may increase the viscosity and density of the aqueous phase which could negatively affect the kinetics of the process and consequently, the extraction efficiency (Fontanals *et al.,* 2006). Further salt addition may increase adsorption on the surface for hydrophobic compounds like PAHs. Figure 6.3 indicate the results of varying the amount of salt added in the sample. The obtained results showed that the optimal extraction is best achieved when no salt was added. These results are in agreement with other studies, where PAHs were extracted using liquid phase micro extraction (LPME) and the addition of salt did not improve the extraction of these compounds (Hou *et al.,* 2003, Charalabaki *et al*., 2005). PAHs are generally very non-polar compounds whose solubility in water is very low (Table 2.1). Addition of salt therefore reduces the solubility further since the solution becomes too

polar and leads to PAHs adsorbing to surface containers thus limiting the amount extracted into the hollow fibre.

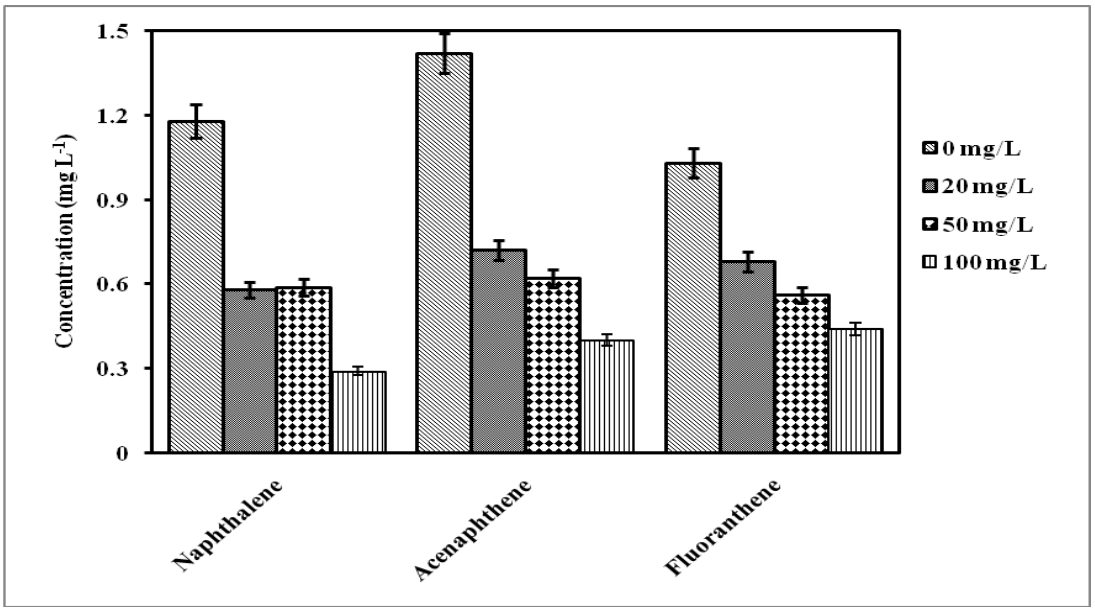


Figure 5.3: Effect of salt added in the sample on amount extracted. Other parameters kept constant were 30 minutes extraction time, 600 rpm as the stirring speed, iso octane as the acceptor solvent and a concentration of 7 µg L^{-1} mixtures of PAHs spiked in deionized water in the sample.

5.2.4 Selection of organic solvent

There are a number of factors that are important in the selection of organic solvent to be used for the acceptor phase and for impregnating the membrane. These factors are; i) high partition coefficient for the target compounds compared to the matrix components to give the desired selectivity ii) low solubility in water so as to prevent dissolution into the aqueous phase, iii) low volatility so as to restrict solvent evaporation during the extraction process and iv) solvent polarity should match that of the fiber so that it can be easily immobilized within the pores of the hollow fiber (Psillakis *et al*., 2003). Figure 5.4 shows the results of the optimization of the organic acceptor solvent while Table 5.1 shows the physical parameters of the solvents used.

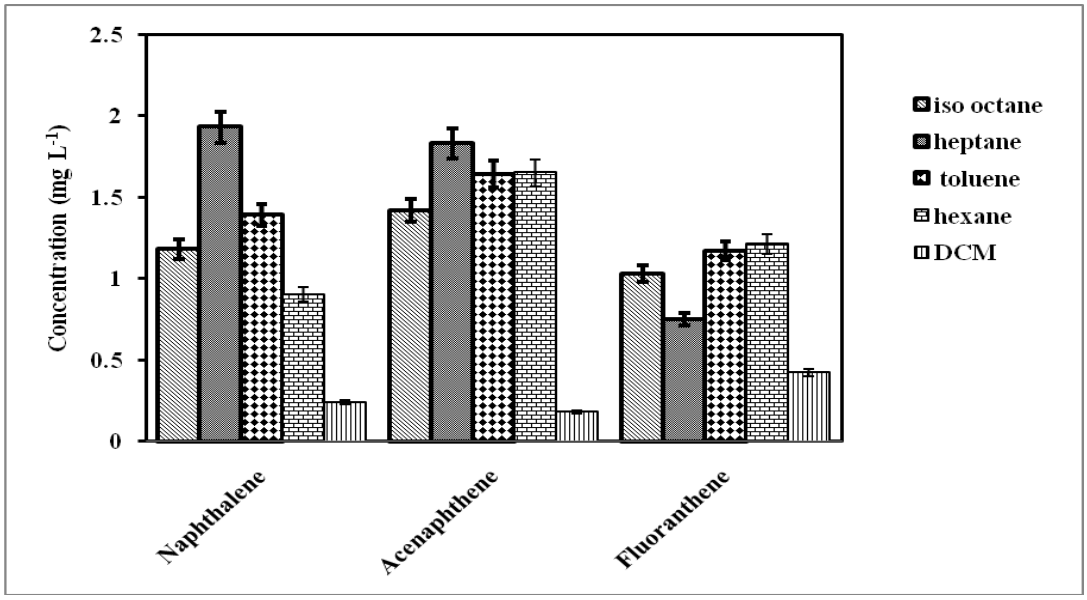


Figure 5.4: Effect of organic acceptor solvent on the concentration extracted. Other parameters kept constant were 30 minutes extraction time, 600 rpm as the stirring speed and a concentration of 7 µg L^{-1} mixture of PAHs spiked in deionized water as sample with no salt added.

Table 5.1: Physical parameters of the solvents used in HF-LPME **(**Knovel, 2008)

Physical Properties					
Solvents	**Boiling Point (^oC)**	**Molar mass (g mol^{-1})**	**Polarity**	**Dielectric constant**	**Water solubility**
DCM	40	84.93	3.1	8.93	1.60
Toluene	111	92.14	2.4	2.38	0.052
Hexane	69	86.18	0.1	1.88	0.014
Heptane	98	100.21	0.1	1.92	0.0003
Iso octane	99	114.23	0.1	1.94	0.0002

According to the results obtained, heptane extracts showed slightly higher response than the other solvents. This was taken as the best solvent. Toluene, iso octane and hexane did also give good results. Dichloromethane is not suitable as it is relatively soluble in water and has high volatility. Since PAHs are neutral non-polar compounds, the major attraction forces are dispersion interaction with non-polar organic solvents. The resulting interaction generally increases with the length of the organic chain of the

solvent or with the molar mass of the solvent (Reemtsma *et al.,* 2006). This is why heptane gave better results than hexane. The resulting interaction tends to decrease with increase in branching of the solvent since this reduces the contact area with PAHs. This may explain why heptane gave slightly better results than iso octane. Heptane was not the best for fluoranthene. This means that apart from the polarity and structure of the extraction solvent, the structure of the PAH also plays a role in determining the strength of interaction between the former and the latter. The optimization of LPME for the extraction of PAHs has been reported (Charalabaki *et al*., 2005; Basheer *et al*., 2003), toluene was chosen as the best solvent. In this case, the amount of sample extracted was 5 mL and extraction time was 15 and 30 minutes, respectively. Octane was found to be the best acceptor solvent in the study by (King *et al*., 2002).

The results of this work (Figure 5.4) show deviation from those of (Charalabaki *et al*., 2005; Basheer *et al*., 2003; King *et al*., 2002) for the similar PAHs as heptane was found to be the best extraction solvent. This could be due to that different analytes bind differently to different matrices. Binding is also a function of time that the PAHs has been in contact with the matrix, hence different solvents with different polarity indices and other chemical and physical properties will perform differently in terms of extraction of samples which will be a function of the stated parameters. The best extraction solvent found in this work has not been previously reported in the literature as the extraction solvent for PAHs.

5.2.5 Addition of acetonitrile (ACN)

PAHs are characterized as hydrophobic compounds with very low water solubility (Table 2.1). This presents a problem as it leads to adsorption problem during sampling, storage and extraction. Their adsorption then results in loses and underestimation of the real concentration in the sample. To increase the solubility of PAHs in the sample during extraction, an organic solvent such as methanol or 2-propanol or acetonitrile or a surfactant is usually added to the sample. The amount of the organic solvent added in the sample is a critical parameter, because if it is too high, PAHs will apparently be too soluble in the donor phase to be efficiently extracted (Barri *et al.,* 2004). Figure 5.5

shows the results on the variation of the amount of organic content in the sample. According to the results obtained, before the addition of acetonitrile, some analytes could not be detected. Amount of PAHs extracted increased with an increase in ACN content up to 20%. This means that the solubility of PAHs is better in 20% acetonitrile in water than in pure water. After 20% acetonitrile, the amount extracted decreased with increase in acetonitrile and some analytes could not be detected. This suggests that the amount of organic solvent was too high, thus analytes became too soluble in the donor phase to be efficiently extracted or the organic solvent in the sample led to acceptor solvent loss. The effect of organic content in the sample in the hollow fibre extraction of PCBs from aqueous sample was also investigated (Barri *et al.*, 2004). In this study, the amount of PCBs extracted increased with acetonitrile content until after 40% when the amount extracted started to decrease. The addition of acetonitrile also improved the repeatability of the extraction and left over effects in the hollow fibre were also reduced. Table 5.2 shows the obtained % RSD in varying the organic content in the sample. At 20% acetonitrile in the sample, the % RSD values were lowest and also this gave the highest amounts of the PAHs extracted in the organic acceptor phase. These findings are consistency with those of (Barri *et al.*, 2004).

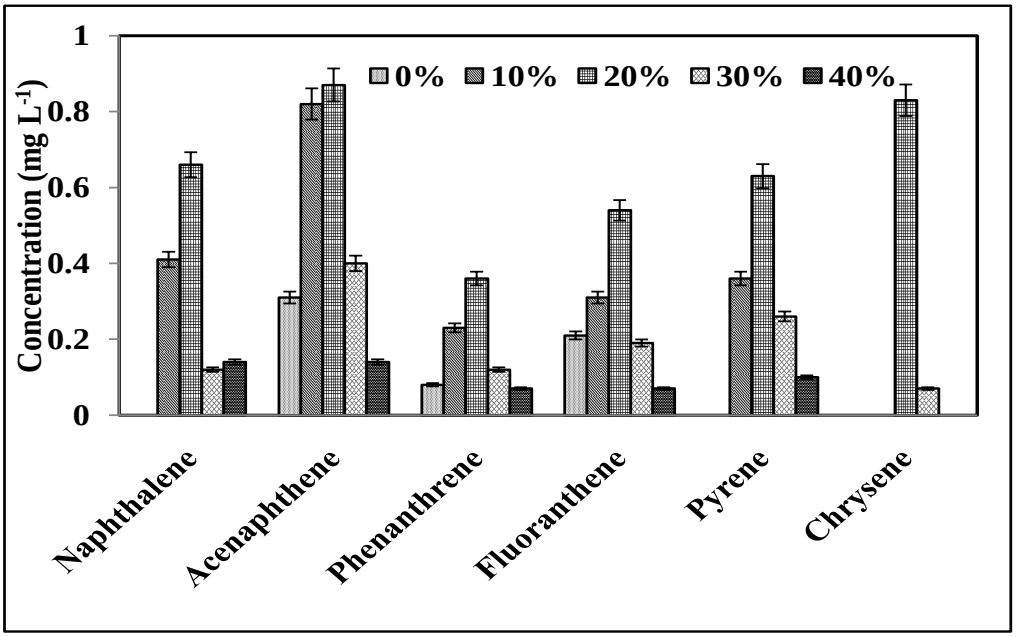


Figure 5.5: Effect of the amount of organic content in the sample on the amount extracted. Other parameters kept constant were 30 minutes extraction time, 600 rpm as the stirring speed, heptane as the acceptor solvent and a concentration of 7 µg L^{-1} mixtures of PAHs spiked in deionized water with no salt added.

Table 5.2: Obtained RSD values at different % of organic content in the sample from results in figure 5.5

	% RSD				
% Organic content	0	10	20	30	40
Naphthalene		2.2	1.5	4	3.7
Acenaphthene	2.9	1.6	1.8	1.8	3.5
Phenanthrene	4	1.7	1.5	2.6	2
Fluoranthene	3.5	1.9	1.6	2	3.7
Pyrene		1.8	1.6	2.2	5.7
Chrysene			1.5	5	

5.2.6 Spiked concentration and detection limit

Since PAHs usually exist in low concentration ranges in the natural water bodies, the developed method should have low detection limits to be able to detect them. This means that the developed method should have high enrichment factors for the target PAHs. For accuracy quantification, the obtained enrichment factors should be independent of sample concentration. This is important because the concentration of the PAHs in the environment is not known. If the method is independent of sample concentration it means the obtained enrichment factors will be constant at any sample concentration. This also means that the amount extracted in the acceptor phase is directly proportional to that in the sample. In order to investigate the effect of spiked water concentration on the concentration enrichment factor, the sample was spiked with 3 and 7 µg L^{-1} levels. Since PAHs are of low solubility, it is impractical to spike too high a concentration. Further, in water samples, the levels of PAHs are generally detected below single µg L^{-1} levels (Manoli and Samari, 1999). According to the results obtained in Table 5.3, the enrichment factor and extraction efficiency was not very much influenced by sample concentration. However, the values were slightly higher with a 7 µg L^{-1} spiked concentration. This could be due to loss from adsorption at lower concentration. Chrysene was not detected at 3 µg L^{-1}. The obtained detection limits shown in Table 5.4 allow detecting trace levels of the PAHs in the environment.

Most reported literature on the extraction of PAHs using LPME rarely varies the spiked concentration in the sample. Only one or two concentrations levels closer to what is expected in real environmental samples are used. To further verify the method, a comparison is made with another well known extraction method such as solid phase extraction or analysis of a certified reference material. Table 5.5 shows a comparison of the developed method with similar ones in the literature in terms of extraction solvent, detection limit and enrichment factor. The literature values of enrichment factor are slightly higher than obtained in this study. This could be attributed to the differences in dimensions of the set-up. In the literature, the hollow fibre length used was 1.3 cm and with acceptor volume of 3-5 µL of organic solvent. The sample volume was 5 mL. These small dimensions might contribute to efficient mass transfer of the PAHs from

the sample into the hollow fibre. The small acceptor solvent also enhances high values of enrichment factor.

Table 5.3: Obtained % extraction efficiency (% E) and concentration enrichment factors (E_n) at two different spiked sample concentrations. % RSD values are given in brackets.

Compound	Spiked concentration (µg L^{-1})			
	3.0		7.0	
	E_n	% E	E_n	% E
Naphthalene	48 (3.1)	4.0 (3.3)	53 (3.5)	4.5 (3.4)
Acenaphthene	60 (2.4)	5.1 (2.0)	94 (1.7)	8.0 (1.7)
Phenanthrene	46 (2.7)	4.0 (2.6)	52 (1.5)	4.4 (1.5)
Fluoranthene	56 (3.1)	4.8 (3.4)	52 (2.1)	4.4 (2.3)
Pyrene	68 (1.8)	5.8 (1.6)	59 (2.2)	5.0 (2.1)
Chrysene			95 (2.0)	8.1 (1.5)

Table 5.4: Target PAHs detection limits in developed HF-LPME-GC-MS method

Detection limits (µg L^{-1})	
Compounds	HF-LPME
Napthalene	0.023
Acenaphthene	0.032
Phenanthrene	0.095
Fluoranthene	0.040
Pyrene	0.027
Chrysene	0.095

Table 5.5 Comparison of the developed HF-LPME method with similar ones in the literature

Sample	Extraction solvent used	Hollow fibre length	Enrichment factors	Detection limits (μg L^{-1})	Determination method	Reference
Wastewater	5 mL of water extracted by 3 μL toluene as acceptor phase for 15 minutes.	1.3 cm	NR	0.006-0.011	GC-MS	Charalabaki *et al.*, 2005
Rainwater	5 mL of water adjusted to pH 9 extracted by 5 μL toluene as acceptor phase for 30 minutes.	1.3 cm	46-160	0.006-0.040	GC-MS	Basheer *et al.*, 2003
Deionised water	20 mL of water extracted by 17 μL heptane as acceptor phase for 30 minutes.	5.0 cm	46-94	0.023-0.095	GC-MS	This method
River water	20 mL of water extracted by 17 μL heptane as acceptor phase for 30 minutes.	5.0 cm	40-82	0.025-0.097	GC-MS	This method

5.3 Application of the HF-LPME method to real river water samples

The optimized HF-LPME method was applied to the extraction of PAHs in real river water samples collected in Jukskei River and the extracts were analyzed by GC–MS. Table 5.6 shows the physical properties of the samples. The pH was basic in all samples and this could be due to contamination of wastewater and solid waste. The conductivities were high which could be attributed to anions such as sulphates, phosphates and nitrates associated with sewage wastewater and solid wastes. Solid waste includes plastics, plastic containers, cans, papers and other household solid waste (Figure 5.6). Wastewater could be mainly from homes especially that Jukskei river passes through Thembisa which is one of the largest informal settlements in Johannesburg, All samples were collected downstream after Themisa. All the studied PAHs were detected in the samples analysed (figure 5.7). Table 5.7 shows the concentrations obtained in real water samples and they were all below the maximum allowable concentrations for PAHs in water which is 3.0 mg L^{-1} (ATSDR, 2006) for the studied PAHs. The low concentrations obtained could be due to low solubility of PAHs in water. The overall order of pollution per PAH compound based on the mean was as follows phenanthrene > acenaphthene > fluoranthene > naphthalene > pyrene.

The stagnant concentrations 0.0490-0.1736 µg L^{-1} were found to be higher than the concentrations in the flowing water 0.0303-0.1193 µg L^{-1} in the same sampling site. The top level concentrations were also found to be higher 0.0611-0.2138 µg L^{-1} than the bottom level concentrations 0.0359-0.1409 µg L^{-1} in the same sampling site. Similar behavior has been reported by (Witt and Matthäus, 2001) where PAHs in water were analysed. They found that the concentration in the surface water 0.0028-0.0056 µg L^{-1} to be higher then the concentration in deep water 0.0012-0.0038 µg L^{-1} in the same sampling site. The effect of depth profile on the concentration of PAHs in water has been investigated (King *et al*., 2004). The results obtained showed that the concentrations varied with depth 0.003-0.742 µg L^{-1}. The concentrations obtained in this work are higher than those obtained by (Witt and Matthäus, 2001), but they are lower than the maximum concentration obtained by (King *et al*., 2004).

