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Comparison of extraction methods efficiency for the extraction of polycyclic aromatic hydrocarbons and phenolics in water matrices, sludge and sediment: sources of origin and ecological risk assessment

By

Sinayo Ndwabu

Supervisor: Dr P Mahlambi

Co-supervisor: Mr M Malungana

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and phenolic compounds (PCs) are persistent and environmentally toxic compounds. This study therefore aimed to determine the levels of both PAHs and PCs in river water, wastewater, sludge and sediment samples. The evaluation of their origin source and ecological risk was also determined. The status of both these contaminants in South African environment is still not fully investigated, which is a gap this study intended to fill together with previous studies that have been carried-out. The PAHs and PCs were extracted using different extraction methods which include a solid phase extraction (SPE) and dispersive liquid-liquid micro-extraction (DLLME) in water matrices. The microwave assisted extraction (MAE) and Ultrasonication (UE) coupled with either filtering (F) or F + SPE as a clean-up technique was used for extraction of solid samples. The analytes extracted form water or sediment were determined using GC-MS.

The PAH %recoveries obtained under optimum conditions in liquid samples were determined to be 72.1 - 118% for SPE and 70.7 – 88.4% for DLLME while the LOD and LOQ were 5.00 – 18.0 ng/L and 10.0 – 44.0 ng/L for SPE while they were 6.00 – 20.0 ng/L and 11.0 – 63.0 ng/L for DLLME. The recovery test for PAHs in solid samples gave a range of 93.7% - 121% for UE and 79.6% - 122% for MAE while the LOD and LOQ ranged from 0.0250 µg/kg to 1.21 µg/kg & 0.0800 µg/kg to 3.54 µg/kg for MAE and from 0.0840 µg/kg to 0.215 µg/kg & 0.0190 µg/kg to 0.642 µg/kg for UE respectively. The LOD and LOQs for PCs in both water and solid matrices were 0.01 – 2.00 µg/L and 0.02 – 6.07 µg/L for SPE, 0.05 – 1.20 µg/kg and 0.17 – 3.17 µg/kg for MAE and 0.09 – 1.33 µg/kg and 0.26 - 3.54 µg/kg for UE correspondingly, their %recovery test gave ranges of 75.2 – 112% (SPE), 80.9 – 110% (MAE) and 79.3 – 119% (UE).The optimization and validation of these methods indicated that they can be used for the extraction of PAHs or PCs in liquid samples, however, SPE when compared to DLLME showed to be more accurate and sensitive. Moreover, in solid samples the clean-up method was a deciding factor, with F + SPE cleaned samples giving higher concentrations of both PCs and PAHs than the filtered ones in both MAE and UE.

The concentrations of PAHs ranged from nd (not detected) to 1046 ng/L in river water and nd to 778 ng/L in wastewater samples with naphthalene showing dominance over all other PAHs in both water matrices. The PC concentrations at 4.12 to 1134 µg/L for wastewater and nd to 98.0 µg/L for river water were high but still within the maximum allowable limit except for 2.4-DCP (2.4 dichlorophenol) at Wdv4. The concentrations obtained from F + SPE cleaned samples were higher for both PAHs and PCs with a range from 95.96 to 926.0 µg/kg and 1.30

to 310 µg/kg compared to concentrations from filtered only samples at 21.61 to 380.6 µg/kg and 0.90 to 266 µg/kg respectively. Pyrene showed dominance over all other PAHs in both sludge and sediments while 2.4-DCP and PCP dominated the sludge and sediment samples respectively. PAHs were determined to be of petrogenic (water matrices) and pyrolytic (solid samples) origin and on average posed low (water matrices) and a medium to high (solid matrices) ecological risk. The $ILCR_{derm}$ values at 4.98×10^{-1} and 2.62×10^{-1} (DahA) and 5.92×10^{-2} and 5.34×10^{-2} (PCP) were highfor adults compared to that of children at 1.92×10^{-1} and 1.01×10^{-1} (DahA) and 1.39×10^{-2} and 1.26×10^{-2} (PCP) for both sediment and sludge samples respectively. The low values of $ILCR_{derm}$ for children indicates that the have a high risk exposure even at low concentrations of the contaminants. The findings of this study showed that both areas (uMsunduzi river and Darvill wastewater works (WWW) of interest are polluted with PAHs and PCs therefore, more regulations such as the National Environmental Management: Waste Act (NEMWA) are needed to ensure environmental, human and animal safety.

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DEDICATION

To my husband and my whole family for supporting and encouraging me to believe in my dreams

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ABBREVIATIONS

2-CP - 2-Chlorophenol

2-MP - 2-Methylphenol

2-M-4.6-DNP - 2-Methyl-4.6-dinitrophenol

2-NP - 2-Nitrophenol

2.3.4.6-TCP - 2.3.4.6-Tetrachlorophenol

2.4-DCP - 2.4-Dichlorophenol

2.4-DMP - 2.4-Dimethylphenol

2.4-DNP - 2.4-Dinitrophenol

2.4.5-TCP - 2.4.5-Trichlorophenol

2.4.6-TCP - 2.4.6-Trichlorophenol

2.6-DCP – 2.6-Dichlorophenol

3-MP - 3-Methylphenol

4C-3-MP - 4-Chloro-3-methylphenol

4-MP - 4-Methylphenol

4-NP - 4-nitrophenol

4-TOP - 4-Tert-octylphenol

ACN – Acetonitrile

ACT - Acetone

An - Anthracene

ATSDR - Agency for Toxic Substances and Disease Registry

B[a]P - Benzo[a]pyrene

BkF - Benzo(k) fluoranthene

BS - Bishopstowe

CD – Camps Drift

CDI - Chronic daily dose/exposure

CR – College road

CSF - Cancer slope factor

DahA - Dibenz(a,h)anthracene

Derm - Dermal

DLLME - dispersive liquid-liquid micro-extraction

DWA - Department of Water Affairs

EA – Ethyl acetate

EEC - Environmental exposure concentrations

ERL - Effects range low

ERM - Effects range median

EU - European Union

EPA - The Environmental Protection Agency

ERA - Ecological risk assessment

F - Filtered

FID - Flame ionisation detector

Fl - Fluoranthene

FL - Fluorescence detectors

FW - Fixed wavelength

GC - Gas chromatography

H_2O - Water

HMW - high molecular weight

HPLC – High performance liquid chromatography

IARC - International Agency for Research on Cancer

ILCR - Increment life cancer risk

LC_{50} - Lethal Concentration 50

LMW - Low molecular weight

LLE – Liquid-liquid extraction

LOD - Limit of detection

LOQ - Limit of quantification

LS - Linear shaker

MAC - Maximum allowable concentrations

MAE - Microwave assisted extraction

Max - Maximum

MEF - Mutagenic equivalency factor

MEQ - Mutagenic equivalency quotient

Min - Minimum

MS - Mass spectrometry

MW - Molecular weight

NEMWA - National Environmental Management: Waste Act

nH – nHexane

nd – Not detected

NOEC - No observed effect concentration

OM - Osborne-Mendel

PAHs - Polycyclic aromatic hydrocarbons

PC - Phenolic compounds

PCP - Pentachlorophenol

PDA - photodiode array detectors

Phe - Phenanthrene

PMB - Pietermaritzburg

PNEC - Predicted no observed effect concentration

POPs – Persistent organic pollutants

Pyr – Pyrene

RQ - Risk Quotient

SBSE - Stir bar sorptive extraction

SE – Soxhlet extraction

SMA - Stone mastic asphalt

SPE - Solid phase extraction

SPME - Solid-phase micro-extraction

SSR - Sample-to-solvent ratio

TEF - Toxic equivalency factor

TEQ - Toxic equivalency quotient

UE - Ultrasonic extraction

UW - Umgeni Water

US EPA - United States Environmental Protection Agency

WH - Woodhouse

WHO - World Health Organisation

WWW - Wastewater works

VD - variable detector

1. CHAPTER ONE - INTRODUCTION

1.1 Background of study

Since the onset of the industrial revolution, pollution of the water, soil and sediment matrices is progressively becoming a major problem all over the world (Srogi, 2007). The level of pollution has been exacerbated by the increase in usage of chemicals in agricultural production and the generation of wastes, some of which are persistent and toxic to environmental ecosystems (Abdel-Shafy & Mansour, 2016a). Also, sludge management and disposal has become a major problem for most municipalities and water boards. Most water treatment plants have always prioritised the treatment of liquid stream over the solid stream and if this is left unaddressed, the disposal landfills could become sinks for both liquid and solid waste containing various contaminants (Parra et al., 2020). Waste produced in any sector, if left uncontrolled does not only become an aesthetic problem, but it also potentially poses health risks especially if hazardous substances or materials are present in the waste (Molewa, 2012). Therefore, the South African government introduced a solid waste management Act which set national norms and standards for the assessment of waste for landfill disposal, National Environmental Management: Waste Act, 2008 (Act No. 59 of 2008)(Molewa, 2013).

The Waste Act, 2008 in South Africa stipulates that there are elements and chemical substances that need to be analysed in waste for total concentrations (TC limits) and leachable concentrations (LC- limits) (Molewa, 2013). These chemicals include metal ions, inorganic anions and organic chemicals. Organic chemicals are compounds that generally contain a carbon, due to carbon's ability to catenate, various organic chemicals are known (Gama et al., 2013). Polycyclic aromatic hydrocarbons (PAHs) and phenolic compounds (PCs) are typical examples of organic chemicals (Gama et al., 2013). PAHs as intermediaries are frequently applied in industries such as pharmaceuticals, agricultural products, thermosetting plastics, lubricating materials, and other chemical industries. PAHs as refined products are used in the field of liquid crystals, functional plastics and electronics (Abdel-Shafy & Mansour, 2016a). Phenols are widely used in household products and as intermediates for industrial synthesis. In industrial synthesis phenols are normally found in petroleum distillates and through combustion conversion of petroleum products and storage stability of these petroleum-derived products is well known and generally recognized as undesirable.

Even though both PAHs and PCs are of benefit, they also have negative effects. The health effects caused by PAHs include cancer, suppression of the immune system, decrease in cognitive function as well as neurobehavioral function, disturbance of sex steroid and thyroid function. Some of these compounds lead to an increased risk of chronic diseases, such as hypertension, cardiovascular disease, and diabetes (Carpenter, 2011). Phenols are also priority pollutants that are enlisted by the United States Environmental Protection Agency (US EPA) and the European Union (EU) due to their toxicity and severe short- and long-term effects on humans and animals well-being (Anku et al., 2017). Both of these pollutants are potentially carcinogenic even at low concentration causing damage to the red blood cells and the liver (Carpenter, 2011). Moreover, the research done on long-term exposure to any concentration levels of PAHs in the water has shown it to cause sub-lethal effects to the aquatic organisms (Baali et al., 2019). Due to the possible dangers that these contaminants pose to the environment, a sensitive and reliable extraction and analysis methods for their determination is necessary.

PAHs are easily deposited into water, soil and sediments through combustion of organic material and industrial processes. They interact with atmospheric particulate material which results in them being transported for long distances by air consequently landing on the soil surface (Parra et al., 2020). These soils particles are often washed away to different water bodies such as rivers, lakes and dams (Parra et al., 2020). As a result of this they linger in the environment leading to negative effects on living organisms and elevated toxicity even at trace levels. In sediments, PAHs can be re-suspended into the water column via bioturbation, degradation or subjection to long-term persistence and threaten the aquatic life (Srogi, 2007). Therefore, there is a need to monitor such contaminants in water, sediments and sludge

PCs have been detected in surface waters, rainwater, sediments, drinking water, groundwater, industrial effluents, urban runoff, and at hazardous waste sites (ATDS, 1997)(Singh *et al*, 2016). Phenolic compounds are discharged with effluents from several industries and enter various environmental matrices including water treatment plants. These industries include Textiles, Woolen Mills, Dye & Dye Intermediate Industries, etc. Chlorinated phenols may be life-threatening to humans even at low concentration. Their presence gives a disagreeable smell and taste even at low ppm concentrations in water (Patel & Vashi, 2015).

This work therefore focused on solid phase extraction (SPE), dispersive liquid-liquid micro-extraction (DLLME), microwave assisted extraction (MAE) and ultrasonic extraction (UE) for the extraction of PAHs and phenols in water, sludge and sediments. The SPE is commonly used due to its high extraction efficiency, and high enrichment factor, easy automation, and less consumption of organic solvents (Rawa-Adkonis et al, 2006). DLLME, MAE and UE are techniques widely used as a result of their minimal extraction time and simplicity of extraction procedure (Banjoo & Nelson, 2005). Gas chromatography-mass spectrometer (GC-MS) was used for detection of PAHs and PCs after extraction due to its ability to separate volatile organic compounds from complex samples.

1.2 Problem Statement

Water, sediments and sludge consists of organic matter and nutrients that are of benefit to living organisms and to the soil. However, they are also heavily polluted with contaminants such as heavy metals, organic compounds and pathogens which potentially result in oxygen depletion, toxins that ultimately lead to diseases and even death in aquatic life. Organic pollutants such PAHs are toxic and recognised by the United States Environmental Protection Agency (EPA) as priority toxic components because of their persistence in the environment (Parra et al., 2020). Wastewater, atmospheric deposition, and petroleum spillage are some of the most important. The PAH sources and their intermediate degradation products have the potential to generate toxic, carcinogenic and mutagenic effects in humans and other living organisms (Baali et al., 2019).

As a result of the toxicity of organic compounds such as PAHs and PCs the South African government set national norms and standards for the assessment of water (final effluent) under the General Authorisation in terms of section 39 of the national Water Act, 1998 (Act No 36 of 1998) (Dep of Water Affairs, 2013). The Department of Water and Sanitation also set the standards and norms for landfill disposal, under section 7(1)(c) of the National Environmental Management: Waste Act, 2008 (Act No. 59 of 2008) (Molewa, 2013), this came about as a result of wastewater treatment plants prioritising the treatment of liquid stream over solid stream. This work therefore aims at developing a method that can sufficiently be applied in the analysis of PAHs and phenols in sludge samples from Darvill disposal landfills. The analysis of PAHs and PCs from the sludge landfill was significant as their presence and harmful effect to humans and other living organisms was revealed for the first time. The sludge is product of

wastewater treatment from Darvill which discharges to uMsunduzi river, therefore also analysing river water was of significance. Furthermore, the comparison of filtration alone and filtration followed by solid phase as the clean-up method after microwave and ultrasonic extraction was undertaken for the first time in this work.

1.3 Aim and objectives

1.3.1. Aim

The main aim of this study was to develop a method that can be efficiently used to detect and measure PAHs and phenolic compounds in both liquid and solid samples.

1.3.2 Objectives

The above aim was achieved by:

- Optimising DLLME and SPE methods for extraction of PAHs and PCs in water samples.
- Optimising MW and UE methods for the extraction of PAHs and PCs in sludge and sediment samples.
- Validating the optimised extraction methods and then applying them for qualitative and quantitative analysis of PAHs and phenols in water, sediment, and sludge samples.
- Investigate if the clean-up methods filtering or filtering + SPE have an effect on the concentration levels determined.
- Determine if the concentration levels of PAHs and PCs are within the allowable concentration levels set by Water and Environmental affairs.

1.4 Research questions

- Which SPE, DLLME, UE and MAE conditions need to optimised in order to improve their extraction efficiencies?
- Are the targeted PAHs and PCs present in Darvill WWW,sludge landfill and uMsunduzi river and are their concentration levels within the accepted limits?
- Which method is more efficient for the extraction of PAHs and PCs in water samples between the SPE and DLLME methods?
- Which method is more efficient for the extraction of PAHs and PCs in sludge and sediment samples between the MAE and UE methods?
- Does a clean-up technique have effect on the extraction?

1.5 Significance and originality this research

Organic contaminants such as PAHs and PCs form part of the persistent organic pollutants which are produced in the agricultural industry and other sectors by means of chemical processes (Cano-Lerida et al, 2009). These compounds are prevalent, potent, they do not degrade quickly in the environment and they can be transported and distributed throughout the environment by rain and wind. PCs and PAHs are said to be priority pollutants by the World Health Organisation (WHO) hence their presence in the environment and exposure to humans and other living organisms is of great concern (Masood et al, 2016). Their source of origin can be petrogenic or pyrogenic and the emission of PAHs into the environment has increased with the increase in demand for petroleum products, wastewater treatment plants, industrial discharge, petroleum spills.

The significance of this study is a result of the South African set norms and standards of waste for landfill disposal under section 7(1)(c) of the National Waste Management: Waste Act of 2008. The Act states that all water boards and municipalities need to conduct research on the particular techniques and analysis required to determine the contaminants of interests which include the PAHs and PCs. Therefore, the analysis of PAHs and PCs was conducted for the first time from the sludge landfill. Thus, the potential human health effect which could result from the presence of these contaminants in this area was revealed for the first time. It is this landfill and the river nearby that needs to be analysed with the assumption that the runoff from the land feeds to uMsunduzi river.

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CHAPTER TWO – LITERATURE REVIEW

2.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a subgroup of persistent organic pollutants (POPs) and an important class of hydrocarbons, which contain mainly carbon and hydrogen atoms in their structures (Figure 2.1). The PAHs can be found as alkylated or substituted derivatives, and they can exist in many isomeric forms (Abdel-Shafy & Mansour, 2016a). However, the most commonly occurring ones do not carry branching substituents on their rings.

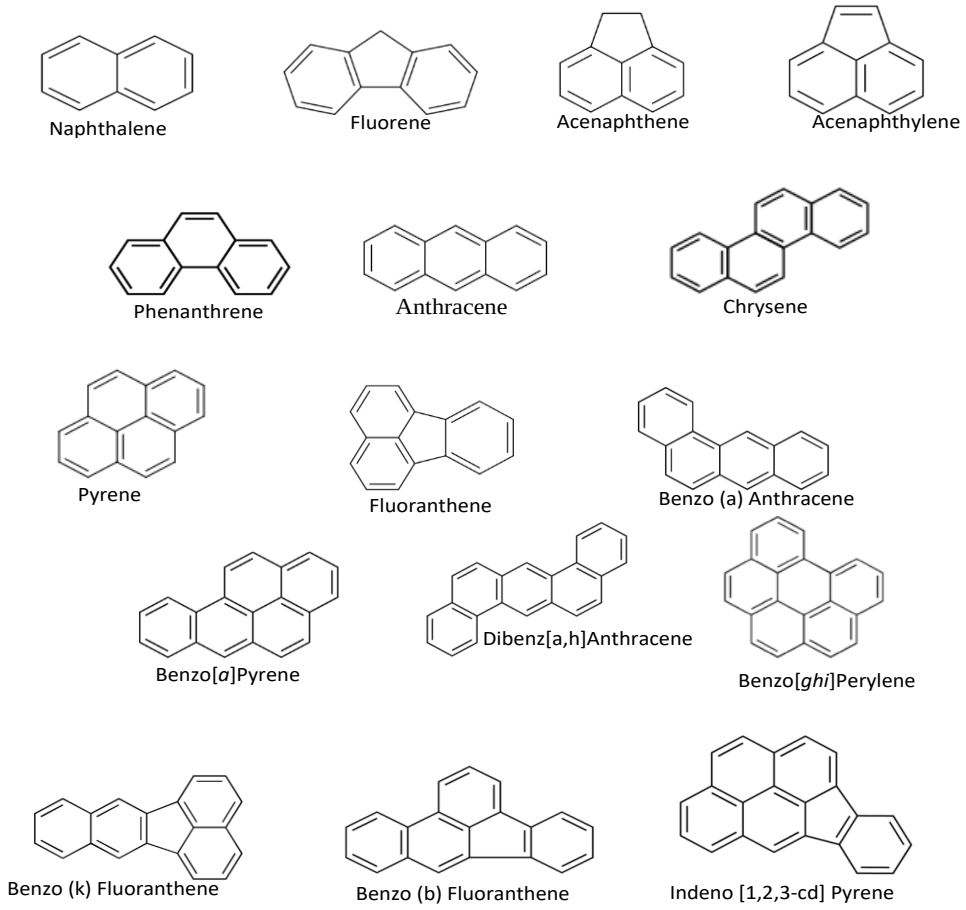


Figure 2.1: Chemical structures of PAHs.

2.1.1 Physicochemical properties of PAHs

As pure chemicals, PAHs usually exist as white, pale yellow or colourless solids. Chemical and physical properties of PAHs vary with the number of rings it possess as well as their molecular weights (Rengarajan et al., 2015). Selected physical and chemical properties of the 16 US EPA-regulated PAHs are presented in Table 2.1. The PAHs are either classified as high molecular weight (HMW) or low molecular weight (LMW) (Samburova & Khlystov, 2017). High molecular weight PAHs contains four or more fused benzene rings (e.g chrysene to

indeno[1,2,3-(c,d)]pyrene) whereas low molecular weight compounds contain two or three fused benzene rings (e.g naphthalene to fluorine) (Lawal, 2017a). PAHs destiny in the environment is mainly governed by their physical and chemical properties. The LMW PAHs have small Log K_{ow} values which makes them slightly soluble in water, less resistant to environmental degradation and highly volatile (Edokpayi *et al* , 2016), while the HMW are less water soluble, more resistant and less volatile.

The HMW PAHs have lower vapour pressures and partition more readily into organic matter than LMW PAHs. Therefore, LMW PAHs are expected to be found more in water samples than HMW PAHs, whereas the HMW PAHs are expected to be found more in soil and sediment samples. Sun et al (2020) reported that PAH composition in soil seepage water was dominated by LMW PAHs, whereas soil matrix was dominated by HMW PAHs. The results revealed that LMW and medium molecular weight (MMW) PAHs, were not as closer to the equilibrium of dissolution than HMW PAHs in soil seepage water.

Table 2.1: Properties of the 16 US-EPA PAHs (Fingas, 2018)

PAH	**Number of rings**	**Molecular Weight (g/mol)**	**Aqueous solubility (mg/L)**	**Vapor pressure (Pa)**	**Log K_{ow}**
Naphthalene	2	128	31	$1.0x10^{2}$	3.37
Acenaphthylene	3	152	16	$9.0x10^{-1}$	4.00
Acenaphthene	3	154	3.8	$3.0x10^{-1}$	3.92
Fluorene	3	166	1.9	$9.0x10^{-2}$	4.18
Phenanthrene	3	178	1.1	$2.0x10^{-2}$	4.57
Anthracene	3	178	0.045	$1.0x10^{-3}$	4.54
Pyrene	4	202	0.13	$6.0x10^{-4}$	5.18
Fluoranthene	4	202	0.26	$1.2x10^{-3}$	5.22
Benzo[*a*]anthracene	4	228	0.011	$2.8x10^{-5}$	5.91
Chrysene	4	228	0.006	$5.7x10^{-7}$	5.91
Benzo[*b*]fluoranthene	5	252	0.0015	-	5.80
Benzo[*k*]fluoranthene	5	252	0.0008	$5.2x10^{-8}$	6.00
Benzo[*a*]pyrene	5	252	0.0038	$7.0x10^{-7}$	5.91
Dibenzo[*a,h*]anthracene	6	278	0.0006	$3.7x10^{-10}$	6.75
Indeno[1,2,3-*cd*]pyrene	6	276	0.00019	-	6.50
Benzo[*ghi*]perylene	6	276	0.00026	$1.4x10^{-8}$	6.50

2.1.2 Uses of PAHs

PAHs are often used as intermediaries in pharmaceuticals, agricultural products, photographic products, thermosetting plastics, lubricating materials, and other chemical industries (Srogi, 2007). Acenaphthene is employed to make, dyes, pigments, plastics, pharmaceuticals and pesticides(Abdel-Shafy & Mansour, 2016a). Anthracene is employed to make dyes and pigments as well as wood preservatives diluent. Fluoranthene is used in the manufacturing of dyes, agrochemicals, and pharmaceuticals. Fluorene is used in the dyes, pharmaceuticals, pesticides, pigments, and thermoset plastic making while phenanthrene makes resins and pesticides and pyrene is needed in the manufacturing of pigments (Tseng, *et al*, 2014). The PAHs such as benzo (b) fluorene, benzo (a) pyrene, indeno (1,2,3 cd) pyrene, dibenzo (a,h) anthracene and benzo (g,h,i) pyrylene are found in asphalt that is employed in roads construction, and roofing tar. Moreover, specific refined products of PAHs are also used in the field of electronics and functional plastics (Abdel-Shafy & Mansour, 2016a).

2.1.3 Sources of PAHs and their identifying techniques

PAHs are introduced into the environment through natural or anthropogenic combustion processes (Figure 2.2). Volcanic eruptions, forest fires or prairie fires, are amongst the major naturally contributing sources for PAHs (Kozak et al., 2017). Anthropogenic PAHs enter aquatic systems through two main sources, namely petrogenic and pyrogenic (Kozak et al., 2017). In general LMW-PAHs are emitted from petrogenic sources such as combustion of diesel fuel, kerosene and gasoline, coking plants and weathering of asphalt surfaces (Stogiannidis & Laane, 2015). They can also enter the environment via oil spills from container wreckages, surface runoff, and atmospheric fallouts. HMW-PAHs are emitted through pyrolysis of organic substances (Stogiannidis & Laane, 2015). Pyrogenic PAHs are dispersed as clusters on nuclei particulates facilitating their dispersal over long distances. They are eventually deposited on terrestrial plants, soil and sediments (Stogiannidis & Laane, 2015). It is extremely important to note that incomplete combustion has been identified as the single largest contributor of PAHs to the environment, either naturally or anthropogenically derived (Abdel-Shafy, *et al*, 2016).

Sewage sludge is another source of PAHs in the environment but the PAH concentration in sludge is dependent mainly on the nature of the wastewater treatment plant and its methods and procedures on sludge treatment (Chen et al., 2019). Hua et al (2008) compared PAH

concentrations in sludge from 12 WWW where 2 out of the 12 treatment works had the anaerobic digester process for treating sludge and the rest of the plants used the direct pressing of settled sludge. Hua et al found that the treatment works equipped with digestion facility produced sludge with relatively lower concentrations of PAHs compared to the plants without the digesters. Advanced oxidation processes including ozone, hydrogen peroxide, UV radiation are processes have also shown to further degrade various PAHs (Zeng et al, 2000). After the treatment process, the sludge is disposed-off through land application, incineration, and landfill, indicating that PAHs can re-enter the environment through air, water, and soil. Therefore, concentration levels and risk evaluation (fate and impact in the environment) of contaminants such as PAHs is of great importance (Chen et al., 2019).

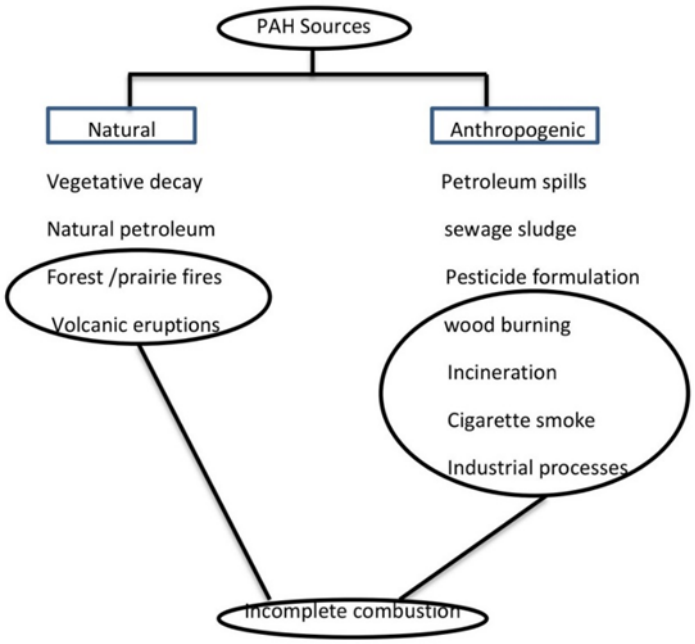


Figure 2.2: Natural and anthropogenic sources of PAHs (Abdel-Shafy & Mansour, 2016).

The PAH content is mainly identified based on real environmental samples using different chemical or analytical techniques. Chemical techniques such as chemical fingerprinting can be used and it includes several analytic techniques which can aid in differentiating between groups of PAH sources such as oil-based, coal-based and wood-based (Stout *et al*, 2015). This fingerprinting examination can be done by looking at specific chemical indicators that are contained in many samples. Chemical fingerprinting can also aid in identifying and assign non-point sources of PAHs to both industrial and residential areas in the environment (Cano-Lerida et al, 2009). The formation temperature is also a key in identifying PAH sources as higher temperatures of formation tend to result in PAHs with fewer alkylated chains than PAHs

produced under lower temperature processes (Abdel-Shafy & Mansour, 2016a). PAHs formed rapidly at elevated temperatures such as in power plant stack effluent will have a different pattern of PAHs than the PAH distribution found at a crude oil spill site. Also, PAHs can be identified by their UV characteristic since they possess distinct absorbance spectra. Each ring structure has a unique UV spectrum; thus each isomer has a different UV absorbance spectrum. Most PAHs are also fluorescent, emitting characteristic wavelengths of light when they are excited (Abdel-Shafy & Mansour, 2016).

Another method used to differentiate between the pyrogenic PAHs and petrogenic PAHs is to investigate the number of five-member hydrocarbon rings in the PAHs (Lawal, 2017). Five-member rings are more abundant in petroleum hydrocarbons than in pyrogenic substances, because the extensive time of petroleum hydrocarbon formation favours the alignment of the rings (Daniela & Magne, 2013). For materials formed pyrolytically the source material is rapidly converted into more stable six-membered rings. Thus, although the sources of PAHs to the environment are many, advances are continuously made in the identification of these sources, and the distinction between pyrogenic PAHs and those not derived from pyrolytic sources (Samburova et al, 2017).

2.1.4 Exposure pathways to PAHs

PAHs are introduced into human and terrestrial animals through inhalation, dermal contact, and ingestion, while absorption is the introduction route in plants from soil via their roots (Lawal, 2017). Generally chemicals administered to the lung as fine particulates, in aerosol form, or orally via drinking water are expected to be more bioavailable than chemicals administered in solid matrices such as food or soil; bioavailability from soil is expected to be less compared to that of food (Benjamin & Baveye, 2003). The toxicological profile for PAHs has shown that some PAHs are carcinogenic because they induce and promote altered DNA by a mechanism described as increased intracellular pro-oxidant production as well as direct adduction to DNA (Morris & Seifter, 1992).

These include benz[a]anthracene, benzo[a]pyrene, benzo[b] fluoranthene, benzo[j] fluoranthene, benzo[k] fluoranthene, chrysene, dibenz[a,h]anthracene, and indenol[1,2,3-c,d]pyrene which have caused tumours in laboratory animals through inhalation and oral exposure, as well as through long periods of skin contact (ASTDR, 1995). Anthracene,

benzo(a)pyrene and naphthalene are direct skin irritants and they have been reported to be skin sensitisers, hence they lead to allergic skin response in animals and humans (Abdel-Shafy & Mansour, 2016).

A study conducted in Finland during the paving seasons in 1999 and 2000, showed that dermal exposure to PAHs was higher in workers who were remixing stone mastic asphalt (SMA) than in SMA paving workers. Skin contamination resulted mostly from the workers not wearing gloves or protective clothing. When dealing with sites that might potentially lead to PAH exposure, skin contamination can be reduced considerably with simple hygienic operations (Alvarez-Pedrerol et al., 2017). Many chemicals carry a skin notation in the occupational exposure limit lists indicating that skin absorption is a possible route of exposure although there are still no standard limits of dermal exposure (Väänänen et al, 2005).

A study on PAH inhalation was conducted in Spain in children between the ages of 8 – 12 years for indoor and outdoor PAHs (benzo[a]pyrene) and BPA levels in the school environment (Alvarez-Pedrerol et al, 2017). Whole-brain volumes and basal ganglia volumes (caudate nucleus, globus pallidus, putamen) associated with ADHD were derived from structural MRI scans using automated tissue segmentation. Total PAHs and BPA were linked to caudate nucleus volume (CNV) and the indoor level of PAH was significantly associated with a decrease in CNV independently of intracranial volume, age, sex, maternal education and socioeconomic vulnerability index at home (Alvarez-Pedrerol et al, 2017). ADHD symptoms and inattentiveness increased in children with higher exposure to BPA. The BPA exposure might have led to subclinical changes on the caudate nucleus, even below the legislated annual target levels established in the European Union, making changes to be of concern for the children's neurodevelopment (Alvarez-Pedrerol et al, 2017).

Food ingestion compared to inhalation is a major route of exposure for a large section of general population exposed to PAHs (Ramesh et al, 2004). Studies conducted on human exposure to B[a]P revealed that the range and magnitude of dietary exposures (2 to 500 ng/day) were larger than for inhalation (10 to 50 ng/day) (Lioy *et al*, 1988). A study was conducted on the transfer of PAHs from food to animals, where milk spiked with phenanthrene (PA) and B[a]P was fed to pigs (Laurent et al, 2001). Portal and arterial blood samples were collected to study the kinetics of PAH absorption, where peak absorption occurred 5 hours and 6 hours after ingestion for PA and BaP, respectively. The absence of a time shifts in absorption for these compounds

indicated that PAHs and milk fat were absorbed during the same time period. Absorption rates were high for phenanthrene (95%) compared to B[a]P (33%), reflecting a high water solubility and low lipophilicity of the PA (Laurent et al, 2001).

2.1.5 Effects of PAHs

The Environmental Protection Agency (EPA) has identified 16 PAHs as one of the priority pollutants, as a result of their recalcitrance and suspected carcinogenicity. PAHs have toxic, mutagenic, carcinogenic and teratogenic properties (Lawal, 2017a). The biologic activity of eight highly purified polycyclic aromatic hydrocarbons widely distributed in the human environment was tested in the respiratory tracts of rats (Deutsch-Wenzel *et al*, 1983). This study investigated carcinogenic properties of ubiquitously distributed PAH and the establishment of dose-related activities by intrapulmonary administration in inbred female Osborne-Mendel (OM) rats. The PAHs in rats induced lung tumours and distant metastases. The most common tumours developed in other organs were mammary adenomas, fibroadenomas, and adenocarcinomas (Deutsch-Wenzel *et al*, 1983). The effects on human health depended mainly on the length and route of exposure, the amount or concentration of PAHs one is exposed to, and the innate toxicity of the PAH.

