Assessment of the Impact of Reforestation on Soil and River Water Quality Based on Organic Chemical Pollutants

by

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PREFACE

The research contained in this dissertation was completed by the candidate while based in the Discipline of Chemistry, School of Chemistry and Physics of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, South Africa. The research was financially supported by the eThekwini municipality.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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Signed: Dr B. Moodley (Supervisor)

Date: 4 December 2018

DECLARATION 1: PLAGIARISM

I, Vishalan Pillay, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

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(v) where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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Signed: Vishalan Pillay

Date: 4 December 2018

Conferences and other participations:

(a) Oral presentation to eThekwini and D'RAP representatives at UKZN, Westville Campus, Durban, South Africa, 22nd April 2016

(b) Poster presentation at the UKZN Research day, Howard College Campus, Durban, South Africa, 2016

(c) Oral presentation at the eThekwini and D'RAP year end symposium, Buffelsdraai, Durban, South Africa, 2016

(d) Poster presentation at the UKZN Research day, Westville Campus, Durban, South Africa, 2017, 1st place in the master's poster section

(e) Presented at the eThekwini and D'RAP year end symposium, Durban, South Africa, 2017

(f) Poster presentation at the Analitika conference, Limpopo, South Africa, 2018

(g) Poster presentation at the UKZN Research day, Westville Campus, Durban, South Africa, 2018

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Abstract

Forests are a natural resource and are influential in most countries as they are a source of food, clothing, and form of shelter for many organisms. These sections of forested land have been sacrificed for the development of urban areas, making way for agriculture, cities and the ever increasing human population. Some of the detrimental effects associated with deforestation are as follows: loss of wildlife and fish habitats, increased nutrient and sediment loads in nearby rivers, and ultimately increases in greenhouse gas emissions. Reforestation refers to the planting of trees so as to replenish an area that was previously a forest but due to anthropogenic effects, such as land deforestation, resulted in its deterioration. This project aimed to assess the impact of the eThekwini Municipality reforestation project on the quality of the soil within the reforestation sites, and water from the nearby rivers (White and Black Mhlasini Rivers) situated at the reforested Buffelsdraai area in KwaZulu-Natal. The levels of organic pollutants were assessed from the analysis of soil, sediment and river water. Selected polyaromatic hydrocarbons (PAHs) and pesticides, which had been previously utilised at this site when it was a sugarcane farm, were analysed. The sixteen PAHs analysed were naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[i]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene which are on the Environmental Protection Agency (EPA) list of priority pollutants. The pesticides analysed were hexazinone, oxamyl, and acetochlor. The soil and sediment samples were extracted using ultrasonication, and liquid-liquid extraction was utilised for the water samples. Gas chromatography mass spectrometry (GCMS) and liquid chromatography mass spectrometry (LCMS) were used to analyse the PAHs and pesticides, respectively. PAH recoveries on the GCMS ranged between 60-110%, and pesticide recoveries on the LCMS were between 83-113%. The PAH LOD values were between 0.30–0.69 μg g^{-1} and between 0.17-0.32 μg g^{-1} for pesticides. PAH LOQ values ranged between 0.99-1.9 μg g^{-1} and between 0.56-1.33 μg g^{-1} for pesticides. The total PAH concentrations determined were between 4.258 – 6.426 μg g^{-1} in the soil samples, 2.210 – 13.900 μg g^{-1} in sediment, and 6.360 – 85.468 ng L^{-1} in river water. The total pesticide concentration was between 1.271 – 1.742 μg g^{-1} in soil, 0.197 – 1.175 μg g^{-1} in sediment, and 0.792 – 12.950 ng L^{-1} in river water. A comparison between the soil samples and the control,

showed that reforestation is potentially reducing the concentration of organic chemical pollutants. The water and sediment samples also provided potential evidence of the positive impact of reforestation, as it revealed the concentration of pollutants to be lower within the reforestation boundaries and higher outside the reforestation boundary. The most abundant PAH determined in the samples was fluoranthene, which could possibly be due to this hydrocarbon being the most abundant aerosol in the atmosphere. Source apportionment analysis showed that most PAHs originated from pyrolytic sources, which was from burning of sugarcane. The total concentration for specific PAHs was above the threshold value for most sampling sites according to Canadian environmental guidelines. However, reforestation was shown to potentially be reducing these pollutant concentrations. The findings from this study will assist the neighbouring communities and eThekwini in future planning for the extension of existing or development of new reforestation sites.