Table 5.6: Physical properties of compounds, pH and conductivity

Samples	Sampling date	pH	Conductivity ($\mu S\ cm^{-1}$)
Jukskei 1 bottom	26 October 2011	8.93	735
Jukskei 2 stagnant	26 October 2011	8.43	651
Jukskei 3 bottom	26 October 2011	8.49	724
Jukskei 4 top	26 October 2011	8.22	726
Jukskei 5 bottom	26 October 2011	8.25	621
Jukskei 6 stagnant	26 October 2011	7.95	623
Jukskei 7 bottom	26 October 2011	8.20	622
Jukskei 8 bottom	26 October 2011	8.20	620

Bottom layer samples were taken at about 10 cm depth of the river. Top layer was taken at the surface of the river.



Figure 5.6: Solid waste in Jukskei River (Sibiya, 2011).

Table 5.7: Levels of PAHs (ng L^{-1}) obtained with HF-LPME for real water samples (n=3) and relative standard deviations expressed in percentage

	Concentration (ng L^{-1})				
	Naphthalene	**Acenaphthene**	**Phenanthrene**	**Fluoranthene**	**Pyrene**
Jukskei 1 (bottom)	113.5 (9.9)	102.6 (5.0)	119.3 (8.5)	46.2 (8.3)	30.3 (11.2)
Jukskei 2 (stagnant)	88.1 (5.3)	173.6 (10.1)	125.6 (2.5)	66.8 (11.9)	49.0 (10.7)
Jukskei 3 (bottom)	76.1 (0.6)	35.9 (8.2)	140.9 (7.7)	130.8 (2.3)	53.2 (3.8)
Jukskei 4 (top)	68.7 (3.8)	213.8 (4.2)	196.7 (1.5)	201.0 (4.0)	61.1 (2.8)
Jukskei 5 (bottom)	25.4 (5.1)	181.3 (1.6)	140.9 (4.8)	53.4 (7.1)	75.4 (2.6)
Jukskei 6 (stagnant)	144.8 (6.2)	239.3 (3.8)	195.6 (5.1)	161.5 (4.1)	74.4 (2.0)
Jukskei 7 (bottom)	114.2 (3.3)	40.2 (3.7)	140.0 (5.1)	92.3 (4.1)	43.4 (2.0)
Jukskei 8 (bottom)	91.1 (7.5)	135.1 (4.8)	192.0 (2.1)	190.4 (2.6)	104.0 (0.8)
Mean	90.7	140.2	156.4	117.8	61.3
Minimum	25.4	35.9	119.3	46.2	30.3
Maximum	144.8	239.3	196.7	201.0	104.0

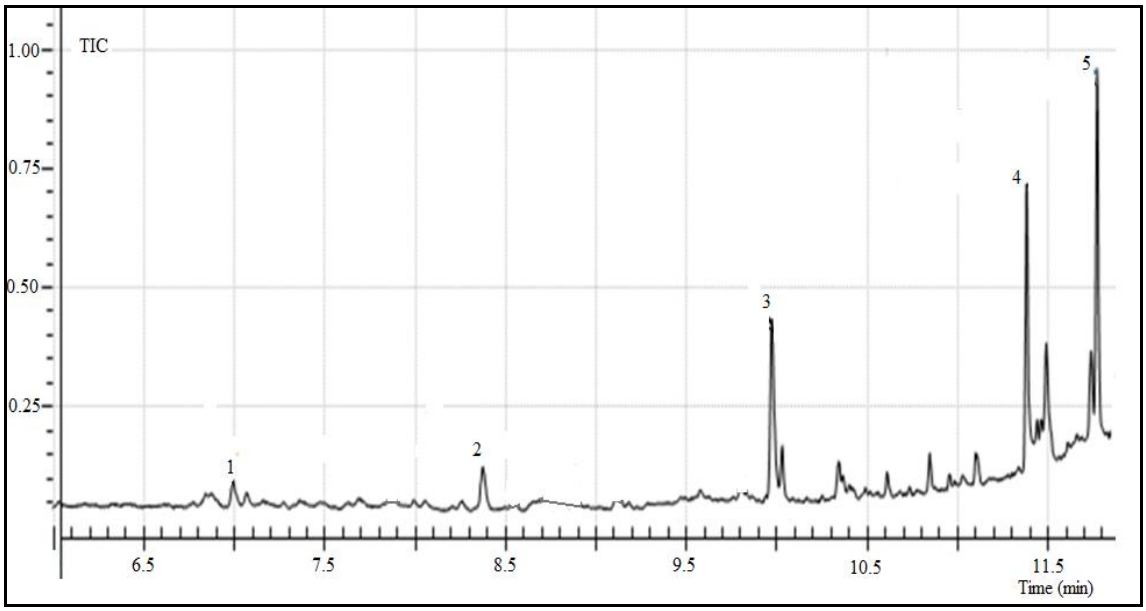


Figure 5.7: A typical chromatogram of Jukskei river water sample where (1) Naphthalene, (2) Acenaphthene, (3) Phenanthrene, (4) Fluoranthene, (5) Pyrene, extracted by HF-LPME and detected by GC-MS with WCOT fused silica capillary column (30×0.25 mm ID, 0.25 µm film thickness).

5.4 Optimization of SPE extraction technique

5.4.1 Sample loaded volume

Sample volume is the key factor that influences the recovery of the analyte in some instances especially those that have low retention in SPE cartridge. Factors that influence sample volume are type and amount of sorbent, type and amount of anaylte and matrix components. It is important to optimize the sample volume to gain high recovery of the analyte. When the sample volume is too high, it results in the decrease of the recoveries due to the fact that breakthrough volume is exceeded. This effect is actually the result of a shift in the adsorption/desorption equilibrium favouring increased desorption from the packings and causing a net loss of adsorbate from the SPE cartridge (Xie *et al*., 2003). Sample volume used in this work was investigated by varying the volume between 100-200 mL, in order to find the optimum volume with high recoveries. The results are shown in Figure 5.8. In general when the amount of sample that passed through the sorbent is increased, it increases the amount of analyte trapped. This explains why the concentration extracted in this work increased with an increase in the sample volume used. The recoveries obtained were almost constant with an increase in sample volume, which indicated that the capacity of the sorbent was not exceeded in the volume range of the sample studied.100 mL sample volume was then chosen to be the optimum volume in this work, taking into consideration the time required for the extraction. Varying the sample volume in extraction of PAHs in aerosol using SPE has been reported (Xie *et al*., 2003). Sample volume was varied between 3-50 mL and the cartridge used contained 500 mg of the sorbent. The recoveries obtained decreased with an increase in sample volume. The decrease in recovery at high sample volume becomes a capacity issue especially for non polar compounds like PAHs. The effect of sample volume on the recoveries of PAHs in water has also been investigated (Kouzayha *et al*., 2011). The sample volume was varied between 500-1500 mL and the cartridge used contained 200 mg of the sorbent. Between 500-1000 mL a decrease in some of the PAHs was observed while there was no apparent decrease for others. With an increase of the volume to 1500 mL there was a significant decrease up to 40% in the recoveries for some of the PAHs while there was no change for others. The most

important factor to predict the recoveries based on the percolated volume is the retention factor of the analyte in water (Kouzayha *et al.*, 2011), which could be the reason for their observations.

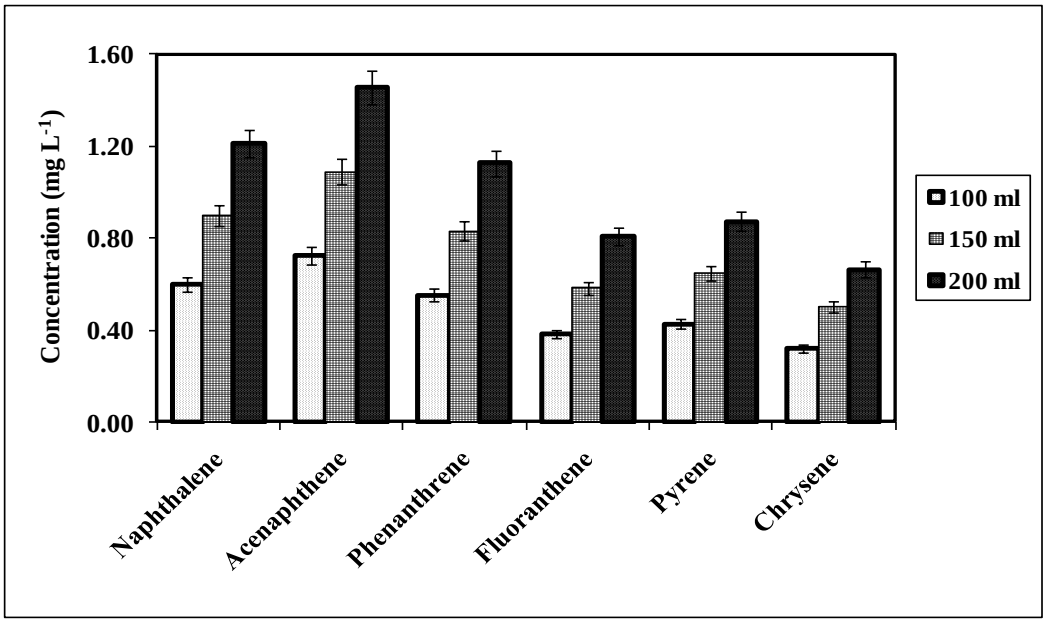


Figure 5.8: Effect of sample loaded volume on the amount extracted. Other parameters kept constant were 20% ACN as the organic modifier, 5 mL of acetone: water (40:60, v/v) as the conditioning solvent, 5 mL of deionized water as the washing solvent, 3 mL of acetone: THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in deionized water was used.

5.4.2 Selection of organic modifier

The low solubility of PAHs which decrease when a molecular weight increases, leads to adsorption problem during sampling and storage. Their adsorption then results in loses and underestimation of the real concentration (Barri *et al.*, 2004). To increase the solubility, an organic solvent or a surfactant is usually added to the sample. The concentration of the organic solvents is a critical parameter, because if it is too low it may not be enough to solubilise the high molecular weight PAHs, whereas if it is too high, the breakthrough volume will be low for the low molecular PAHs (Marce *et al.*, 2000). Organic solvent is also needed to keep the octadecyl chains activated.

Methanol, 2-propanol and acetonitrile were used at 10% in order to investigate the effect of adding organic modifier. According to the results (Figure 5.9) obtained acetonitrile showed the lowest response. This could mean that 10% acetonitrile is not enough to fully activate the octadecyl chains, and therefore penetration of the PAHs in the alkyl chains is still hindered. 2-propanol showed more or less the same results as methanol, but was better for chrysene. Based on the results obtained, methanol was then selected as organic modifier for this work. Other organic modifies that have been used in the literature are 2-propanol, (Kiss *et al*., 1996; Delhomme *et al*., 2007; Marce *et al*., 2000); methanol, (Delhomme *et al*., 2007); acetic acid, (Azevedo *et al*., 2004). Solvents recommended by EPA method 3535a are ethyl acetate, acetonitrile, methanol, acetone, isopropanol.

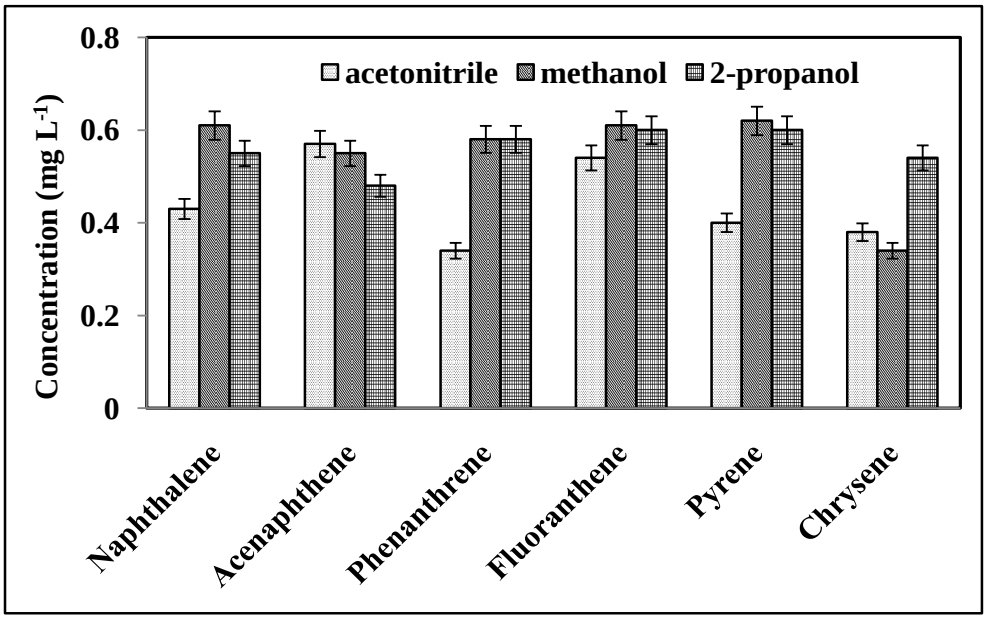


Figure 5.9: Effect of organic modifier on the amount extracted. Other parameters kept constant were 5 mL of acetone:water (40:60, v/v) as the conditioning solvent, 5 mL of deionized water as the washing solvent, 3 mL of acetone:THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water was used.

5.4.3 Amount of organic modifier

Although the efficiency of the sample can be improved by addition of organic modifier, on the other hand the eluotropic strength of the sample increases with increasing modifier content (Kiss *et al*., 1996). The concentration of the organic solvent is a critical parameter, this is because if it is too low it may not be sufficient to solubilise the high molecular weight PAHs, and on the other hand if it is too high, the breakthrough volume will be low for the low molecular PAHs (Marce *et al*., 2000). It also promotes interaction of the hydrophobic C_{18} bonded phase with aqueous sample (Delhomme et al., 2007). In order to investigate this effect, methanol content in the sample was varied between 0-20%. Figure 5.10 shows the results obtained. The results were better at 10% methanol. This means that the solubility of PAHs is better in 10% methanol in water than in pure water. There was no direct correlation between the molar masses and hydrophibicity with the concentrations obtained. Varying the amount of organic modifier on the extraction of PAHs in precipitation (rainwater) using SPE has also been reported (Kiss *et al*., 1996). The amount was varied between 2-40%. The extraction showed to decrease at high amount of the organic modifier. The decrease was attributed to the breakthrough because of the increased eluotropic strength of the sample. Table 5.8 shows the obtained % RSD obtained in varying the organic content in the sample. At 10% methanol in the sample, the highest amounts of the PAHs were extracted and % RSD values were also within the accepted range. The amount of organic modifier that have been used in the literature are 25% of 2-propanol, (Kiss *et al*., 1996; Marce *et al*., 2000; Delhomme et al., 2007) and the sorbent used was C_{18}. This value is not far from what was obtained in this study, this is because 20% methanol in the sample also gave good results close to that of 10% methanol.

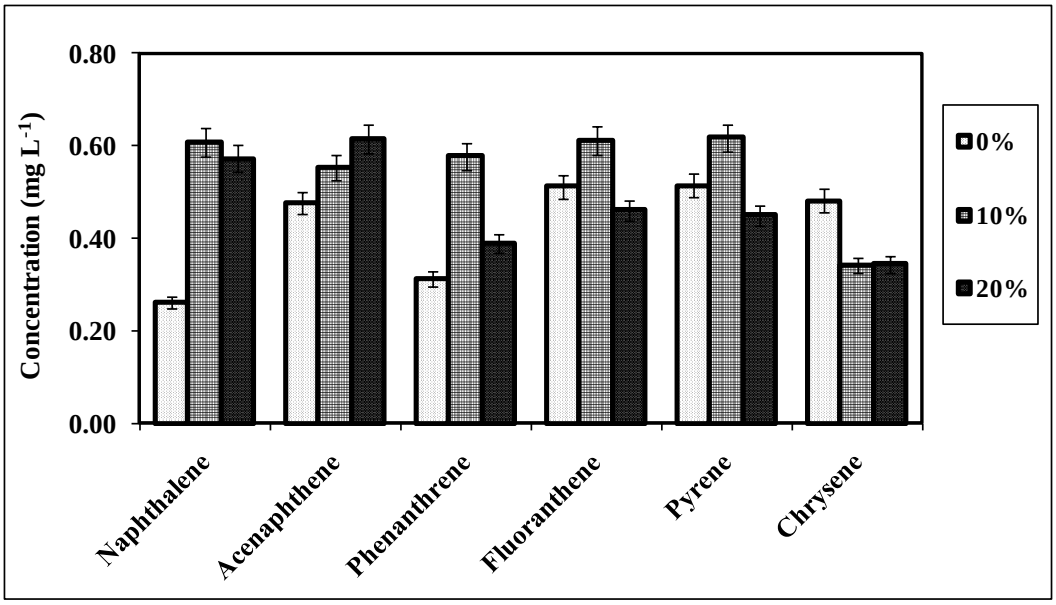


Figure 5.10: Effect of the amount of methanol on the amount extracted. Other parameters kept constant were 5 mL of acetone: water (40:60, v/v) as conditioning solvent, 5 mL of deionized water as washing solvent, 3 mL of acetone: THF (1:1) as eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water was used.

Table 5.8: Obtained % RSD values at different % of organic content in the sample from figure 6.10.

	% RSD		
% Organic content	0	10	20
Naphthalene	1.5	2.6	1.5
Acenaphthene	2.1	2.0	1.2
Phenanthrene	1.1	1.6	2.1
Fluoranthene	1.4	2.1	2.2
Pyrene	1.4	1.9	2.5
Chrysene	1.6	2.1	2.3

5.4.4 Selection of conditioning solvent

Conditioning step is an important step for SPE procedure. The first step of conditioning is to wet the SPE cartridge. Wetting the cartridge opens up the groups of the sorbent surface and thus increases the surface area available for interaction with the analyte. It can also remove the residues from the packing material that might interfere with the analysis. The second step is to wash the sorbent bed with a solvent to prepare the suitable surface for the adsorption of the analyte. The second solvent has to be weaker or equal in eluting strength to that of the sample solution (Xie *et al*., 2003). Acetone, methanol and 2-propanol were evaluated at 40% in water, in order to investigate the effect of the conditioning solvent. Figure 5.11 shows the results obtained. Methanol showed better results than the other solvents. The reason could be due to equal strength close to that on the sample because the sample was modified with methanol. Methanol was then chosen as the conditioning solvent in this work. Other conditioning solvents used in the literature are given in Table 2.7. EPA method 3535a recommended conditioning solvents are methanol and reagent water.