Xue *et al*., (2015) conducted a study to assess the effects of single polycyclic aromatic hydrocarbons on solid tumour initiation, and investigated their roles in immune response regulation. Mice (100) were intraperitoneally injected with 10 daily doses of DMSO (control), anthracene , benzo-(a)-pyrene , benzo-(a)-pyrene , and benzo-[G, H, I])-perylene), (Xue, 2015). Three months later, serum IL-2 and IL-6 levels were assessed and results showed precancerous liver lesions, precancerous stomach lesions, liver cancer incidences and no obvious lung lesions were found (Xue, 2015). Therefore, the results indicated that these PAHs can possibly cause cancer in living organisms such as mice.

A study examining the acute and subchronic oral toxicities of benzo[a]pyrene (BaP) in male and female F-344 rats was conducted by (Knuckles, *et al* (2001). This was done by orally administering single acute BaP doses dissolved in peanut oil for 90 days in the animal diet. The results of the studies indicate that the acute and sub-chronic toxicities of BaP are relatively low, BaP affects specific blood elements and organs, and BaP has a greater effect on males than females. The induction of non-carcinogenic kidney abnormalities in males only may be indicative of renal dysfunction and further substantiates an apparent sex difference in tolerance to BaP (Knuckles *et al*, 2001).

Long-term exposure to high concentrations of PAHs is associated with different health complications including neurotoxicity, infertility and cancer (Ramesh *et al*, 2011). For instance, some PAHs are suspected carcinogens, mutagens and immune-suppressants to different kinds of organisms. The benzo [a] pyrene and benzo [a] anthracene are two of the most common PAHs threatening human health in most urban built environments (Ramesh et al, 2011). These PAHs are said to bio-transform into even more toxic metabolites such as diol epoxide with time. When PAHs adsorbed on particulates are inhaled they pose a serious risk, which include bronchitis and pulmonary cancers. Higher incidence of nasal cancers has been reported in individuals living near heavily industrialized locations compared to rural residents (Yue *et al*, 2015). PAHs lipophilicity is high; therefore, their bioaccumulation in the body after inhalation is significant. Usually high concentration levels of PAHs are usually found in the gastrointestinal tract of mammals and this causes their renal excretion to be low (Harris *et al*, 2013). The adipose tissues are able to retain PAHs for a long period of time only releasing them via metabolic activation by specific enzymes (Harris et al., 2013). Therefore, standards and regulations for PAH exposure in the environment were set by the Agency for Toxic Substances and Disease Registry (ATSDR) in order to safeguard the environment. Table 2.2 shows the maximum allowable concentrations (MAC) which indicate levels not to be exceeded of frequently investigated PAHs in water and soil.

Table 2.2: Maximum allowable concentrations of PAHs in both water and soil (ATDS, 1997)

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

2.1.6 Fate of PAHs in the environment

PAHs are hydrophobic compounds and are resistant to biodegradation in the environment due to their low water solubility and electro-chemical stability. They often remain in the environment for long periods of time due to their high level of conjugation and aromaticity. The resistance to degradability and extractability in soil increases with the time they have been in contact with the soil, a process called aging or weathering (Srogi, 2007). Aging is mainly a result of slow diffusion into the soil organic matter, but other mechanisms involved include the formation of bound residues and physical entrapment within soil micro pores. The processes of sorption and aging limit results in the degradability of contaminants. On the other hand, these processes reduce the toxicity of the soil contaminants, by lowering the fraction available for uptake by living organisms (Ukalska-Jaruga *et al*, 2019)

In the atmosphere, PAHs can react with other contaminants such as chlorine, sulphur dioxide, ozone and nitrogen oxides, producing dinitro- and nitro-PAHs, sulfonated acids, diones, and chlorinated PAHs (Samburova et al, 2017). During pyrolytic processes PAHs can resist thermal degradation which may transform them into their isomers and/or by-products, which informs the fact that all matter gets recycled, it neither can be created nor destroyed. Therefore, PAHs through their resistance remain in the atmosphere in either their original, by-product or isomeric form.

PAHs in the atmosphere can be dispersed over long distances depending upon prevailing atmospheric conditions. PAHs can be transported as adsorbed chemicals, on suspended particulate matter. This global distribution in the atmosphere in other remote locations has been blamed for the occurrence of PAHs in remote locations with temperate climates where they are easily deposited by precipitation. In general, higher molecular weight PAHs are deposited over shorter dispersal distances and are easily removed by precipitation. The relatively lower molecular weight polycyclic aromatic hydrocarbons are dispersed over long distances spanning continents. For example, PAHs have been detected in the sediments and biota from the Bear Island located in the Norwegian Arctic far away from agricultural and industrial activities (Evenset et al, 2004).

In aquatic systems, elevated concentration levels of LMW-PAHs can be found in the pore water and in the column of surface water itself due to their slight solubility (Munyengabe et al, 2018). However, HMW-PAHs, due to their higher hydrophobic nature, are normally transported as

bound complexes to fine particles dissolved in organic matter and can be found in elevated concentrations in the sediments and soils. This was confirmed by the study conducted on the water and sediments from the Baltic Sea investigating the pollution level of PAHs (Witt, 1995). Relatively, higher concentration levels of LMW-PAHs were found in the water than in the sediments while the opposite trend was reported for HMW-PAHs where the surface sediments had elevated levels of PAHs than water, in the environment the incidences of elevated levels of HMW-PAHs (Witt, 1995).

PAHs are semi-volatile compounds under environmental conditions. They move between the atmosphere and the Earth's surface in repeated, temperature-driven cycles of deposition and volatilization (Peeples, 2014). PAHs go through photo oxidation in ambient air, where they are present as vapours, or are absorbed into airborne particulate matter due to the presence of sunlight. Their presence in the atmosphere is subject to complex physicochemical reactions, photochemical transformations, and reactions with other pollutants, which is why their chemical breakdown by photo-oxidation is a process that takes several days and even weeks (Srogi, 2007).

Microbial populations in sediment/water systems degrade some PAHs and reduce their toxicity over a period of time (Srogi, 2007). Bacteria is quick to adapt to any environment and as such have been widely used to degrade environmental hazards. Numerous bacteria have been determined to be able to degrade PAHs including naphthalene and phenanthrene (Ghosal et al, 2016). PAHs can be degraded through two paths in the presence of oxygen (aerobic degradation) or in the absence of oxygen (anaerobic degradation). In the aerobic process, the oxygen is a co-substrate for the hydroxylation and oxygenolytic ring cleavage of the aromatic ring while in the anaerobic catabolism of aromatic compounds the degradation is solely based on the reductive reactions (Ghosal et al., 2016).

Due to the fact that presence of UV light increases toxicity of PAHs the aquatic systems are mostly affected by PAH metabolism and photo-oxidation. Bioaccumulation of PAHs occurs in plants, although certain plant species can synthesize PAHs that act as growth hormones. Since these are persistent contaminants, the concentration of PAHs in marine animals including fish and shellfish is expected to be much higher than in the environment due to bio-magnification (Lawal, 2017a).

2.2 Phenolic Compounds

Phenolic compounds (PCs) are classified by having a hydroxyl group attached directly to one or more aromatic rings in an organic compound (Vermerris & Nicholson, 2007). Phenolic compounds are all derived from the first member of the group called phenol or benzophenol (Figure 2.3). Phenolic compounds are classified as simple, bi- and polyphenols depending on the number of phenolic groups present (Vermerris & Nicholson, 2007). Simple phenols have only one substituted phenolic ring such phenolic acid while biphenols have two and polyphenols have multiple units of the phenolic rings. Phenolic compounds can also either be halogenated or non-halogenated. Examples of halogenated include 2-chlorophenol, 2,4-dichlorophenol and non-halogenated are 2,4- dinitrophenol and 4-methylphenol (Vermerris & Nicholson, 2007).

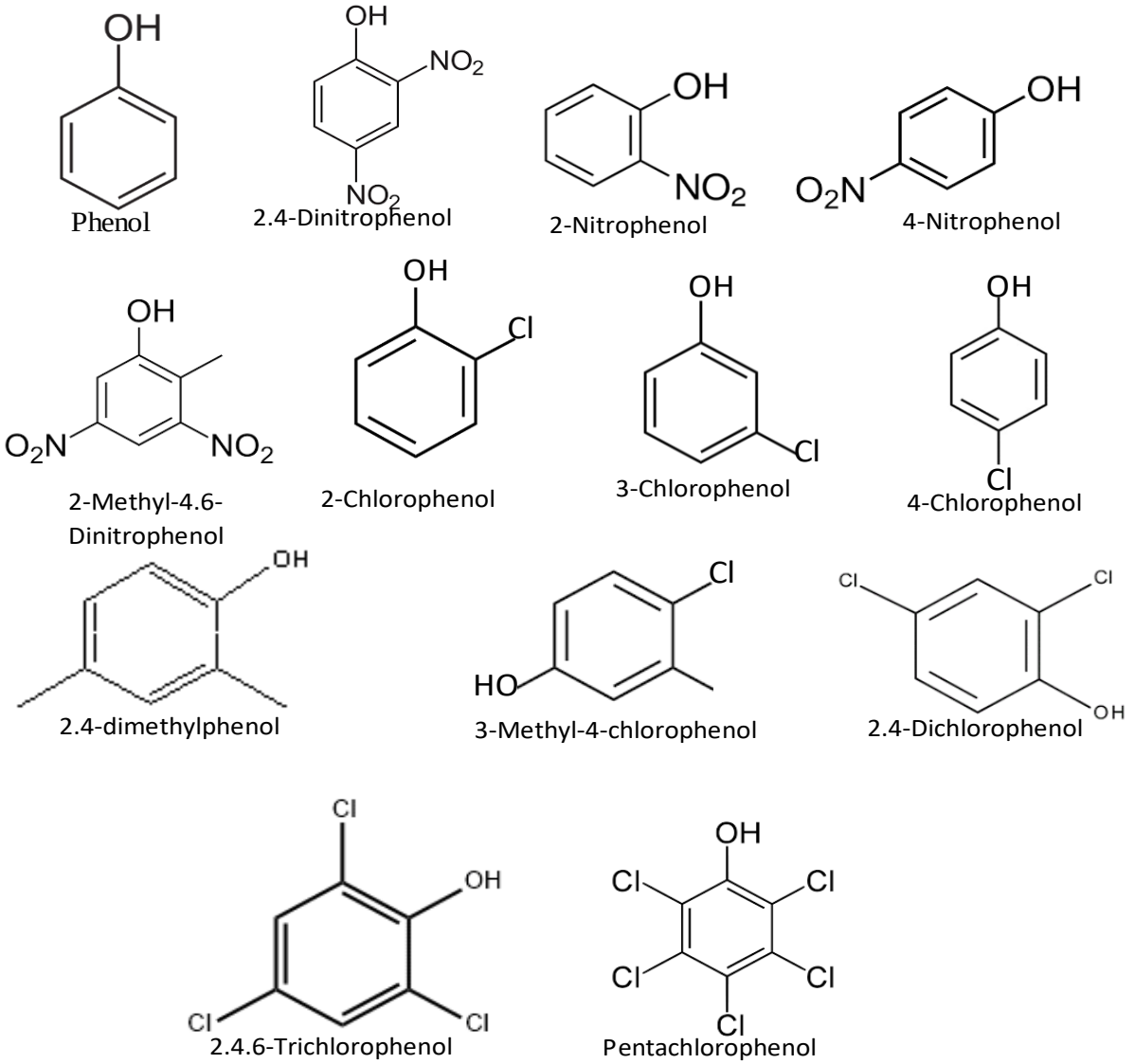


Figure 2.3: Chemical structure of phenolic compounds.

Any organic compound characterized by a hydroxyl (-OH) group attached to a carbon atom that is part of an aromatic ring falls under the family of phenol. These compounds usually exist as either colourless liquids or white solids that maybe highly toxic and caustic at room temperatures (Fini et al, 2021). Phenols form strong hydrogen bonds compared to a normal alcohol thus increasing their solubility in water which results in higher boiling points. Phenolic compounds are present in the effluents of many different industries with some of them being non-biodegradable and persistent in the environment and natural waters except for methylated phenols given their low K_{ow} and low bioconcentration factors, these are not expected to significantly bioaccumulate in organisms. Nitrophenols and chlorinated phenols are often referred as priority pollutants as a result of their persistence and accumulativeness in nature while phenol, methyl-phenols, dimethyl-phenols (Fini et al, 2021), etc are less of a threat in the environment because of their relative ease of biodegradation in activated sludge although they still a danger in groundwater. Some phenolic compounds and their halogenated derivatives can produce dioxin compounds which are infamous for their persistence in the environment and their high toxicity thus, the treatment of phenolic-rich industrial wastes before discharge into the environment is of great importance (Fini et al, 2021).

Table 2.3: Properties of thirteen PCs (Vermerris & Nicholson, 2007)

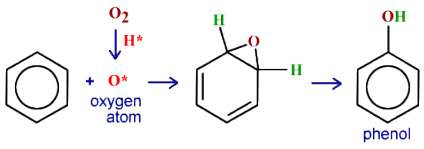
Compound	**MW (g/mol)**	**Log K_{OW}**	**pKa**	**Solubility (mg/L)**
Phenol	94.11	1.50	9.99	$8{,}3 \times 10^{4}$
2,4-Dinitrophenol	184.1	1.53	4.09	$2{,}8 \times 10^{3}$
2-Nitrophenol	139.1	1.78	7.21	$2{,}1 \times 10^{3}$
4-Nitrophenol	139.1	1.90	7.16	$1{,}6 \times 10^{4}$
2-Methyl-4,6-dinitrophenol	198.1	2.12	4.34	1.3×10^{2}
2-Chlorophenol	128.6	2.15	8.55	5.0×10^{4}
4-Chlorophenol	128.6	2.41	8.55	2.4×10^{4}
2,4-Dimethylphenol	122.2	2.42	10.6	1.0×10^{3}
3-Chlorophenol	128.6	2.50	8.55	$2{,}6 \times 10^{3}$
2,4-Dichlorophenol	163.0	3.08	7.85	$1{,}0 \times 10^{2}$
4-Chloro-3-methylphenol	163.0	3.10	9.55	0.4×10^{-1}
2,4,6-Trichlorophenol	197.5	3.69	7.42	5.0×10^{2}
Pentachlorophenol	266.3	5.01	4.93	2.0×10^{1}

2.2.1 Uses of PCs

Phenolic compounds have numerous applications in the day-to-day lives of human beings. In the chemical industry they are used in the production of other derivatives such as alkylphenols, cresols, aniline and resins (Michalowicz & Duda, 2007). Their presence in the oil, gas and coal industries is also noteworthy. Phenols are normally found in petroleum distillates and the conversion products of petroleum on the combustion properties and storage stability of such petroleum-derived products is well known and generally recognized as undesirable (Update, 2003). These compounds in petroleum fractions are the major cause of the corrosion of refinery equipment in which the petroleum distillates are processed and they are of disagreeable odour (Environmental update, 2003). Phenolic resins are used heavily in appliances, timber and construction industries for various purposes. Dyes, textiles and explosive industries also uses phenols as raw material (Michalowicz & Duda, 2007). Non-polymer additives, polycarbonate plastics and epoxy resins are manufactured from phenolic compounds such as bisphenol A (Huang et al, 2012). Phenolic compounds are also constituents of some pesticides and other insecticides (Oliveira et al., 2015). The wide use of PCs imposes a risk to the surrounding areas and their inhabitants therefore great care must be practiced and analysis of such be undertaken religiously.

2.2.2 Sources of PCs

PCs are a result of natural phenomena and they can also be due to the discharge of polluted wastewater from industrial, agricultural and domestic activities (Anku et al, 2017a). Phenol is naturally found in coal tar and creosote and it can be produced during natural fires, and through benzene degradation in the atmosphere under the influence of ultraviolet light radiation via the equation 2.2. Agricultural residues like sugarcane bagasse, corn husk, peanut husk, coffee cherry husk, rice bran and wheat bran are low-value by-products of agriculture that have shown to contain significant levels of phenolic compounds (Vijayalaxmi et al, 2015).



eq (2.2)

Another agricultural contributor to phenolic pollution is the use of pesticides such as insecticides, fungicides and herbicides which through their biodegradation result in the formation of some chlorophenols such as 2-chlorophenol, 2,4-dichlorophenol and some catechols in the aquatic environment (Anku et al, 2017). These pesticides with their degradation

by-products are washed into the water bodies through agricultural runoff. Amongst the chlorinated phenolic pesticides pentachlorophenol is vastly used and it also degrades to chlorophenol (Anku et al, 2017). The compounds are also components of numerous foods including fruits and vegetables.

Phenolic compounds in water can be due to dead plants and animals decomposition in water bodies or as consequence of washing away of decomposed materials into water bodies (Tang et al, 2013). Phenolic compounds are components of many aquatic and terrestrial plant species. Some of these compounds are formed (under ultraviolet light irradiation) from amino acids present in plants hemicelluloses. For example green and red marine algae contain macromolecules of phenolic compounds and hydroxybenzene is a result of decomposed organic matter (Tang et al, 2013). Chlorophenols are formed in industrial activities such as wood distillation, application of chlorine in water disinfection and paper production. Some of these compounds are also released as a result of petroleum products, exhaust fumes and are finally washed into water bodies as rain water (Gabriel de Oliveira et al, 2015).

PCs are also contained in various household chemicals such as disinfectants and antiseptic including varnish removers, toys, perfumes soaps, and paints. Pharmaceutical or medical products including ointments, body lotions also contain phenolic compounds. Oral care products such as oral sprays, toothpastes and mouthwashes some meant for throat treatment or anaesthetic purposes all contain phenols (Larson et al, 2013). Phenol is also a normal metabolic product and the human or animal body excrete in quantities up to 40 mg/L in urine. Thus, the metabolic waste products of humans and animals also contain phenol. It is produced in the gut of mammals as a result of the transformation of tyrosine in the digestive system (Bravo, 2009). Household wastewater, which undoubtedly contains these products, is drained through the sinks or gutter thus gets introduced into wastewater treatment plants.

Another source of phenolic compounds is the effluents released from municipal waste treatment plants and leachates from municipal solid waste landfill sites. Some PCs from municipal waste landfill identified as leachates including p-Cresols, 2,4,6-trichlorophenol, 4-tertrabutyl- phenol and bisphenol A are believed to originate from incineration residues (Kurata et al, 2008). These PCs in landfill leachates originated from incombustibles that lead to the formation of 4-tert-octylphenol. Also in municipal waste landfill sites some chlorophenols, 4-

nonylphenol and phenol have all been identified (Kurata et al, 2008). Thus, the release of untreated waste from landfill sites, residues of incineration such as solid fly ash and well as the release of incombustible materials into the environment results in pollution phenolic compounds (Kurata et al, 2008). Therefore, it is important that waste from water treatment plants is treated and phenol levels be analysed in order to protect the environment.

2.2.3 Exposure pathway to PCs

The exposure pathways to phenols include dermal contact and inhalation exposure. The most likely route of exposure is through dermal contact, where phenols enter human system through exposed skin either in the work environment or at home using ointments and other household products containing phenol. Phenol is a product of combustion of coal wood and municipal solid waste; therefore, residents near coal and petroleum fuelled facilities as well as residents near municipal waste incinerators may have increased exposure to phenol through inhalation (ATSD, 1997). Phenol is also a product of auto exhaust, and therefore, areas of high traffic likely contain increased levels of phenol. Higher phenol concentrations may occur when there is smog or in highly contaminated air (ATSD, 1997).

PCs have been detected in surface waters, rainwater, sediments, drinking water, groundwater, industrial effluents, urban runoff, and at hazardous waste sites (ATDS, 1997) (Singh et al, 2016). Phenols and phenolic compounds are discharged with the effluents from several industries and enter various environmental matrices. These industries include textiles, woolen mills, dye and dye intermediate industries, coke ovens, pulp and paper industries, iron & steel plants, petrochemicals, paint industries, oil; drilling and gas extraction units; pharmaceuticals, coal washeries, refractory industries etc. Chlorinated phenols may be life-threatening to humans even at low concentration. Their presence gives a disagreeable smell and taste even at low ppm concentrations (Patel & Vashi, 2015).

2.2.4 Effects of PCs

Phenolic compounds are amongst the contaminants of major concern as they persist in the environment over a long duration of time, accumulate and have toxic effects on living organisms and humans (National academics of SEM, 2015). Phenolic compounds have been enlisted by the United States Environmental Protection Agency (US EPA) and the European Union (EU) as pollutants of priority concern. This enlistment is due to the fact that these chemicals are noted to be toxic and have severe short- and long-term effects on humans and

animals well-being (Anku et al, 2017). They are carcinogenic even at low concentration causing damage to the red blood cells and the liver (Anku et al, 2017). As aforementioned more toxic substituted compounds or other moieties can form as a result of the original PC interaction with microorganisms, inorganic and other organic compounds in water (Anku et al, 2017).

Phenol contamination of soil and water has raised concerns among people living near phenol-producing factories and hazardous waste sites containing the chemical. Phenol, particularly in high concentrations, is an irritating and corrosive substance, making mucosal membranes targets of toxicity in humans (McCall et al, 2009). An in vitro model employing human intestinal epithelial cells (SK-CO15) cultured on permeable supports on epithelial barrier function with transepithelial electrical resistance and FITC-dextran permeability measurements was used to examine effects of phenol (McCall et al, 2009). Phenol induced changes in cell morphology and expression of several tight junction proteins by immunofluorescence and Western blot analysis as determined by McCall et al(2009). Effects on cell viability were assessed by MTT, Trypan blue, propidium iodide and TUNEL staining. It was discovered that exposure to phenol resulted in decreased TER and increased paracellular flux of FITC-dextran in a dose-dependent manner (McCall et al, 2009). The occurrence of phenolic compounds in the environment is therefore not only horrible and unwelcome but also poses a danger as far as human health and wildlife are concerned (Latimer, 2015)

2.2.5 Fate of PCs in the environment

Phenolic compounds found in surface waters are often a result of pollution from industrial wastes such as petrochemicals, washings from tarmac roads, gas liquors and creosoted surfaces (Brandt et al, 2017). Natural phenols are released from the decaying of algae or higher vegetation into the aquatic environment, whilst traces of phenols and other phenol-like compounds are sometimes available in groundwater surrounded by areas with coal- or oil-bearing strata. Most phenols, even in trace levels of concentrations are able to produce chlorophenols on chlorination. These chlorophenols and their isomers even trace amounts can render the water unacceptable to consumers because of objectionable taste and/or odour (Brandt et al, 2017). These are also powerful and corrosive contact poison; they are rapidly absorbed through skin. Chlorophenols have very low organoleptic thresholds and, if present, are immediately noticed by consumers as an antiseptic taste. Table 2.4 shows the maximum

allowable concentrations for phenolic compounds in both water and soil matrices. The taste thresholds for the most commonly found chlorophenols in drinking water are well below any health-related concerns but they still change the odour and taste of the water, therefore it is important to seek and minimize and/or eliminate the source wherever possible (Brandt et al, 2017).

Table 2.4: Allowable maximum concentrations of phenolic compounds in water and soil (NIEHS, 2006)

Phenolic compounds	**MAC (soil), mg/kg**	**MAC (water), mg/kg**
Phenol	-	107
2,4-Dinitrophenol	120	1.0×10^{-6}
2-Nitrophenol	-	1.0×10^{-7}
4-Nitrophenol	-	1.0×10^{-7}
2-Methyl-4,6-dinitrophenol	-	-
2-Chlorophenol	2100	0.08
4-Chlorophenol	-	0.90
2,4-Dimethylphenol	-	2.1×10^{3}
3-Chlorophenol	-	0.08
2,4-Dichlorophenol	800	0.02
4-Chloro-3-methylphenol	-	1.00
2,4,6-Trichlorophenol	1770	0.005
Pentachlorophenol	-	0.08

The toxic action of phenol is always associated with the loss of the integrity of the cytoplasmic membrane that results in disruption of energy transduction, disturbance of membrane barrier, and related functions and subsequent cell death. Wastewater treatment techniques have been developed as a result, for the removal of contaminants from industrial and domestic wastewater before their disposal so as to minimise or eliminate the devastating effects of these chemicals on human and aquatic lives (Mu'azu et al, 2017). Some of these techniques include electro-Fenton process, polymerisation, photocatalytic degradation, extraction, etc.

2.3 Extraction Techniques of PAHs and PCs

Extraction is a process undertaken to achieve separation and recovery of targeted analytes in matrices of interest, it transforms real matrix samples into samples that are suited for analytical

procedures (Belwal et al, 2018). There are various forms of extraction techniques that are used for separating and recovering analytes in different matrices such as water, soil, sediments, etc. Some of the common extraction techniques for water and solid matrices include, sonication extraction, microwave-assisted extraction , dispersive liquid-liquid micro-extraction and solid phase extraction (Huie, 2002).

2.3.1 Microwave-assisted extraction

Microwave-assisted extraction is an automated extraction technique which employs shorter analysis time (V. Lopez-Avila, 2000) and it is a well-established green extraction techniques. In MAE, microwave energy is applied to heat solvents used to dissolve solid samples, gaining an interaction of the analyte of interest with the solvent. Extractions can either be in a closed or open vessels where the sample is mixed with the solvent and then exposed to microwave energy (Llompart et al, 2018).

An advantage of the MAE is that it significantly reduces extraction time because the microwaves directly heat the sample/solvent mixture, whereas a set period is needed to heat the vessel before heat can be transferred to the solution with classical extraction techniques (Llompart et al, 2018). In MAE multiple samples can be simultaneously extracted, drastically improving sample output (Figure 2.4). MAE is a modern sample preparation techniques as it complies with the minimum criteria required and is a very attractive alternative to conventional approaches for the extraction of a variety of compounds including organic and organometallic from a wide range of matrices (Luque de Castro et al, 2010).

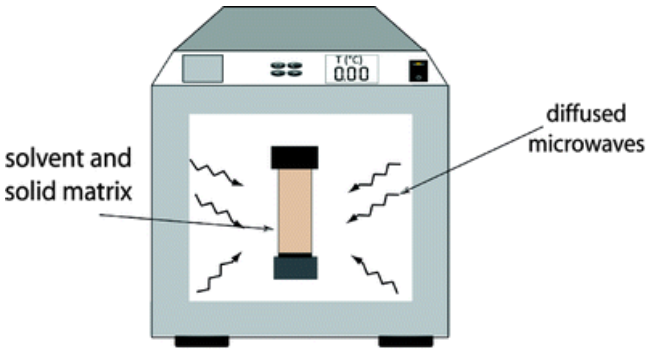


Figure 2.4: The microwave assisted extraction system *(Ying Li et al, 2013).*

Two oscillating fields, a magnetic field and an electrical field make up electromagnetic waves which are microwaves. These two field's direction of propagation varies sinusoidally and they run perpendicular to each other (Llompart et al, 2018). Microwave energy with a frequency range of between 300 and 300,000 MHz is a nonionizing radiation, causing molecular motion by two mechanisms: rotation of dipoles and migration of ions. The magnetic field produces the direct action of waves on the material, which is able to absorb a part of the electromagnetic energy and transform it into heat. The heat up from the microwaves is applied to the molecules by the dual mechanism of dipole rotation and ionic conduction.

The dual mechanism often takes place simultaneously both in the sample and solvent, with which thermal energy is effectively produced from microwave energy (Nour et al, 2021). Heat produced from the resistance of the medium to ion flow is generated from the ionic conduction. The migration of dissolved ions causes collisions between molecules because the direction of ions changes as many times as the field changes the sign. The dipole rotation is related to the alternative movement of polar molecules, which try to line up with the electric field (Nour et al, 2021). Dipolar polarization is the most substantial heating mechanism in microwave extraction.

MAE was used for the extraction of 14 phenols spiked to soil suspensions at 115 °C for 20 min, with recoveries 55% – 80% for alkylphenols and chlorophenols, but low recoveries (10 – 20%) for nitrophenols owing to their degradation (Lopez-Avila et al, 1994).

A study by Sánchez-Uría and Castillo-Busto, (2018) for the routine determination of 16 PAHs in polluted soils usng microwave-assisted solvent extraction followed by GC-MS. The results showed recoveries ranging from 70% to 116% except for anthracene (48%) and benzo (k) fluoranthene (52%). Concentrations of 2.1 – 342 mg/kg were obtained with %RSD of less than 32%, LOD ranging between 10 – 32 ng/mL and LOQ of 32 – 108 ng/mL suggesting that MAE is very efficient in extracting PAHs in soils.

Morales et al (2005) studied phenols in sludge and sediments with the use of microwave-assisted extraction followed by gas chromatography. The results showed quantification limits between 0.4 and 0.8 ng/g and recoveries from 78% to 106% which suggested that this extraction method can be efficiently used for phenols.

Azzouz & Ballesteros (2012) carried out a study based on MAE with continuous SPE, followed by GC-MS, for the simultaneous determination of residues of eighteen pharmaceuticals, three hormones, and one PCP (triclosan) in soils, sediments and sludge. %Recovery for triclosan was found to be 97% and the LOD to be 3 ng/kg. The method was applied to several soils, sediments and sludge, and triclosan was found in all samples in the range 49 – 3100 ng/kg.

2.3.2 Ultra-sonication Extraction

Ultra-sonication extraction mainly involves the use of high-power ultrasound to accelerate solvent penetration into solid materials (Figure 2.5). This is referred to as ultra-sonication because it uses ultrasonic frequencies in the KHz range (Kobus, 2006). This high power or ultrasonic radiation pressure is produced by strong cavitation effect, the disturbance effect, high acceleration, breaking and mixing function of multistage effect. This increases material molecular motion frequency and speed, in turn increasing the solvent penetration, the target component into the solvent and promote extraction (Chukwumah et al, 2009). Ultrasonic is a kind of elastic mechanical vibration wave. Its improvement on extraction efficiency is as a result of the enhancement of cell disruption, solvent penetration and mass transfer. Some critical factors that must be considered for the improvement of the extraction efficiency the compounds from different matrices include the extraction solvent and the sample-to-solvent ratio (SSR) (Chukwumah et al, 2009).

Banjoo and Neslson, (2005) conducted a study on the determination of PAHs using ultrasonication in West Indies sediments. The results showed recoveries ranging from 76% - 120%, the LOD and LOQ were determined to be between 1-2 μg/kg and 3-6 μg/kg respectively. These results suggested that ultrasonication is a suitable method to use for the determination of PAHs as the recoveries fall within the acceptable range of 70% - 120%.

Sun et al (2002) used ultra-sonication extraction and solid phase extraction (SPE) clean-up for determination of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in soils by reversed-phase liquid chromatography (RP-LC) with ultraviolet (UV) absorption detection. Detection limits for all PAHs was determined to be less than 1 mg/ml except for acenaphthene which was 1.05 mg/mL indicating the efficiency of the method.

A study using ultrasonication for the extraction of PCs (octylphenol, nonylphenol, and bisphenol-A) in water and sediments of two major rivers in Lagos, Nigeria was conducted. The

concentrations found were nd - 24.5 ng/g, nd - 79.4 ng/g and nd - 0.4 ng/g respectively in sediments, also proving the ultrasonication efficiency in extraction phenolic compounds in sediments (Oketola & Fagbemigun, 2013).

Oluseyi et al (2011) carried out a study comparing the efficiency of Soxhlet, ultrasonication and mechanical shaking as extraction techniques for the extraction of 16 PAHs in soil samples. The results of the study showed that the ultrasonic is more efficient compared to the others with concentrations averaging at 1.31 – 6.08 µg/g and %recovery range of 64.9 – 119.7%.

A study by Yang et al (2013) for the determination of 21 phenolic compounds in soil by ultrasonic extraction-gas chromatography. This study showed recoveries which ranged from 62.9% to 111.4% with %RSD range of 4.2 – 24% and LOQ ranging from 0.01 – 0.06 mg/kg (Yang et al., 2013). All the above studies on ultrasonication extraction in solid samples suggest it could be successfully used for extracting PAHs and PCs in soils. This could be achieved by paying attention to critical factors such as extraction solvent, extraction time and temperature in order to improve of the extraction efficiency.

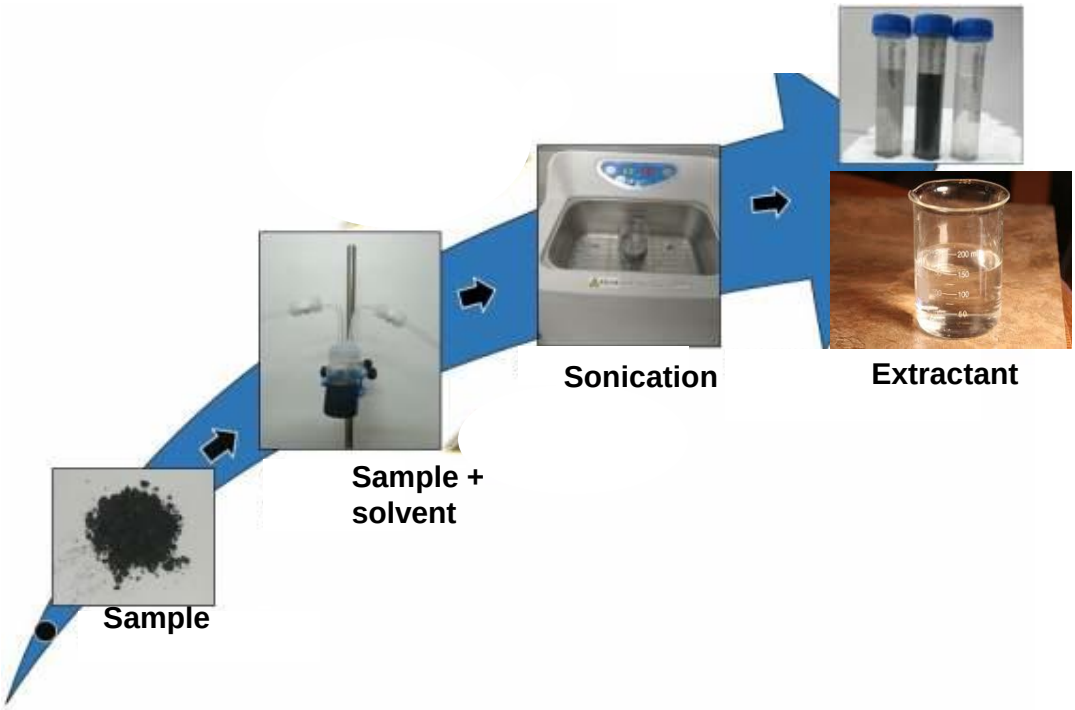


Figure 2.5: Ultrasonication Extraction process

2.3.1 Solid Phase extraction

Solid phase extraction is a type of step-wise chromatography that is intended to extract, partition, and/or adsorb one or more components from a liquid phase sample onto stationary phase, the sorbent or resin. Solid-phase extraction is currently one of the most common forms of extraction techniques used; it is used for concentrating, purifying, and separating of analytes. SPE uses smaller amounts of organic solvents. It generally involves introducing a liquid sample to an extraction cartridge in a small syringe-shaped container (Sibiya et al, 2012). This cartridge constitute of a solid phase which is able to extract the analyte of interest and keeping them on the solid phase (Camel, 2003). This technique produces a cleaner sample and therefore less likelihood of detecting contaminants by the GC or HPLC. Extraction can be performed in various ways including the direct extraction of the analytes from a soil–water suspension or slurry; extraction of the analyte from the sample matrix using hot water; or, headspace extraction (Smith, 2016). The assumption of the first approach is that the analytes are highly soluble in water and that water is the suitable solvent to dissolve the analyte from its matrix. The latter scenario assumes that the analytes of interest are volatile or semi-volatile so that they are available in the headspace above the sample (Smith, 2016).

A characteristic SPE comprises of four basic steps: conditioning, loading, washing, and elution shown in Figure 2.6. The equilibration of the cartridge with a slightly polar or non-polar solvent saturates the surface and permeates the bonded phase (Poole, 2000). A buffer of the same composition as the sample is then passed through the column to wet the silica surface. The sample is then introduced into the cartridge. As the sample passes through the stationary phase, the analytes in the sample will interact and retain on a sorbent while the solvent and other substances pass through the cartridge (Poole, 2000). After the sample is loaded, it is washed out with a polar solvent to remove interferences. The analyte is then removed with a nonpolar solvent such as methylene chloride, hexane, or ethyl acetate (Poole, 2000).
A study using reversed phase SPE for the extraction of PAHs in river water samples, showed recoveries from 81.5 to 98.6% which is indicative of good accuracy of the SPE extraction (Nawaz et al, 2014).

Another study by Sibiya et al., (2012) for the determination of PAHs in aqueous samples using SPE coupled with GC-MS was developed. The study gave %RSD less than 6% while recoveries obtained ranged from 81% to 135% with detection limits range of 20.0 – 52.0 ng/L.

De Almeida Azevedo et al., (2000) used SPE with GC-MS for the determination of priority pesticides and phenols. The results showed that phenols were recovered between 81% - 116% with LOD range of 0.002 – 0.016 µg/L.

Karyab et al., (2013) investigated the distribution and seasonal variation of sixteen priority polycyclic aromatic hydrocarbons (PAHs) in the drinking water of Tehran. The PAHs were extracted using SPE and analysed using GC-MS. Their results showed recoveries ranging from 36.3 - 132.6%, limit of detection of 0.8 – 2.0 ng/L and concentrations ranging from non-detectable levels to 438.96 ng/L. All of the above studies indicated that SPE with GC-MS can be used to reliably quantify PAHs and PCs in both water samples and solid samples under the optimum condition.

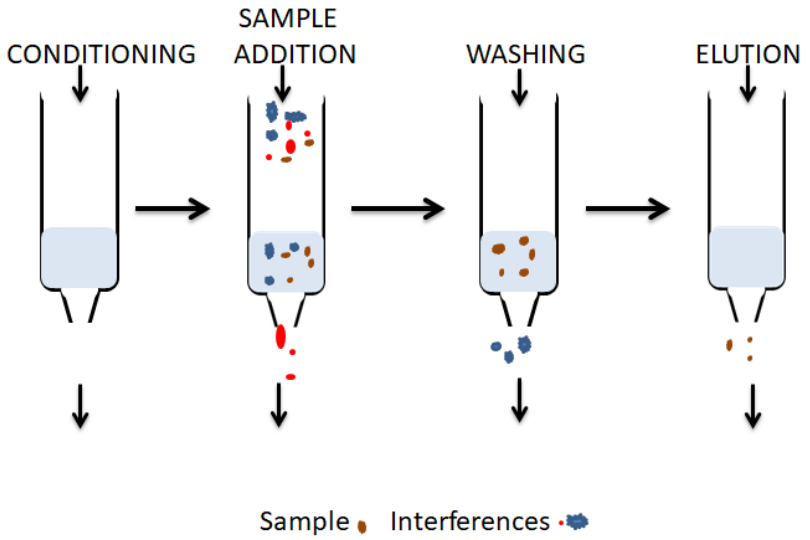


Figure 2.6: The four basic steps of SPE (Huie, 2002)

2.3.3 Dispersive liquid-liquid micro-extraction

Dispersive liquid-liquid micro-extraction (DLLME) is a novel extraction technique that was invented in 2006 (Zhang et al, 2017). This technique renders high enrichment factors that include low volumes of water samples. This sample preparation technique has been widely accepted because of several advantages, including simplicity, low cost and ease of method development, which made it available to virtually all analytical laboratories (Su et al, 2015). This is a modified form of the LLE that needs only a microliter volume of solvent to extract analytes from the aqueous samples. In this technique the sample preparation occurs in two steps

shown in Figure 2.7, where the mixture of extracting and dispersing solvents is rapidly injected to a water sample (Tseng et al, 2014). Dispersion is formed and facilitates fast extraction of analytes from the water sample. Then, the dispersion is removed by centrifugation and the extracting solvent containing analytes is taken for analysis with a micro-syringe.

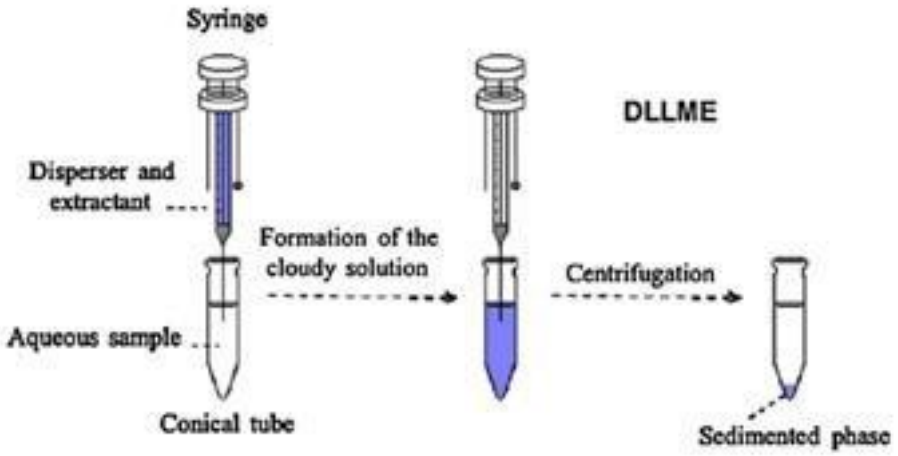


Figure 2.7: Dispersive Liquid–Liquid Micro extraction (Chandewar, 2016)

In general, several requirements have to be met to perform sample isolation using DLLME. The dispersing solvent has to be fully soluble with the water phase. Usually acetone, acetonitrile and methanol are used for this purpose (Bidari et al, 2012). The extracting solvent has to fulfil several requirements in order to have the potential to successfully extract analytes of interest. It has to have high affinity for the analyte of interest, be soluble in the dispersing solvent while its solubility in water has to be low. Finally, the density of the extracting solvent has to differ greatly from the density of water to enable phase separation (Assadi et al, 2012). Such solvents include long chain alcohol (heptanol, octanol, nanol, dedacanol), chlorinated solvents including chloroform, carbon tetrachloride, tetrachloroethene or chlorobenzene and ionic liquids which are limited to the use of liquid chromatography (Tobiszewski et al, 2014). Other important parameters that need to optimisation are sample volume, extraction time, and the salting effect.

Benedé et al., (2018) conducted a study with the employment of DLLME coupled with stir bar sorptive extraction (SBSE) as the extraction technique for accurate and sensitive determination

of 10 PAHs using GC-MS. The study showed good recoveries ranging from 84% - 115% with a %RSD of less than 13%

Luong et al., (2013) carried out a study on the determination of phenols in wastewater using DLLME coupled with GC-FID. The results showed that DLLME is a good technique with recoveries ranging from 85% to 95% with a %RSD range of 1.1 – 3.2% and an LOD range of $3.2 \times 10^{-4} – 1.8 \times 10^{-3}$ µg/mL. The concentrations of phenols obtained in the wastewater ranged from 7.68 µg/mL to 15.3 µg/mL. Not only is DLLME time efficient with low solvent volume, the results obtained in other studies suggest that it can be successfully used as an extraction technique for PAHs and PCs in water samples.

2.4 Separation and Detection techniques

The common separation techniques used for PAHs ad phenols are chromatographic techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC). Upon separation of analytes in the chromatographic column, a detector which is a device located at the end of the column senses the solute vapours in the mobile phase as they exit the column. The GC coupled with flame ionisation detector (FID) and mass spectrometry (MS) are widely used for the analysis of phenolic compounds and PAHs. The common LC detectors are fluorescence, uv visible and mass spectrometry. The MS is the most powerful of all chromatography detectors (Medhe, 2018).

2.4.1 Gas Chromatography

Gas chromatography (GC) is an analytical separation technique used to analyze volatile substances in the gas phase. The sample components are dissolved in a solvent and vaporized in order to separate the analytes by distributing the sample between thea stationary phase and a mobile phase. The mobile phase (carrier gas) is an inert gas that and carries the molecules of the analyte through the column to the detector (Stauffer et al, 2008). The stationary phase must be of high purity and is either a solid adsorbent or a liquid on an inert support. The combination of gas chromatography and mass spectrometry is an invaluable tool in the identification of molecules (Stauffer et al, 2008). The GC is advantageous as a result of its high efficiency in timeously separating components of complex mixtures as well as its wide range of detectors. However, it is limited to volatile and thermally stable compounds.

In comparison to LC-MS another major advantage of GC-MS is its high reproducibility of generated mass spectra using electron ionisation. The electron impact ionization process in GC-MS results in the production of very reproducible mass spectra from one instrument to another (Lynch, 2017). The detection limits for MS have been reported to be 0.6 – 5.4 ng/g (Dong et al, 2012) and 3.64 - 97.64 ng/L (Zhong et al, 2011) for PAHs and phenolic compounds, respectively.

The flame ionization detector is a very common detector used in gas chromatography and it is mostly sensitive to combustible compounds (Stauffer et al, 2008). The functioning principle of this detector is through bringing the sample to the detector via a pumping action, where it is burned in a flame. The flame is usually generated with hydrogen and air. When a chemical compound is burned, it produces ions and electrons. These electrons are located between two electrodes to which a difference of potential of a few hundred volts is applied (Elliott & Hannifin, 2017). As a result, the newly produced ions generate a current that can be recorded. The intensity of the current is directly proportional to the amount of analytes (ions) present in the detector. The detection limits for FID have been reported to be $1.8 \times 10^{-3} – 9.7 \times 10^{-3}$ ng/L (Elliott & Hannifin, 2017) and 1.0 – 6.0 ng/L (Yang et al, 2013) for PAHs and phenolic compounds, respectively.

2.4.2 High-performance liquid chromatography

The HPLC is used to separate non-volatile compounds in a solution. The sample is introduced to a mobile phase which flows through the stationery phase and the separation of the compounds is based on their partition between these two phases. Compounds with higher affinity for the stationery phase will interact more and thus elute later. The compounds in mobile phase are pumped at high pressure through a column which then sends the electronic signal to the detector (Czaplicki, 2013).

Fluorescence detectors (FL) are popular for their sensitivity and specificity; they measure the optical emission light from the analyte molecules after they have been excited to a higher energy state (Swartz, 2010). These detectors are known to be extra sensitive to analytes that have inherent fluorescence light which makes them ideal for trace analysis. A broad spectrum deuterium or xenon flash lamp is usually used as the source of light in these detectors (Swartz, 2010). The excitation wavelength which is always at lower wavelength (higher energy) than the emission wavelength is selected by a filter or monochromator between the lamp and the

flow cell. Extra care in selecting of solvents need to be taken as certain solvents may lead to background fluorescence which in turn affects the sensitivity (Swartz, 2010).

The UV-visible detector is the commonly used HPLC detector since many analytes of interest absorb in the visible region (190 nm to 600 nm) of the spectrum (Swartz, 2010). The detector uses Beer's Law to determine the sample concentration, output as absorbance by the fraction of light transmitted through the detectors cell constant. Beer's Law: $A = \mathscr{E}Bc$, where A = Absorbance, $\mathscr{E}$ = molar absorptivity, b = length of light, C = concentration.

The three different types of UV detectors are fixed wavelength detectors (FW), variable detectors (VD) and photodiode array detectors (PDA). The fixed wavelengths detectors depend on distinct wavelengths while the photodiode array and variable detectors. one or more wavelengths produced from a broad spectrum (Swartz, 2010). Although the FW detectors are economical and simple their use has become redundant. The PDA and Variable detectors are flexible and they can be tuned at a wavelength that increases sensitivity and/or where the analyte's absorbance is at a maximum (Gómez-Alonso et al, 2007).

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3. CHAPTER THREE – STUDY AREA

3.1 Introduction

This chapter discusses the chosen study area, the sampling plan and the analytical procedures that were employed in order to successfully develop a method that can be efficiently used to detect and measure PAHs and Phenolic compounds in both liquid and solid samples. Both the methods involved samplings, extraction using SPE or DLLME, separation and detection with GC-MS for water samples while the solid samples included an extra step of clean-up using filter or filter + SPE after the extraction with MAE or UE.

3.2 Description of Area of study – Kwa-Zulu Natal

Kwa-Zulu Natal (KZN) is a province in South African which has the largest population of 11.5 million people, second to Gauteng province with 15.5 million people, according to Statistics South Africa's mid-year population estimates released in 2020 (Iturralde & Munthree, 2020). KZN covers an area of 94 361 km² which is divided into 10 district municipalities namely: uMgungundlovu District Municipality, Amajuba District Municipality, Ilembe District Municipality, Sisonke District Municipality, Ugu District Municipality, uMkhanyakude District Municipality, uMzinyathi District Municipality, Uthukela District Municipality, Uthungulu District Municipality, Zululand District Municipality and the eThekwini metropolitan municipality. Pietermaritzburg (uMgungundlovu) is the capital city of KZN and the second biggest contributor to the overall economy of the province as a result of its industrial revolution through tertiary sector (accommodation), manufacturing activities, retail sector, transport, construction activities and agriculture & forestry (COGTA, 2019).

Although industrial revolution is great for the economy-, its negative environmental impact which includes the release of contaminants such as PAHs and phenols that can potentially lead to health risks is undeniable. This is exuberated by the fact that South Africa is considered to be an arid to semi-arid country with average rainfall well below the worlds 860 mm rainfall per year (Visser & Verhoog, 2007), therefore all discharged wastes and other contaminants remain in the rivers for a long period of time. uMsunduzi a river in KZN was as an area of study as a result of its proximity to some of the industries in Pietermaritzburg which include production of aluminium, leather manufacturers, iron and steel foundries. There is Darvill a wastewater works (WWW) located in the middle reaches of the river and it discharges of the final effluent treated influents of some of these industries into the river. Also. Another area of

interest in this study was the landfill used by Darvill WWW as a sludge disposal, this forms part of the solid waste management Act which sets national norms and standards for the assessment of waste for landfill disposal.

3.3 Sampling plan

During a sampling process a small area is selected to represent the environment being sampled. In analytical studies, it is of great importance to have a sampling plan, as inaccuracies in sampling can lead to errors in results and improper representation of the environment sampled. When undertaking the process of sampling a method or procedure should be followed. This method should include indications of when, where and how the samples are to be collected, recorded, stored and the preparation of site instruments.

In this study all samples were collected in Pietermaritzburg, Kwa-Zulu Natal. The river water and sediment samples were collected from uMsunduzi river in five different sampling: Camps Drift, College Road, YMCA, Woodhouse & Bishopstowe which are allocation along the river thus different appropriate presentation of the river. The wastewater samples were collected from four different points of the treatment process at Darvill WWW while the sludge samples were collected from Darvill WWW sludge disposal landfill. The river samples collected in April 2021 and wastewater samples in August 2021 were collected in 1L pre-washed glass bottles with Teflon caps on the surface level of water and stored in the refrigerator at 4 ˚C until further analysis were performed. Physical parameters such as pH, conductivity and temperature were recorded on site. The solid samples were collected in ziplock plastic bags which were then transferred to plastic drying bowls and left to dry at ambient temperature for 2 weeks while samples with high water content were left to dry for ±3 weeks. The samples were then grinded and sieved through a 125 μm sieve to fine soil particles of less than 0.125 μm using a soil grinder and stored in plastic honey jars at ambient temperatures until analysis were performed.

3.4 Sampling point description

3.4.1 Camps Drift (CD)

Camps Drift (29°37'22.0"S and 30°22'33.1"E) is not only near the central business district but it is also an industrial hub of Pietermaritzburg with companies such as Hulamin – aluminium semi-manufacturer, Alumicor – aluminium smelters, Somta-tool manufacturers, Agape footwear – footwear manufacture, Leather from Hart - textiles, etc which could significantly contribute to the pollution of the water and sediments of the Msunduzi river. Another source of pollution in this particular area would be the informal settlements located nearby which are

usually overpopulated with no proper sanitation in place which often leads to human and domestic waste possibly leaching into the river.

Image 3.1: Camps Drift sampling point.

3.4.2 College Road (CR)

This sampling point is located at 29°36'50.6"S and 30°22'36.1"E, a short distance from Camps Drift sampling point could be further polluted by automobile exhaust fumes since it is near a popular road with schools that often lead to large amounts of automobile traffic.

Image 3.2: College Road sampling point

3.4.3 YMCA

At the YMCA sampling point situated at 29°36'41.7"S and 30°23'16.2"E pollution is possibly attributed to automobile exhaust fumes since this road is the passing point regularly used by residents from surrounding areas and commuters from Durban to the CBD of PMB. There are also petrol stations in approximation to the river with possible fuel spills that might be introduced to the river as a result of runoff.

Image 3.3: YMCA sampling point.

3.4.4 Woodhouse

The Woodhouse sampling is located 29°36'08.1"S and 30°24'47.7"E which is within a kilometre radius of the N3 highway which carries large volumes of automobile traffic to and from Durban that could contribute to contamination of the river as a result of automobile exhaust fumes. At this point of the river the pollution could also be a result of the Darvill dumpsite nearby, illegal waste dumping from the informal settlement of the Manor area and the effluent from domestic, industrial and commercial activities from Eastwood and the surrounding areas.

Image 3.4: Woodhouse sampling point.

3.4.5 Bishopstowe

Bishopstowe is a secluded agricultural area with the sampling point located at 29°37'07.9"S and 30°26'48.5"E. This point is about 3 Km from Woodhouse and is closely located to Darvill wastewater works responsible for treating domestic waste and some trade effluents from the industries in Pietermaritzburg. The POPs pollution in this area are attributed to the farming activities, automobile exhaust fumes and the effluent discharge that emanates Darvill WWW since the plant is not yet treating POPs.

Image 3.5: Bishopstowe sampling point.

3.5 Darvill wastewater works

The Darvill wastewater works located at 29°36'19.1"S and 30°26'02.9"E is the main wastewater treatment plant servicing the city of Pietermaritzburg (Figure 3.1). It is approximately 1000m away from the Msunduzi River and is centrally located between the residential areas of Bishopstowe, Lincoln meade, Sobantu and the industrial area of Willowtown (Sikhakhane, 2001). Darvill is currently under the construction of a 35 Megalitre per day upgrade which will see the plant handling 100 Ml/d from 65 Ml/d of domestic and industrial waste (DWWW, 2013). The treated wastewater from DWWW is discharged into the Msunduzi River and it is shown in Plate 3.3 where the discharge of treated water from DWWW joins the Msunduzi River. The samples for this study area were taken through the treatment process, starting at the head of works (pre-treatment), primary treatment, secondary treatment, post-treatment (final effluent) and finally the landfill used for the disposal of sludge.

3.5.1 Wdv1

This point is at the head of works (HOW), where the influent-raw sewage from the city of Pietermaritzburg is introduced to the treatment process.

Image 3.6: Influent (ii) grit bars at HOWs for the removal of big particles.

3.5.2 Wdv2

This point is at the primary settling tank, a step after the mechanical treatment where removal of any object that might upset the treatment process such as plastic, papers, etc is removed. This is also where fats, oils, grease and grit have been removed.

Image 3.7: Pre-treated wastewater in the primary settling tank.

3.5.3 Wdv3

This sample was taken at the secondary settling tank, after the biological treatment of the water but before the dosing of chlorine

Image 3.8: Pre-chlorinated water in the secondary settling tank.

3.5.4 Wdv4

This point is located at the end of the maturation pond (used to increase the contact time of chlorine to ensure sufficient disinfectant.), after the chlorine dosing system. This sample is the final effluent from Darvill which ultimately mixes with uMsunduzi river.

Image 3.9: (i) final effluent from DWWW and (ii) final water mixing with river water.

3.5.5 Sludge landfill

The Darvill sludge landfill disposal located adjacent to the treatment plant is 57 ha where a sludge from Darvill WWW is used through sprinkler system for irrigation of turf (lawn grass) by Duzi Turf.

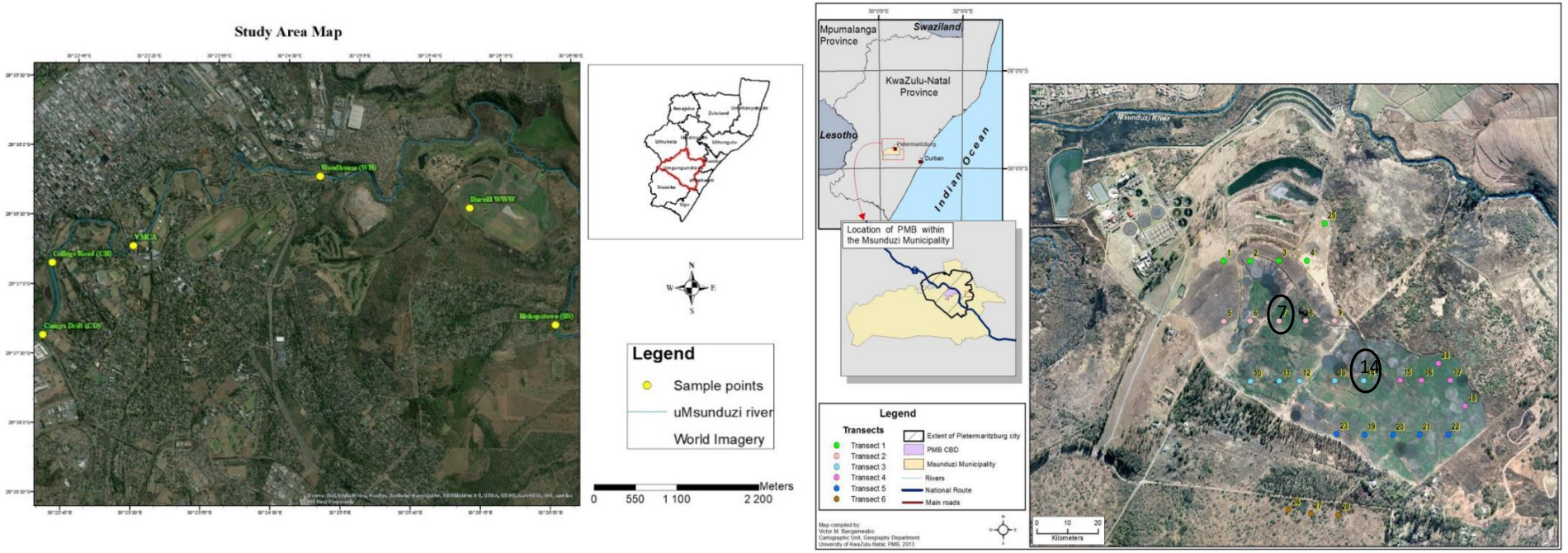

Figure 3.1: Graphic maps showing the location of the 5 sampling points (CD, CR, YMCA, WH & BS) along uMsunduzi river (left) and the 2 sampling points (7A & 14A) in Darvill WWW landfill (right) used for sludge disposal.

3.6 References

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CHAPTER 4:

Determination of PAHs in water matrices: sources of origin and ecological risk assessment

Efficiency comparison of extraction methods for the determination of polycyclic aromatic hydrocarbons levels in river water and wastewater: evaluation of PAH sources of origin and ecological risk assessment

4.1 Abstract

The purpose of this study was to determine the levels of PAHs in river and wastewater samples water and to evaluate their origin source and ecological risk. The status of PAHs in South African environment is still not fully investigated, which is a gap this study aims to fill in the province of KwaZulu-Natal. The PAHs were extracted using a solid phase extraction (SPE) and dispersive liquid-liquid micro-extraction (DLLME). The SPE procedure involved the extraction of PAHs from a 100 mL sample which was passed through a cartridge previously conditioned using 60% of acetonitrile with 40% of water and the analytes elution was achieved with the use of 5 mL acetone:ethyl acetate (1:1). On the DLLME procedure, a centrifuge tube was filled with 5 mL of water sample containing 0.25 g of NaCl then a mixture of extraction (250 µL of 1-heptanol) and disperser solvent (750 µL of acetonitrile) was injected into the sample followed by shaking and the centrifugation to form a cloudy solution. The recoveries were determined to be 72.1 - 118% for SPE and 70.7 – 88.4% for DLLME while the LOD and LOQ were 5.00 – 18.0 ng/L and 10.0 – 44.0 ng/L for SPE while they were 6.00 – 20.0 ng/L and 11.0 – 63.0 ng/L for DLLME. These results indicated that both methods can be used for the extraction of PAHs in liquid samples, however, SPE is more accurate and sensitive than DLLME. The concentrations of PAHs ranged from nd to 1046 ng/L in river water and nd to 778 ng/L in wastewater samples with naphthalene showing dominance over all other PAHs in both water matrices. The PAHs were determined to be of petrogenic origin and on average posed low ecological risk.

4.2 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of environmentally toxic and persistent contaminants that mainly occur as a result of incomplete combustion of organically-derived materials at both domestic and industrial scales (Abdel-Shafy & Mansour, 2016a). The PAHs are introduced into the environment through natural or anthropogenic combustion processes, these anthropogenic PAHs enter aquatic systems mainly through petrogenic and pyrogenic sources (Kozak et al, 2017). In general, low molecular weight polycyclic aromatic

hydrocarbons (LMW-PAHs) are emitted from petrogenic sources such as combustion of diesel fuel, kerosene and gasoline, coking plants and weathering of asphalt surfaces (Stogiannidis & Laane, 2015). They can also enter the environment via oil spills from container wreckages, surface runoff, and atmospheric fallouts. While, the high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) are emitted through pyrolysis (Stogiannidis & Laane, 2015). Pyrogenic PAHs are dispersed as clusters on nuclei particulates facilitating their dispersal over long distances.

The PAHs can also enter the collection system through domestic wastewater, industrial discharge, or surface runoff through urban drainage and reaches the wastewater treatment plants. In wastewater work treatments plants, PAHs may exist in the dissolved phase or in the sorbed form, attached to an organic-rich suspended solid (Liu et al, 2017). The partitioning behaviour of PAHs is expected that the LMW-PAHs are mainly in dissolved form whereas the HMW-PAHs are predominantly bound to organic-rich surfaces or solid (Liu et al, 2017). Beside the PAH molecular weight or number of aromatic rings approach, other diagnostic tools can be used to differentiate between the main sources from which these pollutants originate. These tools include analytic techniques such as structural isomer ratio (Fl/(Fl+Py), An/(An+Ph) and LWM/HWM) which can aid in estimating the origin sources of these contaminants (Montuori et al, 2021).

The PAHs have toxic, mutagenic, carcinogenic and teratogenic properties as a result of these properties, the Environmental Protection Agency (EPA) identified 16 PAHs as one of the priority pollutants (Lawal, 2017a). The PAHs can lead to major risk to human health either through inhalation, dermal contact, ingestion or even absorption an introduction route in plants from soil via their roots (Lawal, 2017a). Any of the exposures routes listed can potentially bring about health challenges of short- and long-term effects, including major respiratory and cardiovascular diseases (Adeniji et al, 2019). Long-term exposure to high concentrations of PAHs is associated with different health complications including neurotoxicity, infertility and cancer (Ramesh et al, 2011). Due to the persistence, negative effects on living organisms as well as the toxicity of these compounds even at trace levels, it is important that they are monitored and analysed in the environment (Adeniji et al, 2018). Moreover, the research done on long-term exposure to any concentration levels of PAHs in the water has shown it to cause sub-lethal effects to the aquatic organisms.

There are 16 PAHs that have been classified as priority pollutants by the International Agency for Research on Cancer (IARC) and selected as genotoxic human carcinogen and mutagenic by US Environmental Protection Agency (EPA) (Yang et al, 2014). These include naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene and dibenz(a,h)anthracene. Thus, it is important to monitor the presence and the potential ecological risk of PAHs in all compartments of the environment including the water treatment plants (Edokpayi et al, 2017).

Ecological risk assessment (ERA) is an important process used to characterize the ecological risk of PAHs on the surrounding environment. This process is used in the evaluation (using site-specific data) of the potential negative ecological effects that may transpire as a result of organism exposure to one or more environmental stressors (Wu et al., 2011). The ERA processes generally include the characterization of exposure, characterization of ecological effects and risk characterization. PAHs are hydrophobic compounds and are resistant to biodegradation in the environment due to their low water solubility and electro-chemical stability. They often remain in the environment for long periods of time due to their high level of conjugation and aromaticity (Srogi, 2007).

The PAHs are often present as a result of pollution source such as fuel spillage and atmospheric deposition although their concentrations in water are usually at trace levels (Liu et al., 2017). Therefore, very sensitive analytical procedures are needed for their accurate determination. Techniques for extraction and pre-concentration of PAHs from environmental water samples such as solid-phase extraction (SPE), liquid-liquid extraction (LLE), dispersive liquid-liquid microextraction (DLLME), solid-phase micro-extraction (SPME) and stirring bar sorptive extraction (SBSE) are often used (Kissoudi & Samanidou, 2018). However, SPE and LLE are two most commonly used techniques, which are also recommended by the USEPA for pre-concentration of PAH in liquid samples (Adeniji et al, 2018). SPE is often used for the preparation of liquid samples as it is an excellent tool in sample extraction, pre-concentration and also clean-up. The SPE also offers many advantages compared to LLE and these include simultaneous extraction of samples, high analytes recoveries, high sample clean up, easy automation and compatibility with instrumental analysis (Sibiya et al, 2012). Even though LLE has its drawbacks such as the use of large volumes of solvents of high purity, which in turn increases the pollution of the environment, it continues to be used as it gives good recoveries

and it is simple to operate (Rawa-Adkonis et al, 2006). Some of the LLE drawbacks were mitigated by the use of DLLME, a method known to be rapid due to small sample handling and low cost method since the volume of organic solvent used is typically in the microliter range (Cheng et al, 2010).

Compared to the DLLME, the SPE is a slow process, uses larger amounts of solvents and also requires the purchase of solid phase extraction cartridges which come at a cost. However, the SPE is a well-studied method that has been vastly used for extraction of different organic compounds (Notardonato et al, 2021). Once the PAHs have been extracted from the sample matrix, they may be determined satisfactorily using the high-performance liquid chromatography (HPLC) and gas chromatography (GC) technique with various detection techniques. However, the GC coupled with mass spectrometer is preferred as it offers robust identification of the analyte compounds both by retention time and mass spectrum, with additional structural information (Adeniji et al, 2018). Therefore, the aim of this work was to develop and compare the efficiency of the two developed methods, SPE and DLLME for extracting polycyclic aromatic hydrocarbons in water matrices and also to assess their ecological risks.

4.3 Experimental

4.3.1 Chemicals and Reagents

The sodium chloride (NaCl), acetonitrile (99.9%), acetone (99.8%), ethyl acetate (99.9%),1-heptanol (99.5%), dedacanol and the PAH mixture (100% purity) containing naphthalene, acenaphylene, acenaphthene, fluorene, Phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene and dibenz(a,h)anthracene was purchased from Sigma Aldrich (South Africa, JHB).

4.3.2 Instrumentation

Supelco SPE Vac-Elut unit, purchased from Sigma Aldrich (Germany, Darmstadt) was used to extract PAHs in liquid samples. Oasis hydrophilic-lipophilic balance (HLB) cartridge (60 mg, 3 mL) used for the extraction of PAHs was purchased from Waters (Ireland, Dublin). A vacuum pump from Edwards and Holdoph-Basis Hei-VAP Value rotor evaporator purchased from Holdoph (Germany, Berlin). Quantitative analysis of PAHs was conducted using a Shimadzu Gas Chromatography (GC) coupled to a Mass Spectrometry (MS) QP-2010 series (Japan,

Kyoto). A capillary column InertCap 5MS/Sil 30 m (I.D. = 0.25 mm, film thickness = 0.25 µm, Japan) was used for separation of PAHs. Helium was used as the carrier gas with a flow rate of 1.32 ml/min and an injection temperature of 260 °C. The injection volume was 3 µL performed in a splitless mode. The oven 480 ˚C temperature was held at 40 °C for 1 min, then programmed to 100 °C at a rate of 15°C/min, 2nd ramp at 10 °C/min to 210 °C, then held for 2 min. Final ramp at a rate of 5 °C/min to 310 °C, held for 8 min. For data acquisition, MS selected ion monitoring (SIM) mode was used to identify the analytes. The target mass ions (m/z) together with the boiling points were used for quantification and these were 128 (naphthalene), 152 (acenaphthylene), 154 (acenaphthene), 166 (fluorene), 178 m/z, 330 ℃ (phenanthrene), 178 m/z, 340 ℃ (anthracene), 202 m/z, 375 ℃ (fluoranthene), 202 m/z, 404 ℃ (pyrene), 228 m/z (benz(a)anthracene), 252 m/z, 475 ℃ (benzo(k)fluoranthene), 252 m/z, 495 ˚C (benzo(a)pyrene), 278 m/z, 524 ˚C (dibenz(a,h)anthracene) and Benzo(g,h,i)perylene (278 m/z, 550 ℃)

4.3.3 Preparation of calibration standards

The mixture of thirteen PAHs (naphthalene, acenaphylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene and dibenz(a,h)anthracene) was used for the calibration of the instrument. This mixture was diluted in acetonitrile to give a stock solution of 10 ppm. The stock solution was then further diluted in a 100 mL volumetric flask to give 1.0×10^4 ppm – 8.0×10^5 ng/L calibration standards.