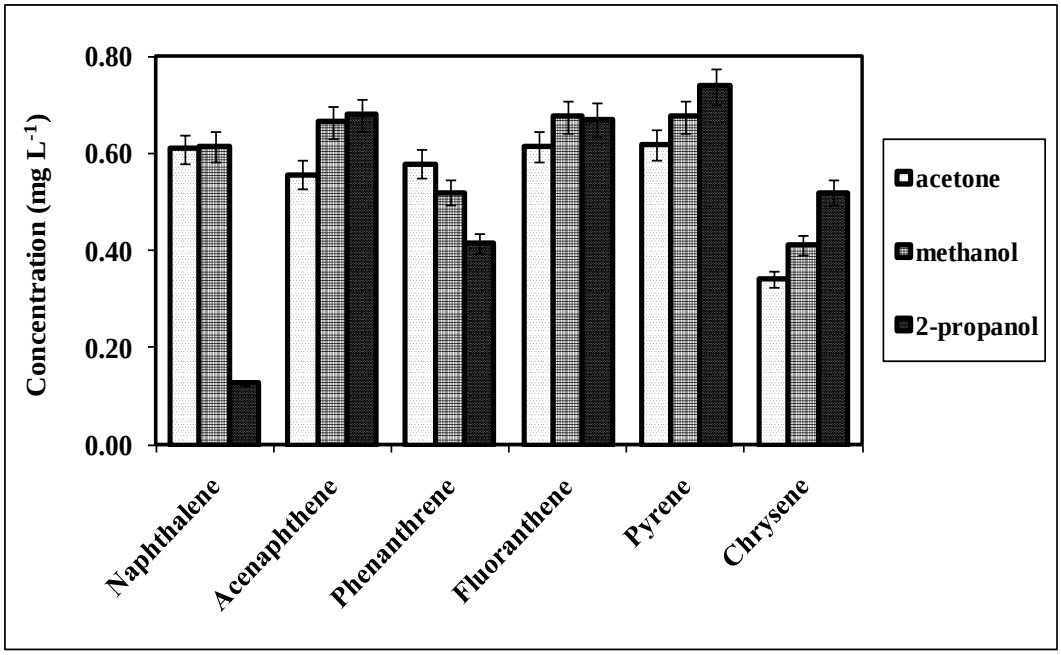


Figure 5.11: Effect of the conditioning solvent on the amount extracted. Other parameters kept constant were 5 mL of deionized water as the washing solvent, 3 mL of acetone: THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water and 10% methanol as the organic modifier.

5.4.5 Amount of conditioning solvent

Conditioning solvent is used to activate the octadecyl chains in the cartridge. It is therefore important to use enough amounts to be able to activate it. Methanol showed better results than the other solvents and it was then investigated further. In order to investigate its proper volume for extraction, it was evaluated using 20-40% in water. Figure 5.12 shows the results obtained. At 20-30% methanol chrysene was not detected, although the results were better for the lower molecular weight. At 40% all compounds were detected, it was then suggested to be the amount of conditioning solvent used in this work.

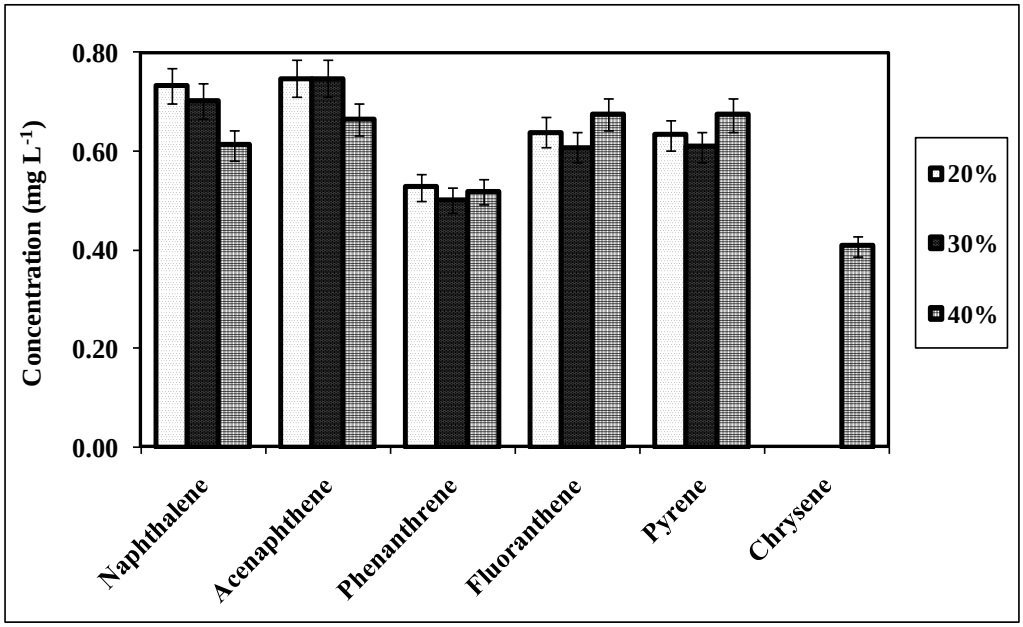


Figure 5.12: Effect of the amount of the conditioning solvent on the amount extracted. Other parameters kept constant were 5 mL of deionized water as the washing solvent, 3 mL of acetone: THF (1:1) as the eluting solvent, and a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water and 10% methanol as the organic modifier.

5.4.6 Selection of eluting solvent

Once the analytes are retained on the cartridge they are eluted by an appropriate organic solvent. The choice of eluting solvent should be carefully considered. If the solvent is too powerful, more interference will be eluted out. If the elution strength of the solvent is not enough, a larger elution volume will be needed, and then it will dilute the sample giving lower detection sensitivity (Xie *et al*., 2003). Recoveries of low molecular weight PAHs are higher with less strong solvents, but most of the PAHs are eluted with stronger solvent. Therefore to ensure high recoveries of all PAHs, mixtures of solvents are usually recommended (Marce *et al*., 2000). A suitable strength of the eluting solvent was determined by examining 3 mL acetone in tetrahydrofuran (1:1), 3 mL methanol in tetrahydrofuran (1:1) and 3 mL acetonitrile in tetrahydrofuran (1:1). Figure 5.13 shows the results obtained on the selection of eluting solvent. The performance of acetonitrile:THF was the worst compared to methanol:THF and acetone:THF. Although the lower molecular weights were better eluted, chrysene was not detected.

Methanol:THF showed average response. The reason could be that methanol saturated (too much) the compounds and thus reduced its eluting strength as it was also used as a modifier and a conditioning solvent. Acetone:THF showed the highest recovery for all analytes and it was then considered as the suitable eluting solvent for this work. This was due to stronger elution strength of acetone:THF combination, (Table 5.9). Other eluting solvents that have been used in the literature are given in Table 2.7. EPA method 3535a recommended eluting solvents are 5 mL acetone or 20 mL acetonitrile. Large volume of acetonitrile is used because of less strength.

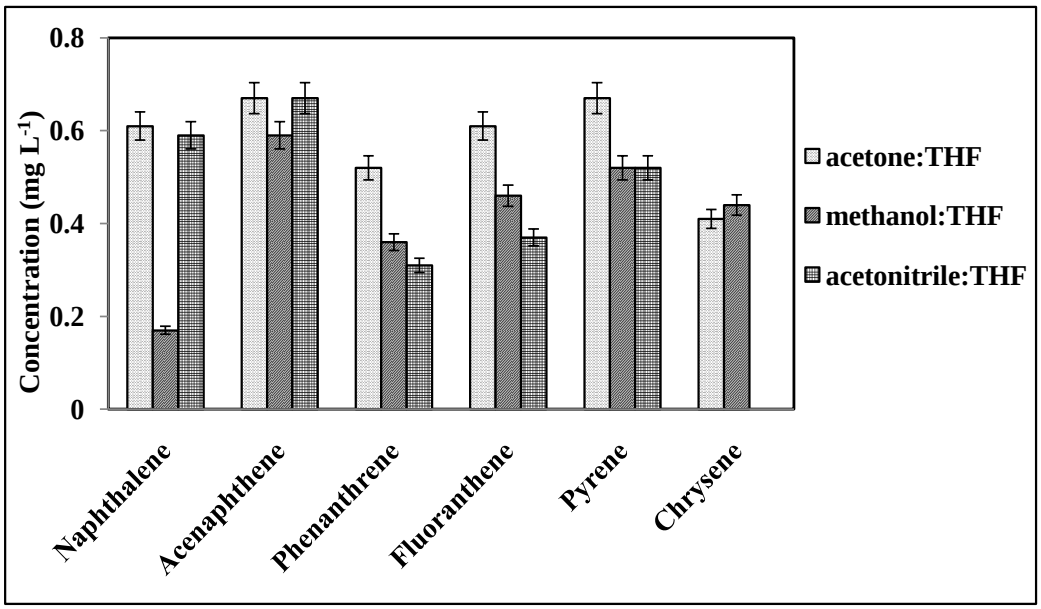


Figure 5.13: Effect of the eluting solvent on the amount extracted. Other parameters kept constant were 5 mL of acetone: water (40:60, v/v) as conditioning solvent, 5 mL of deionized water as washing solvent, a concentration of 5 µg L^{-1} mixture of PAHs spiked in 100 mL deionized water, 10% methanol as the organic modifier and 40% methanol in water as the conditioning solvent.

Table 5.9: Physical parameters of the solvents used in SPE **(**Knovel, 2008)

Physical Properties					
Solvents	**Boiling Point (^oC)**	**Molar mass (g mol^{-1})**	**Polarity**	**Dielectric constant**	**Water solubility**
Acetonitrile	81.6	41.05	5.8	37.5	100
Methanol	64.7	32.04	5.1	32.7	100
Acetone	56.3	58.08	5.1	20.7	100
THF	66.0	72.11	4.0	7.58	100

5.4.7 Spiked concentration and detection limits

Since PAHs usually exist in low concentration ranges in natural water due to their low solubility (Zuydam, 2007), the method developed should have low detection limits to be able to detect them. This means that the developed method should have high enrichment factors for the target PAHs. For accurate quantification, the enrichment factors should be independent of sample concentration. This is important because the concentration of the PAHs in the environment is not known. The constancy of enrichment factors at any sample concentration means that the method is independent of sample concentration. It also assures the direct proportionality of the amount extracted to that in the sample. In order to investigate the effect of spiked water concentration on the concentration enrichment factor, the sample was spiked between 3, 5 and 7 µg L^{-1} levels of PAHs. Since PAHs are of low solubility, it is impractical to spike too high a concentration. Figure 5.14 shows the results obtained on varying spiked concentration in the sample. The amount extracted increased with the spiked amount. This is expected and shows that loss due to adsorption is minimal. Table 5.10 shows the obtained enrichment factors and extraction efficiencies at different concentrations.

According to the results obtained, the enrichment factors and extraction efficienies were not very much influenced by sample concentration. However, the values were slightly higher with a 7 µg L^{-1} spiked concentration. The values obtained for the enrichment factors and extraction efficiencies are similar. Some of the extraction efficiencies were higher then 100 percent. The reason could be factors such as matrix effect, operation

error, method errors, but they are still acceptable (Ma *et al*., 2010).The obtained detection limits shown in Table 5.11 allow detecting trace levels of the PAHs in the environment. Table 5.12 shows a comparison of the developed method with similar ones in the literature in terms of extraction solvents, detection limits and enrichment factors. The detection limits and recovery results obtained by (Kiss *et al*., 1996) and (Azevedo *et al*., 2004) are comparable to those obtained in this.

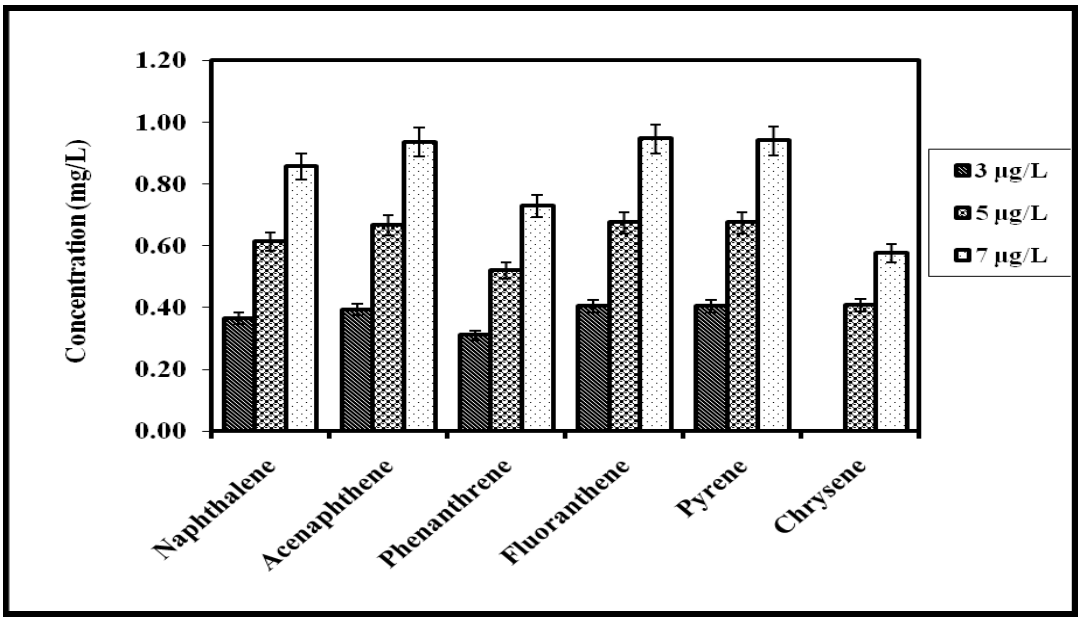


Figure 5.14: Effect of spiked concentration on the amount extracted. Other parameters kept constant were 5 mL of acetone: water (40:60, v/v) as conditioning solvent, 5 mL of deionized water as the washing solvent, 40% methanol in water as conditioning solvent, 3 mL of acetone: THF (1:1) as the eluting solvent, 100 mL deionized water and 10% methanol was used as the organic modifier.

Table 5.10: Obtained extraction efficiency and concentration enrichment factors at three different spiked sample concentration

Spiked concentration [µg L^{-1}]						
	3.0		5.0		7.0	
Compounds	**Enrichment Factor**	**% Extraction Efficiency**	**Enrichment Factor**	**% Extraction Efficiency**	**Enrichment Factor**	**% Extractio Efficiency**
Naphthalene	122 (2.0)	122 (2.0)	122 (1.6)	122 (1.6)	123 (1.1)	123 (1.1)
Acenaphthene	130 (2.3)	130 (2.3)	133 (1.6)	133 (1.6)	134 (2.6)	134 (2.6)
Phenanthrene	103 (2.6)	103 (2.6)	104 (1.8)	104 (1.8)	104 (5.7)	104 (5.7)
Fluoranthene	135 (1.6)	135 (1.6)	135 (1.9)	135 (1.9)	135 (6.0)	135 (6.0)
Pyrene	135 (5.2)	135 (5.2)	135 (1.9)	135 (1.9)	134 (4.0)	134 (4.0)
Chrysene			82 (4.9)	82 (4.9)	82 (5.1)	82 (5.1)

Table 5.11: Detection limits of the developed SPE-GC-MS method

Detection limits (µg L^{-1})	
Compounds	SPE
Napthalene	0.020
Acenaphthene	0.030
Phenanthrene	0.052
Fluoranthene	0.025
Pyrene	0.021
Chrysene	0.091

Table 5.12: Comparison of the developed SPE method with similar ones in the literature

Sample	Extraction solvent used	Organic modifier	% Recovery	Enrichment factors	Detection limits (µg l^{-1})	Determination method	Reference
Rain water	50 mL of water extracted in a C_{18} cartridge, conditioned with 4 mL 2-propanol-water (25-75, v/v), washed with 3 mL deionised water and then eluted with 2 mL DMC: THF.	25% 2-propanol	78-92	NR	<1	Column liquid chromatography	Kiss *et al*.,1996
River water	200 mL of water extracted in a C_{18} cartridge, conditioned with 6mL methanol and 6 mL water washed with 1 mL water and then eluted with 6 mL methanol.	Acetic acid	56-78	NR	0.0004-0.2	GC-MS	Azevedo *et al*., 2004
Deionised water	100 mL of water extracted in a C_{18} cartridge, conditioned with 5 mL methanol-water (40-60, v/v), washed with 5 mL deionised water, and then eluted with 3 mL acetone: THF.	10% methanol	81-135	81-135	0.020-0.095	GC-MS	This method
River water	100 mL of water extracted in a C_{18} cartridge, conditioned with 5 mL methanol-water (40-60, v/v), washed with 5 mL deionised water, and then eluted with 3 mL acetone: THF.	10% methanol	102-133	102-133	0.027-0.095	GC-MS	This method

5.5 Validation of SPE method with HPLC-Fluorescence

The SPE method was also validated using reference standard material under the optimized conditions described above. The analyses were carried out in triplicates. The results are presented in Table 5.13 below. The concentrations obtained for all analytes were within the acceptable limits. The precision (relative standard deviations) were less than 4%. The wide range of acceptable limits from certified materials underlines the difficulties in extraction and determining these compounds at trace levels. Quality assurance procedure should be applied at all steps of the analytical procedure.

Table 5.13: Concentrations and relative standard deviations of standard reference materials under optimal conditions

Compounds	**Certified values ($\mu g\ L^{-1}$)**	**Obtained values ($\mu g\ L^{-1}$)**	**Acceptable limits ($\mu g\ L^{-1}$)**
Naphthalene	4.83 (0.71)	6.99 (2.27)	2.49 to 7.17
Acenaphthene	2.90 (0.58)	2.24 (0.96)	1.25 to 4.54
Fluoranthene	0.51 (0.06)	0.49 (2.54)	0.30 to 0.73
Pyrene	1.20 (0.17)	0.78 (3.76)	0.23 to 2.17

5.6 Application of the SPE method to real river water samples

The optimized conditions were applied to real liquid samples in and around Johannesburg. Table 5.14 shows samples physical properties, while Table 5.15 below shows the results obtained. Centurion Lake, Hennops River, Blaauwpan Dam, Jukskei River and Homestead lake pH values were close to neutral. Middle Lake, Kleinfontein Lake, Hartbeespoort river pH values were basic which could be due to contamination of wastewater and solid waste. The conductivity of Centurion Lake, Hennops River, Jukskei River and Hartbeespoort River were high which could be attributed to anions such as sulphates, phosphates and nitrates associated with sewage wastewater and solid wastes. Middle Lake, Kleinfontein Lake, Blaauwpan Dam and Homestead Lake conductivity values were low which could mean that they are not polluted with metals

and anions. In all the samples analyzed all compounds were detected as shown in Figure 5.15. The concentrations obtained in all samples were lower than the maximum allowable concentration levels in water which is 3.0 mg L^{-1} for all of the analysed PAHs (ATSDR, 2006). Even though the concentrations were low, Blaauwpan Dam was the most polluted followed by Kleinfontein Lake, Homestead Lake, Hartbeespoort Dam (West of Johannesburg), Middle Lake, Centurion Lake, Jukskei River and Hennops River. The Blaauwpan Dam sampling point is the dam that flows through Homestead Lake, Middle Lake and Kleinfontein Lake in the Benoni area in the East rand/East of Johannesburg. The low concentrations may have resulted in the low solubility of PAHs in water, which is due to their removal by adsorption on particles (Charalabaki *et al*., 2005). The other reason for low concentrations may be due to the sources that are responsible for their presence in the rivers.