4.3.4 Sampling of the water samples

The water samples were collected along uMsunduzi river and Darvill wastewater works (WWW) at Umgeni Water in Pietermaritzburg, Kwa-Zulu Natal. These samples were collected from five different sampling points namely: Camps Drift, College Road, YMCA, Woodhouse & Bishopstowe at uMsunduzi river which were chosen as result of being potentially high contamination points. Darvill WWW samples were collected from four different points of the treatment process. The samples were collected in 1L pre-washed glass bottles with Teflon caps on the 28th of April 2021 in Msunduzi river and 30 August 2021 at Darvill WWW. The samples were collected on the surface level of water. These samples were then stored in the refrigerator at 4 ˚C until further analysis.

4.3.5 Optimisation of SPE

The SPE parameters that were optimised include conditioning solvent, and eluting solvent in order to enhance the extraction efficiency of the SPE. Different conditioning and eluting solvents were chosen based on their low toxicity compared to commonly used solvents for these compounds such as hexane. The student t-test (p) was used for statistical analysis in determining the probability of significant difference between the recoveries of any of the parameter pairs used shown in Table S4.1. The conditioning solvents were (i) methanol:water, (ii) acetone:water, (iii) acetonitrile:water and (iv) ethyl acetate:water and the eluting solvents were (i) ethyl acetate:acetone, (ii) acetonitrile and (iii) acetonitrile:acetone. The other parameters such as washing solvent and sample load volume were kept constant while changing the conditioning or eluting solvent separately. A 100 mL water sample spiked with the mixture of the PAHs of interest to make a final concentration of 10 ppb was used during the optimization.

Under optimum conditions, the SPE cartridge was conditioned by 5 mL of acetonitrile and distilled water (60%:40% ratio), respectively. The 100 mL wastewater sample was then loaded through the cartridge followed by washing with 5 mL of de-ionised water. The analyte were then eluted by 3x1 ml of acetone: ethyl acetate (1:1). The extractant volume was then reduced to 1mL under nitrogen flow. The eluates collected were injected into GC-MS for analysis (Limam & Driss, 2013).

4.3.6 Optimisation of DLLME

Parameters that were optimized included extraction solvent, disperser solvent, volume of the extraction solvent, disperser solvent, and the salting effect to achieve the cloudy phase which ensures the increased mass transfer between two phases. Different extracting and dispersing solvents were chosen based on their solubility and toxicity. Extraction and disperser solvents pairs investigated included (i) Propanol and acetonitrile, (ii) dedacanol and acetonitrile and (iii) 1-heptanol and acetonitrile & (iv) octanol and acetonitrile respectively, while the salting effect was kept constant. Under optimum conditions, a 5 mL water sample containing NaCl (0.25 g) was filled in a 10 mL centrifuge tube and then a mixture of extraction solvent (1-heptanol, 250 µL) and dispersing solvent (acetonitrile, 750 µL) was rapidly injected into the sample solution by a syringe. The tube was shaken at 50 rpm for 5 min to emulsify the solution and form the cloudy state. After centrifugation at 5000 rpm for 5 min, the organic phase floating on the surface of the solution was then collected by a micro-syringe. The floating phase was further

centrifuged at 12,000 rpm for 1 min. The organic phase (1mL) was then collected and injected into the GC–MS for analysis (Tseng et al, 2014).

4.4 Methods Validation

4.4.1 Quality assurance

To ensure the validity of the method and quality of the results, a variety of parameters such as linearity, accuracy, recoveries, limit of detection, limit of quantification, sensitivity and selectivity/specificity were accounted. Linearity was determined by analysing the calibration standards and obtaining the correlation coefficient (r^2) of ≥0.9900. Sensitivity is the capability of the method to discriminate between small differences of concentration of analytes (SANAS, 2017). From the regression equation for a straight line: $y = mx + c$, a method is calibration-sensitive if the slope (m) of the calibration graph is $\neq 0$. Specificity/selectivity is the ability of a method to respond to a particular analyte of interest in the presence of possible interferences such as impurities, degradants and matrix effects. This was ensured by using the mass spectrometer as a detector where the detector was set to selectively detect only ions of the targeted PAHs (Ngubo et al., 2021). Specificity of the method was conducted by comparing spiked and non-spiked samples under SIM mode to ensure that the electron ionization detector source on the GC-MS instrument is set to target and identify only the PAHs of interest by using specific m/z values.

4.5 Results and Discussion

4.5.1 Effect of the conditioning solvent of SPE

Effective adsorption of the analyte onto the sorbent is dependent on the type of solvent selected for conditioning. Therefore, the effect of the conditioning solvent was investigated using different solvent-water mixtures at 60%:40% respectively, these mixtures included ACN + Water, EA + Water and ACT + water. 100 mL was used as the sample volume. Significant difference was observed between the recoveries of all conditioning solvent pairs with a p value of 4.08×10^{-10} (shown in Table S4.1) with ACN + water mixture giving the highest recoveries with percentages between 71.0% -110% for all the compounds of interest while ACT + water mixture gave recoveries of 10.0% to 50.6% and the EA + water mixture gave recoveries ranging between 28.4% - 72.6% (Figure 4.1). These results were obtained when ethyl acetate: acetone (1:1, v/v) was used as the eluting solvent. The recoveries obtained from the use of the ACN + water mixture indicated that it was more effective removing soluble matrices adsorbed

on the packaging material that may possibly interfere with the cleaning up process and thus promoted adsorption of the analytes compared to the acetone + water mixture and ethyl acetate + water mixture. This could be a result of higher polarity of acetonitrile compared to acetone and ethyl acetate and also the fact that the sample solution was prepared in acetonitrile (Munyengabe et al, 2018). This led to an effective activation of the surface functional groups of the sorbent with which resulted in effective interaction with the analytes and consequently increased the amount of analytes recovered. These findings suggest that low toxicity solvents can be used for a successful extraction of PAHs, therefore, acetonitrile:water mixture was then selected as the optimum conditioning solvent. (Oluseyi et al(2011)compared dichloromethane, cyclohexane, acetone, acetonitrile, 2-propanol and methanol cyclohexane for the extraction of PAHs and the extraction efficiency of these solvents followed methanol> acetonitrile > 2-propanol> acetone> dichloromethane > cyclohexane, an order of decreasing polarity.

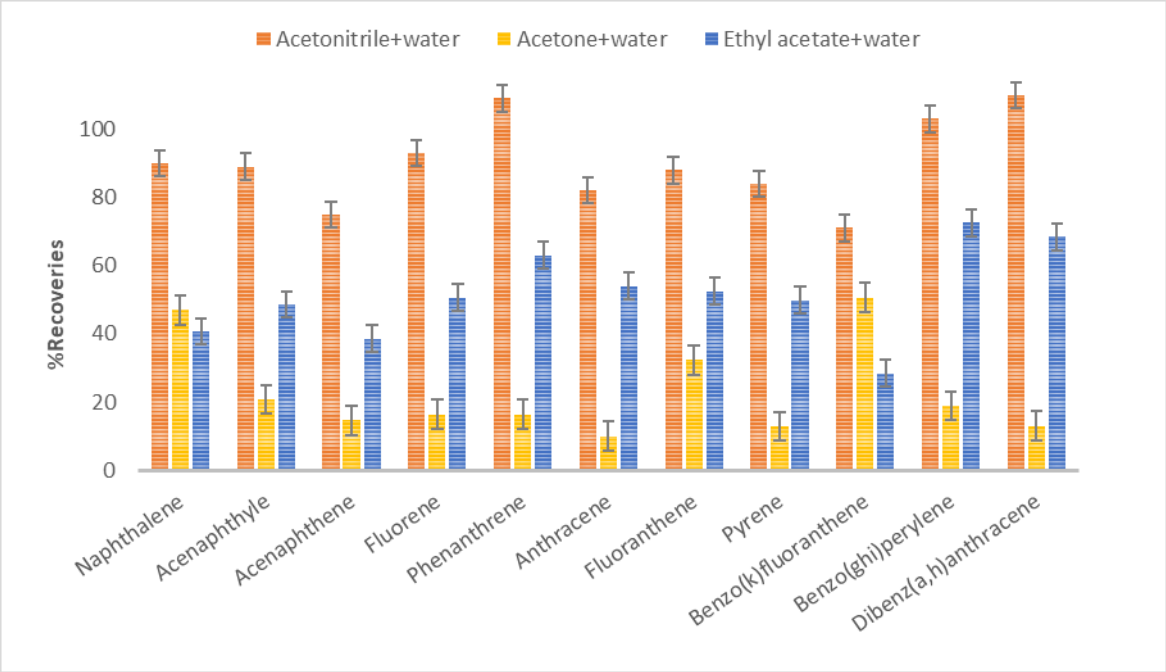

Figure 4.1: Effect of conditioning solvent on PAHs recoveries.

4.5.2 Effect of sample loading volume of SPE

Sample loading is done to determine the breakthrough volume and it involves passing a liquid sample containing the analyte through the cartridge so as to adsorb the analyte onto the sorbent bed. The SPE sorbent is unable to retain the analyte when the breakthrough is reached and due to this reason the analyte can possibly be washed away from the sorbent before the eluting step due to sample overloading (Dujaković et al, 2010). Therefore 50 mL, 100 mL and 200 mL of

sample load volumes of distilled water spiked with 10ppb of analytes were used in order to evaluate its effect on the recoveries of the analytes (Figure 4.2). Lower recoveries were obtained from 50 mL sample load volume which could be a result of insufficient amount of the analyte available for interaction with the sorbent. The lower recoveries attained with 200 mL sample load volume could be attributed to over saturation as the sample could be sipping through before the elution step leading to insufficient amount of the PAH compounds available for interaction with the sorbent therefore leading to lower recoveries being observed. Therefore, 100 mL sample volume was adopted as the optimum volume with recoveries ranging between 75% - 103% which is within the acceptable recovery range of 70 – 120% (SANAS, 2017) and when statistical evaluation was carried out between the recoveries of these sample loading volume efficiencies significant difference was observed for all of them with p values ranging from 5.75×10^{-8} to 1.0×10^{-2} shown in Table S4.2.

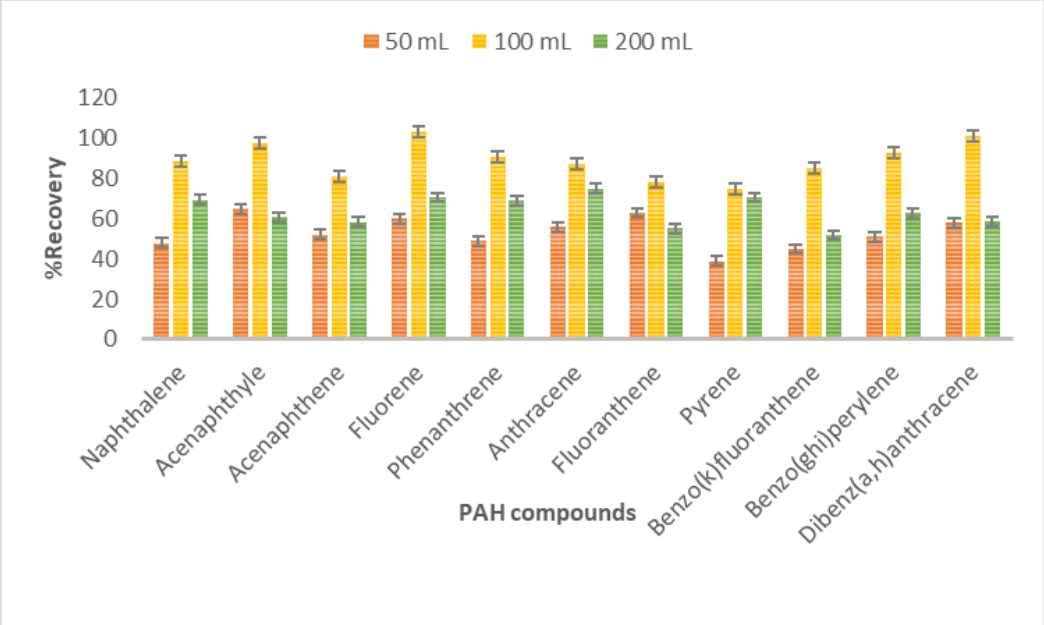

Figure 4.2: Effect of loading volume on the PAH's recoveries.

4.5.3 Effect of the extraction solvent of DLLME

The extraction solvent in DLLME is selected based on its low solubility with water and miscibility with the dispersive solvent. The extraction solvent also needs to have high affinity for the analyte of interest and have good chromatographic behaviours. Contrarily, the dispersive solvent has to be miscible with both the extraction solvent and the aqueous sample (Quigley et al, 2016). Therefore, the effectiveness of the extraction solvent was investigated using (i) propanol and acetonitrile, (ii) dodecanol and acetonitrile and (iii) 1-heptanol and

acetonitrile and (iv) ethanol and acetonitrile. Statistical evaluation for the extraction of PAHs from water samples using these extraction solvents showed significant difference ($p<0.05$) except for ethanol + acetonitrile and propanol + acetonitrile which showed no significant difference ($p = 0.08$) with low recoveries between the extraction solvent pairs shown in Table S4.3. The 1-heptanol and acetonitrile combination gave the highest recoveries ranging between 70.3% - 88.5% while dodecanol + ACN gave comparable recoveries of 70.0% - 81.0%. Propanol + ACN gave recoveries ranging between 1.07% - 31.8% while 2.99% - 16.9% recoveries were obtained when EtOH + ACN was used (Figure 4.3) These results were obtained with a constant salting effect of 0.25g using sodium chloride (NaCl). The recoveries obtained using 1-heptanol and dodecanol each mixed with acetonitrile as the extraction solvent were high compared to propanol and EtOH, this could be due to their low solubility in water, high solubility with the desired analytes and their miscibility in acetonitrile. The 1-heptanol was chosen as the optimum extraction solvent because the dodecanol recrystallizes which is not good chromatographic behaviour. Rezaee et al assessed carbon disulfide, carbon tetrachloride, and tetrachloroethylene as extraction solvents based on their solubility in water and the study showed that the least soluble solvent (tetrachloroethylene) in water had the highest extracting efficiency of PAHs in water (Rezaee et al., 2006)

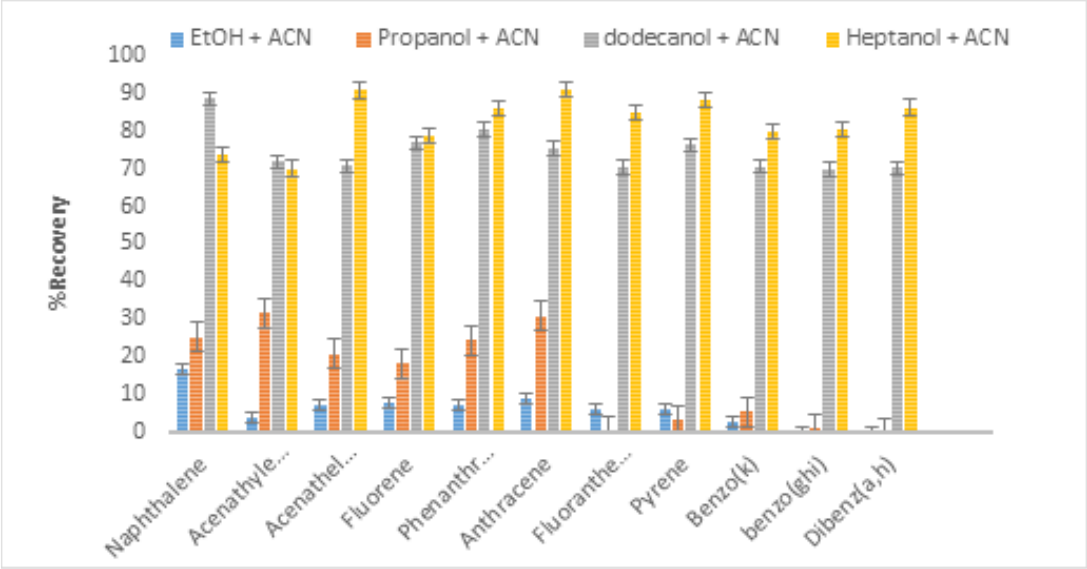

Figure 4.3: efficiency of different extraction solvents with acetonitrile as the dispersing solvent and salting effect of 0.25g

4.5.4 Effects of the extraction solvent volume of DLLME

Different extraction solvent (1-heptanol) volumes of 10 µL, 100 µL, 250 µL and 500 µL were studied in order to determine its influence on the recoveries. The 10 µL and 100 µL as extraction volumes did not recover the analytes well with recoveries of 0.49% - 23.2% and 0.19% - 43.1% respectively (Figure 4.4), also their recoveries were not significantly different with a p value of 0.16 (Table S4.4). The low recoveries could be a result of the small amount of the sedimented phase collected which potentially did not carry sufficient amounts of the analyte (Yang et al, 2012). Extraction volume of 10 µL with 250 µL or 500 µL and 100 µL with 250 µL or 500 µL gave low recoveries with a significant difference ($p<0.05$) between them. Volume 250 µL and 500 µL gave comparable results of recoveries at 70.9% - 87.6% and 70.7% - 86.8% respectively, with no significant difference ($p = 0.63$) between their recoveries. Therefore, 250 µL was selected as the optimum extraction solvent volume as means of cost effectiveness.

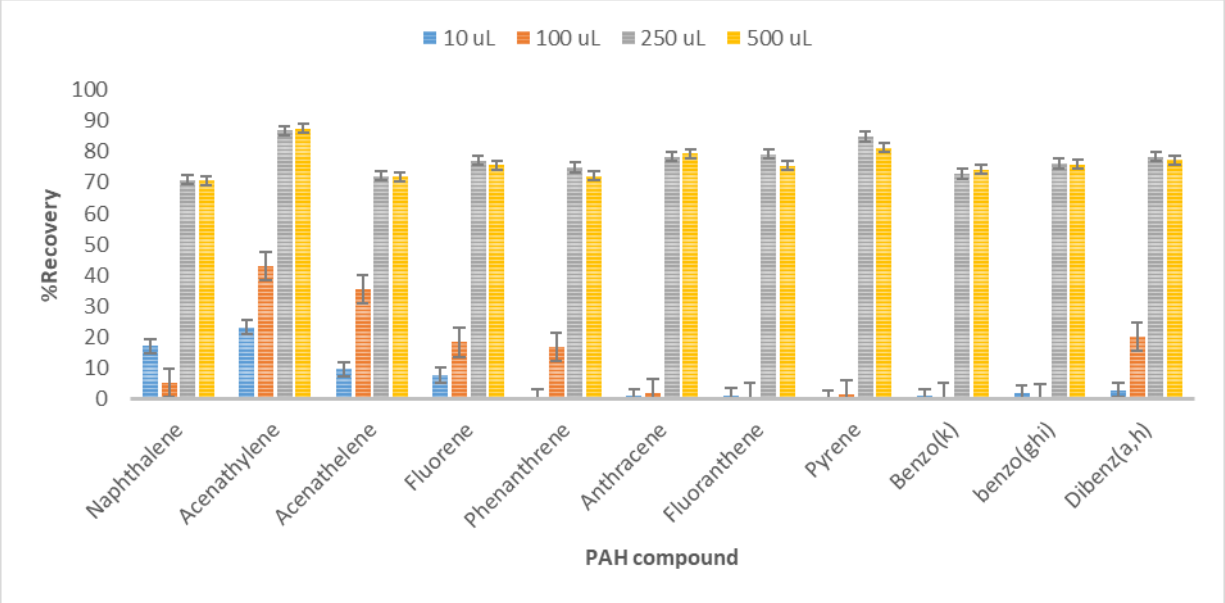

Figure 4.4: effects of extraction solvent volume on recoveries with 750 µL of acetonitrile as the dispersing volume and salting effect of 0.25g

4.6 Methods validation

Method validation is an important factor in ensuring both qualitative and quantitative analysis is achieved for the compounds of interest. The method validation for the developed SPE and DLLME methods was achieved by investigating accuracy through a recovery test, linearity, specificity, limit of detection (LOD) and limit of quantification (LOQ). The recovery test (n=5) gave a %recovery of 72.1 - 118% for SPE and 70.7 - 88.4% for DLLME with a %RSD that is

less than 10% (Table1) which are within the acceptable recovery range of 70 – 120% (Steiner et al, 2020). The statistical evaluation shown in Table S5 between the recoveries of SPE and DLLME show significant difference with a p value of 0.002. Linearity was achieved through the analysis of PAHs standards and coefficient (r^2) of ≥ 0.9900 were obtained for all PAHs. The LOD and LOQ were calculated by using signal-to-noise ratios of 3 and 10, which ranged from 5.00 to 18.0 ng/L and 6.00 – 20.0 ng/L for SPE and from 10.0 to 44.0 ng/L and 11.0 to 63.0 ng/L for DLLME respectively (Table 4.1), there was significant difference observed between the LODs ($p = 2.49 \times 10^{-6}$) and LOQs ($p = 1.42 \times 10^{-5}$) of both methods (Table S4.5). The results obtained indicated that SPE is more sensitive and accurate compared to DLLME towards all PAHs accept for naphthalene.The low sensitivity of DLLME could be attributed to low extraction solvent or the inadequacy of micro-emulsion formed between extractant and solution (Notardonato et al, 2021). Naphthalene could be compatible with DLLME due to its high aqueous solubility which could result in it oversaturating the sorbent in SPE causing it to be washed away thus decreasing its concentration. The results obtained for SPE were comparable to results obtained by Sibiya et al (2012) while results for DLLME are in par with the ones obtained byTobiszewski et al (2014).

Table 4.1: Recoveries, LOD and LOQ for SPE and DLLME and correlation coefficient R^2

PAHs	LOD (ng/L)		LOQ (ng/L)		%Recovery ±%RSD		R^2 Values
	SPE	**DLLME**	**SPE**	**DLLME**	**SPE**	**DLLME**	
Naphthalene	14.0	10.0	15.0	11.0	87.0 ±1.22	88.4 ± 2.68	0.9992
Acenaphthylene	12.0	34.0	14.0	38.0	77.1 ± 2.29	71.7 ± 8.67	0.9970
Acenaphthene	10.0	35.0	11.0	39.0	83.0 ± 3.19	70.7 ± 7.09	0.9969
Fluorene	5.00	36.0	6.00	40.0	102 ± 2.60	76.8 ± 4.15	0.9969
Phenanthrene	10.0	40.0	11.0	42.0	109 ± 2.23	80.4 ± 4.81	0.9988
Anthracene	7.00	35.0	8.00	39.0	118 ± 1.98	75.3 ± 5.54	0.9970
Fluoranthene	9.00	44.0	10.0	63.0	83.3 ±1.52	70.3 ± 8.26	0.9991
Pyrene	8.00	40.0	9.00	42.0	93.4 ± 1.72	76.3 ± 2.71	0.9993
Benzo(k)fluoranthene	11.0	37.0	13.0	41.0	72.1 ± 1.45	70.4 ± 6.59	0.9982
Benzo(ghi)perylene	18.0	26.0	20.0	30.0	100 ± 1.46	79.7 ± 5.45	0.9989
Dibenz(a,h)anthracene	12.0	40.0	14.0	42.0	117 ± 0.17	72.2 ± 5.41	0.9977

4.7 Determination of PAHs in water

4.7.1 PAH concentration in Darvill WWW

Table 4.2 shows the concentrations of PAHs obtained with the use of both SPE and DLLME as extractions methods at four (4) different sampling points in the treatment process of Darvill WWW. Sampling point WDV1 is the influent, WDV2 is pre-treated wastewater, WDV3 is pre-chlorinated wastewater and WDV4 is the effluent where the chlorinated waste water discharge from Darvill mixes with uMsunduzi river. The concentrations ranged from 44.9 to 778 ng/L with SPE as an extraction method while for DLLME concentrations ranged from 30 to 617 ng/L with only naphthalene, anthracene and fluoranthene detected (Table 4.2). The concentration at WDV2 are lower than WDV1, the decrease could be attributed to the preliminary treatment that occurs between these two points, where removal of untreatable solids through screening (rags, paper, plastic, and vegetable matter) and grit (sand, gravel and coffee grounds) take place (Clay et al, 1996). The concentrations then increase at WDV3 and WDV4, this is could be a result of the vehicles and concrete used in the construction currently underway (DWWW, 2013). High levels of naphthalene with a total concentration of 76% was obtained in all four sampling points with the highest concentrations at WDV4 (778 ng/L) which is within the maximum allowable concentration of 3.0×10^6 ng/L, followed by fluoranthene at 18% then anthracene at 7% (ASTDR, 1995).

The high concentration levels of PAHs in the effluent is due to the fact that Darvill is designed to biologically treat inorganic nutrients and trace metal to meet the discharge limits, and as a result of this residual organic compounds including PAHs are discharged back to the river after the treatment process. The high levels of naphthalene could be a result of their various applications which include their use in the leather industry as tanning agents, a feedstock for naphthalene sulfonic acids often employed in the making of plasticizers for concrete, a plasterboard ingredient and also their use in synthetic and natural rubbers as dispersants (Buckpitt et al, 2010) (Munyengabe et al, 2018). Darvill is currently under the construction of a 35 Megalitre per day upgrade which employs a lot of concrete which might be the reason for the high concentration levels of naphthalene (DWWW, 2013; Jia & Batterman, 2010). Anthracene and fluoranthene high concentration levels at Darvill could be attributed tobacco smoke since it has been found in cigarettes, fumes from vehicle exhaust especially diesel fuelled engines and coal tar (Chanda & Mehendale, 2005) (USEPA, 2010). This could

possibly end up in wastewater as a result of surface runoff through urban drainage. The underestimation of concentrations by DLLME could be a result of small extraction solvent employed which might have been saturated resulting to inefficient extraction of the PAHs from the sample. Also, the inadequacy formation of the micro-emulsion between extractant and solution may have an impact on the final concentration obtained (Notardonato et al, 2021).

Table 4.2: Concentrations of PAHs in Darvill wastewater works

Concentration (ng/L)									
	WDV1		**WDV2**		**WDV3**		**WDV4**		
Compounds	**SPE**	**DLLME**	**SPE**	**DLLME**	**SPE**	**DLLME**	**SPE**	**DLLME**	**Total %PAHs**
Naphthalene	563	543	506	410	763	658	778	617	75.0
Anthracene	45.5	nd	44.9	nd	49.0	nd	90.1	15.8	7.00
Fluoranthene	130	37.0	114	30.0	186	59	184	67.0	18.0

4.7.2 PAH concentration detected in river water using SPE and DLLME

The river samples were collected along uMsunduzi River from five different sampling points and in the treatment process of Darvill WWW in Pietermaritzburg (PMB). All the studied PAHs were detected in all the collected river samples except for phenanthrene. The PAHs were detected at varied concentrations which informs the permeating nature of the pollutants. The concentrations of PAHs obtained using SPE (Table 4.3) were higher than those obtained with the use of DLLME, this highlights the sensitivity of SPE which is shown by low LOD and LOQ values (Table 4.1) which in turn informs its extraction efficiency for the determinants. The concentrations of PAHs obtained in Msunduzi could possibly be attributed to discharge of effluents from the manufacturers and industries which include production of aluminium, leather manufacturers, iron and steel foundries that are in close proximity to the river. PAHs such as naphthalene are used in the leather industry as tanning agents while in the aluminium production industry, a process called Soderberg leads to combustion of some materials which may lead to PAH formation (Borgulat, 2018).

All five sampling points had high concentrations of naphthalene in comparison to other PAHs with a total concentration of 32% (Table 4.3), which could be a result of its high solubility in water and out of all sampling points, YCMA had the highest naphthalene concentration of 1046

ng/L which could be a result of incomplete combustion from motor vehicle exhaust fumes, spills from the nearby fuel stations, cigarette smoke, etc. The other sampling points also showed high naphthalene concentrations with CR at 1035 ng/L, CD (580 ng/L), WH (427 ng/L) and BS at 346 ng/L which could be a result of tar fabrication, illegal dumping, landfill waste burning and industrial activities (Munyengabe et al, 2018). YMCA and CR were observed to have the highest concentration of all the other PAHs studied and this is attributed to the large volume of motor vehicle traffic as well as asphalts degradation which can be introduced to the nearby soils through run-off and eventually leaching into the river water (Ngubo et al, 2021). Benzo(ghi)perylene was the least detected pollutant in water with a total concentration of 0.3% and this could be due to the fact that it is a HMW PAH and therefore has low solubility in water, low vapour pressure and partitions easily to organic matter (Lawal, 2017a). Although the concentrations in this study were high they are still within the maximum acceptable concentration of 3.0×10^6 ng/L for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benzo(ghi)perylene and 5.0×10^3 ng/L for benzo(k)fluoranthene and dibenz (a,h) anthracene in all sampling sites (ASTDR, 1995).

Table 4.3: Concentrations of PAHs and diagnostic ratios obtained in uMsunduzi river samples

PAH concentration (ng/L)											
PAH	**BS**		**CD**		**CR**		**WH**		**YMCA**		
	SPE	**DLLME**	**SPE**	**DLLME**	**SPE**	**DLLME**	**SPE**	**DLLME**	**SPE**	**DLLME**	**SPE - Total %PAHs**
Naphthalene	346	230	580	290	$1.04x10^3$	491	427	279	$1.05x0^3$	569	30.7
Acenaphthylene	nd	nd	nd	nd	343	82.7	21.0	nd	nd	nd	3.06
Acenaphthelene	176	34.5	nd	nd	226	93	197	68.3	253	111	7.60
Fluorene	128	11.8	118	43.3	409	25.7	123	55.9	161	101	8.38
Phenanthrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Anthracene	91.4	nd	156	84.2	974	367	146	nd	304	105	12.5
Fluoranthene	nd	nd	nd	nd	790	460	nd	nd	139	46.9	9.93
Pyrene	98.8	nd	101	31.8	984	452	98.8	38.8	206	50.8	9.18
Benzo(k) fluoranthene	230	99.0	279	nd	268	130	256	101	238	68.6	11.3
Benzo (ghi) perylene	34.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.31
Dibenz (a,h) anthracene	92.1	43	91.9	48.0	95.8	49	90.9	42.8	115	50.7	4.33
Fl/(Fl+Py)	-	-	-	-	0.44	-	-	-	0.40	-	
LMW/HMW	1.63	-	1.81	-	1.77	-	2.05	-	2.72	-	

There are few studies that have been done on the concentrations of PAHs in water and wastewater in South Africa. The average concentration of PAHs in river water obtained in this study and other studies done in other parts of Africa (10.1 – 1046 ng/L) are higher than those obtained in countries outside of Africa such as Mexico (0.5 – 680 ng/L) (Jaward et al, 2012) and China (32.6 – 108 ng/L) (Hu et al, 2016). While studies done in other African countries such as Ghana (550 – 13.5 x 10^2 ng/L) (Essumang et al, 2009) and Nigeria (1. 0x10^3 – 50.7 x 10^4 ng/L) (Asagbra et al, 2018) showed lower average concentrations of PAHs compared to those studies conducted in South Africa, this emphasises the importance of monitoring the concentrations of PAHs in the environment. In South Africa, a study in the Limpopo province by Edokpayi et al (2016) showed high PAH average concentrations at 1.32 x10^7 – 2.64x10^7 ng/L followed by a study in the Eastern Cape (14.91 x10^2 – 20.6 x 10^3 ng/L), carried out by Adeniji et al(2019)while low average PAH concentrations were obtained in a study carried out in Gauteng (22.0 to 1040 ng/L) by Sibiya et al (2012) compared to the studies conducted in KZN () by Munyengabe et al (2018) with concentrations ranging from 6.07x10^5 to 1.3 x10^6 ng/L and by Ngubo et al (2021) with 330 to 10.4x10^2 ng/L concentration range. This study also in KZN, showed high concentrations of naphthalene at 580 ng/L to Munyengabe's 24.5 ng/L at the same sampling point, Camps Drift in Msunduzi river (Munyengabe et al, 2018). The significant increase in the concentration could be attributed to the burning of waste, new tar road construction, increase of motor vehicles on the roads which might possibly escalate the degrading of infrastructure such as tar roads leading to high concentration levels in the surrounding environment.

The focus on the study of PAHs has been more on other water matrices (i.e river water) than wastewater that means that more focus/emphasis is needed to this part of the environment (wastewater) which is why this study is significant. The concentration obtained in this study from wastewater samples were lower (30.0 to 778 ng/L) than those obtained by both Ngubo et al (2021) at 90 – 2700 ng/L and Munyengabe et al (2018) at208.4 to 1750 ng/L in KZN. While a study carried-out in the Limpopo province by Edokpayi et al (2016) showed higher concentrations at 1.32 x 10^7 to 2.64 x 10^7 ng/L than those obtained in KZN province. There average concentrations obtained in Africa were higher than those obtained in other countries such as China reported by Qi et al (2013) at WWTP (245 to 404 ng/L) and small sewers at 431 to 2860 ng/L. The lower concentrations in China could be attributed to the fact their treatment plants use sophisticated technology which allows a higher standard of treatment (Water Technology, 2022.).

4.8 Potential source Identification

To identify the origin of PAHs and separately identify petrogenic from pyrolytic inputs, chemical profiling and different diagnostic ratios on isomeric relations were used: An/(An+ Phe), Fl/(Fl + Pyr) and LMW/HMW. The petrogenic PAHs are a result of petroleum products while the pyrolytic are due to incineration processes. Typically, when the Fl/(Fl+Pyr) ratio is less than 0.40 the PAHs are said to be a result of petroleum contaminants and ratios that are greater than 0.40 are typical combustion of grass, wood, or coal (Table 4.4) (Montuori et al, 2021). Each source pyrolytic or petrogenic provides typical PAH patterns and typically, HMW compounds with four or more condensed aromatic rings are more abundant in combustion products, while LMW with two and three aromatic rings are more abundant in fossil fuels.