Table 5.14: Physical properties of samples analyzed by SPE

Samples	**Date of sampling**	**pH**	**Conductivity (µS cm^{-1})**
Blaauwpan Dam (East rand)	19 May 2011	6.66	187
Homestead Lake (East rand)	19 May 2011	6.66	306
Middle Lake (East rand)	19 May 2011	8.67	332
Kleinfontein Lake (East rand)	19 May 2011	8.44	241
Hennops River (Centurion)	3 May 2011	7.30	600
Centurion Lake (Centurion)	3 May 2011	7.15	874
Harbeespoort Dam (West Johannesburg)	19 April 2011	7.88	571
Jukskei River	7 July 2011	7.50	700

Table 5.15: Levels of PAHs (ng L^{-1}) obtained with SPE for real water samples (n=3) and relative standard deviations expressed in percentage

	Concentration (ng L^{-1})					
	Naphthalene	**Acenaphthene**	**Phenanthrene**	**Fluoranthene**	**Pyrene**	**Sum**
Blaauwpan Dam	128.5 (5.3)	406.6 (13.0)	615.7 (4.3)	89.0 (7.7)	89.8 (6.0)	1329.6
Homestead Lake	202.8 (4.3)	53.0 (6.7)	279.7 (4.1)	125.8 (4.2)	41.8 (9.3)	731.0
Middle Lake	62.6 (9.9)	168.0 (5.3)	105.6 (11.9)	125.7 (5.6)	38.8 (7.2)	500.7
Kleinfontein Lake	21.6 (0.9)	278.6 (1.9)	419.3 (4.9)	281.4 (2.7)	43.7 (9.5)	1044.6
Hennops River	46.5 (9.9)	75.8 (8.3)	53.5 (5.8)	56.4 (7.2)	28.5 (9.2)	260.7
Centurion Lake	33.4 (8.8)	115.7 (7.7)	168.8 (4.2)	29.7 (7.3)	23.6 (10.3)	371.2
Hartbeespoort Dam	238.6 (2.3)	112.4 (8.8)	64.3 (8.2)	37.6 (9.9)	82.3 (4.2)	535.2
Jukskei River	64.7 (7.8)	136.8 (6.3)	74.0 (7.4)	21.4 (10.7)	35.7 (10.9)	332.6

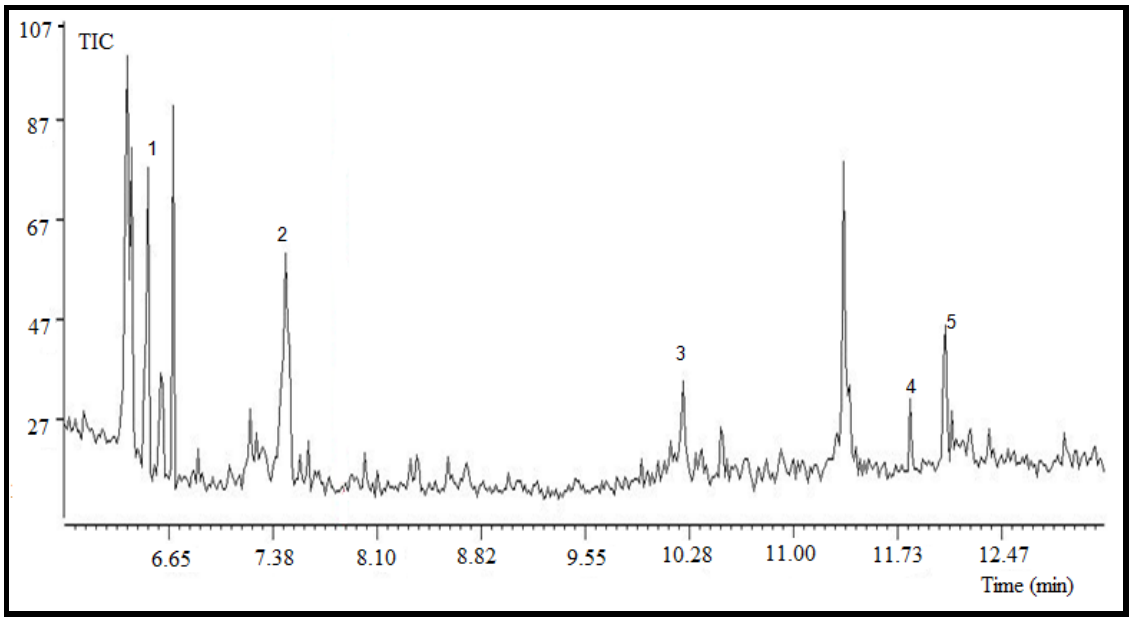


Figure 5.15: A typical chromatogram of Blaauwpan Dam water sample where (1) Naphthalene, (2) Acenaphthene, (3) Phenanthrene, (4) Fluoranthene, (5) Pyrene, extracted by SPE and detected by GC-MS with WCOT fused silica capillary column (30×0.25mm ID, 0.25µm film thickness).

5.7 Comparison of HF-LPME and SPE methods

The extraction methods were compared with each other in-terms of the extraction time, sample and organic solvent used as shown in Table 5.16. Even though solvent consumption is reduced in both methods, HF-LPME performed better compared to SPE because less organic solvent was used, and the sample volume was 10-50 mL (Reemtsma *et al.*, 2006). Compared to LPME, SPE consist of more manual steps (conditioning, sample loading, washing, elution of the analyte, evaporation), and much more glass ware is needed. The whole extraction process was longer than HF-LPME. On the other hand a higher sample volume used in SPE is advantageous, since it enhances the amount of target analyte transferred which improves the sensitivity (Pinxteren *et al.*, 2009). Solvent evaporation is a critical step in SPE as analyte losses may occur during the evaporation. LPME uses low amounts of organic solvent and there is no need of reducing by evaporation. There is a possibility of loss of organic solvent during extraction especially if it is too volatile and less non-polar, which can be even worse if the extraction time is too long.

Table 5.16: Comparison of the SPE and HF-LPME parameters

Parameter	**SPE**	**HF-LPME**
Extraction time per sample	115 minutes	30 minutes
Total organic solvent used	15 mL	17 µL
Sample volume	100 mL	20 mL
Extraction volume (organic)	3 mL	17 µL

Comparison of methods extraction efficiency, enrichment factor and detection limits

Important method parameters such as extraction efficiency (*Ee*), enrichment factor (*E*f) and detection limits (DL) were calculated, and then compared for both methods.

i) Enrichment factors

The enrichment factor is the ratio of concentration that is found in the acceptor phase to that in the original sample. It also determines the detection limits of the method (Jönsson and Mathiasson, 1999). Figure 5.16 shows the results on the comparison of the enrichment factors obtained for both methods. The results obtained for SPE are higher than those obtained for the HF-LPME. The reason why HF-LPME has lower enrichment factors is due to lower extraction efficiency explained below. Not all analytes in the sample are extracted as opposed to SPE technique. This could be the reasons why the enrichment factors obtained for LPME are much smaller than those obtained for SPE in this work.

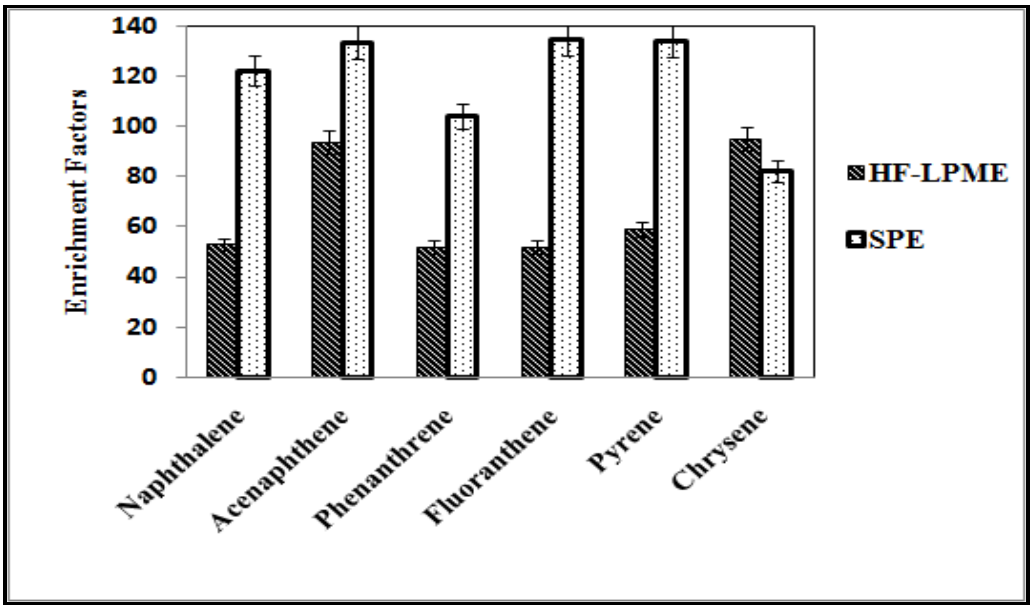


Figure 5.16: Enrichment factors obtained for HF-LPME and SPE

ii) Extraction Efficiency

The extraction efficiency is defined as the fraction of analyte in the extracted sample that is found in the acceptor phase (Msangati *et al.*, 2008; Jönsson and Mathiasson, 1999). It is also a measure of mass transfer between the donor and acceptor phase and

is constant under specified extraction conditions. High extraction efficiency represents fast mass transfer, while low extraction efficiency represents slow mass transfer.

The extraction efficiency was obtained under optimized conditions as obtained in the procedure. Table 5.17 shows the results on the comparison of the extraction efficiency and relative standard deviations (RSD) obtained for both methods. The extraction efficiency results of PAHs obtained using HF-LPME compared to SPE are very low. The cause of low extraction efficiency in HF-LPME is due to slow diffusion of analyte from the organic solvent in the pores of the hollow fiber to the bulk of the acceptor solution. This step has been identified as the rate limiting step (Chimuka *et al.,* 2004). This also makes HF-LPME a non exhaustive technique, whereas SPE is an exhaustive technique, which means that it extracts everything that is in the sample and gives 100% recoveries. The RSD obtained for both methods are lower that 6%, which means that the methods are reproducible. Low RSD values for HF-LPME allows the method to be used for quantitative purpose just like SPE. The first condition for the method to be acceptable for quantitative analysis is that it is reproducible at trace levels of expected concentrations. Both HF-LPME and SPE methods thus fulfill this requirement.

Table 5.17: Comparison of the percentage extraction efficiency and relative standard deviations (RSD, n=3) obtained for SPE and HF-LPME methods

Compounds	**SPE**	**HF-PME**
Naphthalene	122 (1.1)	5 (3.3)
Acenaphthene	134 (2.6)	8 (1.7)
Phenanthrene	104 (5.0)	4 (1.5)
Fluoranthene	135 (5.0)	4 (2.2)
Pyrene	134 (4.0)	5 (2.2)
Chrysene	82 (5.1)	8 (2.0)

iii) Limits of detection

The limits of detection LOD is the lowest concentration that can be detected. One way of determining this concentration is by determining the concentration that is three times greater than the background noise (3 x S/N). Table 5.18 shows the detection

limits obtained for both methods. The results obtained for HF-LPME are slightly higher compared to those obtained for SPE, which corresponds to the enrichment factors obtained. For both methods the, detection limits are low which means that the methods can be used for the detection of PAHs at trace levels. The calibrations gave good level of linearity with a correlation coefficient (r^2) between 0.9757 - 0.9972. (See quality assurance section).

Table 5.18: Detection limits for SPE and H F-LPME methods in river samples using GC-MS

	Detection limits (µg L^{-1})	
Compounds	SPE-GC-MS	HF-LPME-GC-MS
Napthalene	0.020	0.023
Acenaphthene	0.030	0.032
Phenanthrene	0.052	0.095
Fluoranthene	0.025	0.040
Pyrene	0.021	0.027

From comparison of extraction efficiencies, enrichment factors and detection limits, it does suggest that SPE is superior to HF-LPME. However, HF-LPME has other advantages in that it is much simple and cheaper than SPE. It is also less demanding compared to SPE. In terms of selectivity, LPME is also much better since the membrane acts as barrier excluding a number of potential interfering compounds.

Comparison of SPE and HF-LPME on real water samples

The optimized HF-LPME and SPE methods were applied for the extraction of PAHs in real river water samples. Samples were collected from Centurion Lake, Hennops River, Hartbeespoort Dam and Jukskei River and the extracts analyzed by GC–MS. Table 5.19 shows the physical properties of the samples which have been discussed previously. Table 5.20 shows the concentrations obtained in real water samples and they were all below the maximum allowable concentrations for PAHs in water which is 3.0 mg L^{-1}. Figure 5.17 and Figure 5.18 shows the compounds detected. Naphthalene was found to have the highest concentrations in all samples. Pyrene has

the lowest concentrations except in Hartbeespoort Dam. Fluoranthene was not detected in Hartbeespoort Dam. The low concentrations obtained could be due to low solubility of PAHs in water. Centurion Lake was found to be the most polluted followed by Jukskei River and then Hennops River. Hartbeespoort Dam was the least polluted. The trend was similar for both extraction methods. The results are comparable for the two methods. Major difference was with naphthalene in all samples where concentrations obtained using the SPE method were much higher. The reason for these differences is not clear. Other PAHs determined were comparable for both methods

Table 5.19: Physical properties of compounds, pH and conductivity

Samples	**Sampling date**	**pH**	**Conductivity ($\mu S\ cm^{-1}$)**
Hennops river	3 May 2011	7.33	650
Centurion lake	3 May 2011	7.21	890
Harbeespoort dam	19 April 2011	8.17	570
Jukskei river	7 July 2011	7.98	714

Note: These samples are not from the same site as those analysed by SPE in Table 5.16.

Table 5.20: Levels of PAHs (ng L^{-1}) obtained with SPE and HF-LPME for real water samples (n=3) and relative standard deviations expressed in percentage

	Hennops River		Centurion Lake		Hartbeespoort Dam		Jukskei River	
Compounds	**SPE**	**HF-LPME**	**SPE**	**HF-LPME**	**SPE**	**HF-LPME**	**SPE**	**HF-LPME**
Naphthalene	276.1 (7.2)	131.1 (2.5)	430.1 (14.7)	406.6 (4.6)	354.9 (11.6)	66.0 (7.8)	385.9 (2.5)	63.9 (4.0)
Phenanthrene	57.3 (2.7)	91.9 (4.4)	244.7 (3.9)	168.0 (1.6)	39.9 (10.9)	52.5 (9.5)	48.3 (4.1)	46.7 (8.1)
Fluoranthene	77.0 (1.9)	74.4 (2.1)	105.6 (12.6)	278.6 (3.3)	nd	nd	16.5 (9.8)	21.5 (6.2)
Pyrene	55.2 (4.4)	36.5 (7.3)	85.2 (11.6)	163.9 (3.1)	12.0 (6.1)	17.7 (9.9)	10.7 (5.6)	12.2 (5.6)
Sum	465.6	333.9	865.6	1017.1	406.8	76.8	461.4	143.3

Note: Another GC-MS instrument (from University of Johannesburg) was used which gave slightly lower detection limits that those reported earlier.

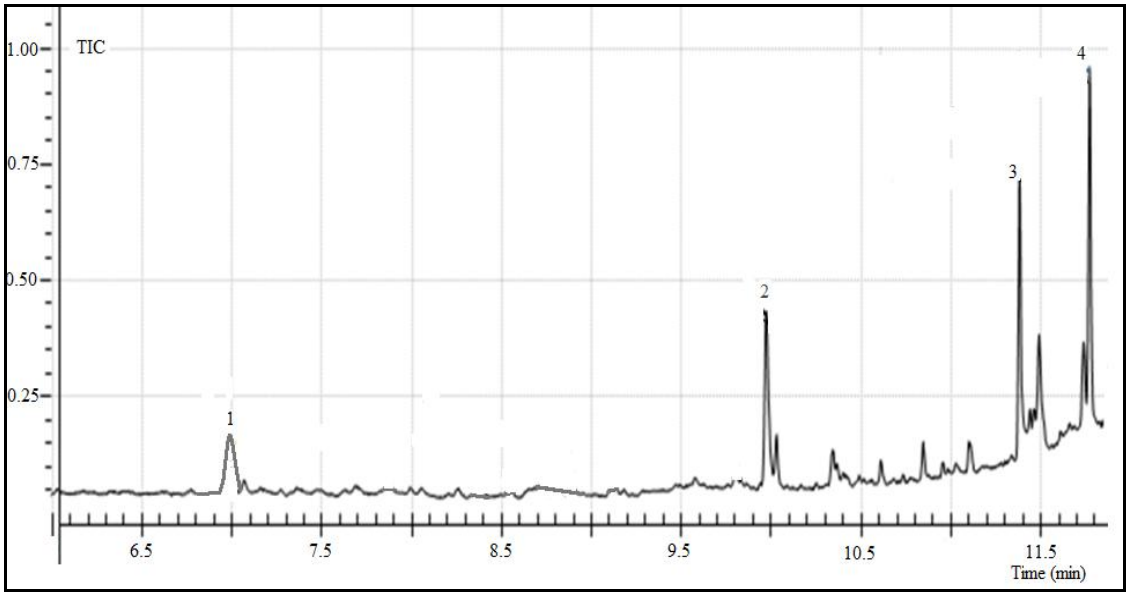


Figure 5.17: A typical chromatogram of centurion lake water sample where (1) Naphthalene, (2) Phenanthrene, (3) Fluoranthene, (4) Pyrene extracted by HF-LPME and detected by GC-MS with WCOT fused silica capillary column (30×0.25mm ID, 0.25µm film thickness).