Table 4.4: Diagnostic ratios for PAHs

Diagnostic Ratio	Petrogenic	Pyrogenic	References
An/(An+Phe)	<0.1	>0.1	(Brändli et al., 2007)
Fl/(Fl+Pyr)	≤0.4	≥0.4	(Montuori et al., 2021)
LMW/HMW	>1	<1	(Nasher et al, 2013)

The ratio An/(An+Phe) was not used in this study as no phenanthrene concentrations were detected in all the studied sites. The Fl/(Fl + Pyr) shown in Table 4.3 was found to be 0.4 for both the sites (CR and YMCA) where both isomers were detected indicating that the PAHs originated as a result of petroleum contaminants. The LMW/HMW ratio was also determined for all sampling sites in uMsunduzi river and found to be greater than 1 for all sites which also is indicative of the petrogenic sources of PAHs in this area. Figure 4.5 exhibits that there were high concentration levels of LMW PAHs in all the sampling points compared to HMW PAHs which also supports the evidence that PAHs in the river system originated from petrogenic sources. These ratios were not applied to the effluents from Darvill WWW as only one of the isomers were detected in the samples. Generally, the source of PAHs in both water bodies (the river & wastewater works) can be attributed to petrogenic activities although pyrolytic input cannot completely be ruled out (Edokpayi et al, 2016).

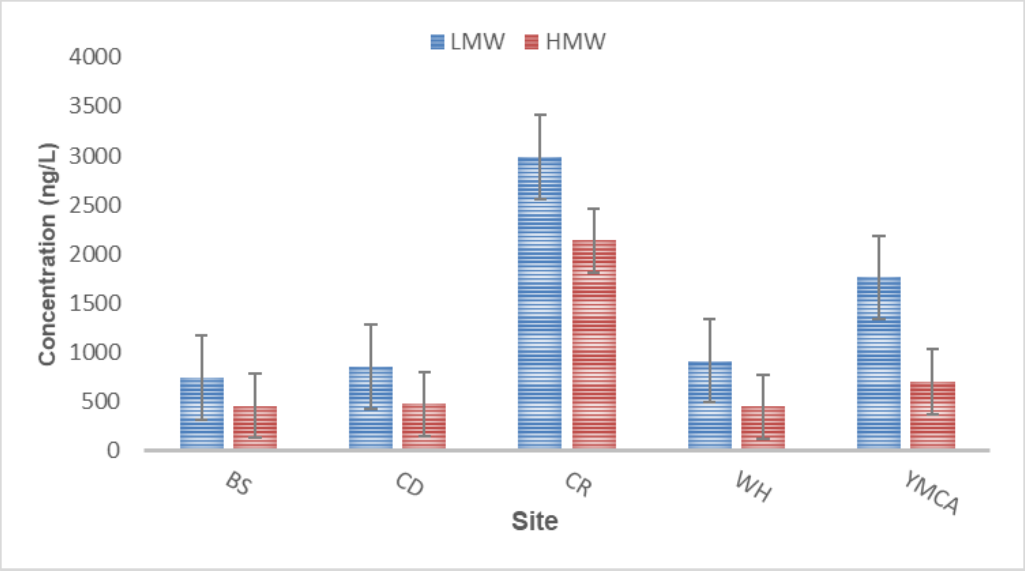

Figure 4.5: LMW PAHs vs HMW PAHs in uMsunduzi river

4.9 Ecological Risk Assessment (ERA)

A risk assessment of the 11 PAHs investigated in this study (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(k) fluoranthene, benzo (ghi) perylene and dibenz (a,h) anthracene) was carried out to examine their ecological risk to the surrounding environment. The PAH concentrations obtained from uMsunduzi river and Darvill WWW was used as the environmental exposure concentrations (EEC). The predicted no observed effect concentrations (PNECs) of PAHs, obtained from the Oil Spill Prevention, Administration and Response data base, were used as the toxicity assessment endpoint. A Risk Quotient (RQ) expressed as the ratio of EEC and PNEC (ECC/PNEC) was applied to characterize a potential risk from PAH exposure (OSPAR, 2014)(Wu et al, 2011). The risk is then ranked as negligible if RQ <0.1, low if RQ is 0.1≤RQ>1, and high if RQ ≥1. All the LWM PAHs exhibited low ecological risk in all the sampling points with RQ values less than 1 except for acenaphthylene and fluorene in CR with RQ values of 2.638 and 1.636, respectively and anthracene in all sampling sites (Table 4.5). While low concentrations of HWM PAHs were obtained in the river samples they showed high ecological risk (RQ >1) in all sampling points, this evidence emphasises the importance of monitoring the PAHs in the environment.

Table 4.5: Risk Quotient (RQ) for PAHs

PAHs	PNECs (ng/L)	BS	RQ -BS	CD	RQ-CD	CR	RQ-CD	WH	RQ-WH	YMCA	RQ-YMCA
Naphthalene	2.00×10^3	346	0.17	580	0.29	1.04×10^3	0.52	427	0.21	1046	0.52
Acenaphthylene	130	nd	-	nd	-	343	2.64	21.0	0.16	nd	-
acenaphthelene	380	176	0.46	nd		226	0.60	197	0.52	253	0.67
fluorene	250	128	0.51	118	0.47	409	1.64	123	0.49	161	0.64
phenanthrene	1.30×10^3	nd	-	nd	-	nd	-	nd	-	nd	-
anthracene	100	91.4	0.91	156	1.56	974	9.74	146	1.46	304	3.04
fluoranthene	6.30	nd	-	nd	-	790	125	nd	-	139	22.1
pyrene	23.0	98.8	4.30	101	4.39	984	42.8	98.8	4.30	106	4.61
benzo(k) fluora	0.17	230	1.35×10^3	279	1.64×10^3	268	1.58×10^3	256	1.5×10^3	238	1.40×10^3
benzo (ghi) perylene	0.17	34.5	203	nd	-	nd	-	nd	-	nd	-
dibenz (a,h) perylene	0.14	92.1	658	91.9	656	95.8	684	90.9	649	115	821

4.10 Conclusion

The optimised SPE and DLLME method coupled with GC-MS were found to be suitable extraction methods for the determination of PAHs in river water and wastewater samples. Although they both can quantitative and qualitative analyse trace levels of PAHs in water, SPE showed to be more accurate and sensitive than DLLME. The river water and the wastewater were found to be contaminated with trace levels of PAHs with the river showing high levels of contamination than the wastewater and naphthalene dominating both environments. The concentrations obtained in this study were all within the acceptable levels in all the sampling sites. The PAHs in the investigated areas are a result of petrogenic sources such as combustion of diesel fuel (vehicle exhaust fumes), weathering of asphalt surfaces, oil spills and surface runoff. All the PAHs in the respective sampling sites demonstrated a low ecological risk to the surrounding environment except for acenaphthylene and fluorene in CR and anthracene in all the sampling sites.

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CHAPTER FIVE:

Determination of PAHs from sediment and sludge and ecological risk assessment

Determination of concentrations, origin and ecological risk of polycyclic aromatic hydrocarbons, in sediment and sludge samples

5.1 Abstract

This study evaluated the concentrations, origin and ecological risk of polycyclic aromatic hydrocarbons (PAHs), a group of environmentally toxic and persistent chemicals in sediment and sludge samples. This is a requirement by the South African government (The Department of Water and Sanitation) which has set national norms and standards for the assessment of waste for landfill disposal through the National Environmental Management: Waste Act, 2008 (NEMWA) license (Act No. 59 of 2008). The PAHs were extracted using an ultrasonic extraction (UE) and microwave assisted extraction (MAE) followed by filtration or filtration then a clean-up with solid phase extraction (SPE). These results indicated that both methods can be used for the extraction of PAHs with relative accuracy and sensitivity, however higher concentrations were obtained with SPE cleaned samples (95.96 – 926.0 µg/kg) compared to filtered samples (21.61 – 380.6 µg/kg) with pyrene showing dominance over all other PAHs. Although the concentrations obtained were high for these PAHs, they were still within the total acceptable concentration levels of 5.0×10^4 µg/kg as prescribed by the NENWA standards for sludge (Molewa, 2013) and 3000 µg/kg for sediment samples (ASTDR, 1995).

5.2 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic contaminants that are derived from both domestic and industrial scale as a consequence of incomplete combustion (Abdel-Shafy & Mansour, 2016b) and can be classified as low molecular weight (LMW) or high molecular weight (HMW). The LMW-PAHs contain two or three fused aromatic rings, they are emitted from petrogenic and pyrogenic sources and they can be dispersed as clusters on nuclei particulates facilitating their dispersal over long distances with which in case of rain they end up in water through run-off (Stogiannidis & Laane, 2015). The HWM-PAHs contain four or more fused aromatic rings and are a result of pyrolytic sources. Due to their higher hydrophobic nature, they are normally transported as bound complexes to fine particles dissolved in organic matter and can be found in elevated concentrations in the sediments and soils than in water (Munyengabe et al, 2018). The PAHs are hydrophobic compounds (hydrophobicity is proportional to molecular mass) and are resistant to biodegradation in the environment due to their low water solubility and electro-chemical stability. They often remain in the environment for long periods of time due to their high level of conjugation and aromaticity. The resistant to

degradability and extractability of PAHs in soil increases with the time they have been in contact with soil particles, a process called aging or weathering (Srogi, 2007). This process reduce the toxicity of the soil contaminants, by lowering the fraction available for uptake by living organisms (Ukalska-Jaruga et al, 2019).

The PAHs have toxic, mutagenic, carcinogenic and teratogenic properties consequently, the Environmental Protection Agency (EPA) identified 16 PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(k)fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(ghi)perylene and dibenz(a,h)anthracene) as one of the priority pollutants (Lawal, 2017b). As a result of the PAH toxicity, exposure to PAHs can lead to various health issues including neurotoxicity, infertility and cancer (Knuckles et al, 2001). Due to the potential negative effects on the environment, it is endorsed through the NEMWA of 2008 that contaminants including PAHs are monitored in all compartments of the environment including the water treatment plants (Edokpayi et al, 2017). The NEMWA of 2008, ensures the protection of the human health and environment through regulating waste management by providing reasonable measures for the prevention of pollution and ecological degradation in order to secure ecological sustainable development. This Act stipulates the set national norms and standards for the assessment of waste management for landfill disposal in all spheres of government in order to ensure compliance (Gama et al, 2013).

Techniques for extraction and concentration of PAHs from environmental solid samples such as soxhlet extraction (SE) microwave assisted extraction (MAE), ultra-sonication extraction (UE) and linear shaker (LS) are often used for the extraction organics in solid samples. Soxhlet has been largely used as the benchmark of extraction of PAHs in solid samples as it is a well-studied method. Basically in SE, extraction is achieved via reflux cycle with the use of an appropriate solvent. Although SE has been used successfully, it has its disadvantages including the use of large volumes of solvents (~300mL), it is also time-consuming (~24 hours) and labour intensive (Becker et al, 2015). Extraction with linear shaker can be used in order to reduce the amount of solvent required (~20 mL) for PAHs in solid samples but like SE, extraction with the use of a linear shaker is time consuming, its sensitivity is only attainable when long shaking times (~20 hours) are applied in order to extend the contact time with solvent. Techniques such as MAE and UE have been used in order to reduce extraction times and promote ease of sample preparation. Microwave assisted extraction is an automated

extraction technique which offers shorter analysis time. In MAE, microwave energy is applied to heat solvents in contact with samples (mainly solid samples) achieving the partition of the target compounds of interest from the sample into the solvent (Llompart et al, 2018).

The MAE technique is vastly used because it is a modern sample preparation techniques as it complies with the minimum criteria required and it also a very attractive alternative to conventional approaches for the extraction of various compounds including organic and organometallic from different matrices (Luque de Castro & Priego-Capote, 2010). MAE also renders the possibility of simultaneously extracting multiple samples, drastically improving sample output. Ultrasonication extraction mainly involves the use of high-power ultrasound to accelerate solvent penetration into solid materials and it is a good alternative to linear shaker extraction. The ultrasonic uses mechanical vibration as the linear shaker extraction but with a shorter timer. Once the PAHs have been extracted from the sample matrix, they may be determined satisfactorily using the high-performance liquid chromatography (HPLC) and gas chromatography (GC) technique with various detection techniques.

However, the GC coupled with mass spectrometer is preferred as it offers robust identification of the analyte compounds both by retention time and mass spectrum, with additional structural information (Adeniji et al, 2018). GC is also compatible with both polar and non-polar molecules for as long they can evaporate (David et al, 2021). Therefore, the aim of this work was to develop UE and MAE followed by either filtration or filtered then SPE cleaned-up methods for determining concentrations of PAHs in sludge and sediments from the uMsunduzi river and Darvill sludge landfill. The source of origin and ecological risks of polycyclic aromatic hydrocarbons in solid matrices were also determined. The filtration and cleaning with SPE was performed as to ensure that impurities are sufficiently removed as they tend to camouflage the analyte of interest leading to underestimation of concentrations.

5.3 Experimental

5.3.1 Chemicals and Reagents

Acetonitrile (99.9%), acetone (99.8%), ethyl acetate (99.9%), and n-hexane were purchased from Merck (Johannesburg, South Africa), while the PAHs mixture (100% purity) containing naphthalene, acenaphylene, acenaphthene, fluorene, Phenanthrene, anthracene, fluoranthene,

pyrene, benz(a)anthracene, benzo(k)fluoranthene, benzo(a)Pyrene, benzo(ghi)perylene and dibenz(a,h)anthracene was purchased from Sigma-Aldrich (Johannesburg, South Africa).

5.3.2 Instrumentation

Multiwave 5000 microwave purchased from Anton Paar (United Stated of America) and Eumaxultrasonic cleaner bath purchased from LABOTEC (Singen, Germany). Supelco SPE Vac-Elut unit, purchased from Sigma Aldrich (Darmstadt, Germany) was used to extract PAHs in solid samples. Oasis hydrophilic-lipophilic balance (HLB) cartridge (60 mg, 3 mL) used for the extraction of PAHs was purchased from Waters (Dublin, Ireland). A vacuum pump from Edward 8 and Holdoph-Basis Hei-VAP Value rotor evaporator purchased from Holdoph (Berlin, Germany). PAHs were quantitatively analysed with the use of Gas Chromatography (GC), Mass Spectrometry (MS) combination (QP-2010 series (Kyoto, Japan)). Separation of PAHs was successfully carried out using the capillary column InertCap 5MS/Sil 30 m (I.D. = 0.25 mm, film thickness = 0.25 µm, Japan). Helium at a flow rate of 1.32 ml/min and an injection temperature of 260 °C was used as a carrier gas. 3 µL was the injection volume performed in a splitless mode. The temperature of an oven (480 ˚C) was held at 40 °C for 1 min, then increased to 100 °C at a rate of 15°C/min, 2nd ramp at 10 °C/min to 210 °C, then held for 2 min. Final ramp programmed at a rate of 5 °C/min to 310 °C, held for 8 min. To identify the analytes, the MS selected ion monitoring (SIM) mode was used. Due to some target ions having the same mass ions (m/z),boiling points were also used for quantification and these were 128 (naphthalene), 152 m/z (acenaphthylene), 154 m/z (acenaphthene), 166 m/z (fluorene), 178 m/z, 336 ˚C (phenanthrene), 178 m/z, 340 ˚C (anthracene), 202 m/z, 375 ˚C (fluoranthene), 202 m/z, 404 ˚C (pyrene), 228 m/z (benz(a)anthracene), 252 m/z, (benzo(k)fluoranthene), 252 m/z, 495 ˚C (benzo(a)pyrene), 278 m/z, 524 ˚C (dibenz(a,h)anthracene) and Benzo(g,h,i)perylene (278 m/z, 550 ˚C)(Ngubo et al, 2021).

5.3.3 Preparation of calibration standards

The mixture of thirteen PAHs at 2000 µg/L (naphthalene, acenaphylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene and dibenz(a,h)anthracene) was used for the calibration of the instrument. This mixture was diluted in acetonitrile to give a stock solution of 10 ppm. The stock solution was then further diluted in a 100 mL volumetric flask to give 0.01 ppm – 0.8 ppm calibration standards.

5.3.4 Sampling of sediments and sludge samples

The solid samples were collected from along uMsunduzi river (Figure 1.1) and Darvill wastewater works (WWW) sludge landfill (Figure 1.2) at Umgeni Water in Willowtown, Pietermaritzburg, Kwa-Zulu Natal. These samples were collected from four different sampling points namely: Camps Drift (CD), College Road (CR), Woodhouse (WH) & Bishopstowe (BS) at uMsunduzi river which were chosen as result of being high contamination points. Darvill WWW samples were collected from two different points (7A and 14A) of the Darvill landfill for the disposal of treated sludge. The sludge samples were collected near surface using a spade and stored in 1000 mL honey jars on the 30 August 2021 at Darvill WWW while the sediment samples were collected on the basin of the river on the 28th of April 2021 in Msunduzi river. The samples were then air dried in a fume hood for 14 days before grinding using a soil grinder and sieving through a 125 µm sieve to fine soil particles of less than 0.125 µm. These samples were then stored at room temperature until further analysis were performed.

5.4 Optimization of extraction methods

5.4.1 Ultrasonication extraction

An UE method by Oluseyi et al (2011) was optimized prior application to real sample in order to increase recoveries for PAHs. The UE parameters that were investigated included the extraction solvents on 1:1 ratio (n-hexane:acetone, n-hexane:water, n-hexane:ethyl acetate and n-hexane) and extraction solvent volumes (10 mL, 25 mL and 50 mL) at 30 min, 60 min and 90 min extraction times. A statistical evaluation was also carried out in order to determine the probability of significant difference between the parameters that were optimised. This optimisation was imperative to determine the optimum extraction solvent and the volume needed to maximise the penetration of the solvent into the solids in order to sufficiently break their surfaces, thus allowing efficient extraction of the targeted determinants.

Under optimum conditions, 0.5g of the soil/sludge sample was dissolved in 50 mL of 1:1 ratio of acetone:nhexane. The sample was then ultra-sonicated in the sonication bath for 60 min with occasional swirling to prevent sticking on the bottom of the flask and then rested for 5 min. The extraction solution was filtrated into a beaker using filter paper. A 1 mL of the filtrate was collected while the rest (45 mL) was cleaned up using SPE and then the eluate was reduced with nitrogen gas to give a final volume of 1 mL.

5.4.2 Microwave assisted extraction

Extraction of PAHs by the use of a microwave was achieved through the use of the EPA 3546 method (Coker, 2007). The parameter that was investigated on the MAE was the extraction solvents, the solvent mixture used was based on the fact that at least one component is able to absorb microwave energy. The extraction solvent mixture chosen gave optimum, reproducible recovery of the analytes of interest from the solid samples. The extraction solvent mixtures that were chosen were (i) nhexane:acetone, (ii) nhexane:acetonitrile and nhexane:ethyl acetate.

Under optimum conditions, 2g of the soil/sludge sample was diluted in 50 mL of equal parts (1:1) of nhexane:acetone in an extraction vessel. The extraction vessel containing the sample and solvent mixture was heated to 110 ˚C for 5 min and then extracted for 15 min, the mixture was then allowed to cool down. The vessel was then opened and the contents filtered, 1 mL was collected for analysis while the rest of the filtrate (45 mL) was cleaned-up using SPE and the eluate reduced with nitrogen gas to 1 mL.

5.5 Methods Validation

5.5.1 Quality assurance

To ensure the validity of the method and quality of the results, a variety of parameters such as accuracy, recoveries, limit of detection (LOD), limit of quantification (LOQ), sensitivity and selectivity/specificity were accounted. Accuracy was determined by the recovery test through spiking the sample with standard of interest with a known concentration of 10ppb. Sensitivity is the capability of the method to discriminate between small differences of concentration of analytes (SANAS, 2017). The LOD is the smallest concentration of an analyte in the test sample that can be reliably distinguished from zero concentration and it is calculated at concentrations given by 3 times the standard deviation of signal to noise ratio. LOQ is defined as the lowest concentration of an analyte that can be determined with acceptable precision and accuracy and it is given by concentrations 10 times the standard deviation of signal to noise ratio. Specificity/selectivity is the ability of a method to respond to a particular analyte of interest in the presence of possible interferences such as impurities, degradants and matrix effects. This was ensured by using the mass spectrometer as a detector where the detector was set to selectivity detects only ions of the targeted PAHs (Hinshaw, 2010). Specificity of the method was conducted by comparing spiked and non-spiked samples under SIM mode to ensure that the electron ionization detector source on the GC-MS instrument is set to target and identify only the PAHs of interest by using specific m/z values

5.6 Results and Discussion

5.6.1 Optimization of ultrasonication

5.6.1.1 Effect of the extraction solvent

Effective extraction of the determinants depended on the efficiency of the solvent in penetrating the solids in order sufficiently break down and obtain enough surface area to extract from. In order to sufficiently extract the target analyte, extraction time needs to be controlled so as to prevent excessive exposure to the irradiation which may degrade the contaminants in the sample and reduce the extraction rate. Therefore, choosing the correct extraction solvent, extraction time was of high importance and the effect of the extraction solvent was investigated using different solvent mixtures giving 1:1 ratio and these were (i) n-hexane (nH) + acetone (ACT), (ii) n-hexane + water (H_2O) (iii) n-hexane + ethyl acetate (EA) and (iv) n-hexane. The n-hexane + acetone mixture gave highest recoveries with percentages between 93.7% -121%,

while nhexane + ethyl acetate gave low recoveries ranging between 69.8% - 99.8% (Figure 5.1). This could be attributed to small dielectric constant compared to acetone and ethyl acetate thus leading to their inadequacy in extracting the target analyte. The recoveries in Table S5.1 were not significantly different for nH + ACT to nH + EA ($p = 0.18$) and nH + H_2O ($p=0.06$) while significant difference was observed to nH ($p = 0.007$). Also no significant difference in recoveries was observed for nH + EA to nH + H_2O ($p = 0.12$) and nH to nH + H_2O ($p = 0.20$) while the opposite is true for nH + EA to nH with a p value of 0.01. These results were obtained when the extraction time was at 60 min. The high recoveries obtained from the use of the nhexane + acetone showed that is an effective extraction solvent and the order of the extraction efficiencies for most of the PAHs was as follows: nhexane + acetone > nhexane + water > nhexane + ethyl acetate > nhexane. Its efficiency could be attributed to high polarity of acetone in comparison to water and ethyl acetate which led to high penetration power of the solvent thus breaking down the solid samples increasing their surface area (Ngubo et al, 2021). In turn this aided hexane (non-polar) to sufficiently dissolve the analyte through contact which ultimately led to a successful extraction (Ncube et al, 2018) (Scaramboni et al, 2021). The n-hexane–acetone solvent mixture has been used in other studies to extract quantitative amounts of PAHs in matrices such as soil, sediment and plant material (Banjoo & Nelson, 2005).

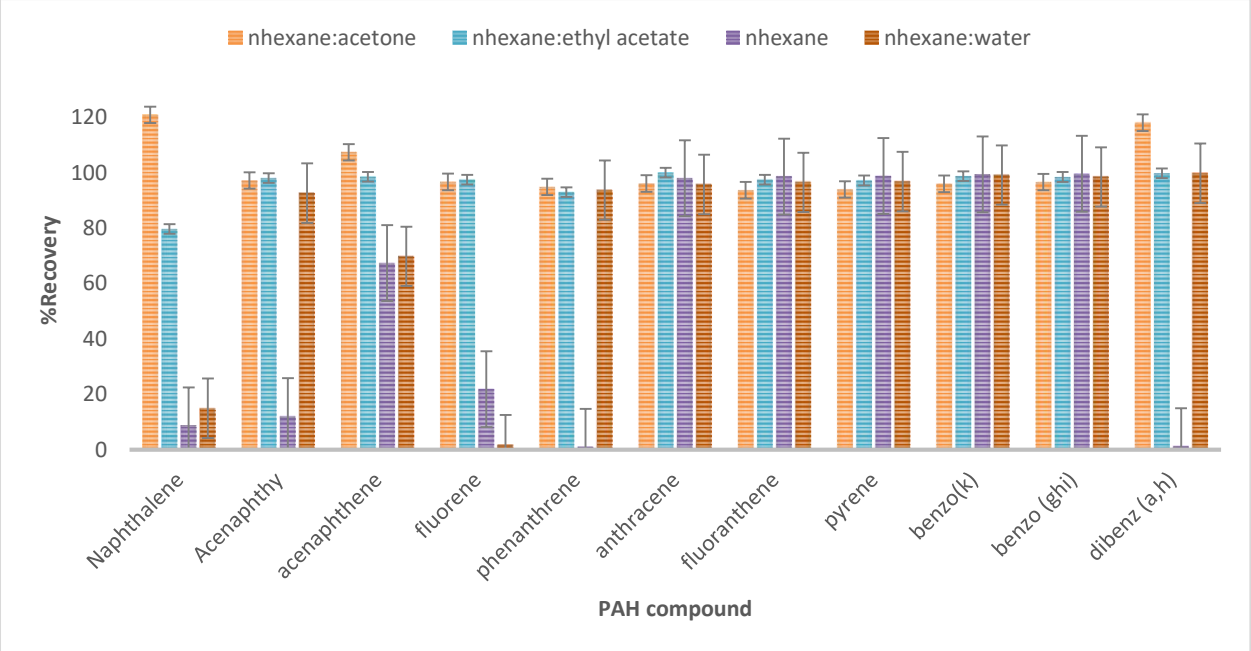

Figure 5.1: Effect of extraction solvent on PAHs %recovery.

5.6.1.2 Effect of the extraction solvent volume

The extraction solvent volumes of 25 mL, 50 mL and 75 mL were used in the investigation of their efficiency. The percentage recovery increased with an increase in solvent volume from 25 mL to 50 mL for most of the PAHs which attributed to the fact more solids were dissolved in a larger amount of the solvent. The statistical evaluation (student t-test) in Table S5.2 showed that there was a significant difference between the recoveries of these volumes with a p value of 0.02. When the solvent volume was increased from 50 mL to 75 mL, the recoveries obtained a showed a slight decrease with a significant difference (p = 0.005) in recoveries. This could mean that the solution was saturated, a point of equilibrium where no more solute could dissolve in the solvent. Therefore 50 mL was chosen as the extraction solvent volume as it gave the highest recoveries (86.7% - 123%), (Figure 5.2). The recoveries obtained in this study were comparable to the recoveries obtained by Aydin et al (2007) of 48 – 100% with 25 mL, 67 – 100% (50 mL), 70 – 112 % (75 mL) and 76 – 114% (100 mL).

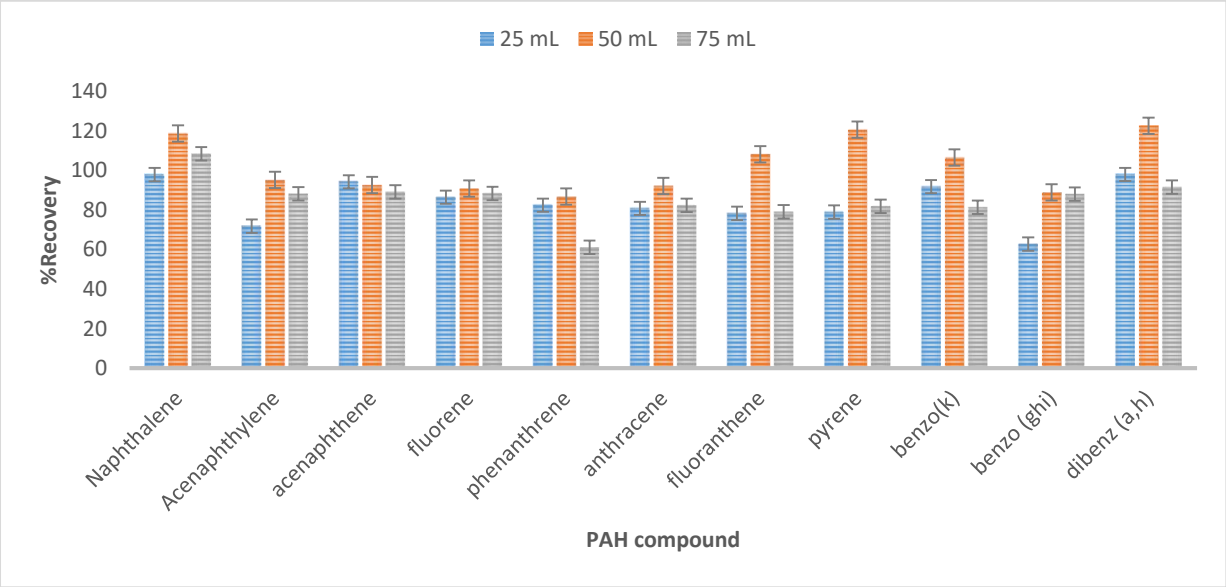

Figure 5.2: Effect of the extraction solvent volume on the PAH's %recovery.

5.6.1.3 Effect of extraction time

The extraction time was also optimised to ensure sufficient extraction without degradation of target analyte, the investigated times were 30 min, 60 min and 90 min. It was found that percentage recovery increased from 30 min to 60 min with a significant difference (p= 0.02) in recovery, while further increase of extraction time from 60 min to 90 min showed a slight decrease in the percentage recovery (Figure 5.3) with no significant difference (p = 0.37) in recovery, a pattern followed by 30 min recoveries when compared to that of 90 min (p = 0.05) shown in Table S5.3. The decrease in recoveries could indicate that the possible degradation

of the analytes by radiation, therefore, 60 min was used as the optimal extraction time. The results obtained were comparable to those obtained by Aydin et al, who optimised extraction times of 15 - 60 min and obtained higher recoveries (70 to 112%) at 45 minutes (Aydin et al, 2007).

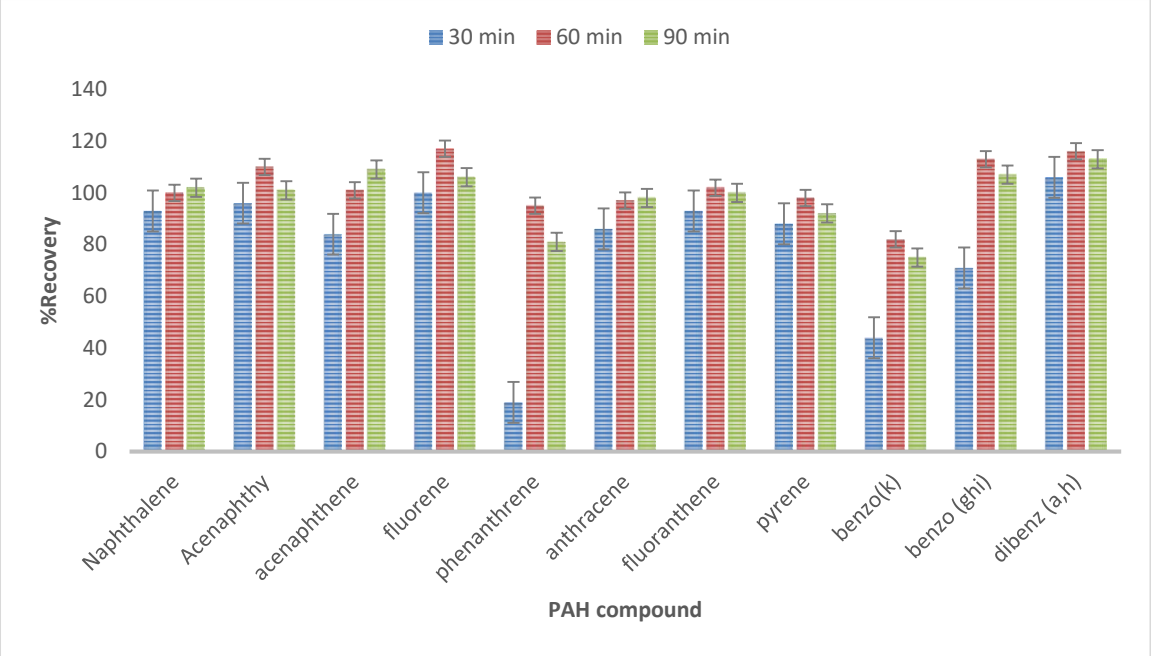

Figure 5.3: Effect of extraction time on the PAH's %recovery

5.6.2 Optimization of microwave assisted extraction

5.6.2.1 Effect of the extraction solvent on recoveries

The selection of the extraction solvent when using MAE is based on the solvent's dielectric constant which is a solvent's ability to absorb microwave energy. This constant also known as the relative permittivity measures a solvent's ability to store electric charges and is directly propositional to polarity (Scaramboni et al, 2021). One solvent is often immiscible while the other one is miscible with water, the miscible solvents helps the mixed solvent to penetrate the layer of water on the surface of the solid in order to successfully facilitate extraction (Coker, 2007). Therefore, the effectiveness of the extraction solvent was determined shown in Figure 5.4 using (i) n-hexane + acetone, (ii) n-hexane + acetonitrile and (iii) n-hexane + ethyl acetate. The n-hexane and acetone combination gave the highest recoveries ranging between 79.6% & 120% while nhexane + acetonitrile gave comparable recoveries that ranged from 71.8% to 101% with no significant difference ($p = 0.15$) observed between the mixture's recoveries, therefore the solvent with higher recoveries was used as the optimum. The nhexane + ethyl

acetate mixture gave low recoveries (12.8% - 92.6%) and the p value of the mixture to nH + ACN (0.03) and nH + ACT (0.007) suggested significant difference in their recoveries (Table S5.4). The recoveries obtained using acetone and acetonitrile were high compared to ethyl acetate this could be a result of their high dielectric constants compared to that of ethyl acetate, which allows the penetration of the surface layer of the solid leading to a successful extraction as the microwave directly binds and polarise the molecules that are available in the mixture (Fecher et al, 2014), The results obtained above are in agreement with results obtained by Sanchez-Uria et al where nhexane/acetone as an extracting solvent was compared to acetone alone for the same purpose (Sánchez-Uría & Del Castillo-Busto, 2018).