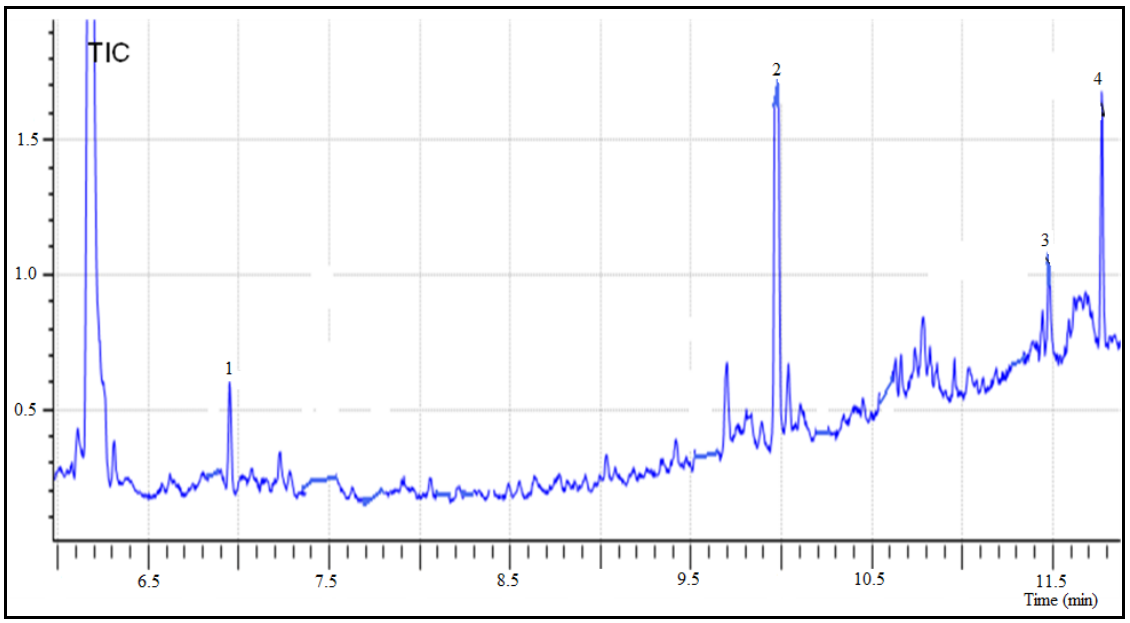


Figure 5.18: A typical chromatogram of centurion lake water sample where (1) Naphthalene, (2) Phenanthrene, (3) Fluoranthene, (4) Pyrene extracted by SPE and detected by GC-MS with WCOT fused silica capillary column (30×0.25mm ID, 0.25µm film thickness).

MASE and SPE have been compared for the extraction of pesticides in water (Pinxteren *et al*., 2009). The performance of HF-LPME and SPME has also been compared for extraction of pesticides in water (Frenich *et al*., 2011). These are shown in Table 5.21.

Table 5.21: Comparison of MASE, SPE, HF-LPME and SPME extraction methods

Extraction method	% RSD	LOD (ng L^{-1})	% Recovery	Concentration (ng L^{-1})	Reference
MASE	6-13	0.5-3.5	71-100	2-1440	Pinxteren *et al*., 2009
SPE	5-12	0.5-5.5	60-80	1-1410	
HF-LPME	4.3-27.3	0.2-47.1	70-119.5	35-51	Frenich *et al*., 2011
SPME	2.1-21.5	0.1-28.8	70.2-113.5	32-50	

5.8 Optimization of the MAE extraction technique

5.8.1 Extraction power

Microwave power and irradiation time are two factors which influence each other to a great extent. The extraction power is an important parameter that needs to be chosen correctly to avoid excessive temperature which could lead to solute degradation and overpressure inside the vessel (Raner *et al*., 1993). If power is too high with prolonged exposure, there is a risk of thermal degradation of the target compounds (Mandal *et al*., 2007). In order to investigate this effect, extraction power was varied between 200 and 300 W. Figure 5.19, shows the results obtained. According to the results obtained, the extraction efficiency was improved for most of the PAHs by raising extraction power from 200 to 250 W. It then decreased at 300 W. The reason for the decrease may be that extraction power became too high and led to degradation of analyte. This was however never proved. Other unknown factors could contribute to low amount extracted at high power. This is because no evidence was seen to support the possibility of the compounds degrading at 300 W.

There was a general trend on the influence of extraction power on the amount of PAHs extracted except for pyrene. Pyrene decreased with an increase in extraction power from 200 W to 250 W, it then increased at 300 W. The reason for this behavior is not clear, but could be due to homogeneity issues. Even though 200 W was better for pyrene. 250 W was then chosen as the best compromise optimum power for this work. Temperature which relates to these powers was between 45 ^{o}C and 92 ^{o}C, the pressure can not determined by the instrument used. The effect of extraction power (300 W-700 W) on the extraction of PAHs in sediments has been investigated (Blanco *et al.*, 2000). They found that the time required to achieve quantitative recoveries was a function of irradiation power. At 300 W, their optimum time was 9 minutes, while at 500 W to 700 W it was 6 minutes. At 700 W, acceptable results were achieved in 3 minutes. All the PAHs they studied followed the same trend of increasing with the extraction power. The power of 500 W was selected in order to avoid the use of high power which could lead to deterioration of the PTFE reactors and analyte losses. The power investigated in this work was lower compared to those used by Blanco, while the extraction time was higher than what they used. This could be the reason why in this work high power did not give good results.

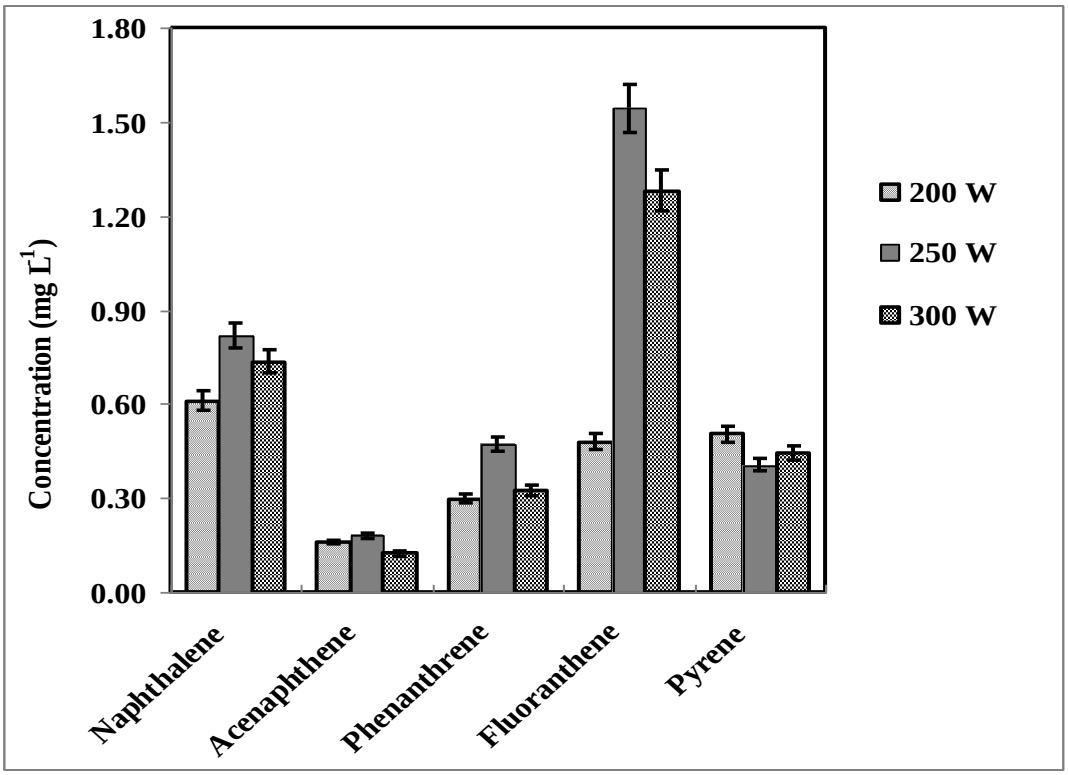


Figure 5.19: Effect of extraction power on the amount extracted. Other parameters kept constant were 20 mL hexane:acetone as extraction volume and solvents, 10 minutes as extraction time and a concentration of 5 mg L^{-1} mixture of PAHs spiked in 1 g sediment sample.

5.8.2 Selection of extraction solvent

A correct choice of solvent is essential for obtaining optimal extraction process. The choice of the solvent is based upon the solubility of the desired analyte, the solvent-matrix interaction and the ability of the solvent to absorb microwaves (Chen *et al.*, 2008). The selected solvent should have a high selectivity towards the analyte of interest than the other matrix components and also a good compatibility with further chromatographic analytical steps. Solvents which are transparent to microwaves, do not heat up under microwave and those with good microwave absorbing capacity get heated up faster and enhance the extraction process. Polar solvents such as acetone will absorb microwave efficiently, as they have permanent dipole moment that can interact with microwaves. Non-polar solvents such as hexane will not be heated when exposed

to microwaves, but can instead be used in mixtures with polar solvents in order to obtain the desired heating properties (Mandal *et al.,* 2007). In this work for the selection of the optimum MAE solvent, extraction efficiency was evaluated by testing three solvents namely, hexane: water (1:1 v/v), hexane:acetone (1:1 v/v) and hexane alone. The solvent mixture hexane: acetone was included since it has been proven to be efficient solvent for the extraction of different pollutants from environmental samples such as PAHs, PCBs, phenols etc (Castro *et al.,* 2009). Water was included because it is the most polar solvent but with poor solubility for the PAHs (Castro *et al.,* 2009). Water also has high dielectric constant (Table 5.23) and it is easily heated by MAE (Wang, 1997).

Figure 5.20, shows the results obtained on varying the extraction solvent. With hexane only, the results were the lowest, which could be due its disability of being heated when exposed to microwaves as it is non-polar, even though it is a good extraction solvent for aromatic compounds (Bangkedphol *et al*., 2010). The results with hexane: water showed average response, this could be due the extra liquid-liquid extraction step (Shu *et al*., 2000). Hexane: acetone showed better results of the three solvents, this could be due to both being good extraction solvent for aromatic compounds and acetone's efficiency in absorbing microwaves. This mixture was then selected as the correct choice of extraction solvent for this work. The effect of organic solvent (hexane: acetone, cyclohexane: water and hexane: dichloromethane) on the extraction of PAHs in sediments has been investigated (Blanco *et al.,* 2000). Hexane: acetone was obtained to be the best extraction solvent. EPA method 3546 recommended extraction solvent is haxane: acetone (1:1,v/v).

Table 5.22: Physical parameters of the solvents used in MAE **(**Knovel, 2008)

	Physical Properties				
Solvents	**Boiling Point (^oC)**	**Molar mass (g mol^{-1})**	**Polarity**	**Dielectric constant**	**Water solubility**
Hexane	68.7	86.18	0.1	1.8	0.014
Acetone	56.3	58.08	5.1	20.7	100
Water	100.0	18.02	10.2	80.1	100

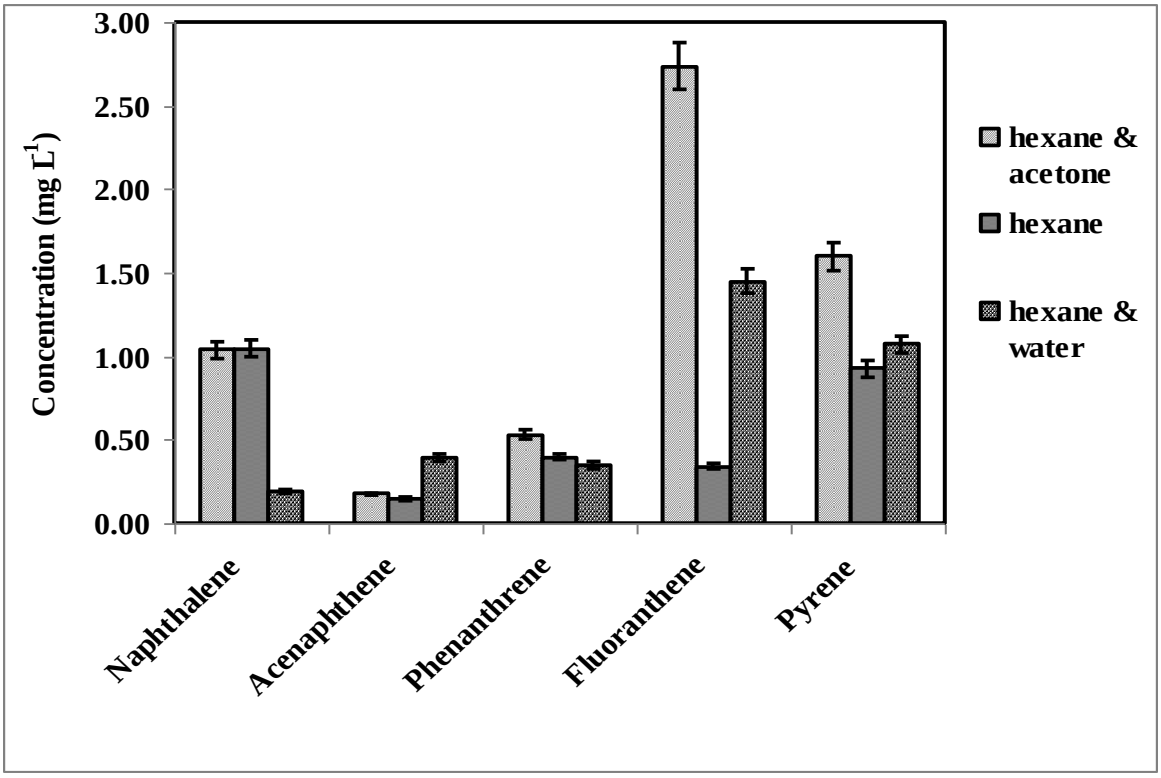


Figure 5.20: Effect of extraction solvent on the amount extracted. The other parameters kept constant were 20 mL as extraction volume, 20 minutes as extraction time, 250 W as extraction power and a concentration of 5 mg L^{-1} mixture of PAHs spiked in 1 g sediment sample.

5.8.3 Extraction solvent volume

The solvent volume is a critical factor and it should be sufficient enough to immerse the matrix completely in the solvent throughout the entire irradiation process. In conventional extraction methods, a higher ratio of solvent volume to solid matrix gives

better extraction yields, whereas in case of MAE a higher solvent: matrix ratio may not give better yield due to inadequate stirring of the solvent by the microwaves (Luque-Gracia, 2003). Too much extraction solvent means more energy and time required for extraction. The effect of extraction solvent volume was investigated by varying it between 20-40 mL. Figure 5.21 shows the results obtained. The amount extracted for naphthalene, acenaphthene, phenanthrene and fluoranthene decreased from 20 mL to 30 mL and then increased at 40 mL. The reason could be due to inadequate stirring of the solvent by microwaves at high volumes (Eskilsson *et al*., 2000).

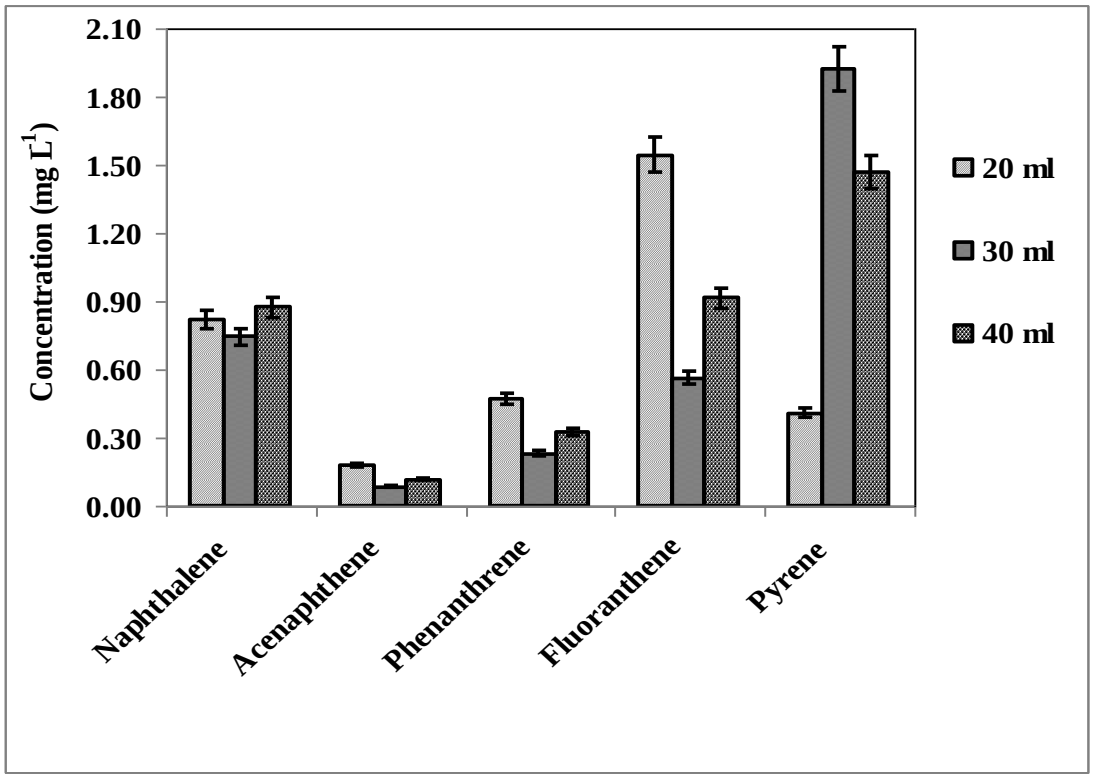


Figure 5.21: Effect of the extraction solvent volume on the amount extracted. The other parameters kept constant were hexane:acetone as extraction solvents, 10 minutes as extraction time, 250 W as extraction power and a concentration of 5 mg L^{-1} mixture of PAHs spiked in 1 g sediment sample.

Pyrene did not follow this trend as the amount extracted increased from 20 mL to 30 mL and then decreased at 40 mL. This deviation could be attributed to differences in compounds physical-chemical behaviour during the extraction, and on how strongly

they are bound to sediment particles (Landrum *et al*., 1992). 20 mL was chosen as the extraction solvent volume for this work. Several groups have reported low recoveries in MAE due to high solvent volume. PAHs and PCBs have been extracted from the sewage sludge where recoveries decreased when solvent volume was increased from 30 to 56 mL (Eskilsson *et al*., 2000). Also on extraction of PAHs from 5 g sediment, the recoveries were higher for 30 mL than 45 mL, (Eskilsson *et al*., 2000). The solvent volume to be used also depends on the amount of sample used (Mandal *et al*., 2007). According to Mandal, it was established that lower volume of solvent led to higher yield on the study of the effect of solid-liquid ratio on MAE of pectin, but this fact may not always be true as there exists reports which claim the reverse, (Mandal *et al*., 2000). The effect of solvent volume between 10-30 mL was investigated (Blanco *et al*., 2000). It was found that all the volumes were optimum since they were not exeeding 30% w/v, to the amount of sample mass used. Mandal as well as Blanco's findings may explain why in this work no major differences in the amount extracted between 30-40 mL. However EPA method 3546 recommended solvent volume of 25 mL with 2-20 g of sample.

5.8.4 Extraction time

Extraction time is another parameter whose influence needs to be taken into account in MAE optimization. Generally by increasing time, the quantity of analyte extracted is increased, although there is a risk that degradation may occur (Mandal *et al*., 2007). To determine the time needed to obtain high recovery, extractions were performed at different times between 10-30 minutes. Figure 5.22 shows the results obtained. The results showed an increase in the amount of analyte extracted with an increase in extraction time from 10 to 20 minutes for most analytes, followed by a decrease at 30 minutes. The reason might have been that the analyte degraded due to longer extraction time though this was not proved. For other analytes (naphthalene and pyrene), this trend was not followed. 20 minutes was chosen as sufficient extraction time for this work. For analytes to be extracted, the solvent has to penetrate the soil particle pores. The analytes has to be extracted from its particle and diffuse into the bulk solvent. All these processes are time dependent. Higher extraction time should eventually lead to

more amounts extracted until a plateau is reached. However too long extraction could degrade the compound, which could lead to less amount extracted. The effect of extraction time (15-30 min) on the extraction of PAHs in sediments was investigated (Purcaro *et al.*, 2009). They observed that there was no significant difference that was found on varying the extraction time for most of the PAHs and there was no trend on the results obtained. EPA method 3546 recommended extraction time is 10 minutes.

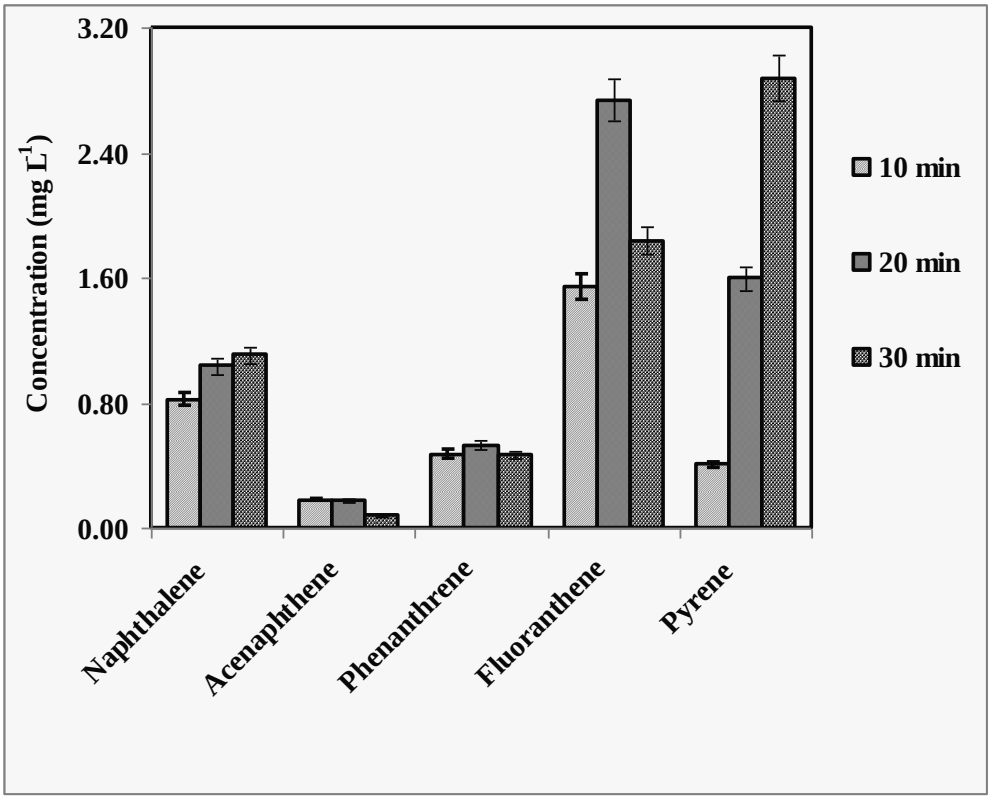


Figure 5.22: Effect of extraction time on the amount extracted. The other parameters kept constant were 20 mL hexane: acetone as extraction volume and solvents, 250 W as extraction power and a concentration of 5 mg L^{-1} mixture of PAHs spiked in 1 g sediment sample.

5.8.5 Amount of sample

Optimization of the amount of sample is important in order to assess the homogeneity of the material (Pensado *et al*., 2000). In general, the increase of the sample amount increases the amount of the analyte being extracted. But when it is too much it will

require more extraction time as well as extraction solvent (Lou *et al*., 1997). To check the effect of the sample amount, the sample mass was varied between 0.5 and 2 g. Figure 5.23, shows the results obtained on varying the sample amount. On the results obtaned, 0.5 g sample gave better results for acenaphthene and pyrene. 1 g gave better results for naphthalene and fluoranthene, while 2 g was better for phenanthrene. 1 g was then chosen as the sample amount for this work because it was the best compromise. The effect of sample mass (1-3 g) on the exctraction of PAHs on meat samples was also investigated (Purcaro *et al.*, 2009). They reported that there was no significant difference on the range investigated nor was there any trend observed and a sample amount of 2 g was chosen as the best compromise increase the extraction efficiency. EPA methos 3546 recommended sample mass is 2-20 g, but other parameters have to be verified especially for large sample masses (Lou *et al*., 1997).

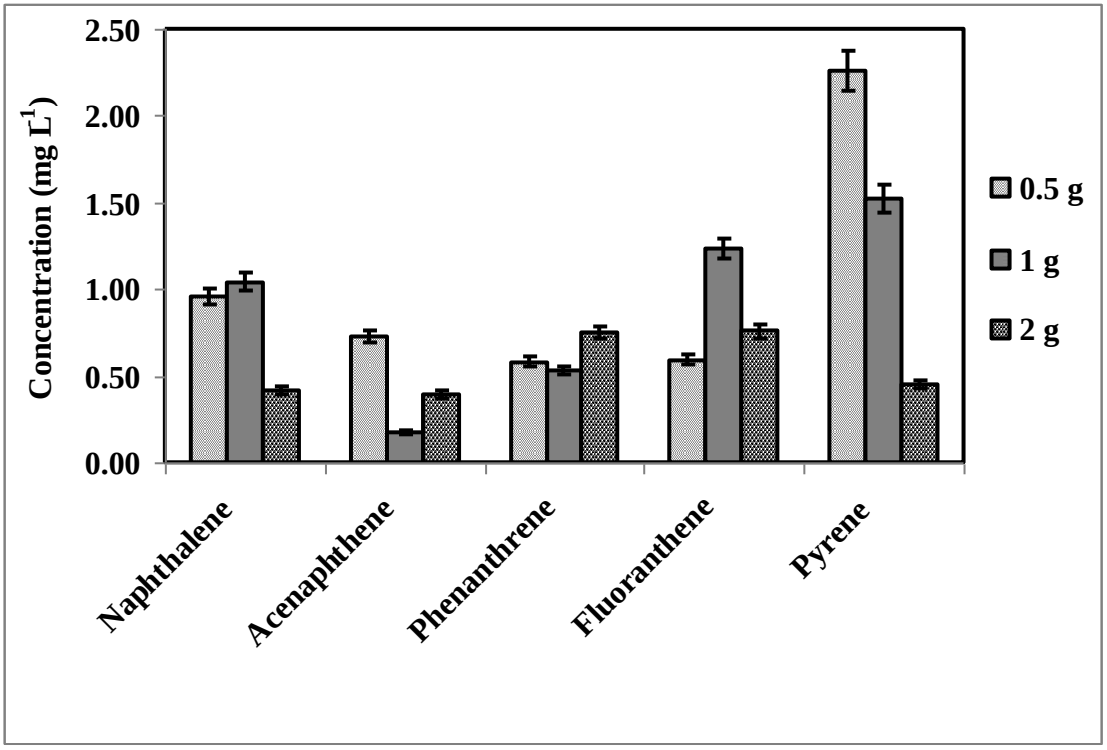


Figure 5.23: Effect of sample mass on amount extracted. The other parameters were kept constant, 20 mL hexane:acetone as extraction volume and solvent, 20 minutes as extraction time and 250W as extraction power.

The recoveries and LOD for MAE are given in Table 5.23.

Table 5.23: Recoveries and LODs for MAE

Compounds	% Recovery	LOD (µg L^{-1})
Naphthalene	93.4	0.85
Acenaphthene	80.9	0.8
Fluoranthene	92.6	1.7
Pyrene	61.2	0.1

5.9 Validation of MAE method

The MAE method was also validated using reference standard material under the optimized conditions described above. The analyses were carried out in triplicates. The results are presented in Table 5.24 below. The concentrations obtained for all analytes were within the acceptable limits. The precision (relative standard deviations) were less than 5%. The validation of MAE method with certified reference materials has also been reported (Flotron *et al.*, 2003). They found that the mean concentrations were lower than the certified values and said the reason was that PAHs were not quantitatively extracted from the sample matrix.

Table 5.24: Concentrations and relative standard deviations obtained from standard reference materials

Compounds	Certfied values (µg kg^{-1})	Obtained values (µg kg^{-1})
Naphthalene	752 (113)	594 (1.24)
Acenaphthene	526 (82.2)	435 (4.09)
Fluoranthene	588 (90.7)	440 (4.68)
Pyrene	327 (47.2)	285 (2.90)

5.10 Application of the MAE method to real sediment samples

The application of MAE to real samples was performed using HPLC-Fluorescence. The physical properties for all the sediments samples that were analysed are shown in Table 5.25. The pHs for the PIT (Natalspruit River) samples were acidic. That could be due to the acid mine drainage in the river. pH of sediment samples from Hartbeespoort Dam was slightly basic perhaps due to contamination of sewage wastewater and solid waste from feeding streams and rivers. Kempton Park Dams sediments were almost of neutral pH except for the last dam that was more on the acidic side. This is the last dam where the river flows and low pH could indicate other processes occurring in the dam including acid mine drainage. The pH of the Hennops River sediments that forms part of the Centurion Lake was similar to the sediments and was close to neutral. The conductivity was high in the PIT sediment samples due to reduced pH that releases a lot of metal ions and other ions in solution. Conductivity of the sediment samples from Hartbeespoort Dam was the second highest and that can be attributed to anions such as sulphates, phosphates and nitrates associated with sewage wastewater and solid wastes. Kempton Park sediments have the lowest conductivity. This means that it is less polluted with metals and other anions.

Table 5.26 shows the concentrations of PAHs obtained on real sediment samples. All studied compounds were detected in all samples except fluoranthene and pyrene which were not detected in Hennops River. The results obtained were below the maximum allowable concentrations in soil except for naphthalene in Blaauwpan Dam, Kleinfontein Lake, Centurion Lake and Hennops River. For the Jukskei River, they were all above the maximum allowable concentrations (Table 2.3; ATSDR, 2006). Jukskei River was found to be the most polluted while Hartbeespoort Dam was found to be the least polluted river. The trend was as follows Jukskei River > Blaauwpan Dam > Centurion Lake > Middle Lake > Homestead Lake > Kleinfontein Lake > Hennops River > Hartbeespoort Dam > PIT. Hartbeespoort Dam is quiet big and sediment samples collected may not be representative of the scenario. Since Jukskei and Hennops Rivers flows into the Harbeespoort Dam one would expect it to be the sink of PAHs found in these rivers. Further investigation is still required. There is a

posibity that the results obtained for December could be different to those obtained in May due to seasonal effects.

Determination of organic carbon is used to identify the presence of organic substances in sediments. The content of organic carbon depends on geographical location, pollutants entering rivers and layer depth of tested sediments (Niemirycz *et al*., 2006). Dissolved organic matters which are ubiquitous in aquatic systems are largely composed of humic substances and tend to bind to PAHs owing to their high content of organic carbon. Therefore it can be considered as both the mobile phase for enhancing the solubility of PAHs and sorbed phase itself on soil particle for retarding the mobility of them (Moon and Park, 2001). There was no clear trend in the organic content except that sediment samples with lowest pH have least amount of organic content. According to the results obtained (Figure 5.24), it is shown that there is no direct correlation between % organic content and the concentrations obtained. PIT and Homestead Lake samples, even though they have the lowest organic content have higher concentrations of PAHs. On the other hand Hennops River has the highest organic content but it has lower concentration. The reason for these differences in concentrations could be due to the sources of PAHs into the river.

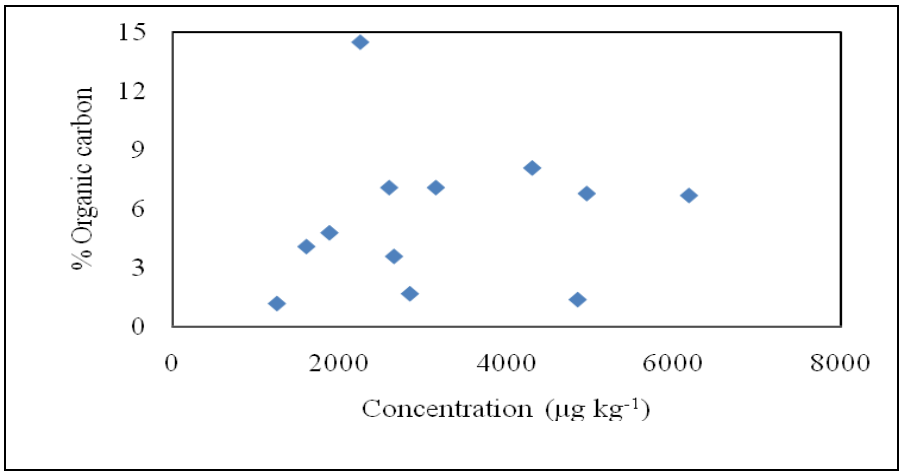


Figure 5.24: % Organic carbon obtained in Jukskei River, Kempton Park, Centurion River, Natalspruit River samples. 1 g sample analysed using MAE-HPLC-Fluorescence**.**

Table 5.25: pH, conductivity and organic carbon content

Sample	Sampling date	pH	Conductivity (µS)	%Organic carbon
Hartbeespoort Dam1	19 April 2011	7.80	598	4.1
Hartbeespoort Dam 2	19 April 2011	7.87	602	4.8
PIT 1	12 December 2010	3.96	750	1.2
PIT 2	12 December 2010	4.02	768	1.4
PIT 3	12 December 2010	4.0	761	1.7
Centurion Lake 1	3 May 2011	7.57	872	6.8
Centurion Lake 2	3 May 2011	7.60	890	7.1
Hennops River	3 May 2011	7.76	593	14.5
Blaauwpan Dam	19 May 2011	7.85	375	6.7
Homestead Lake	19 May 2011	7.37	276	3.6
Middle Lake	19 May 2011	7.54	117	8.1
Kleinfontein Lake	19 May 2011	5.97	443	7.1
Jukskei River 1	7 May 2011	6.01	100.8	3.5
Jukskei River 2	7 May 2011	7.48	484	5.6
Jukskei River 3	7 May 2011	7.61	153.3	1.2
Jukskei River 4	7 May 2011	7.82	143.1	2.3

Table 5.26: Levels of PAHs (µg kg^{-1}) obtained with MAE for real sediment samples from Hartbeespoort dam, Natalspruit, Centurion, Kempton Park and Jukskei (n=3) and relative standard deviations expressed in percentage

Compounds	HPB1	HBP2	PIT1	PIT2	PIT3	Hennops River	Centurion Lake 1	Centurion Lake 2
Naphthalene	858 (9.6)	970 (7.4)	630 (9.4)	844 (5.8)	558 (7.3)	1299 (8.5)	1159 (10.7)	1180 (3.6)
Acenaphthene	756 (3.1)	636 (8.9)	523 (6.5)	639 (6.7)	452 (6.7)	950 (8.1)	1690 (9.0)	1635 (3.2)
Fluoranthene	135 (6.8)	175 (10.3)	nd	888 (8.8)	214 (6.6)	Nd	904 (8.3)	276 (10.7)
Pyrene	129 (5.0)	99 (5.6)	100 (7.2)	2475 (2.1)	1590 (3.2)	Nd	1202 (2.2)	61 (10.8)
Sum	1605	1880	1253	4846	2814	2249	4955	3152

Compounds	Blaauwpan Dam	Homestead Lake	Middle Lake	Kleinfontein Lake	Jukskei 1	Jukskei 2	Jukskei 3	Jukskei 4
Naphthalene	1553 (7.8)	1546 (8.4)	904 (2.6)	1037 (9.5)	17349 (0.5)	23689 (4.7)	30439 (3.3)	27293 (9.6)
Acenaphthene	2454 (8.7)	771 (0.6)	770 (4.9)	993 (9.7)	16620 (1.0)	24026 (1.1)	25038 (2.9)	35919 (10)
Fluoranthene	1940 (8.0)	250 (5.6)	875 (2.7)	340 (7.7)	18616 (9.9)	35298 (3.0)	17259 (9.2)	45281 (9.4)
Pyrene	230 (7.0)	84 (3.5)	1756 (10.9)	224 (8.7)	24637 (0.8)	16980 (1.1)	23155 (10)	12310 (0.7)
Sum	6177	2652	4304	2594	77222	99993	95891	12080

5.11 Comparison of MAE and SE for PAHs

The methods parameters for MAE and SE were compared to each other for this work (Table 5.27). The results shows that MAE uses little solvent as well as sample volume compared to SE. Figure 5.25 gives the recoveries and relative standard deviations obtained for the extraction of PAHs using MAE and SE extraction techniques. The recovery of PAHs from the MAE compared to SE are slightly lower. This is common in such a technique since extraction is matrix dependent. What is important is that they are very reproducible and therefore can easily be used for quantitative analysis. From comparison of recovery and relative standard deviations, it does suggest that both methods are comparable. However, MAE has other advantages in that it is faster and uses little organic solvent than SE. In terms of selectivity, SE is much better since after extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded, which is not the case for MAE. MAE has been used for the extraction of PAHs in sediments (Blanco *et al*., 2000), with hexane: acetone at an extraction power of 500 W for 6 minutes and 1.5 g sample mass. The obtained recoveries were between 91-104%. The use of MAE for the extraction of PAHs in 1 g sediment sample with hexane: acetone at an extraction power of 500 W for 10 minutes has been reported (Shu *et al*., 2000). The obtained recoveries were between 89-109%. In this MAE was used for the extraction of PAHs in sediments with hexane: acetone at an extraction power of 250 W for 20 minutes, and the recoveries obtained were between 61-92%. The % recoveries obtained in this work are slightly lower compared to what Blanco and Shu obtained, the reason could be due to the lower extraction power used and different instruments. SE has also been used for the extraction of PAHs in soil samples with 150 mL of hexane: acetone for 24 hours and 2.5 g samples mass (Gfrerer *et al*., 2002). Obtained recoveries were between 69-102%. PAHs in 5 g sediment samples with 250 mL dichloromethane: hexane for 24 hours has also been extracted (Guo *et al*., 2009). The obtained recoveries were between 60-120.7%. In this work SE was used to extract PAHs in sediment samples with 250 mL dichloromethane: hexane for 24 hours. The obtained recoveries were between 84-98%. The results obtained in this work are comparable to those obtained by (Guo *et al*.,

2009), since the procedure used is similar. The results obtained (Gfrerer *et al*., 2002) are slightly lower than those obtained in this work, the reason could be due to the difference solvent type and amount used.