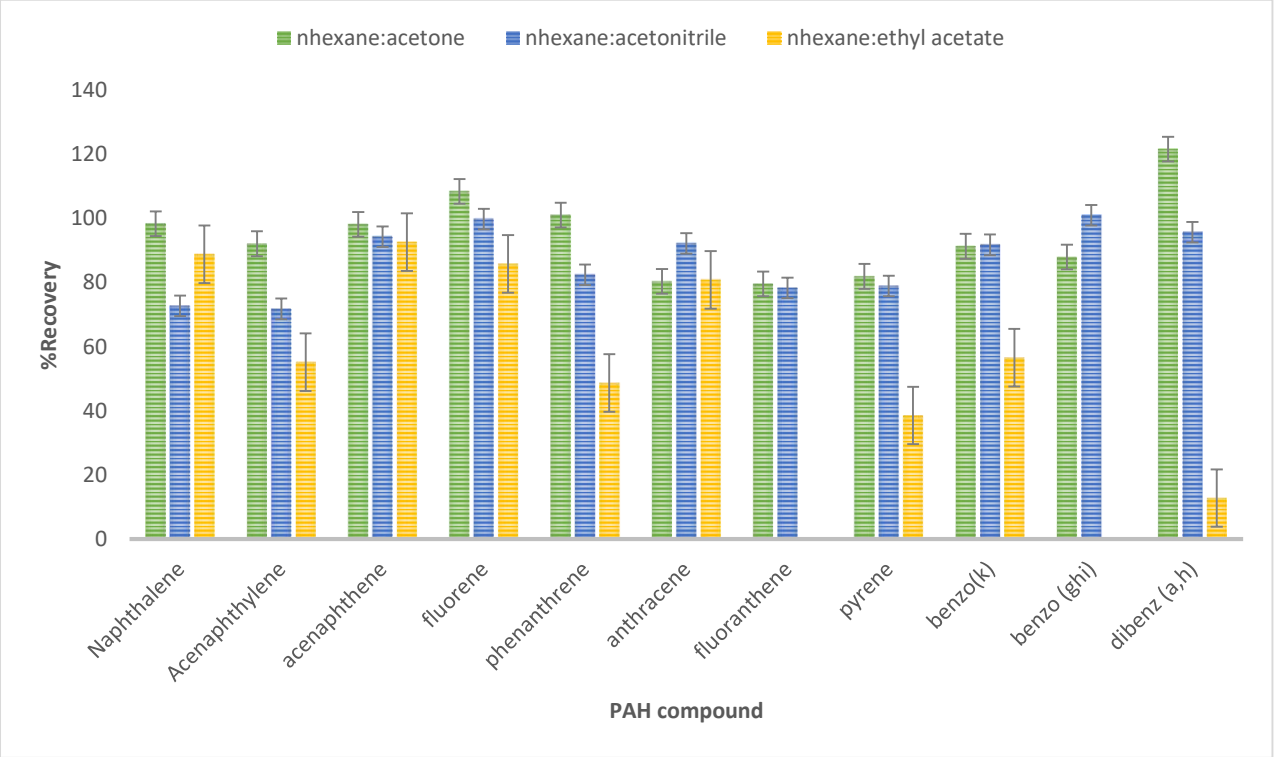

Figure 5.4: efficiency of different extraction solvents

5.6.3 Methods validation

Method validation is an important factor in ensuring both qualitative and quantitative analysis is achieved for the compounds of interest. The method validation for the developed UE and MAE methods was achieved by investigating accuracy, recoveries, limit of detection LOD, LOQ, sensitivity and selectivity/specificity (SANAS, 2017). The recovery test (n=5) gave a %recovery of 93.7% - 121% and 79.6% - 122% for UE and MAE respectively with a %RSD that is less than 10% (Table1) which are within the acceptable recovery range of 70 – 120% (Steiner et al, 2020). The student t-test showed that the was no significant difference (p = 0.24)

between the recoveries of both UE and MAE as shown by Table S5.5. The LOD and LOQ were calculated by using signal-to-noise ratios of 3 and 10, which ranged from 0.0250 µg/kg to 1.21 µg/kg & 0.0800 µg/kg to 3.54 µg/kg for MAE and from 0.0840 µg/kg to 0.215 µg/kg & 0.0190 µg/kg to 0.642 µg/kg for UE respectively (Table 5.1). The p values obtained shown in Table S5 for both LODs and LOQs showed no significant difference between their concentrations at 0.18 and 0.10 respectively. The results obtained showed that both microwave and ultrasonic extraction methods can be effectively used although UE was more sensitive and accurate for most compounds.

Table 5.1: Recoveries, LOD and LOQ for MWE and USE and correlation coefficient R^2

PAH Compound	LOD (µg/kg)		LOQ (µg/kg)		%Recovery ±%RSD		R^2 Values
	MAE	UE	MAE	UE	MAE	UE	
Naphthalene	0.162	0.095	0.434	0.245	92.3 ± 0.04	121 ± 0.02	0.9992
Acenaphthylene	0.113	0.051	0.323	0.136	98.1 ± 0.03	97.2 ± 0.01	0.9970
Acenaphthene	0.034	0.145	0.080	0.394	98.1 ± 0.01	107 ± 0.04	0.9969
Fluorene	0.762	0.084	1.238	0.234	88.4 ± 0.21	96.7 ± 0.02	0.9969
Phenanthrene	1.211	0.960	3.536	1.259	101 ± 0.33	94.9 ± 0.02	0.9988
Anthracene	0.138	0.019	0.310	0.049	113 ± 0.21	96.1 ± 0.004	0.9970
Fluoranthene	0.964	0.093	2.795	0.266	79.1 ± 0.26	93.7 ± 0.02	0.9991
Pyrene	0.968	0.091	2.814	0.259	81.9 ± 0.26	93.9 ± 0.02	0.9993
Benzo(k)fluoranthene	0.058	0.183	0.118	0.546	81.3 ± 0.29	96.0 ± 0.05	0.9982
Benzo(ghi)perylene	0.025	0.215	0.445	0.642	87.9 ± 0.26	96.6 ± 0.06	0.9989
Dibenz(a,h)anthracene	0.062	0.127	0.245	0.382	122 ± 0.13	118 ± 0.04	0.9977

5.6.4 PAHs concentration detected in uMsunduzi river sediment samples

The optimised MAE and UE conditions were also applied in sediment samples collected from uMsunduzi River at four different sampling namely: CD, CR, WH & BS. The samples from both the microwave and ultrasonic extraction methods were either filtered only or filtered then passed through SPE for further sample purification. The extraction methods and the effects of filtering and SPE-cleaning of samples were then compared to determine their efficiency. All the studied PAHs were detected in all the collected sediment samples at varied concentrations except for phenanthrene and fluorene (Table 5.2). Pyrene, a four-ring PAH showed high total concentration dominance at 24% followed by a five-ring (Dibenz (ah) anthracene) at 17% then fluoranthene, another four-ring PAH at 13%. All the PAH concentrations obtained were within the maximum allowable concentration of 3.0×10^3 µg/kg (for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benzo[ghi]perylene) except for dibenz (ah) anthracene which showed concentrations above the limit of 300 µg/kg in all four sampling sites. The anticipated dominance of these high molecular weight PAHs suggests the predominance of pyrolytic (incineration processes) contamination sources (Lucas et al, 2016).

The high pyrene concentrations were obtained in sampling site CD followed by WH, CR then BS, which is expected since CD is the industrial hub of Pietermaritzburg, WH is close to New England dumpsite and Willowtown factories. The HWM-PAHs are more hydrophobic than low molecular weight, which makes them adsorbed to soil matrix easily since they do not dissolve in water (Jorfi et al, 2013). The results obtained when using MAE and UE with the same purification technique (filtering or SPE-cleaning) were comparable while the results with the same extraction but different purification technique were different. The SPE-cleaned results gave high concentrations compared to the concentrations obtained when filtering only. The MAE-SPE gave concentrations ranging from 95.96 to 919.0 µg/kg while the UE-SPE gave concentrations at ranges of 96.26 - 926.0 µg/kg. The MAE-F and UE-F gave low concentration in comparison to the aforementioned with ranges from 21.75 to 380.6 µg/kg and 21.61 to 308.3 µg/kg respectively. The low concentrations obtained from filtered samples could be attributed to interferences in the sample as a result of inadequate purification of the sample that could possibly camouflage the analyte of interest (Dabrowska et al, 2003).

Table 5.2: Concentrations of PAHs in uMsunduzi river sediments

	Concentration (µg/kg)															
Sampling points	**CD**				**CR**				**WH**				**BS**			
Extraction method	**MW-F+SPE**	**UE-F+SPE**	**MW-F**	**UE-F**	**MW-F+SPE**	**UE-F+SPE**	**MW-F**	**UE-F**	**MW-F+SPE**	**UE-F+SPE**	**MW-F**	**UE-F**	**MW F+SPE**	**UE-F+SPE**	**MW-F**	**UE-F**
Naphthalene	95.96	96.26	96.06	96.14	99.27	100.4	121.6	119.03	138.3	173.2	96.53	96.95	102.7	122.7	97.34	97.56
Acenaphthylene	112.4	99.5	100.2	98.88	120.4	138.6	102.2	103.5	212.6	259.5	96.00	96.33	113.7	132	98.73	91.99
Acenaphthelene	185.5	156.5	98.00	98.14	220.0	231.6	174.9	183.5	412.6	319.8	318.9	275.3	171.5	179.7	nd	nd
Fluorene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Phenanthrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Anthracene	240.6	270.5	187.20	194.1	361.3	432.9	77.3	70.8	445.6	431.2	71.54	71.69	381.7	449	75.79	81.37
Fluoranthene	763.6	497.3	214.4	219.3	787.2	650.4	235.2	237.9	757.0	709.4	115.3	203.9	644.6	684.8	251.9	123.8
Pyrene	926.0	620.2	177.1	172.6	894.1	775.8	248.2	260.9	919.0	657.5	223.3	222	780.7	850.8	184.0	250.5
Benzo(k) fluoranthene	nd	nd	nd	nd	229.1	199.5	29.0	25.82	221.5	235.2	21.75	21.61	186.0	195.6	30.3	28.49
Benzo (ghi) perylene	162.3	200.9	81.78	95.27	421.0	414.5	109.8	110.5	301.4	254.6	241.4	243.1	221.7	223.5	110.5	110.4
Dibenz (ah) antharacene	353.6	346.2	238.0	298.4	411.0	411.3	380.6	301.4	725.1	729.6	269.7	308.3	355.8	353.4	181.5	170.3
Fl/(Fl+Py)	0.45	0.45			0.47	0.46			0.45	0.52			0.45	0.45		
LMW/HMW	0.29	0.39			0.29	0.39			0.43	0.46			0.37	0.40		

*F – Filtered

5.6.5 PAH concentration detected in Darvill sludge landfill

Table 5.3 shows the concentrations of PAHs obtained from filtered or SPE cleaned, microwaved or ultra-sonicated sludge samples at sampling points 7A and 14A of the Darvill sludge landfill used for sludge disposal by Darvill wastewater works. These two sampling points are the centre of the sludge land which makes them likely to be more concentrated that the rest of the points. All the targeted PAHs were detected except for phenanthrene. Pyrene dominated with a total concentration of 34% followed by fluoranthene and dibenz (ah) anthracene both at 12%, their concentrations were all within the maximum allowable limits of 5.0×10^4 µg/kg. Since the sampling sites were also dominated by HMW-PAHs, similar pollution sources are expected and also the fact that these two sampling are close to the New England dumpsite and Willowtown. The concentrations for microwave filtered and SPE-cleaned ranged from 55.79 to 728.4 µg/kg and 71.62 to 1656 µg/kg respectively, while for UE-filtered ranged from 54.39 to 535.4 µg/kg and UE SPE-cleaned 70.9 to 1443 µg/kg. The SPE cleaned samples in both sampling points gave higher concentrations compared to the filtered ones with both extraction methods giving relatable concentrations. The high concentration levels of PAHs in the sludge land could be a result of the fact that Darvill is designed to biologically treat inorganic nutrients and trace metal to meet the discharge limits, and therefore whatever contaminant entered the treatment process including PAHs can still be detected after the treatment has taken place (DWWW, 2013).

Table 5.3: Concentrations of PAHs in Darvill sludge landfill

PAH Concentration (µg/kg)									
Sampling point	**14A**				**7A**				
Extraction method	**MAE-F+SPE**	**MAE-F**	**UE-F+SPE**	**UE-F**	**MAE-F+SPE**	**MAE-F**	**UE-F+SPE**	**UE-F**	**Total %PAHs**
Naphthalene	102.0	113.2	101.9	124.1	286.1	154.6	361.5	166.0	7.5
Acenaphthylene	98.57	55.79	96.6	54.39	143.4	30.98	121.7	27.53	3.3
Acenaphthelene	178.0	88.8	178.6	75.3	182.8	93.8	176.1	69.8	5.6
Fluorene	173.5	149.4	189.8	146.0	244.4	167.7	248.2	153.2	7.8
Phenanthrene	nd	nd	nd	nd	nd	nd	nd	nd	0.0
Anthracene	71.62	98.14	70.86	69.50	411.2	89.2	506.5	99.8	7.5
Fluoranthene	1428	829.2	1194	402.5	651.7	257.9	693.7	461.1	12
Pyrene	1656	728.4	1443	535.4	798.5	249.1	837.6	219.5	34
Benzo(k) fluoranthene	nd	nd	146.3	212.1	nd	nd	nd	nd	1.9
Benzo (g,h,i) perylene	159.5	72.62	159.0	60.50	268.8	172.0	378.4	251.4	8.1
Dibenz (a,h) anthracene	295.2	233.9	291.3	246.9	451.6	158.5	422.9	161.8	12
Fl/(Fl+Py)	0.46		0.45		0.45		0.45		
LMW/HMW	0.30		0.61		0.21		0.63		

There are only a few studies done on PAHs in sediments, even fewer in sludge matrix especially in South Africa. A comparison of average concentration of PAHs obtained in the present study with other countries in Africa and the world is presented in Table 5.4. The maximum concentration obtained from sediment analysis of this study is higher (926 µg/kg) than the concentrations obtained by Ngubo et al (2021) at 42 µg/kg but lower than the ones obtained by Munyengabe et al (2018) at 69070 µg/kg at the same area of study (uMsunduzi river). However, pyrene was found at the highest average concentration in all three studies. The maximum concentration obtained in Kwa-Zulu Natal (69070 µg/kg) is higher than the ones obtained in other South African Provinces like Limpopo at 21600 µg/kg (Edokpayi et al, 2016) and Eastern Cape at 22310 µg/kg (Adeniji et al, 2019). The maximum concentration PAHs observed in South African studies is higher than those obtained in other African countries like Nigeria by Asagbra et al (2015) at 6561 µg/kg. The maximum concentrations obtained in African countries is higher than those reported in overseas countries like Mexico by Jaward et al (2012) at maximum concentration of 68 µg/kg. The PAHs obtained in the sludge analysis of this study showed lower concentrations than a study done in China (Hua et al, 2008) and Italy (Torretta & Katsoyiannis, 2013). The high concentration of PAHs in South Africa and other parts of the world emphasises the importance of monitoring the concentrations of PAHs in the environment.

Table 5.4: PAHs concentration in SA and other countries in the world

Region	Sediments (µg/kg)	Sludge (µg/kg)
Torretta & Katsoyiannis (2013) Italy	-	nd - 2645
Hua et al (2008) in China	-	33730 - 82580
Jaward et al (2012) Mexico	0.6 - 68	-
Asagbra et al (2015) Nigeria	11 - 6561	-
Edokpayi et al (2016) Limpopo	206 - 21600	-
Adeniji et al (2019) Eastern Cape	1107 - 22310	-
Munyengabe et al (2018) KwaZulu Natal	200 - 69070	-
Ngubo et al (2021) KwaZulu Natal	2.8 - 42	-
This study, KwaZulu Natal	21.6 – 926	27.5- 1656

5.7 PAH origin identification: diagnostic isomer ratio

Different diagnostic ratios (Table 5.5) such as Fl/(Fl + Pyr) and LMW/HMW were used to identify the original sources of PAHs, either petrogenic or pyrogenic. The pyrolytic sources are a result of incineration processes while petrogenic PAHs are a result of incomplete combustion of petroleum products. When the ratio (Fl/(Fl+Pyr)) is less than 0.40 the PAHs are said to be due to petrogenic sources and greater than 0.40 are a result pyrogenic sources (Montuori et al, 2021). When the LMW/HMW ratio is greater than 1 the PAHs are typical a result of combustion of petroleum contaminants and when it is less than 1 is due to combustion of grass, wood, or coal.

Table 5.5: Diagnostic ratios for PAHs

Diagnostic Ratio	Petrogenic	Pyrogenic	References
Fl/(Fl+Pyr)	≤0.4	≥0.4	(Montuori et al., 2021)
LMW/HMW	>1	<1	(Nasher et al, 2013)

The Fl/(Fl + Pyr) was determined to be greater than 0.4 for all sites indicating that the PAHs originated from incineration process. The LMW/HMW ratio was also found to less than 1 for all sampling sites in uMsunduzi river and Darvill sludge which suggests that the PAH were indeed a result of emissions of high-temperature combustion processes, which typically gives PAH mixtures with higher proportion of HMW PAHs.

5.8 Toxicity studies

5.8.1 Risk assessment

To characterize and assess the toxicity of PAHs, a number of factors were evaluated which included the effects range low (ERL), effects range median (ERM), the benzo(a)pyrene toxic equivalency quotient (TEQ) together with mutagenic equivalency quotient (MEQ) and the increment life cancer risk (ILCR). The ERL which corresponds to the 10^{th} percentile of data (concentration below with which effects that infrequently occur) and the effects range ERM corresponding to the 50^{th} percentile of data (effects that frequently occur) was used (Howard et al, 2021). The ERL and ERM values (Table 5.6) were used as guidelines where the average acceptable concentration levels of total PAH in sediments should be below the ERL, while the unacceptable concentration levels are the ones above the ERM. Average concentrations above ERM are indicative of toxic effects relative to the area of investigation (Howard et al., 2021). The TEQ and MEQ were calculated using equation 1 and equation 2. where C*i* is the PAH

concentration, TEF is the toxic equivalency factor and MEF is mutagenic equivalency factor (Adeniji et al., 2019). The ILRC was evaluated based on the dermal exposure risk (equation 3) since uMsunduzi river is used for recreational purposes such the Dusi canoe marathon and the sludge land is used for planting landscaping grass (lawn). The contamination by the PAHs in the studied sites was classified into 3 different groups, (1) not contaminated when the total PAH concentration of a site was less than 200 µg/kg, (2) weakly contaminated when the total concentration was between 200 – 600 µg/kg and heavily contaminated when it was greater than 1000 µg/kg (Bandowe et al., 2021).

$$TEQ = \sum Ci \cdot TEF \quad (1)$$

$$MEQ = \sum Ci \cdot MEF \quad (2)$$

Table 5.6: Min, max, average concentrations, ERL, ERM, TEQ and MEQ

PAHs	Min	Max	Mean	ERL	ERM	TEF	MEF	∑TEQ	∑MEQ	Total %PAHs
Naphthalene	96.3	173	123	97.5	112	0.001	-	0.49	-	3.52
Acenaphthylene	112	260	161	118	135	0.001	-	0.64	-	4.60
Acenaphthelene	180	413	252	181	209	0.001	-	1.01	-	7.22
Anthracene	271	449	400	319	439	0.01	-	16.0	-	11.4
Fluoranthene	685	787	748	706	760	0.001	-	2.99	-	21.4
Pyrene	851	926	897	864	907	0.001	-	3.59	-	25.7
Benzo(k) fluoranthene	nd	235	220	202	229	0.1	0.11	66.0	72.6	4.72
Benzo (ghi) perylene	201	421	287	208	262	0.01	0.19	11.5	180	8.20
Dibenz (ah) antharacene	354	730	463	354	384	1	0.29	1850	434	13.2

The average concentration levels were above the ERM for all PAHs in uMsundizi river sediments except for anthracene, fluoranthene, pyrene and benzo(k)fluoranthene, which had values between the ERL and ERM indicating mild toxic effects to the organisms are likely to occur. The average concentrations above the ERM especially for the HMW (benzo (ghi) perylene and dibenz (ah) anthracene) are indicative of toxic effects to the surrounding environment. The TEQ and MEQ were calculated by multiplying the PAH compound concentration with their corresponding TEF and MEF values respectively (CCME, 2010). The PAH TEQ and MEQ average ranged from 0.49 to 1850 µg/kg and 72.6 – 434 µg/kg respectively. The sum of toxic equivalency quotient (∑TEQ) of all PAH compounds was below the safe level of 600 µg/kg except for the ∑TEQ of dibenz(ah)anthracene indicating its high toxicity potency in the study area (CCME, 2010), therefore consideration of regulating combustion and ensuring all activities that cause these contaminants in the environment are controlled to ensure the safety of human and aquatic lives is vital. All the sites investigated were heavily contaminated with concentrations ranging from 2909 µg/kg (CD) - 4326 µg/kg (14a).

5.8.2 Increment life cancer risk

The increment life cancer risk was evaluated for the PAHs with probable carcinogenic potentials to humans as listed by the International Agency for Research on Cancer (IARC) and U.S Environmental Protection Agency (US EPA) which include Benzo(k) fluoranthene (BkF) and Dibenz(a,h)anthracene (DahA) (IARC, 2020). The BkF and DahA contributed 4.7% and 13% respectively with the highest concentration of DahA (730 µg/kg) recorded at WH while for sludge the highest concentration of DahA was recorded at 7a (452 µg/kg). Due to the high contribution of DahA to the total carcinogenic PAHs concentration it was used in the calculation of ILCR. The model equation and parameters used for the evaluation of ILCR were taken from literature and are tabulated in Table 5.7. The regulatory guidelines of the New York State Department of Health provided the classification of ILCR values where it was suggested that if the ILCR is less than 10^{-6} it is very low or negligible risk, between 10^{-6} to 10^{-4} it is low risk, greater than 10^{-4} to 10^{-3} it is moderate risk while greater than 10^{-3} to 10^{-2} is high risk and $\geq 10^{-1}$ is classified as very high risk (NYSDOH, 2007). The $ILCR_{derm}$ represents the increment life cancer risk via dermal contact (µg/kg/day) and was calculated using equation 3, the variables used are tabulated in Table 7.

$$ILCR_{derm} = \frac{Ci \times SA \times Kp \times ET \times EF \times ED \times CF}{BW \times AT} \qquad (3)$$

Table 5.7: Parameters used in the estimation of human cancer risk

Variables	Child	Adult	Reference
Concentration, Ci (µg/kg)	-	-	
Skin area exposed, SA (cm^2)	2800	5700	(Adeniji et al., 2019)(Howard et al., 2021)
Exposure duration, ED (years)	6	30	(Adeniji et al., 2019)
Body weight, BW (kg)	15	71.9	(World Data Info, 2019)
permeability coefficient, Kp (cm/h) (DahA)	2.3		(Adeniji et al., 2019)
Exposure time, ET (h/day)	8		(Howard et al., 2021)
Exposure frequency, EF (days/year)	313		(Howard et al., 2021)
Averaging time, AT (day)	64.63*365days = 23590		(Macrotrends, 2020)
Conversion factor, CF	1×10^{-6}		(Howard et al., 2021)
$ILCR_{derm}$ (µg/kg/day) -sediments	1.92×10^{-1}	4.98×10^{-1}	This study
$ILCR_{derm}$ (µg/kg/day) - sludge	1.01×10^{-1}	2.62×10^{-1}	

Life expectancy of an average South African is 65 years.

The $ILCR_{derm}$ values calculated with DahA concentrations for sludge and sediments were 1.01×10^{-1} and 1.92×10^{-1} for children and 2.62×10^{-1} and 4.98×10^{-1} for adults correspondingly. Both the sites exhibited very high potential risk of cancer to the inhabitants of the area, this suggests that the study area is not entirely safe for use even for recreational purposes. An article titled "Msunduzi River being flushed ahead of Dusi marathon" published by Mercury News also echoed the unsafeness of being exposed to the water of uMsunduzi river where Henley dam water was released in order to flush toxins in the river water prior to the annual Dusi canoe maranthon of 2022 (Magubane, 2022). It is worth to note that although adults have a higher $ILCR_{derm}$ values the probability of being dermally exposed to these carcinogenic contaminants for children is high even at trace level concentrations. Therefore, such pollution should be regulated in order to ensure the safety of human beings and the living organisms in these areas.

5.9 Conclusion

The optimised MAE and UE were found to be suitable extraction methods as they gave relatable concentrations (although UE gave slightly higher concentrations for majority of the sites) in the determination of PAHs in sediments and sludge samples, with the type of clean-up method (SPE or filter) being the deciding factor. Therefore, both methods can quantitative and qualitative analyse trace levels of PAHs in sediments and sludge, with SPE-cleaned samples giving higher concentration than filtered samples with pyrene dominating both environments. All the PAHs were within the maximum allowable concentration except for DahA in all sampling siteexcept for 14a. These PAHs in the investigated areas were proven to be a result of pyrolytic sources which is incineration of biomass such as burning of waste (New England

dumpsite), fumes from industries, etc. All the sampling sites showed to be highly contaminated with PAHs, with TEQ and MEQ of DahA suggesting carcinogenic and mutagenic risk. The ILCR values of DahA also suggested high potential carcinogenic risk through dermal exposure for children than adults . Therefore, the use of regulations such as NEMWA as a guideline for proper monitoring is significant.

5.10 References

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CHAPTER SIX:

Determination of PCs in water, sediment and sludge and ecological risk evaluation

Phenolic compounds occurrence in water, sediment and sludge, and ecological risk evaluation

6.1 Abstract

Ten phenolic compounds (PC) concentration levels were determined in river water, wastewater, sediments and sludge using solid phase extraction (SPE) microwave assisted extraction (MAE) and ultrasonic extraction (UE) coupled with GC-MS and their ecological risk was evaluated. All the extraction methods showed good sensitivity with limit of detection and limit of quantification values at 0.01 – 2.00 µg/L and 0.02 – 6.07 µg/L for SPE, 0.05 – 1.20 µg/kg and 0.17 – 3.17 µg/kg for MAE and 0.09 – 1.33 µg/kg and 0.26 - 3.54 µg/kg for UE correspondingly. The %recovery ranges of 75.2 – 112% (SPE), 80.9 – 110% (MAE) and 79.3 – 119% (UE) were obtained. The PC concentrations in wastewater matrix were within the maximum allowable limit except for 2.4-DCP at WDV4. In river water, CD showed the highest concentrations compared to other sampling points, however, they were all within the acceptable limits. The risk quotient values exhibited high ecological risk in majority of the sites, with ERM values indicating toxic effects to the surroundings likely to occur frequently. The probable carcinogenic risk potential was notably in the same risk category for both children and adults with $ILCR_{derm}$ values at 5.34×10^{-2} and 5.92×10^{-2} and1.26×10^{-2} and 1.39×10^{-2} for both sludge and sediment samples respectively.

6.2 Introduction

Phenolic compounds can either be halogenated or non-halogenated. Examples of halogenated include 2-chlorophenol, 2,4-dichlorophenol and non-halogenated are 2,4- dinitrophenol and 4-methylphenol (Vermerris & Nicholson, 2007). These phenolic compounds have numerous applications in the day-to-day lives of humans. In the chemical industry it is used in the production of other derivatives such as alkylphenols, cresols, aniline and resins (Michalowicz & Duda, 2007). Their presence in the oil, gas and coal industries is also noteworthy (Update, 2003). Phenolic resins are used heavily in appliances, timber and construction industries for various purposes. Dyes, textiles and explosive industries also uses phenols as raw material (Michalowicz & Duda, 2007). Non-polymer additives, polycarbonate plastics and epoxy resins

are manufactured from PCs such as bisphenol A (Huang et al., 2012). The PCs are also constituents of some pesticides and other insecticides (Gabriel de Oliveira et al, 2015). Therefore, phenolic compounds are discharged with effluents from such industries to wastewater treatment plants and they can also leach to various environmental matrices including ground waters, surface water bodies, air and soil which ultimately can lead into humans and other living organisms where it can cause endocrine disruption (Oketola & Fagbemigun, 2013).

The most likely route of exposure to phenol is through dermal contact either in the work environment or at home using ointments and other household products containing phenol. Phenol is a product of combustion of coal wood and municipal solid waste; therefore, residents near coal and petroleum fuelled facilities as well as residents near municipal waste incinerators may have increased exposure to phenol (Agency for Toxic Substances and Disease, 1997). Phenol is also a product of auto exhaust, and therefore, areas of high traffic likely contain increased levels of phenol (Agency for Toxic Substances and Disease, 1997).

The PCs are amongst the contaminants of major concern as they tend to persist in the environment over a long period of time, accumulate and exert toxic effects on living organisms and humans (National academics of SEM, 2015). PCs have been enlisted by the United States Environmental Protection Agency (US EPA) and the European Union (EU) as pollutants of priority concern. This enlistment is due to the fact that these chemicals are noted to be toxic and have severe short- and long-term effects on animals and humans well-being (Anku et al, 2017). They are carcinogenic even at low concentration causing damage to the red blood cells and the liver (Anku et al, 2017). More toxic than the original PC can form as a result of their interaction with microorganisms, inorganic and other organic compounds in water. (Anku et al, 2017). Chlorinated phenols may be life-threatening to humans even at low concentration, their presence gives a disagreeable smell and taste even at low concentrations (Patel & Vashi, 2015) and they are also powerful and corrosive contact poison as they rapidly absorbed through skin.

A study on Buffalo river monitoring conducted by Eastern Cape River Health Programme revealed that bio-accumulation of organic pollutants in the aquatic system leading to death of living organisms such as fish is a result of industrial effluents discharged from a textile industry

in King William's Town. This is a concern as the pollutants could possibly pass to human beings through the food chain (Coastal & Environmental Services, 2004). Another study on the Buffalo river in New York, USA reported that phenols in the water at concentrations above 5 µg/L may taint fish flesh, while a concentrations above 1 µg/L of chlorinated phenols are food-tainting (Date, 2012). It is therefore important that the presence of PCs is monitored in all environmental compartments. This work therefore aimed at determining the concentrations of PCs in water, sludge and sediment and assess their ecological risk to the environment.

6.3 Experimental

6.3.1 Chemicals and Reagents

The acetonitrile (99.9%), acetone (99.8%), ethyl acetate (99.9%), methanol (99.9%), nhexane (95%) and the EPA phenol mixture (100% purity) containing 4-chloro-3-methylphenol (4C-3MP), 2-chlorophenol (2CP), 3-methylphenol (3MP), 4-methylphenol (4MP), 2.4-dichlorophenol (2.4DCP), 2.6-dichlorophenol (2.6DCP), 2.4-dimethylphenol (2.4DMP), 2.4-dinitrophenol (2.4DNP), dinoseb, 2-methyl-4.6-dinitrophenol (2M-4.6DNP), 2-methylphenol (2MP), 2-nitrophenol (2NP), 4-nitrophenol (4NP), pentachlorophenol (PCP), phenol, 2.3.4.6-tetrachlorophenol (2.3.4.6TCP), 2.4.5-trichlorophenol (2.4.5TCP), 2.4.6-trichlorophenol (2.4.6TCP), 4-tert-octylphenol (4TOP) were all purchased from Sigma Aldrich (South Africa, JHB).

6.3.2 Instrumentation

Supelco SPE Vac-Elut unit, purchased from Sigma Aldrich (Germany, Darmstadt) was used to extract phenols in liquid samples and to clean-up extract from solid samples. A SPE was connected to a vacuum pump from Edwards, and Holdoph-Basis Hei-VAP Value rotor evaporator used to reduce sample volumes were bought from Holdoph (Germany, Berlin). Oasis hydrophilic-lipophilic balance (HLB) cartridge (60 mg, 3 mL) used as SPE sorbent was obtained from Waters (Ireland, Dublin). The quantitative analysis of the phenolic compounds was conducted using a Shimadzu Gas Chromatography (GC) coupled to a Mass Spectrometry (MS) QP-2010 series (Japan, Kyoto). A capillary column InertCap 5MS/Sil 30 m (I.D. = 0.25 mm, film thickness = 0.25 µm, Japan) was used for separation of the phenolic compounds. Nitrogen was used as the carrier gas with a flow rate of 1.32 mL/min and an injection temperature of 260 °C. The injection volume was 3 µL performed in a splitless mode. The oven 480 ˚C temperature was held at 50 °C for 0 min, then programmed to 180 °C at a rate of 25°C/min held for 1 min. Final ramp at a rate of 20 °C/min to 240 °C held for 1 min (Onyekwere

et al, 2019). For data acquisition, mass spectrum (MS) selected ion monitoring (SIM) mode was used to identify the analytes, the MS was operated in accordance to the mass (m/z) range of the analytes. For the target compounds, a normal qualitative and quantitative analysis was applied, using the characteristic fragment ions of each compound at the correct retention time.

6.3.3 Preparation of calibration standards

The mixture of the PCs 4-chloro-3-methylphenol, 2-chlorophenol, 3-methylphenol, 4-methylphenol, 2.4-dichlorophenol, 2.6-dichlorophenol, 2.4-dimethylphenol, 2.4-dinitrophenol, dinoseb, 2-methyl-4.6-dinitrophenol, 2-methylphenol, 2-nitrophenol, 4-nitrophenol, pentachlorophenol, phenol, 2.3.4.6-tetrachlorophenol, 2.4.5-trichlorophenol, 2.4.6-trichlorophenol, 4-tert-octylphenol was used for the calibration of the instrument. This mixture was diluted in acetonitrile to make a stock solution of 50 mg/L. The stock solution was then further diluted in a 50 mL volumetric flask to give 1.0 mg/L – 5.0 mg/L calibration standards.

6.3.4 Sampling of the water samples

The water and the solid samples were collected from along uMsunduzi river and Darvill wastewater works (DWWW) at Umgeni Water in Pietermaritzburg, Kwa-Zulu Natal. The water and sediment samples were collected from five different sampling points namely: Camps Drift, College Road, YMCA (no sediment sample), Woodhouse & Bishopstowe at uMsunduzi river. Darvill WWW samples were collected from four different points of the treatment process (WDV1-WDV4) and the sludge samples were collected in sludge landfill at two points (7A & 14A). The water samples were collected in 1L pre-washed glass bottles with Teflon caps between the 28th of April 2021 and 30th August 2021. The water samples were collected on the surface level (1-5 cm) of water and stored in the refrigerator at 4 ˚C until further analysis were performed. The sludge samples were collected near surface using a spade and stored in 1000 mL honey jars, while the sediment samples were collected on the basin of the river. The solid samples were then air dried in a fume hood for 14 days before grinding using a soil grinder and sieving through a 125 µm sieve to fine soil particles of less than 0.125 µm. These samples were then stored at room temperature until further analysis were performed.

6.4 Optimization of extraction methods

6.4.1 Solid phase extraction

In order to get optimum efficiency for SPE, parameters such as the conditioning solvent, and sample load volume were optimised. Different pairs of conditioning solvents were selected

based on their low toxicity. The conditioning solvent pairs tested for the optimisation (i) acetone:water, (ii) acetonitrile:water and (iv) ethyl acetate:water. The sample load volumes was assessed at 50 mL, 100 mL and 250 mL. The washing solvent (water) and eluting solvent (acetone:ethyl acetate) were kept constant while optimising the conditioning solvent or sample load volume separately. A 50 mL water sample spiked with the mixture of the PAHs of interest to make a final concentration of 1 mg/L was used during the optimization process.

Under optimum conditions, the SPE cartridge was conditioned by 5 mL of acetonitrile (3 mL) and de-ionised water (2 mL). The 50 mL water sample was loaded through the cartridge which was then followed by running 5 mL of de-ionised water through the cartridge to wash off impurities. The elution was achieved by running 3x1 mL of acetone: ethyl acetate (1:1) which was followed by reducing the extractant volume to 1mL under nitrogen flow. The eluates collected were injected into GC-MS for analysis.

6.4.2 Ultrasonication extraction

An optimisation of an UE method by Oluseyi et al (2011) to increase its efficiency was performed before its application to real sample to improve the recovery of the phenolic compounds from solid samples. The parameters optimised were the extraction solvents: n-hexane:acetone, n-hexane:water and n-hexane, and extraction time at 30 minutes, 45 minutes and 60 minutes. This optimisation was important in determining the optimum extraction solvent and time required for the maximum penetration of the solvent into the solids, so as to breakdown their surfaces, thus enabling efficient extraction of the targeted analytes.

Under optimum conditions, 0.5g of the soil/sludge sample was dissolved with 50 mL of 1:1 ratio of acetone:nhexane. The sample was then ultra-sonicated in the sonication bath for 30 min with occasional swirling to prevent the sample from sticking on the bottom of the flask and then rested. The extraction solution was filtered and 1 mL of the filtrate was collected while the rest was cleaned up using SPE and then the eluate was reduced with nitrogen gas to give a final volume of 1 mL. The eluate and filtrate collected were then injected into GC-MS for analysis. A student t-test was used for statistical analysis in determining the probability of significant difference between the recoveries of any of the parameter pairs used shown in Table S.

6.4.3 Microwave assisted extraction

The EPA 3546 method (Coker, 2007) was used for the extraction of phenolic compounds in solid samples. The optimisation in the MAE included the extraction solvents and time. The solvent mixtures were selected based on their ability to absorb microwave energy and the time it takes to breakdown the sample surface. The extraction solvent mixtures investigated were (i) nhexane:acetone, (ii) nhexane:water and (iii) nhexane:ethyl acetate, and the extraction times were at 10 minutes, 15 minutes and 30 minutes.

Under optimum conditions, 50 mL of equal parts (1:1) of nhexane:acetone was used to dilute 2g of the sediment/sludge sample in an extraction vessel. The extraction vessel was heated to 110 ˚C for 5 minutes, extracted for 15 minutes and then allowed to cool down. The vessel was then opened and the contents filtered, 1 mL was collected for analysis while the rest of the filtrate was cleaned-up using SPE and the eluate reduced with nitrogen gas to 1 mL. The filtrate and eluate collected were then injected into GC-MS for analysis.

6.5 Method Validation

6.5.1 Quality assurance

The validity of the method and quality of the results were determined through various parameters such as limit of detection, limit of quantification, linearity, accuracy, recoveries, sensitivity and selectivity/specificity. Linearity was determined through the correlation coefficient ($r^2 \geq 0.9900$) by analysing the calibration standards. Sensitivity was assessed to determine the capability of the method to discriminate between small differences of concentration of analytes (SANAS, 2017). The specificity/selectivity was done to evaluate the method's ability to respond to a specific analyte of interest in the presence of possible interferences. The selectivity was achieved through the use of mass spectrometer as a detector where it selectivity detected ions or fragmentations of the targeted phenolic compounds (Zhong et al, 2011). Specificity was determined by comparing spiked and non-spiked samples under SIM mode to make sure electron ionization detector source on the GC-MS instrument is set to target and identify only the compounds of interest through the use specific m/z values.

6.6 Results and Discussion

6.6.1 Effect of conditioning solvent of SPE

The effect of conditioning solvent was determined using organic solvent + H_2O mixtures, (acetonitrile + H_2O, acetone + H_2O and ethyl acetate + H_2O), this is because effective adsorption of the analyte onto the sorbent is dependent on the type of solvent selected for conditioning. Significant difference was observed between the recoveries of all conditioning solvent pairs with a p value ranging from 5.55 x 10^{-6} to 0.01 (Table S6.1). Acetonitrile + H_2O mixture was observed (Figure 6.2) to give the highest percentage recoveries ranging from 75.2 to 112% while acetone + H_2O gave 47.4 - 94.7% and ethyl acetate + H_2O gave 20.7 - 97.5% (Figure 6.1). These results were obtained when 50 mL was used as a sample loading volume. These findings indicated that acetonitrile with water was more effective in activating the functional groups of the sorbent surface with which interaction with the analytes was achieved. This promoted the adsorption of the analytes to the sorbent thus increasing the amount of analytes recovered. This could be attributed to the high polarity of acetonitrile compared to methanol and ethyl acetate (Munyengabe et al, 2018). Alonso et al also found that increasing the polarity of the solvent generally led to increased recoveries of phenols as a result of strong solvation power from the solvent that enables release of phenols from the matrix (Alonso et al, 1998).

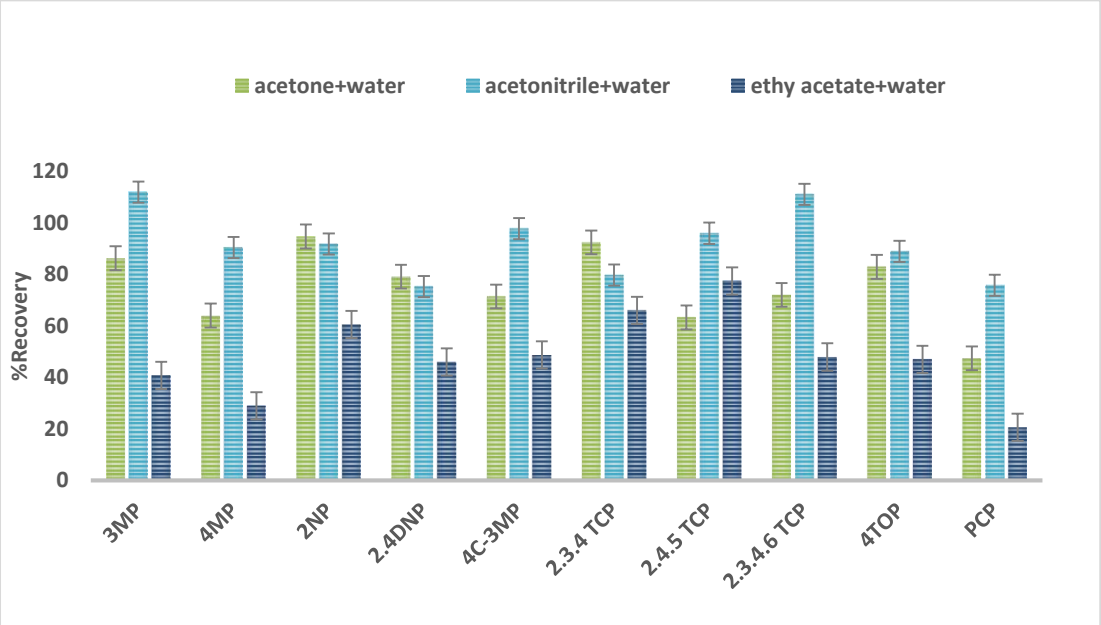

Figure 6.1: Effect of conditioning solvent on the recovery of phenolic compounds.

6.6.2 Effect of sample loading volume of SPE

The sample loading volume was assessed in order to obtain the volume that will allow high recoveries of the analytes without reaching the breakthrough volume. This is important because when the breakthrough is reached the sorbent is unable to retain the analyte and as a result the analyte is possibly washed away before the eluting step due to sample overloading (Dujaković et al, 2010). Therefore, sample volume of 50 mL, 100 mL and 250 mL spiked with 1 mg/L of the analyte was used in order to evaluate the effect on the recoveries of the analytes. The recoveries obtained while using a 50 mL sample volume were 75.2 - 112%, a slight decrease in recovery (70.0 – 106%) was observed when a 100 mL sample volume was used (Figure 6.2). When a 250 mL was used recoveries (27.0 - 66.4%) decreased significantly indicating that a breakthrough volume has been reached. Hence, an over saturation of the sorbent resulted in sample sipping through before the elution step leading to insufficient amount of the analytes available for interaction with the sorbent which then caused the low recoveries. Therefore, 50 mL sample volume was used as the optimum volume. Sample loading volume's t-test showed significant difference between 100 mL and 250 mL ($p = 8.75 \times 10^{-7}$) and 50 mL and 250 mL ($p = 3.26 \times 10^{-6}$) while no significant difference was observed between 50 mL and 100 mL with a p value of 0.52 (Table S6.2). Different sample volumes (10 - 100 mL) were tested by Masque et al, to determine the breakthrough volume for phenolic extraction by SPE and found 100 mL as the optimum volume although 50 mL gave relatable recoveries (Masqué et al, 1997).

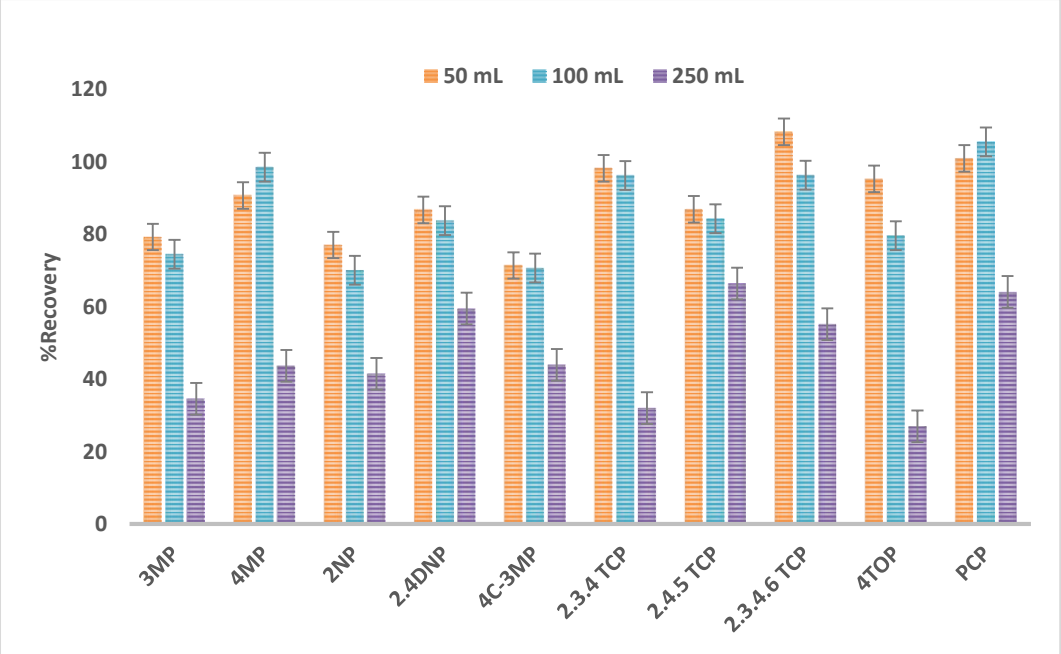

Figure 6.2: Effect of loading volume on recoveries of phenolic compounds

6.6.3 Effect of extraction solvent of UE

The extracting solvent and time are important factors when optimising UE as their effectiveness directly informs how much analyte is recovered from the matrix. Therefore, solvents (i) nhexane + acetone, (ii) nhexane + H_2O and (iii) nhexane + ethyl acetate were tested as the extraction solvents to assess their effects of the analytes recoveries. The nhexane + acetone gave high recoveries ranging from 79.3% to 119% (Figure 6.3). This indicates that this solvent was able to penetrates the solids, sufficiently broke it down and obtained enough surface area to extract the analytes from. The difference in the recoveries between the two pairs could be a result of acetone's high dielectric constant and polarity compared to that of ethyl acetate, which enables acetone to effectively extract while nhexane (non-polar) dissolves the analyte leading to high recoveries (Ncube et al, 2018) (Scaramboni et al, 2021). Kurata et al (2008), also successful in extracting PCs using the nhexane + acetone solvent pair. The recoveries (Table S6.3) were found to be significantly different for all solvent pairs with nH + ACT to nH + H_2O ($p = 0.001$), nH + ACT to nH + EA ($p = 8.02 \times 10^{-6}$) and nH + H_2O to nH + EA ($p = 0.002$).

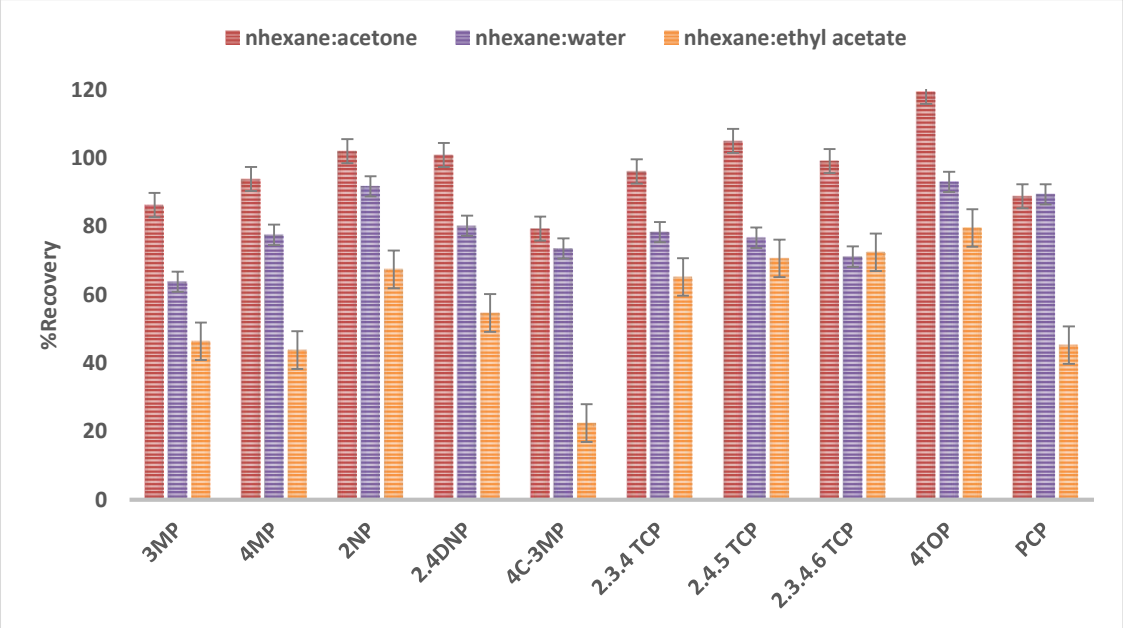

Figure 6.3: effect of extraction solvent on recoveries of phenolic compounds by UE

6.6.4 Effect of extraction time of UE

Different extraction times (30 minutes, 45 minutes and 60 minutes) were tested to ensure maximum extraction. It was found that 30 minutes gave the highest recoveries with a range of 88.3% to 116% (Figure 6.4). This indicates that this extraction time was long enough to

sufficiently extract the target analyte without excessive exposure to the irradiation which may degrade the contaminants in the sample and reduce the extraction rate. When the time was increased to 45 minutes a slight decrease (78.4% - 108%) in recovery with no statistical difference ($p = 0.19$), (Table S6.4) was observed. A further decrease in recovery (71.7% - 100%) was observed when the extraction was further increased to 60 minutes also with an insignificant difference ($p = 0.20$). Altemimi et al investigated 10 - 30 minutes extraction time for UE in extracting total phenolics where 25.67 minutes was found to be the optimum (Altemimi et al, 2016).

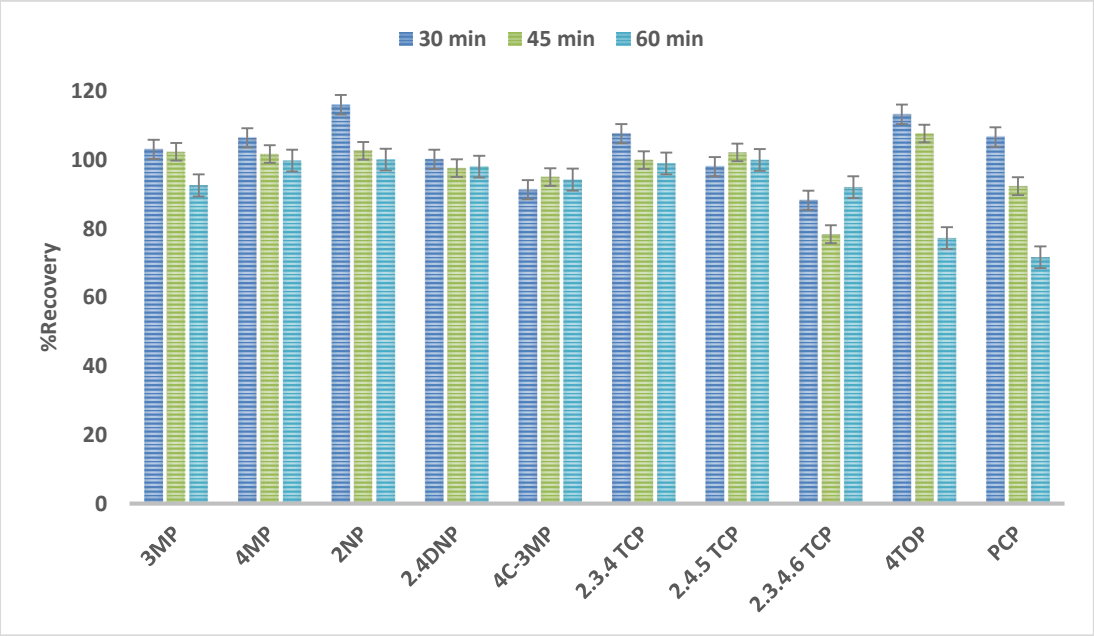

Figure 6.4: Effect of extraction time on the recoveries of phenolic compounds by UE.

6.6.5 Effect of extraction solvent of MAE

A number of factors are important to consider when selecting the correct extraction solvent in MAE, namely the solvents dielectric constant, polarity, solubility in water. The dielectric constant enables the solvent to absorb microwave energy and is directly proportional to its polarity which also affords the solvent strong solvation power (Scaramboni et al, 2021). The extraction solvent's solubility in water is also important, one solvent should be miscible in water which aids in penetrating the surface of the solid leading to a successful extraction. While the other solvent should be immiscible in water which will help in dissolving the extracted analyte in the liquid medium (Coker, 2007). Therefore, extraction solvent pairs of miscible-immiscible solvents ((i) n-hexane + acetone, (ii) n-hexane + water and (iii) n-hexane + ethyl acetate) were tested to access their effect on the recoveries of the analytes. The nhexane + acetone pair (i) give the highest percentage recovery (80.9% - 110%) (Figure 6.5). This was

attributed to its high polarity compared to other pairs where pair (ii) recoveries were 59.4% - 101% and (iii) were 18.9% - 67.1%, with significant statistical difference amongst all 3 pairs with p values at 0.04 (i to ii), 8.15 x 10^{-8} (i to iii) and 3.89 x 10^{-6} (ii to iii) (Table S6.5).

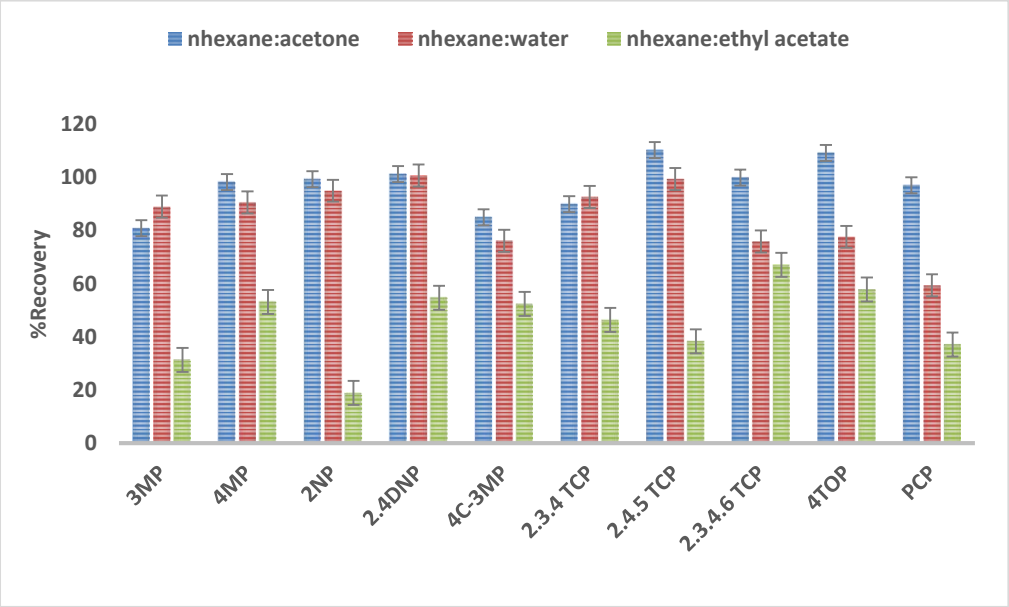

Figure 6.5: Effect of extraction solvents on the recovery of phenolic compounds by MAE

6.6.6 Effect of extraction Time of MAE

Extraction time is another important parameter considered in the optimisation of MAE, and 10 minutes, 15 minutes and 30 minutes were the times investigated to ensure sufficient extraction of phenolic compounds. To avoid underestimation of the analyte in the sample, time should be controlled appropriately, as short extraction time leads to low recoveries as a result of insufficient interaction between the solvent and solute, a case observed when 10 minutes (12.0% - 55.0%) was used as the extraction time (Figure 6.6). Increasing extraction to 15 minutes increased the recoveries of the analytes (75.6% to 120%), while a slight decrease was observed with further increasing time to 30 minutes (72.2% - 100%) except for 2-NP which showed about 20% decrease at 30minutes. This indicates that 30minutes was too long for 2-NP and possibly led to its degradation attributed to long-time exposure of analyte to radiation. Therefore, 15 minutes was chosen as the optimum extraction time with a significant difference from both extraction times (10 min, p = 2.06 x 10^{-8} and 30min, p = 3.58 x 10^{-8}) (Table S6.6). Valcarcel et al tested 1 - 15 minutes as extraction times where it was found that the recoveries increased with increasing time but decreased beyond 10 min (Valcarcel et al, 2000).

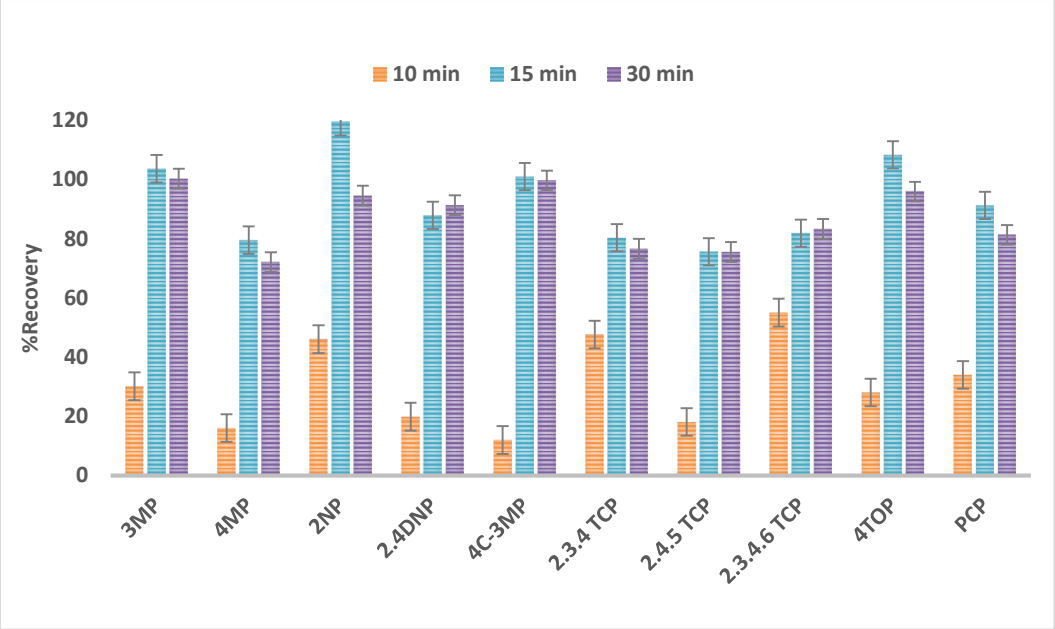

Figure 6.6: Effect of extraction time on the recoveries of phenolic compounds by MAE

6.7 Methods validation

In ensuring that both qualitative and quantitative analysis is accurately achieved for the phenolic compounds, method validation was performed. This was done through investigation of various parameters that include linearity, accuracy through a recovery test, specificity, limit of detection (LOD) and limit of quantification (LOQ) for all three methods (SPE, UE & MAE). Linearity was achieved through the analysis of phenolic compounds standards with a concentration range of 1.0 – 5.0 mg/L and linear coefficient (r^2) of ≥ 0.9900 were obtained for all compounds (Table 6.1). The recovery test (n=3) gave a %recovery of 75.2% - 112% for SPE, 79.3% - 119% for UE and 80.9% - 110% for MAE with a %RSD of less than 10% which are within the acceptable recovery range of 70.0% – 120% (Steiner et al, 2020). The LOD and LOQ were calculated by using signal-to-noise ratios of 3 and 10, which ranged from 0.01 to 2.00 µg/L and 0.02 to 6.07 µg/L for SPE, 0.08 to 1.33 µg/L and 0.24 to 3.54 for UE and 0.05 to 1.20 µg/L and 0.17 to 3.17 µg/L for MAE respectively.

The results obtained indicated that SPE, UE and MAE are sensitive and accurate for the analysis of phenolic compounds in the respective matrices. The MAE showed more sensitivity and accuracy than UE in the detection of PCs in solid samples. However, there was no significant difference between their LODs ($p = 0.97$) and LOQs ($p = 0.98$), and %recoveries ($p = 0.99$), (Table S7).

Table 6.1: Recoveries, LOD and LOQ and correlation coefficient R^2 for SPE, MAE and UE.

Phenolic compounds	SPE (µg/L)		UE (µg/kg)		MAE (µg/kg)		%Recovery ± %RSD			R^2
	LOD	LOQ	LOD	LOQ	LOD	LOQ	SPE	UE	MAE	
3-methylphenol	0.18	0.54	0.08	0.24	0.07	0.22	112 ±1.32	86.2 ±0.53	80.9 ±1.03	0.9922
4-methylphenol	0.01	0.02	0.54	1.65	0.58	1.74	90.4 ±7.41	93.8 ± 3.61	98.3 ±1.33	0.9919
2-nitrophenol	0.64	1.93	0.35	1.05	0.52	1.58	91.8 ±7.10	102 ±2.04	99.3 ±2.01	0.9903
2.4-dichlorophenol	0.33	0.99	0.20	0.62	0.29	0.76	65.2 ±6.90	101 ± 3.49	101 ±9.51	0.992
4-chloro-3methylphenol	0.37	1.12	0.83	2.50	0.76	2.29	97.8 ±2.59	79.3 ±0.21	85.1 ±0.55	0.9938
2.3.4-trichlorophenol	0.77	2.34	0.63	1.91	0.63	1.91	69.8 ±4.96	96.1 ±1.56	90.0 ±6.53	0.9921
2.4.5-trichlorophenol	0.11	0.34	0.49	1.49	0.53	1.62	95.9 ±1.19	105 ±1.92	110 ±6.66	0.9906
2.3.4.6-tetrachlorophenol	0.01	0.02	0.09	0.26	0.05	0.17	74.4 ±7.54	99.1 ±5.40	100 ±0.81	0.9915
4-tert-octylphenol	2.00	6.07	0.21	0.65	0.18	0.55	88.9 ±9.98	119 ±8.74	109 ±0.96	0.9946
Pentachlorophenol	0.51	1.55	1.33	3.54	1.20	3.17	111 ±7.41	88.9 ± 7.20	97.1 ±2.03	0.9963

6.8 Determination of phenolic compounds in water matrices

6.8.1 River and wastewater samples

The wastewater sample were collected at DWWW, Wdv1 (the influent), Wdv2 (pre-treated wastewater), Wdv3 (pre-chlorinated wastewater) and Wdv4 (the effluent where the chlorinated wastewater discharge from Darvill mixes with uMsunduzi river). The river samples were collected along Msunduzi river bank from five different sampling points, Camps Drift (CD), College road (CR), YMCA, wood house (WH) & Bishopstowe (BS) and they were extracted using SPE. The concentrations obtained in wastewater ranged from 4.00 to 1134 µg/L having 2-NP at 28.6%, 2.4DCP at 26.1% and 4C-3MP 9.83% total concentration (Table 6.2). The concentration range from 0.3 to 98.0 µg/L was observed in river samples with high total concentrations of 3-MP (19.9%), 2-NP (19.3%) and 4-TOP (13.8%). Although the PC concentrations were high, they were still within the maximum allowable limit except for 2.4-DCP in the final effluent (Wdv4) were it was detected at 50.1 µg/L surpassing the limit of 20.0 µg/L (U.S Dept of Health, 2002)(Australia DCCEEW, 2022). The limits were only applied to the final effluent, since it is the water that is discharged to the environment. The presence of PCs in water could be a result of pollution by an agricultural source as they are key ingredients in pesticides, insecticides and herbicides production (Anku et al, 2017)(Australia DCCEEW, 2022).

The biodegradation of pesticides such as pentachlorophenol, 2,4-dichlorophenoxyacetic acid, 4-chloro-2-methyl- phenoxyacetic acid leads to the formation of PCs that include 2-chlorophenol, 2,4-dichlorophenol and other chlorinated phenols while the methylated phenols such as 3-MP could be attributed to incineration residues and 4-tert-octylphenol a result of combustibles (Anku et al, 2017). The concentrations of PCs in wastewater were all observed to decrease as the treatment process progressed, this is because the biological treatment (microbial) employed at DWWW is able to break down the PCs into harmless products such as carbon dioxide, methane and water (Sikhakhane, 2001)(Kargi et al, 2005). In uMsunduzi river high concentrations of PCs were obtained in CD , this could be because CD is an industrial hub of Pietermaritzburg where there could be PCs varied applications in the chemical industry as they are used to make other chemicals, oil, gas and coal industries, dyes, textiles, etc (Luong et al, 2013).

Table 6.2: PC concentrations in the water matrices

Compounds	Concentration (µg/L)										
	Wastewater					River water					
Sample point	**WDV1**	**WDV2**	**WDV3**	**WDV4**	**%Total PCs**	**CD**	**CR**	**YMCA**	**WH**	**BS**	**%Total PCs**
3-methylphenol	561	206	114	48.2	**8.67**	98.0	5.73	2.61	2.41	3.08	**19.9**
4-methylphenol	89.8	38.2	43.9	39.6	**1.98**	19.5	nd	nd	nd	0.29	**3.52**
2-nitrophenol	1084	1018	894	67.0	**28.6**	96.9	7.48	nd	nd	3.96	**19.3**
2.4-dichlorophenol	1134	898	712	50.1	**26.1**	8.5	nd	7.26	5.98	11.2	**5.85**
4-chloro-3-methylphenol	345	334	304	70.1	**9.83**	47.8	nd	2.81	nd	nd	**9.00**
2.3.4-trichlorophenol	446	356	78.1	83.8	**8.99**	28.9	28.9	3.23	nd	nd	**10.9**
2.4.5-trichlorophenol	173	154	110	50.7	**4.55**	1.50	3.49	7.31	2.20	3.50	**3.21**
2.3.4.6-tetrachlorophenol	360	354	210	11.2	**8.73**	46.5	5.15	nd	7.32	4.66	**11.3**
4-tert-octylphenol	10.5	10.4	13.6	7.58	**0.39**	26.9	12.4	14.5	9.60	14.1	**13.8**
Pentachlorophenol	104	100	22.7	4.12	**2.16**	nd	3.80	3.54	3.76	7.37	**3.29**

*nd – not detected

6.8.2 PCs concentration detected in Darvill sludge landfill

The sludge samples were collected from Darvill sludge landfill, from sampling points 7A and 14A and extracted by means of microwave or ultra-sonication extraction which was followed by either filtering or filtering then SPE clean-up. These two sampling points are most likely to be more concentrated than the rest as they are the centre of the sludge. All the targeted PCs were obtained at varying concentrations in different sampling points using different extraction and clean-up methods. Both sampling points were found to have high concentrations of 2.4-DCP at 40.6% and 31.7%, PCP at 18.4% and 33.4% and 4-TOP 8.15% and 8.39% for sample point 7A and 14A respectively. The presence of PCs such as 2.4-DCP and PCP in this area could be attributed to the residual pollutants left after the wastewater treatment process since they are used in various products including detergents, dyes, resins, pharmaceutical drugs and wood preservatives. The presence of 4-TOP could be due to the fact that the sludge landfill is in close proximity to the New England dumpsite which hosts many combustible materials such as paper, wood, plastics which when ignited 4-TOP is one of the phenols formed. Also, anthropogenic activities which include processing of petroleum products, deforestation and steel manufacturing could be another reason for the presence of to these PCs (Michalowicz & Duda, 2007). The phenolic compounds were all detected at concentrations below the maximum allowable concentrations in the sludge landfill (U.S Dept of Health, 2002),(Department of Water Affairs-South Africa, 2013).