Table 5.27: Comparison of MAE and SE methods parameters

Parameter	MAE	SE
Extraction time per sample	20 minutes	24 hours
Total organic solvent used	20 mL	250 mL
Sample mass	1 g	15 g
Extraction volume	1 mL	1 mL

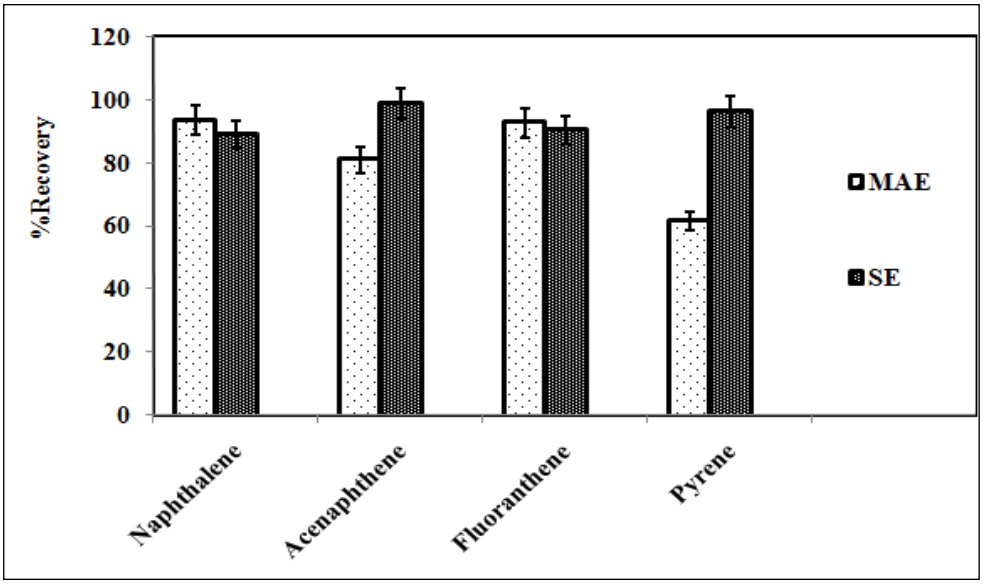


Figure 5.25: Recovery of MAE and SE for PAHs analysis under optimized condition.

Comparison of MAE and SE methods on real samples

Both methods were applied to real samples. The results of correlation coefficient obtained on comparing the methods are closer to 1 (Figure 5.26), which means that both of the methods are capable of analysing the PAHs in sediments and are comparable. The concentrations obtained are shown in Table 5.28.

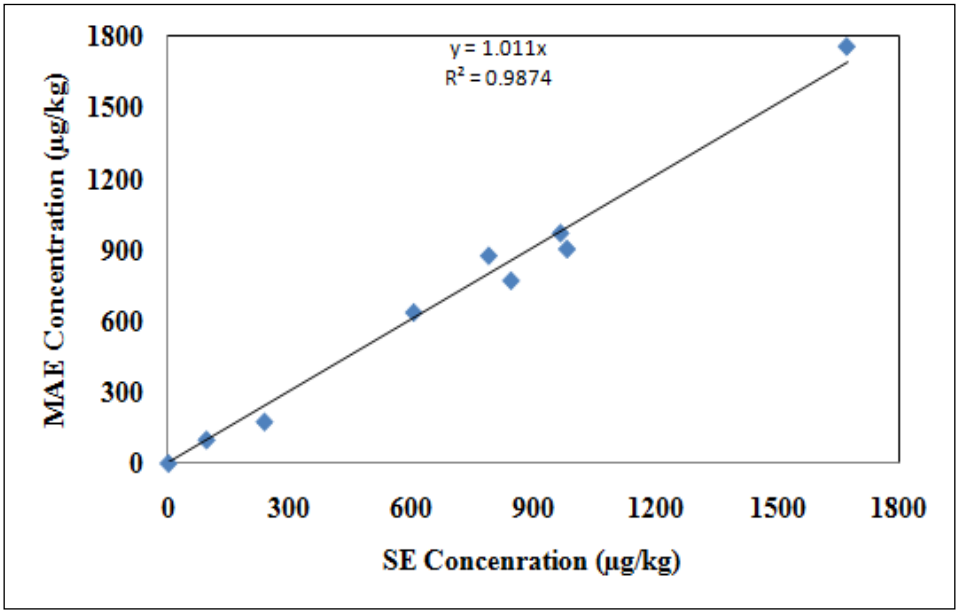


Figure 5.26: Comparison of the MAE and SE methods performance from Hartbeespoort Dam and Middle Lake.

Table 5.28: Comparison of MAE and SE on levels of PAHs (µg kg^{-1}) for real sediment samples (n=3) and relative standard deviations expressed in percentage

	HBP2		Middle Lake	
Compounds	**MAE**	**SE**	**MAE**	**SE**
Naphthalene	970 (7.4)	967 (3.1)	903 (2.6)	983 (3.2)
Acenaphthene	636 (8.9)	605 (6.7)	770 (4.9)	845 (9.5)
Fluoranthene	175 (10.3)	237 (4.5)	875 (2.7)	790 (1.1)
Pyrene	99 (5.6)	94 (1.8)	1756 (10.9)	1673 (2.9)

The comparison of MAE and SE on extraction of PAHs in soil has been reported (Wang *et al*., 2007). MAE and SE methods on PAHs have also been compared (Shu *et al.*, 2003). The results are given in Table 5.29. These comparisons prove that MAE gives same results as SE and is much faster besides using less organic solvent.

Table 5.29: Comparison of MAE and SE methods in the literature

Extraction method	% RSD	% Recovery	Reference
MAE	2.84-9.02	60.73-109.23	Wang *et al*., 2007
SE	0.92-4.53	76.18-100.15	
MAE	1.2-7.3	35-67.5%	Shu *et al.*, 2003
SE	0.2-3.3	27.4-59.6%	

5.12 Correlation studies of PAHs in water and sediment samples

The correlation of PAHs in all samples was examined in order to investigate if they originate from the same source. For the liquid samples few of the PAHs showed a positive correlation (Figure A1-A3 in appendix A). In East rand area (Blaauwpan Dam, Homestead Lake, Middle Lake and Kleinfontein Lake) and West Johannesburg area (Hartbeespoort Dam), there was no positive observed. In Centurion area (Centurion Lake and Hennops River), the positive correlation was observed for naphthalene/pyrene (R^2 = 0.9798) and fluoranthene/pyrene (R^2 = 0.7296). In South Johannesburg area (Jukskei River), its only fluoranthene/phenanthrene which showed a positive correlation (R^2 = 0.8526). These positive correlations may suggest similar source of PAHs but generally results indicate that PAHs are coming from various sources.

For sediment samples also few positive correlations were observed (Figure A4-A6 in appendix). In East rand area, it was positive correlation for acenaphthene/fluoranthene (R^2 = 0.8268). In South Johannesburg area only fluoranthene/naphthalene (R^2 = 0.8227) and acenaphthene/pyrene (R^2 = 0.7595) gave positive correlations. In West Johannesburg area and Centurion area there was no positive correlation observed. The correlation of PAHs with the suspended solids has been investigated as a transport route from source (Kim *et al*., 2009) and obtained poor correlation coefficient (R^2 = 0.54). The investigation was also done on the correlation of sorption of PAHs by bacteria with the cell surface as possible sink (Stringfellow *et al*., 1999) and observed a

positive correlation (R^2 = 0.950). Sediments give long term pollution as they act as sink for PAHs.

5.13 The effect of depth profile studies on PAHs in sediment samples

The effect of depth profile on PAHs was investigated by examining three different depths. The pH of the sediment was slightly neutral and increased with an increase in the sediment depth profile for all samples. The conductivity was low and it decreased with an increase in sediment depth per sample. The organic content also slightly decreased with an increase in sediment depth as shown in Table 5.30. Table 5.31 shows the concentrations obtained. The concentration increased with an increase in sediment depth and then decrease on further increase in depth for both samples. For Jukskei1 sample, naphthalene, phenanthrene and pyrene were above the acceptable limits at all depths. Fluoranthene was above the acceptable limits at 0-24 cm. Acenaphthene was below the acceptable limits. In Jukskei 2 sample all compounds were below the acceptable limits at all depths except for naphthalene at 0-18 cm. The concentration increase with an increase in the molecular weight in each depth for most PAHs but naphthalene did not follow that trend. The reason could be that low molecular weight PAHs biodegrade more rapidly (thus reduce concentration) than the higher molecular weight compounds (Cerniglia, 1992). The results for Jukskei 1 sample were 10 times higher than Jukskei 2 except for naphthalene where they were comparable. The reason for these higher results could be that Jukskei 2 was sandy while Jukskei 2 was muddy. The accumulation of PAHs depends on characteristic of the sediment with soil sand accumulating less PAHs than mud or peat (Trapido and Veldreg, 1996). The reason for the lower concentration at the lower depth could be due to the photo oxidation on the surface and microbial enzymatic production at the bottom (Pawloska *et al*., 2007). The concentration of PAHs in sandy and muddy sediments was measured (Trapido and Veldreg, 1996). It was observed that the concentration was higher in muddy (14.3 µg kg^{-1}) than in sandy sediment (2.89 µg kg^{-1}), which was also observed in this work. The decrease in concentration with further increase in depth (18-24 cm) and 27-36 cm) could be due to other processes that might be occurring in the sediment. The effect of sediment depth on PAHs was also studied (Choudhary *et*

al., 2010). There was no direct correlation between the depth and the concentration of PAHs that was obtained. In this study it was impossible to get depth profiles below 36 cm because of the rocks found at the bottom.

Table 5.30: Physical properties of samples analysed for depth profile

	Depth (cm)	Sampling date	Organic content	pH	Conductivity (µS cm^{-1})
Jukskei 1	12	11 October 2011	2.99	6.38	332
	24	11 October 2011	2.75	6.70	216
	36	11 October 2011	2.73	6.93	54
Jukskei 2	9	11 October 2011	3.71	7.27	161
	18	11 October 2011	2.74	7.41	152
	27	11 October 2011	1.58	7.47	113

Table 5.31: Levels of PAHs (µg kg^{-1}) obtained with MAE for real sediment samples from Jukskei River depth (n=3) and relative standard deviations expressed in percentage

	Depth (cm)	Naphthalene	Acenaphthene	Phenanthrene	Fluoranthene	Pyrene	Sum
(J 1)	0-12	1280 (2.1)	520 (1.1)	4630 (0.9)	7990 (0.8)	13850 (1.4)	15800
	12-24	2250 (1.2)	760 (2.9)	5580 (3.7)	8640 (3.6)	15740 (3.2)	32970
	24-36	2110 (1.2)	430 (0.7)	8640 (2.8)	2560 (0.1)	4190 (0.1)	17930
(J 2)	0-9	1300 (2.4)	60 (4.7)	210 (2.3)	160 (2.6)	460 (4.2)	2190
	9-18	1360 (1.9)	220 (3.6)	300 (4.1)	350 (1.1)	660 (2.3)	1910
	18-27	950 (2.4)	20 (9.8)	170 (1.5)	170 (0.9)	460 (2.0)	1770

Note: J = Jukskei River

5.14 Status of PAHs in South Africa

The status of PAHs in South Africa is not well known because not much work has been done (Nekhavhambe, 2008; Nieuwoudt *et al.*, 2011, Cele, 2005; Tikilili, 2004).

Comparison of PAHs levels in South African water samples

The levels of PAHs obtained in this work ranged from 1.8-615.7 ng L^{-1} for liquid samples, where naphthalene, acenaphthene and phenanthrene were the most dominant.

On comparing PAHs levels with those reported in the literature it was found that Western Cape is the most polluted province, the most dominant PAHs were acenaphthene and naphthalene. In Gauteng, naphthalene, acenaphthene and naphthalene were the most dominant. In Limpopo, fluoranthene and pyrene were dominant. These differences could be attributed to the diverse sources of PAHs in each region. So far there are no detailed studies that have been done in trying to locate major sources in these regions. To our best knowledge no data has been reported on PAHs levels in water in Gauteng, North West and KwaZulu Natal province. Table 5.32 shows the summary of obtained results in water samples in South Africa.

Table 5.32: Levels of PAHs (ng L^{-1}) obtained from water samples around South Africa ([1]Tikilili, 2004; [2]Nekhavhambe, 2008)

Compounds	**[1]Western Cape**	**[2]Limpopo**	**Ours/Gauteng**
Naphthalene	1600	-	21.6-430.1
Acenaphthene	25100	-	35.9-406.6
Phenanthrene	390	-	39.9-615.7
Fluoranthene	Nd	3.4-200	1.8-278.6
Pyrene	10	0.1-2500	12.0-278.6

Comparison of PAHs levels in South African sediment samples

The levels of PAHs obtained in this work ranged from 0.06-45.2 mg kg^{-1} for sediment samples. Fluoranthene, acenaphthene, naphthalene were the most dominant. On comparing our results with those reported for Gauteng province in the literature it was found that our levels are higher. The reason for this could be sampling areas analyzed, whereby in the literature they have not analyzed the Jukskei River where the higher levels in our results were obtained. The other reason could be time and seasons in which the analyses were done, method of sample preparation and analysis and also the difference of the original sources of these compounds.

On comparing with other provinces, it was found that Gauteng is the most polluted province. In Western Cape, naphthalene was the most dominant PAH. In Limpopo and Gauteng Provinces, fluoranthene and pyrene were the most dominant PAHs in sediments. In North West, acenaphthene and pyrene were most dominant. In KwaZulu Natal, fluoranthene and pyrene were most dominant. These differences could be due to diverse sources. In Western Cape Province, naphthalene was the most dominant PAH. Table 5.33 shows the summary of the reported levels.

Table 5.33: Levels of PAHs (mg kg^{-1}) obtained from solid samples around South Africa ([1]Cele, 2005; [2]Nekhavhambe, 2008; [3]Nieuwoudt *et al.*, 2011)

Compounds	**[1]Western Cape**	**[2]Limpopo**	**[1]North West**	**[1]KwaZulu Natal**	**[3]Gauteng**	**Ours/Gauteng**
Naphthalene	0.42	-	nd	1.6	0.059	0.5-30.4
Acenaphthene	nd	-	1.62	1.18	0.080	0.4-35.9
Fluoranthene	nd	0.41-15.1	nd	5.84	3.6	0.1-45.2
Pyrene	nd	0.53-34.4	0.6	18.6	2.9	0.06-24.6

Cele- measured sludge samples

Nekhavhambe and Nieuwoudt *et al*- measured sediment samples

Comparison of PAHs levels in worldwide water and sediment samples

A review on the levels of PAHs around the world has been reported (Manoli and Samara 1999). The levels of PAHs around the world have also been reported (Pereira *et al*., 1999; Blanco *et al*., 2000). On comparing the reported PAHs levels with those in South Africa it was found that Nigeria is the most polluted and the dominant PAHs were pyrene, acenaphthene and phenanthrene. In Germany, the most dominant were naphthalene, pyrene and fluoranthene. In Greece, pyrene, phenanthrene and fluoranthene were dominant. In Denmark, pyrene and phenanthrene were dominant. In South Africa, naphthalene, acenaphthene and phenanthrene were most dominant. The results are shown in Table 5.34.

Table 5.34: Levels of PAHs (µg L^{-1}) obtained from water samples around the world (Manoli and Samara, 1999)

Compounds	**Germany**	**Denmark**	**Greece**	**Nigeria**	**South Africa**
Naphthalene	0.007-0.051	0.0055	-	1910	0.0216-1.6
Acenaphthene	0.0085-0.046	0.0004	0.010-0.064	6630	0.0359-25.1
Phenanthrene	0.0041-0.022	0.021	0.030-0.132	8380	0.0399-0.6157
Fluoranthene	0.0076-0.040	0.0054	0.010-0.065	-	0.0018-0.2786
Pyrene	0.0073-0.033	0.015	0.010-0.140	10780	0.0001-0.2786

On comparing the sediment levels in South Africa and worldwide it was found that South Africa is the most polluted country with fluoranthene, acephthene and naphthalene being the most dominant PAHs. In Germany, Spain and California fluoranthene and pyrene were the most dominant. In Hong Kong, fluoranthene, acenaphthene and pyrene were the most dominant (Table 5.35).

Table 5.35: Levels of PAHs (mg kg^{-1}) obtained from solid samples around the world ([1]Manoli and Samara, 1999; [2]Blanco *et al*, 2000; [3]Pereira *et al*, 1999)

Compounds	[1]Germany	[3]California	[1]Hong Kong	[2]Spain	South Africa
Naphthalene	0.002-0.010	0.0021-0.033	0.0116	-	0.42-30.4
Acenaphthene	0.001-0.005	0.0005-0.041	0.0439	-	0.08-35.9
Fluoranthene	0.082-0.266	0.0021-0.773	0.0442	0.695-2.520	0.1-45.2
Pyrene	0.067-0.237	0.0022-0.980	0.0552	0.546-2.577	0.06-24.6

Comparison of PAHs levels in water and sediment samples

The levels of PAHs were found to be high in sediment/solid samples (Michiga, New York) than in water samples, this is because they prefer to partition in sediments due to their hydrophobicity (Kannan *et al*., 2005). In South Africa they are between 0.0018-25.1 µg L^{-1} in water and 0.06-45.2 mg kg^{-1} in solid samples. Around the world they are between 0.0001-1078 µg L^{-1} and 0.001-45.2 mg kg^{-1}. PAHs concentrations in sediment and water samples (Izmit Bay, Turkey) have been measured (Trapido *et al*., 1996). Obtained concentrations in sediments were 103 -106 times higher than in water. The concentrations of PAHs in water and sediment have also been compared (Telli-karakoc *et al.*, 2001). The concentrations obtained were higher in sediment (30-1670 µg g^{-1}) than in water (1.16-13.68 µg L^{-1})

Chapter Six – Conclusion and Recommendations

6.1 Conclusion

Optimization of extraction method parameters is important before any application of the method. Parameters such as extraction efficiency and enrichment factors need to be known. Even when applying an aexisting method, optimization still needs to be done because the reported parameters might not be applicable because of factors such as differences in set-up, analytical separation systems, detection etc. This study has been demonstrated the applicability of HF-LPME and SPE techniques followed by GC-FID or GC-MS for the detection of PAHs in water samples at trace levels. On the bases of the experiments discussed above, the optimum HF-LPME conditions were heptane as extraction solvent, an extraction time of 20 minutes, a stirring speed of 600 rpm and a sample volume of 20 mL with 20% ACN as organic modifier and without salt addition. The optimum SPE conditions were 10% methanol as organic modifier, 100 mL sample volume, 40% methanol in water as conditioning solvent, 3 mL acetone:THF as elution solvent.

SPE and HF-LPME techniques are very attractive because they are simple and they also give high enrichment factors for the target PAHs and low detection limits. This makes them suitable for the extraction of these compounds, since PAHs usually exist in low concentration ranges in natural water bodies. They are also concentration independent, which is important because the concentration of PAHs in the environment is not known. A modifier in water prevented PAHs from adsorption into the glass container and influenced analyte enrichment.

Even though solvent consumption is reduced in both methods, HF-LPME is better compared to SPE because it uses less organic solvent and sample. Compared to HF-LPME, SPE consist of more manual steps (conditioning, sample loading, washing, elution of the analyte, evaporation), and much more glass ware is needed. The whole extraction process is longer than HF-LPME. On the other hand higher sample volumes used in SPE is advantageous, since enhances the amount of target analyte transferred

which improves sensitivity. Many steps also enhance the enrichment and recovery of analytes. Solvent evaporation is a critical step in SPE as analyte losses may occur during the evaporation. HF-LPME uses low amount of organic solvent and there is no need of reducing by evaporation. Despite the simplicity of HF-LPME, it still needs an experienced analyst who will ensure that the fiber is properly filled with appropriate organic solvent and is not lost during extraction.

MAE is influenced by many factors such as extraction time, solvent type, solvent volume, sample amount, extraction power. With these factors interacting with one another a statistical optimization strategy needs to be adopted for determining the optimum operating conditions. Therefore optimization of extraction method parameters is important before any application of the method. Parameters such as extraction efficiency and enrichment factors need to be known. This study, has demonstrated the applicability of MAE technique followed by GC-FID or HPLC-Fluorescence for the detection of PAHs in sediment samples. On the basis of the experiments discussed above, the optimum MAE conditions were hexane:acetone as extraction solvents, an extraction time of 20 minutes, extraction volume of 20 mL, 1 g as sample mass and 250 W as extraction power. The MAE technique is very attractive because it is simple and cheap. The optimized and validated MAE method was successfully applied to real sediment samples in and around Johannesburg area.

Most of the river water analyzed were contaminated with PAHs even though they were all below the maximum allowable concentrations in water. Blauuwpan Dam was the most contaminated. All the river sediments analyzed were contaminated with PAHs. Jukskei River has concentrations higher than the maximum allowable concentration levels in soil. For the other rivers, the concentration levels were lower than the maximum allowable levels, except for naphthalene.

Concentrations of PAHs were found to be more in sediments samples than in water. Despite PAHs being persistent organic pollutants (POPs) that are of interest globally, very little studies have been done in South Africa. In contrast with other provinces reported PAHs concentrations in water Western Cape is most polluted, while in river

sediments Gauteng is the most polluted province. However, more data is needed for proper comparison. Comparing with other countries in the world, Nigeria is themost polluted with PAHs in river water, while in river sediment South Africa is the most polluted country. This is from current available literature. Generally in Africa, the study of PAHs in the environment is still behind and more data is still needed.

6.2 Recommendations for future work

- For future work, more application on real water and sediments samples of the optimized methods still needs to be conducted so that a valid conclusion can be drawn on the behaviour of the PAHs.

- Continued montoring has to be conducted in order to investigate long term effects of PAHs, to see if their concentration remains the same as they are knowto be persistent.

- Studies on seasonal variation of PAHs in water and sediment samples have to be done in order to have an idea of the seasonal effect.

- Studies have to be conducted on the depth profile of PAHs in sediments in order to investigate the effect as a result of photo oxidation at the surface and microbial enzymic production at the bottom.

- More studies have also to be done in water and sediments in areas such as Pretoria and its surrounding areas, in order to have overall overview on the pollution of PAHs in Gauteng as a whole.

- Continual studies needs to be conducted in water and sediment samples on the road or near the roads in order to investigate the effect of traffic density with time on PAHs.

- More studies have to be done on fish samples since levels and presence of pollutants in fish have a direct bearing on human health risk. The concentration

of pollutants in fish also presents the bioavailable fraction of the pollutants in water bodies. Since PAHs are capable of undergoing bioconcentration and biomagnifications, comprehensive risk assessment can only be performed by monitoring the levels of these compounds in common freshwater fish of the region.

- Studies have to be conducted in sediment-pore water as its interaction is one of the most dominant processes controlling the distribution and behavior of PAHs in the river.

- Dust particles need to be evaluated since small particles are associated with the higher pollutants concentrations. Soil ingestion has been recognized to be as an important exposure route of PAHs to human. Soil studies have to be conducted in different soil types since the accumulation of PAHs depends on characteristic of the soil.

- Further optimization of extraction and detection methods needs to be done in order to improve their applicability and performance.

Chapter Seven – Presentations and Publications

7.1 Conference presentations

1. P.N. Sibiya, L. Chimuka, E. Cukrowska, J. Å Jönsson. Modification and development of hollow fiber- liquid phase micro extraction method for PAHs in River Water. ChromSA Postgraduate Student Seminar 2009, 10th September. University of the Witwatersrand (South Africa). *Oral presentation*

2. P.N. Sibiya, L. Chimuka, E. Cukrowska, J. Å Jönsson. Modification and development of solid phase extraction method for PAHs in Surface Water. ChromSA Student Seminar 2010, 18th August. University of the Witwatersrand, Johannesburg (South Africa). *Oral presentation.*

3. P.N. Sibiya, L. Chimuka, E. Cukrowska, J. Å Jönsson. Comparison between solid phase extraction (SPE) and liquid phase micro-extraction (LPME) for the extraction of polycyclic aromatic hydrocarbons in aqueous samples. International Conference on Analytical Sciences 2010, 5-9th December. Stellenbosch University, Cape Town (South Africa). *Oral presentation.*

4. P.N. Sibiya, L. Chimuka, E. Cukrowska, H. Tutu. Development and application of microwave assisted extraction method for PAHs in South African solid samples. 40th SACI Convention 2011, 16-21st January. University of the Witwatersrand, Johannesburg (South Africa). *Oral presentation.*

5. P.N. Sibiya, L. Chimuka, E. Cukrowska. Development and application of microwave assisted extraction method for PAHs in South African solid samples. SACI Convention 2011, 22nd September. University of Pretoria (South Africa). *Oral.*

6. P.N. Sibiya, L. Chimuka, E. Cukrowska, J. Å Jönsson. Modification and development of extraction methods for PAHs extraction in surface water. Young

water symposium 2011, 3-5th July. Pretoria (South Africa). *Poster presentation.*

7.2 Publications emanating from this project

1. Precious Sibiya, Ewa Cukrowska and Luke Chimuka (2011), Prevention is better than cure: An alternative approach to sample preparation of complex samples. *LCGC North America*, 29, 11, 104-110. www.chromatographyonline.com

2. 4. P.N. Sibiya, L. Chimuka, E. Cukrowska, H. Tutu. Development and application of microwave assisted extraction (MAE) for the extraction of polycyclic aromatic hydrocarbons in sediments samples (Submitted to Environmental Monitoring and Assessment).

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Appendix A

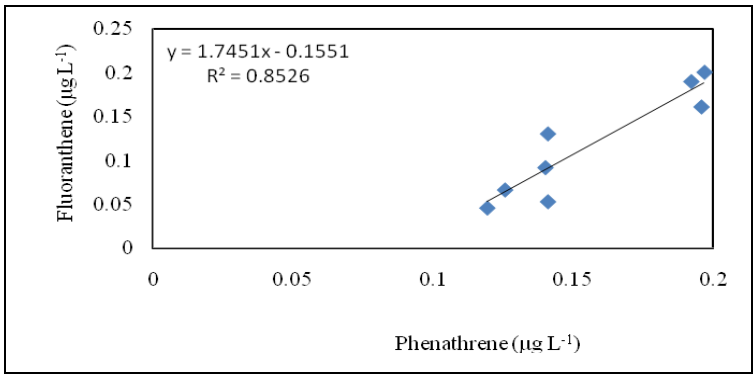


Figure A1: Pair-wise correlation of concentration of phenanthrene and fluoranthene measured in Jukskei River water sample

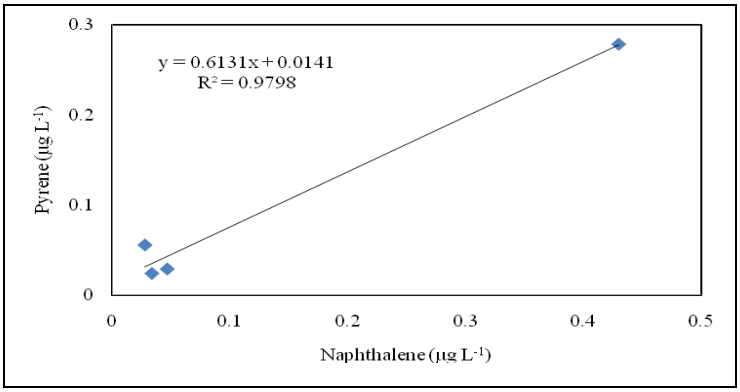


Figure A2: Pair-wise correlation of concentration of fluoranthene and pyrene measured in Centurion River water sample.

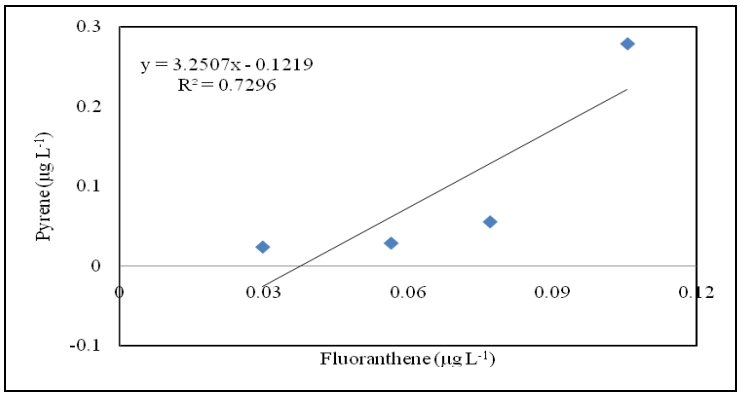


Figure A3: Pair-wise correlation of concentration of fluoranthene and pyrene measured in Centurion River water sample.

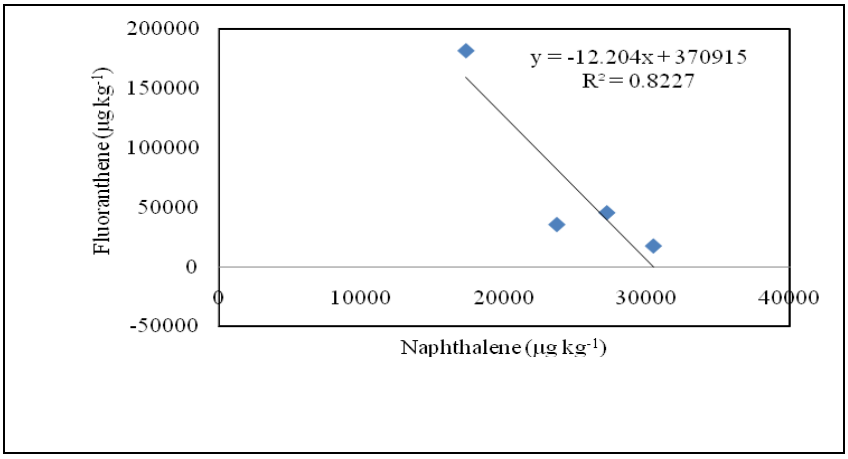


Figure A4: Pair-wise correlation of concentration of naphthalene and fluoranthene measured in Jukskei River sediment sample.

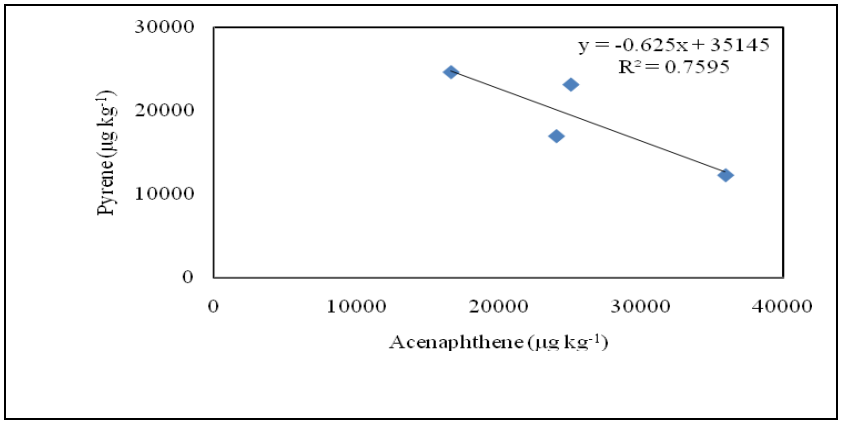


Figure A5: Pair-wise correlation of concentration of acenaphthene and pyrene measured in Jukskei River sediment sample.

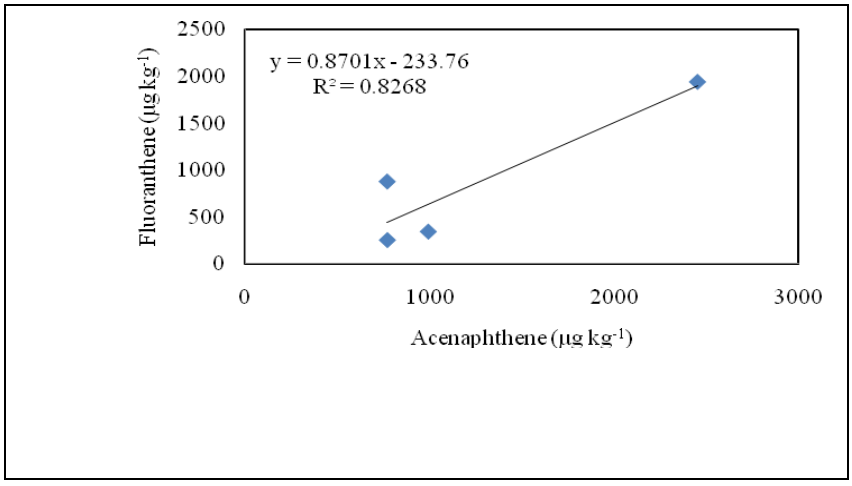


Figure A6: Pair-wise correlation of concentration of fluoranthene and pyrene measured in Centurion River water sample.

APPENDIX B

HF-LPME

Calculation of concentration from the peak areas from calibration curve (for stardands)

Naphthalene
y = 10796x y-peak area
x = 4043 / 10796
= 0.3744 mg L^{-1}

Calculation for the enrichment factors (EF)

EF = C_A / C_D
= 0.3744 mg L^{-1} / 0.007 mg L^{-1}
= 53.4
C_A- concentration in the acceptor phase, C_D-concentration in the donor phase

Calculation for the recovery (R)

$$R = \frac{(C_A V_A)}{(C_D V_D)} \times 100$$
$$= \frac{(0.3744 \text{ mg } L^{-1} \times 17 \text{ µL})}{(0.007 \text{ mg } L^{-1} \times 20\,000 \text{ µL})} \times 100$$
= 4.5%
V_A- volume in the acceptor phase, V_D-volume in the donor phase

Calculations of the concentration from peak areas from calibration curves (for the sample)

Naphthalene
y = 843.45x
x = 5.1 / 843.45
= 0.006 mg L^{-1}

Calculation of the amount extracted

n = conc. x volume
= (0.0060 mg L^{-1} x 0.000017 L)
= 0.000000102 mg

Determination of concentration in the sample from the volume and recovery

$$c = \frac{(\text{amount} / \text{recovery})}{\text{volume}} \times 100$$
$$= \frac{(0.0000000102 \text{ mg} / 4.5\%)}{0.02 \text{ L}} \times 100$$
= 0.0001133 mg L^{-1} or 0.1133 µg L^{-1}

SPE

Calculation of concentration from the peak areas

Naphthalene
y =10796x
x = 6617 / 10796
= 0.6129 mg L^{-1}

Calculation for the enrichment factors (EF)

$EF=C_A / C_D$
= 0.6129 mg L^{-1} / 0.005 mg L^{-1}
= 53.4
C_A- concentration in the acceptor phase, C_D-concentration in the donor phase

Calculation for the recovery (R)

$$R= \frac{(C_A V_A)}{(C_D V_D)} \times 100$$
$$= \frac{(0.6129 \text{ mg } L^{-1} \times 1 \text{ mL})}{(0.005 \text{ mg } L^{-1} \times 100 \text{ mL})} \times 100$$
= 122.5%
V_A- volume in the acceptor phase, V_D-volume in the donor phase

Calculations of the concentration from peak areas from calibration curves (for the sample)

Naphthalene
y = 843.45x
x = 13.3/843.45
= 0.0157 mg L^{-1}

Calculation of the amount (n) extracted

n = Conc. x volume
= (0.0157 mg L^{-1} x 0.01 L)
= 0.0000157 mg

Determination of concentration in the sample from the volume and recovery

$$c = \frac{(\text{amount} / \text{recovery})}{\text{volume}} \times 100$$
$$= \frac{(0.0000157 \text{ mg} / 122.5\%)}{0.01 \text{ L}} \times 100$$
= 0.000128 mg L^{-1} or 0.128 µg L^{-1}

MAE

Calculation of concentration from the peak areas from calibration curves

Naphthalene
y =16.914x
x = 79 / 16.914
= 4.671 mg L^{-1} (concentration in the sample)

Calculation of the amount (n) extracted in the sample

n = conc. in the sample x mass
= 4.671 mg kg^{-1} x 0.001 kg
= 0.004671 mg

Calculation of recovery (R)

$$R = \frac{\text{amount extracted in the sample (mg)}}{\text{0riginal amount in the sample (mg)}} \times 100$$

$$= \frac{0.004671 \text{ mg}}{0.005 \text{ mg}} \times 100$$

= 93.5%

Calculation for concentration from peak area from calibration curve (for sample)

Naphthalene
y = 3521.6x
x = 1953.3/3521.6
= 0.5545 mg kg^{-1}

Calculation of the amount (n) extracted

n = conc. x mass
= (0.5545 mg kg^{-1} x 0.001 kg)
= 0.0005545 mg

Determination of concentration in the sample from the volume and recovery

$$c = \frac{\text{(Amount / recovery)}}{\text{mass}} \times 100$$

$$= \frac{(0.0005545 \text{ mg} / 93\%)}{0.001 \text{ kg}} \times 100$$

= 0.594 mg kg^{-1} or 594 µg kg^{-1}