When microwave extraction was used with both filtering and filtering + SPE-clean-up, concentrations ranged from 1.00 to 198 µg/kg and 1.30 to 310 µg/kg respectively, while for UE-filtered ranged from 0.90 to 266 µg/kg and UE SPE-cleaned 1.30 to 132 µg/kg. When comparing the two methods used for extraction and clean-up, an underestimation of concentration was observed when the samples were only filtered, this could be due to interferences in the sample which possibly camouflages the analyte of interest (Dabrowska et al, 2003). The SPE cleaned samples in both sampling points gave higher concentrations with both extraction methods giving relatable concentrations.

Table 6.3: Concentrations of PC s in Darvill sludge landfill

Sample point	Concentration (µg/kg)									
	7A					14A				
Compounds	**UE F**	**UE-F +SPE**	**MW-F**	**MW-F + SPE**	**%Total PCs**	**UE-F**	**UE-F + SPE**	**MW-F**	**MW-F + SPE**	**%Total PCs**
3-methylphenol	2.60	2.69	3.03	6.08	**0.81**	4.77	5.92	6.10	13.73	**5.22**
4-methylphenol	11.8	12.64	2.75	4.67	**1.59**	0.86	0.88	1.00	1.28	**0.57**
2-nitrophenol	5.6	41.1	3.98	39.8	**7.44**	12.0	12.5	12.8	17.3	**7.93**
2.4-dichlorophenol	29.0	132	198	310	**40.6**	30.5	58.4	58.8	60.9	**31.7**
4-chloro-3-methylphenol	22.7	30.1	2.16	11.6	**3.84**	4.91	10.9	16.1	12.9	**6.33**
2.3.4-trichlorophenol	nd	nd	nd	20.2	**1.85**	nd	nd	nd	4.98	**1.32**
2.4.5-trichlorophenol	nd	5.04	nd	78.3	**7.67**	nd	nd	nd	nd	**0.00**
2.3.4.6-tetrachlorophenol	9.1	9.24	8.90	95.29	**9.62**	4.18	9.42	8.20	9.90	**5.13**
4-tert-octylphenol	266	13.2	13.1	75.4	**8.15**	8.7	8.8	19.0	22.8	**8.39**
Pentachlorophenol	34.9	105	16.8	94.7	**18.4**	66.8	69.0	33.6	56.6	**33.4**

*nd- not detected

6.8.3 Concentrations of PCs detected in uMsunduzi sediments

The sediment samples were collected from four different sampling namely: CD, CR, WH & BS at uMsunduzi river, and they were extracted with both MAE and UE. The samples after extraction were either filtered only or filtered then passed through SPE for further sample purification and their effects were then compared to determine their efficiency. The targeted PCs were detected in the collected samples at varied concentrations which is evident to the permeating nature of the pollutants. Pentachlorophenol showed high total concentration dominance at 32.5% with the highest concentration of 134 µg/kg detected at BS, followed by 4-tert-octylphenol at 18.4% with 54.3 µg/kg also at BS then 2.3.4-trichlorophenol at 13.7% with 122 µg/kg at WH. Although the concentrations were high they were still within their maximum allowable concentration of 21 000 µg/kg, 100 µg/kg and 800 µg/kg for pentachlorophenol, 4-tert-octylphenol and 2.3.4-trichlorophenol, respectively. However, the 134 µg/kg of 4-tert-octylphenol in BS exceeds the maximum concentration of 100 µg/kg. These high concentrations could be attributed to discharge of effluents from the manufacturers of resins, ethoxylates and ether sulphates which are used in basic products such as rubber for tyres, water-based paints, pesticide formulations. While 2.3.4-trichlorophenol and other chlorinated phenols are formed from the biodegradation of other chlorinated PCs such as pentachlorophenol and their use in pesticides, or wood, leather, or glue preservatives. The PCs detected in the sediments were mostly of low solubility in water which is evident to their recalcitrant nature to biodegradation, moreover, highly chlorinated phenols such as pentachlorophenol, usually persist longer in the environment, even more in soils and sediments (Santana et al, 2009).

The comparisons of the extraction and purification technique followed the same pattern as the analysis of PCs in sludge samples where the SPE-cleaned results gave higher concentrations compared to the concentrations obtained when filtering only. The MAE-F+SPE gave concentrations ranging from 3.44 to 134 µg/kg while the UE-F+SPE gave concentrations at ranges of 1.01 - 108 µg/kg. The filtered only samples gave low concentration in comparison to the aforementioned with ranges from 0.92 to 113 µg/kg for MAE-F and 1.73 to 98.5 µg/kg for UE-F which again necessitates the importance of the correct clean-up method. The instances where low concentrations were obtained with F+SPE compared to F only (i.e 2-NP at CD & CR using MAE) could be a result of oversaturation of the sorbent where the analyte is possibly washed away as a result. Morales et al study in extracting chlorophenols from sediment and

sludge emphasized the importance of having exhaustive clean-up, where SPE was used to remove co-extracted compounds (Morales et al, 2005)

Table 6.4: Concentrations of PCs detected in uMsunduzi river sediments

Sampling point	Concentration (µg/kg)																
	CD				CR				WH				BS				MW-SPE
PCs	UE-F	UE-F+SPE	MW-F	MW-F+SPE	UE-F	UE-F+SPE	MW-F	MW-F+SPE	UE-F	UE-F+SPE	MW-F	MW-F+SPE	UE-F	UE-F+SPE	MW-F	MW-F+SPE	%Total PCs
3-methylphenol	nd	nd	3.40	3.44	nd	nd	nd	15.9	nd	nd	6.27	8.12	3.74	4.07	nd	6.15	3.26
4-methylphenol	nd	nd	nd	nd	nd	nd	nd	11.3	nd	nd	3.48	3.75	nd	nd	nd	nd	1.46
2-nitrophenol	nd	nd	8.67	nd	nd	nd	14.6	3.71	nd	nd	3.06	5.48	nd	nd	6.62	6.78	1.55
2.4-dichlorophenol	nd	nd	nd	20.4	nd	nd	nd	31.0	nd	nd	16.6	16.1	nd	nd	nd	nd	6.55
4-chloro-3-methylphenol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.34	10.4	1.01
2.3.4-trichlorophenol	nd	nd	4.22	8.00	nd	nd	32.8	10.8	nd	nd	nd	122	nd	nd	nd	nd	13.7
2.4.5-trichlorophenol	nd	nd	48.5	72.4	nd	nd	nd	nd	3.47	3.57	nd	15.9	nd	nd	nd	nd	8.56
2.3.4.6-tetrachlorophenol	3.77	6.11	61.3	88.1	1.73	3.34	22.4	16.3	1.82	1.01	0.92	19.41	9.19	9.21	6.52	10.5	13.0
4-tert-octylphenol	16.9	7.30	9.71	34.5	6.44	31.4	46.6	53.1	23.3	13.2	23.5	48.3	26.4	29.6	24.7	54.3	18.4
Pentachlorophenol	31.3	32.4	30.0	32.9	22.5	24.5	61.8	77.2	16.9	17.5	64.4	91.0	98.5	108	113	134	32.5

*nd – not detected

A comparison of PCs concentrations of obtained in this study in river water, wastewater, sediments and sludge with studies in other South African provinces, African countries and the world. The results exhibited that the concentrations of PCs in river water of this study is higher (1.5 - 98 µg/L) than all the studies done in SA, including Western Cape (1.063×10^{-3} – 106×10^{-3}) reported by Olujimi et al, (2012), Eastern Cape (Buffalo river), with concentration levels of nd to 4.74 µg/L in Buffalo (Yahaya et al, 2019) and nd to 0.737 µg/L in Alice (Farounbi & Ngqwala, 2020). The concentrations obtained in South Africa (nd - 98 µg/L) was higher than the ones obtained in other African country Nigeria (Delta, Lagos and Anambra states) at nd-90.4 µg/L (Onyekwere et al, 2019) and 0.70 µg/L (Inam et al, 2019) also in Nigeria at New Calabar River. The concentration obtained in African countries (nd- 98 µg/L) are higher than countries overseas with concentrations ranging from 0.0065 to 0.0308 µg/L in Hungary, Danube River (Faludi et al, 2015), 0.06 to 14.1 µg/L in Northwest China, Weihe River (Y. Chen et al., 2021) and 0.053 to 0.339 µg/L in China, Yinma River Basin (Zhou et al, 2017). However, a study done by Faludi et al in Hungary (Lake Balaton) revealed higher concentrations of 20.6 - 888 µg/L than those obtained in African countries ((Faludi et al, 2015).

In wastewater, the concentrations observed in this study (4.12 - 83.3 µg/L) are higher than those obtained in other South African provinces like Eastern Cape (nd – 2.20 µg/L) as reported by Farounbi & Ngqwala, (2020). However, they are lower than those obtained by Olujimi et al, (2012) in Western Cape nd - 34.52×10^3. The South African country's wastewater is more polluted by PCs than Hungary with concentrations at nd – 0.074 µg/L (Faludi et al, 2015),

The sediment analysis of this study gave a maximum concentration of 134 µg/kg which was higher than concentrations of 2.20 – 24.5 µg/kg obtained by (Oketola & Fagbemigun, 2013) in Ogun and Ibeche River and 0.90 µg/kg (Inam et al, 2019) at New Calabar River both in Nigeria. The concentrations of PCs obtained in African countries are lower than a study done in Hungary (nd – 2910 µg/kg) by (Faludi et al, 2015) and in China at Weihe River (3.54×10^3 - 34.09×10^3 µg/kg) by (Y. Chen et al., 2021) but higher than those reported in China (0.0963 - 0.448 µg/kg) at Yinma River Basin by (Zhou & Sun, 2017).

In sludge sample, the concentrations obtained in this work (0.86 – 310 µg/kg) are lower than those reported in Japan (0.003 – 3600 µg/kg) by Kurata et al., (2008), but higher that those reported in Taiwan (2.72 – 6.35 µg/kg) by (Chung & Lee, 2008). The difference in

concentrations in the various matrices from different countries could be due to the increase in industrialisation which continues to cause pollution which has a direct negative impact on the ecosystem. The presence of PCs in various matrices from different countries indicate that these compounds are a worldwide problem and thus need to be continuously monitored. A study done by Olisah et al, (2021) on the "state of persistent organic pollutants in South African" indicated that it is difficult to understand the fate and behaviour of persistent organic pollutants as there is no enough data, therefore, more studies of such nature should be prioritized.

6.9 Ecological Risk Assessment (ERA)

The risk assessment of the PCs in this study in both water and solid matrices was undertaken in order to examine their ecological risk to the surrounding environment. A risk quotient, a ratio of measured environmental concentration (MEC) against the predicted no observed effect concentrations (PNECs) was determined for the water samples from both uMsunduzi river and Darvill WWW. The PNEC is the concentration of a chemical marking the limit at which below no adverse effects of exposure in an ecosystem are measured and it was calculated from the ratio between the acute toxicity data (Lethal Concentration 50 (LC50) or no observed effect concentration (NOEC) data) and an assessment factor (AF) of 1000 (Chen et al., 2021). The risk is then ranked as low if RQ <0.1, medium if RQ is 0.1≤RQ>1, and high if RQ ≥1. Most of the PCs exhibited high ecological risk in all the sampling points with RQ ≥1 values while some showed medium ecological risk including 3-MP in all sampling sites except in CD, 2-NP in BS, 4C-3MP and 2.3.4-TCP in YMCA and 2.4.5-TCP in all sampling sites except for CD and WH (Table 6.5). The 4-MP and 2.4.5 TCP at BS, CD and WH had RQ <0.1 values, indicating low risk. This potential risk evidence puts further emphasises on the importance of monitoring such contaminants in the environment.

The assessment of the ecological risk in regards to solid sample sites was evaluated by means of effects range low (ERL) and effects range median (ERM), this is due to the absence of toxicity data for phenolic compounds in sediment and sludge. The ERL which directly corresponds to the data's 10th percentile, concentration below with which effects that rarely occur while the effects range ERM corresponds to the data's 50th percentile with which effects that frequently occur (Howard et al, 2021). These values used as guidelines are indicative of acceptable concentration levels in both sludge and sediments which should be below the ERL, while the unacceptable concentration levels are the ones above the ERM. Any concentration

levels above ERM are indicative of toxic effects relative to the area of investigation. The average concentration levels were above the ERM for most of the PCs in uMsunduzi river sediments and the sludge landfill. The 2.4.5-TCP, 4-TOP and PCP had values between the ERL and ERM indicating that mild to high toxic effects to the organisms are likely to occur.

Table 6.5: Risk Quotient (RQ) for PCs in water matrices and min, max, average concentrations, ERL, ERM for solid matrices

PCs	PNECs (ug/L)	CD	CR	YMCA	WH	BS	WDV4	MIN	MAX	MEAN	ERL	ERM
3-MP	10.0	9.80	0.57	0.26	0.24	0.31	4.82	3.44	15.9	8.90	4.76	7.14
4-MP	10.0	1.95	-	-	-	0.03	3.96	1.28	11.3	5.25	2.02	4.21
2-NP	4.89	19.8	1.53	-	-	0.81	13.7	3.71	39.8	14.6	4.42	6.78
2.4-DCP	0.97	8.74	-	7.48	6.16	11.50	51.7	16.1	310	87.6	17.8	31.0
4C-3MP	15.0	3.18	-	0.19	-	-	4.67	10.4	12.9	11.6	10.6	11.6
2.3.4-TCP	1.00	1.11	1.11	0.12	-	-	83.8	4.98	122	33.2	6.19	10.8
2.4.5-TCP	1.00	0.06	0.13	0.28	0.08	0.13	50.7	15.9	78.3	55.5	27.2	72.4
2.3.4.6-TCP	0.80	58.0	6.40	-	9.20	5.80	14.0	9.90	95.3	39.9	10.2	17.9
4-TOP	0.01	2690	1240	1449	960	1414	758	22.8	75.4	48.1	28.6	50.7
PCP	0.02	-	190	177	188	368	206	32.9	134	81.1	44.7	84.1

6.10 Increment life cancer risk

The increment life cancer risk (ILCR) of the PCs with probable carcinogenic potentials to humans as listed by the International Agency for Research on Cancer (IARC) and U.S Environmental Protection Agency (US EPA) was evaluated with PCP concentrations. Only PCP was considered due to the absence of some parameters for other PCs. The ILCR a fraction of chronic daily dose/exposure (CDI) and cancer slope factor (CSF) was determined for children and adults. The equations (1 and 2) and parameters (Table 6.6) applied for ILCR evaluation were taken from literature. The ILCR of less than 10^{-6} is considered as negligible risk, between 10^{-6} to 10^{-4} is low risk, greater than 10^{-4} to 10^{-3} is moderate risk while greater than 10^{-3} to 10^{-2} is considered high risk and $\geq 10^{-1}$ is classified as very high risk (NYSDOH, 2007). The $ILCR_{derm}$ proved that both sludge landfill and sediments have a high potential risk of cancer through dermal exposure to the inhabitants of the area with values at 1.26 x 10^{-2} and 1.39 x 10^{-2} for children and 5.34 x 10^{-2} and 5.92 x 10^{-2} for adults respectively. This indicates that the study area is not safe for prolonged exposure even for recreational purposes. The low ILCRderm values for children shows that they have a higher probability of being dermally exposed to these carcinogenic contaminants even at trace level concentrations than adults. Therefore, regulation of such pollution should be a priority in order to ensure the safety of human beings and other living organisms in such areas.

$$CDI = \frac{Ci \text{ x SA x Kp x } EF \text{ x } ED}{BW \text{ x } AT} \qquad (1)$$

$$ILCR_{derm} = CDI \text{ x } CSF \qquad (2)$$

Table 6.6: ILCR parameters

Variables	Child	Adult	Reference
Average concentration, Ci (µg/kg)	-	-	
Skin area exposed, SA (cm^2)	2800	5700	(Howard et al., 2021)
Exposure duration, ED (years)	6.0	30.0	(Yahaya et al., 2019)
Body weight, BW (kg)	30.0	71.9	(World Data Info, 2019)
permeability coefficient, Kp (cm/h) (PCP)	0.48 x 10^{-3}		(Baynes et al, 2002)
Cancer slope factor, CSF (PCP)	4.00 x 10^{-2}		(Zhou et al., 2017)
Exposure frequency, EF (days/year)	365		(Howard et al., 2021)
Averaging time, AT (day)	65*365days = 23590		(Macrotrends, 2020)
$ILCR_{derm}$ (µg/kg/day) -sediments	1.39 x 10^{-2}	5.92 x 10^{-2}	This study
$ILCR_{derm}$ (µg/kg/day) - sludge	1.26 x 10^{-2}	5.34 x 10^{-2}	

* An average South African has a life expectancy of 65 years.

6.11 Conclusion

The SPE, UE and MAE methods coupled with GC-MS for determination of PCs in water, sludge and sediment were found suitable. SPE showed accuracy and sensitivity in detecting PCs even at trace concentration levels. Both MAE and UE gave higher concentrations when coupled with SPE as a clean-up method compared to the same method with filtering as a clean-up method. The PCs were all within the maximum allowable concentration except for 2.4-DCP in WDV4. All the sampling sites showed medium to high ecological risk in all the sampling points except for 4-MP and 2.4.5 TCP in BS, CD and WH. The $ILCR_{derm}$ values also suggested high potential carcinogenic risk through dermal exposure for fro children than adults. It is therefore, important to properly monitor levels of contaminants in the environment and using regulations such as NEMWA as guidelines to safeguard human, animal and environmental health.

6.12 References

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CHAPTER SEVEN: CONCLUSION AND FUTURE WORK RECOMMENDATION

7.1 Conclusion

The SPE, DLLME, UE and MAE methods coupled with GC-MS for the determination of PAHs and PCs in river water, wastewater, sludge and sediment were found suitable for this purpose. SPE proved to be more accurate and sensitive than DLLME for all the PAH (except for naphthalene) extraction in water matrices and was able to extract both analytes even at trace concentration levels. Both MAE and UE gave higher concentrations when coupled with F + SPE as a clean-up method compared to the same method with filtering only as a clean-up method. The water matrices were found to be contaminated with trace levels of PAHs with the river water showing high levels of contamination than the wastewater with LMW-PAH (naphthalene) dominating both environments. All PAHs in solid samples were found to be within the maximum allowable concentration excect for DahA in all sampling site but 14A with HMW PAH (pyrene) dominating. The PCs were all within the maximum allowable concentration except for 2.4-DCP in WDV4 in wastewater.

The PAHs in the water matrices were found to be a result of petrogenic sources such as combustion of diesel fuel (vehicle exhaust fumes), weathering of asphalt surfaces, oil spills and surface runoff. While in solid samples the PAHs were observed to be of pyrolytic sources which is incineration of biomass such as burning of waste (New England dumpsite), fumes from industries, etc. All the PAHs in the water matrices sampling sites demonstrated a low ecological risk to the surrounding environment except for acenaphthylene and fluorene in CR and anthracene in all the sampling sites. The solids were determined to be highly contaminated with PAHs, with TEQ and MEQ of DahA suggesting carcinogenic and mutagenic risk. The PCs in all the sampling sites showed medium to high ecological risk in all the sampling points except for 4-MP and 2.4.5 TCP in BS, CD and WH. The ILCR values of both DahA (PAHs) and PCP (PC), also suggested high potential carcinogenic risk through dermal exposure for children than adults. It is therefore, important to properly monitor levels of such contaminants in the environment in order to understand their fate and behaviour and using regulations such as NEMWA as guidelines is important so as to safeguard human, animal and environmental health.

7.2 Future work suggestions

- Further optimisation of DLLME to improve extraction in order to overcome the underestimation of concentrations.
- Further investigation of both PAHs and PCs to get a full picture on the concentration levels, fate and behaviour in all environmental spheres, i.e chemical fingerprinting to differentiate the sources of each PAH or PC
- Study different seasons to understand the changes in PAH or PC concentration
- Include toxicological tests to compensate for the analytical data
- Further investigation study that involves the fish in the river and its surrounding vegetation and the grass grown on the sludge landfill to prove the ecological and toxicity risk.
- Proper monitoring of industrial effluents which are the main sources of these contaminants.
- Extending the scope of wastewater monitoring to include PAHs and other POPs
- Continuous analysis of PAHs and PCs to increase the database available for PAHs and PCs to allow policy making and regulation of the PAHs and PCs in all the different environmental compartments in KZN and the African continent as a whole.
- Ensuring a proper application of the already existing regulation (i.e NEMWA) by municipalities and water boards

APPENDIX

Table S4.1: SPE conditioning solvents

t-Test: Two-Sample Assuming Unequal Variances						
Solvents	Acetonitri	Acetone+v	Acetonitri	Ethyl acet	Acetone+v	Ethyl acet
Mean	90.4	20.526	90.4	52.66	20.526	52.66
Variance	180.9333	149.2774	180.9333	174.6182	149.2774	174.6182
Observati	10	10	10	10	10	10
Hypothesi	0		0		0	
df	18		18		18	
t Stat	12.15962		6.329226		-5.64628	
P(T<=t) on	2.04E-10		2.89E-06		1.17E-05	
t Critical o	1.734064		1.734064		1.734064	
P(T<=t) tw	4.08E-10		5.78E-06		2.34E-05	
t Critical t	2.100922		2.100922		2.100922	

Table S4.2: SPE sample loading volume

t-Test: Two-Sample Assuming Unequal Variances						
Volume	*50 mL*	*100 mL*	*50 mL*	*200 mL*	*100 mL*	*200 mL*
Mean	53.8	89.2	53.8	63.26	89.2	63.26
Variance	66.84444	93.51111	66.84444	57.72933	93.51111	57.72933
Observati	10	10	10	10	10	10
Hypothesi	0		0		0	
df	18		18		17	
t Stat	-8.84018		-2.68027		6.670156	
P(T<=t) on	2.87E-08		0.007639		1.97E-06	
t Critical o	1.734064		1.734064		1.739607	
P(T<=t) tw	5.75E-08		0.015278		3.95E-06	
t Critical t	2.100922		2.100922		2.109816	

Table S4.3: DLLME extraction solvent

t-Test: Two-Sample Assuming Unequal Variances												
Solvent	EtOH + AC	Propanol -	EtOH + AC	dodecano	EtOH + AC	Heptanol -	Propanol -	dodecano	Propanol -	Heptanol -	dodecano	Heptanol -
Mean	6.45875	15.25	6.45875	73.18	6.45875	83.5	15.25	73.18	15.25	83.5	73.18	83.5
Variance	4.161698	162.4095	4.161698	13.86844	4.161698	41.82222	162.4095	13.86844	162.4095	41.82222	13.86844	41.82222
Observati	8	9	8	10	8	10	9	10	9	10	10	10
Hypothesi	0		0		0		0		0		0	
df	8		14		11		9		12		14	
t Stat	-2.0403		-48.315		-35.5273		-13.1414		-14.4762		-4.37309	
P(T<=t) on	0.037819		2.81E-17		5.28E-13		1.77E-07		2.92E-09		0.000318	
t Critical o	1.859548		1.76131		1.795885		1.833113		1.782288		1.76131	
P(T<=t) tw	0.075638		5.62E-17		1.06E-12		3.54E-07		5.83E-09		0.000637	
t Critical t	2.306004		2.144787		2.200985		2.262157		2.178813		2.144787	

Table S4.4: DLLME extraction solvent volume

t-Test: Two-Sample Assuming Unequal Variances												
Volume	10 uL	100 uL	10 uL	500 uL	10 uL	250 uL	100 uL	500 uL	100 uL	250 uL	500 uL	250 uL
Mean	4.9683	12.67045	4.9683	78.11	4.9683	77.083	13.9179	77.45455	13.9179	76.50273	78.11	77.083
Variance	51.14883	240.6019	51.14883	22.389	51.14883	22.28747	248.3162	24.87593	248.3162	23.7626	22.389	22.28747
Observati	10	11	10	10	10	10	10	11	10	11	10	10
Hypothesi	0		0		0		0		0		0	
df	14		16		16		11		11		18	
t Stat	-1.48262		-26.9718		-26.6114		-12.2066		-12.0463		0.485882	
P(T<=t) on	0.080167		4.56E-15		5.63E-15		4.88E-08		5.59E-08		0.316456	
t Critical o	1.76131		1.745884		1.745884		1.795885		1.795885		1.734064	
P(T<=t) tw	0.160335		9.12E-15		1.13E-14		9.76E-08		1.12E-07		0.632913	
t Critical t	2.144787		2.119905		2.119905		2.200985		2.200985		2.100922	

Table S5.1: UE extraction solvent

t-Test: Two-Sample Assuming Unequal Variances												
Solvents	*nH:ACT*	*nH:EA*	*nH:ACT*	*nH*	*nH:ACT*	*nH:H2O*	*nH:EA*	*nH*	*nH:EA*	*nH:H2O*	*nH*	*nH:H2O*
Mean	101.0283	96.22349	101.0283	55.16246	101.0283	78.13198	96.22349	55.16246	96.22349	78.13198	55.16246	78.13198
Variance	97.136	33.40754	97.136	2062.694	97.136	1265.621	33.40754	2062.694	33.40754	1265.621	2062.694	1265.621
Observati	11	11	11	11	11	11	11	11	11	11	11	11
Hypothesi	0		0		0		0		0		0	
df	16		11		12		10		11		19	
t Stat	1.394746		3.273226		2.057088		2.974541		1.664799		-1.32049	
P(T<=t) on	0.091078		0.003711		0.031045		0.006969		0.062073		0.101179	
t Critical o	1.745884		1.795885		1.782288		1.812461		1.795885		1.729133	
P(T<=t) tw	0.182156		0.007423		0.06209		0.013937		0.124147		0.202357	
t Critical t	2.119905		2.200985		2.178813		2.228139		2.200985		2.093024	

Table S5.2: UE extraction solvent volume

t-Test: Two-Sample Assuming Unequal Variances						
Volume	*25 mL*	*50 mL*	*25 mL*	*75 mL*	*50 mL*	*75 mL*
Mean	83.91633	102.0557	83.91633	85.36306	102.0557	85.36306
Variance	123.3827	186.8975	123.3827	127.1979	186.8975	127.1979
Observati	11	11	11	11	11	11
Hypothesi	0		0		0	
df	19		20		19	
t Stat	-3.41541		-0.30312		3.12386	
P(T<=t) on	0.001451		0.382465		0.002795	
t Critical o	1.729133		1.724718		1.729133	
P(T<=t) tw	0.002902		0.764931		0.00559	
t Critical t	2.093024		2.085963		2.093024	

Table S5.3: UE extraction time

t-Test: Two-Sample Assuming Unequal Variances						
Time	*30 min*	*60 min*	*30 min*	*90 min*	*60 min*	*90 min*
Mean	80	102.8182	80	98.54545	102.8182	98.54545
Variance	688.4	109.3636	688.4	137.0727	109.3636	137.0727
Observati	11	11	11	11	11	11
Hypothesi	0		0		0	
df	13		14		20	
t Stat	-2.67942		-2.14083		0.902712	
P(T<=t) on	0.009461		0.025185		0.188711	
t Critical o	1.770933		1.76131		1.724718	
P(T<=t) tw	0.018922		0.050369		0.377423	
t Critical tv	2.160368		2.144787		2.085963	

Table S5.4: MAE extraction solvent

t-Test: Two-Sample Assuming Unequal Variances						
	nH: ACT	*nH:CAN*	*nH: ACT*	*nH:EA*	*nH:CAN*	*nH:EA*
Mean	94.59753	87.14299	94.59753	62.18319	87.14299	62.18319
Variance	164.0235	113.4567	164.0235	725.475	113.4567	725.475
Observati	11	11	11	9	11	9
Hypothesi	0		0		0	
df	19		11		10	
t Stat	1.484229		3.316584		2.617612	
P(T<=t) on	0.077075		0.003436		0.012851	
t Critical o	1.729133		1.795885		1.812461	
P(T<=t) tw	0.154149		0.006873		0.025703	
t Critical tv	2.093024		2.200985		2.228139	

Table S5.5: Method Validation

t-Test: Two-Sample Assuming Unequal Variances						
	LOD MAE	*LOD UE*	*LOQ MAE*	*LOQ UE*	*%R MAE*	*%R UE*
Mean	0.408818	0.187545	1.121636	0.401091	94.82727	101.0091
Variance	0.214208	0.068753	1.659497	0.110042	182.1262	96.72091
Observati	11	11	11	11	11	11
Hypothesi	0		0		0	
df	16		11		18	
t Stat	1.379625		1.796502		-1.22781	
P(T<=t) on	0.09334		0.049949		0.117667	
t Critical o	1.745884		1.795885		1.734064	
P(T<=t) tw	0.18668		0.099897		0.235335	
t Critical tv	2.119905		2.200985		2.100922	

Table S6.1: SPE conditioning solvent

t-Test: Two-Sample Assuming Unequal Variances						
Solvents	*CT + Wate*	*CN + Wate*	*CT + Wate*	*EA + Wate*	*CN + Wate*	*EA + Water*
Mean	75.35219	91.84943	75.35219	48.42365	91.84943	48.42365
Variance	214.9719	168.349	214.9719	279.378	168.349	279.378
Observati	10	10	10	10	10	10
Hypothesi	0		0		0	
df	18		18		17	
t Stat	-2.66459		3.829972		6.489944	
P(T<=t) on	0.007897		0.000613		2.77E-06	
t Critical o	1.734064		1.734064		1.739607	
P(T<=t) tw	0.015794		0.001227		5.55E-06	
t Critical t	2.100922		2.100922		2.109816	

Table S6.2: SPE sample load volume

t-Test: Two-Sample Assuming Unequal Variances						
sample lo	*50 mL*	*100 mL*	*50 mL*	*250 mL*	*100 mL*	*250 mL*
Mean	89.49701	85.97331	89.49701	46.78161	85.97331	46.78161
Variance	133.3317	158.2701	133.3317	191.4046	158.2701	191.4046
Observati	10	10	10	10	10	10
Hypothesi	0		0		0	
df	18		17		18	
t Stat	0.652535		7.49582		6.627688	
P(T<=t) on	0.261152		4.38E-07		1.6E-06	
t Critical o	1.734064		1.739607		1.734064	
P(T<=t) tw	0.522303		8.75E-07		3.2E-06	
t Critical t	2.100922		2.109816		2.100922	

Table S6.3: UE extraction solvent

t-Test: Two-Sample Assuming Equal Variances						
solvents	*nH:ACT*	*nH:wtr*	*nH:ACT*	*nH:EA*	*nH:wtr*	*nH:EA*
Mean	97.07189	79.54612	97.07189	56.78174	79.54612	56.78174
Variance	123.6631	88.33536	123.6631	303.1559	88.33536	303.1559
Observati	10	10	10	10	10	10
Pooled Va	105.9992		213.4095		195.7456	
Hypothes	0		0		0	
df	18		18		18	
t Stat	3.806368		6.167044		3.638269	
P(T<=t) or	0.000646		4.01E-06		0.00094	
t Critical o	1.734064		1.734064		1.734064	
P(T<=t) tw	0.001293		8.02E-06		0.00188	
t Critical t	2.100922		2.100922		2.100922	

Table S6.4: UE extraction time

t-Test: Two-Sample Assuming Unequal Variances						
	30 min	*45 min*	*30 min*	*60 min*	*45 min*	*60 min*
Mean	103.095	97.99376	103.095	92.48334	97.99376	92.48334
Variance	78.57531	65.96704	78.57531	100.9449	65.96704	100.9449
Observati	10	10	10	10	10	10
Hypothes	0		0		0	
df	18		18		17	
t Stat	1.34178		2.504542		1.348781	
P(T<=t) or	0.098175		0.011049		0.097551	
t Critical o	1.734064		1.734064		1.739607	
P(T<=t) tw	0.19635		0.022098		0.195102	
t Critical t	2.100922		2.100922		2.109816	

Table S6.5: MAE extraction solvent

t-Test: Two-Sample Assuming Unequal Variances						
Solvents	*nH:ACT*	*nH:wtr*	*nH:ACT*	*nH:EA*	*nH:wtr*	*nH:EA*
Mean	97.97189	85.67515	97.97189	45.72035	85.67515	45.72035
Variance	139.4151	170.1539	139.4151	204.3506	170.1539	204.3506
Observati	10	10	10	10	10	10
Hypothesi	0		0		0	
df	18		17		18	
t Stat	2.210097		8.911849		6.528906	
P(T<=t) on	0.020145		4.07E-08		1.94E-06	
t Critical o	1.734064		1.739607		1.734064	
P(T<=t) tw	0.040289		8.15E-08		3.89E-06	
t Critical t	2.100922		2.109816		2.100922	

Table S6.6: MAE extraction time

t-Test: Two-Sample Assuming Unequal Variances						
Time	*10 min*	*15min*	*10 min*	*30 min*	*15 min*	*30 min*
Mean	30.68833	92.93372	30.68833	87.11028	92.93372	87.11028
Variance	219.2146	213.1972	219.2146	111.4924	213.1972	111.4924
Observati	10	10	10	10	10	10
Hypothesi	0		0		0	
df	18		16		16	
t Stat	-9.46582		-9.8113		1.021987	
P(T<=t) on	1.03E-08		1.79E-08		0.160996	
t Critical o	1.734064		1.745884		1.745884	
P(T<=t) tw	2.06E-08		3.58E-08		0.321992	
t Critical t	2.100922		2.119905		2.119905	

Table S6.7: UE/MAE method validation

t-Test: Two-Sample Assuming Unequal Variances						
MV	*LOD UE*	*LOD MAE*	*LOQ UE*	*LOQ UE*	%R UE	%R MAE
Mean	0.475671	0.480957	1.391276	1.401763	97.07189	97.15046
Variance	0.150772	0.121811	1.119831	0.936662	123.6631	90.27784
Observati	10	10	10	10	10	10
Hypothesi	0		0		0	
df	18		18		18	
t Stat	-0.03202		-0.02312		-0.01699	
P(T<=t) on	0.487405		0.490903		0.493317	
t Critical o	1.734064		1.734064		1.734064	
P(T<=t) tw	0.97481		0.981805		0.986634	
t Critical t	2.100922		2.100922		2.100922	