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Table of Contents

List of Tables

List of Figures

Abbreviations

ARP	Acetochlor Registration Partnership
ATSDR	Agency for Toxic Substance and Disease Registry
BM	Black Mhlasini
CDM	Clean Development Mechanism
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DSW	Durban Solid Waste
GCMS	Gas chromatography and mass spectrometry
HMW	High molecular weight
HPLC	High performance liquid chromatography
LCMS	Liquid chromatography mass spectrometry
LLE	Liquid-liquid extraction
LMW	Low molecular weight
LOD	Limit of detection
LOQ	Limit of quantitation
MCL	Maximum contaminant level
MRL	Minimum risk level
OCPs	Organochlorine pesticides
PAHs	Polyaromatic hydrocarbons
PCBs	Polychlorinated biphenyls
POPs	Persistent Organic Pollutants
RT	Retention time
SPE	Solid-phase extraction
TDS	Total dissolved solids
UNEP	United Nations Environment Programme
UNFCC	United Nations Framework Convention on Climate Change
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation
WM	White Mhlasini

Chapter 1: Introduction

1.1 General overview

Forests are a natural resource, serving as an influential source of food, clothing, medicine and shelter for many organisms throughout the world (Wangpimool et al., 2013). However, forested land is continuously sacrificed for the development of agricultural lands, cities and the ever increasing human population (Cunningham et al., 2015). This is evident in the Philippines, where approximately 59% of the official forest lands are presently covered in grass or shrubs (Le et al., 2014). The loss of Philippino forest cover is attributed to heavy logging and agricultural expansion over the last century. Widely referred to as deforestation, the removal of trees, primarily affects the climate, hydrology, soil and biodiversity of the surrounding environment, while secondary impact is on societies and the economy (Cunningham et al., 2015). Deforestation impacts on the environment through the loss of wildlife and fish habitats, increased nutrient and sediment loads in nearby rivers, increases in greenhouse gas emissions, and changes to the hydrological cycle (Cunningham et al., 2015). Furthermore, it allows for increased surface erosion, resulting in pollutants and other potentially hazardous substances entering water systems leading to the degradation of water quality (Pilgrim et al., 2015).

One way of curtailing the effects brought about by the removal of trees is through reforestation. It promotes biodiversity and reduces topsoil erosion that occurs from storm water runoff (Ku et al., 2016). Reforestation refers to the planting of trees, as a means to replenish an area that was previously a forest but had deteriorated as a result of anthropogenic activities, such as land deforestation (Schirmer and Bull, 2014). Forest restoration is motivated by increasing concerns surrounding climate change as reforestation allows for the sequestering of carbon, which is part of the Clean Development Mechanism (CDM) of the United Nations Framework Convention on Climate Change (UNFCC) (Schirmer and Bull, 2014). The CDM covers two objectives of the Kyoto Protocol, which includes the reduction of greenhouse gas emissions and the sustainable development of the host country (Sutter and Parreño, 2007). However, reforestation cannot proceed without adequate funds or technical expertise (Marliana and Rühe, 2014). In 2011, a National Greening Program became a government priority as the Philipino president implemented

a plan to plant an estimated 1.5 billion trees during 2011-2016, resulting in 1.5 million ha of forest cover (Le et al., 2014). Largescale reforestation in South Africa has not taken place as compared to global projects, but presently there are smaller projects such as the Platbos indigenous forest and KwaNibela community sand reforestation programme.

One of the main problems associated with restoration projects is that research is not conducted to benefit local communities, especially in rural areas where planted trees will be removed if the reforestation project fails to meet the needs of the community (Le et al., 2014). Some of the other challenges accompanying reforestation projects include: poor quality of planting sites; incorrect species matching; objections from local communities; lack of interest of stakeholders in the reforestation planning, and occurrence of forest fires (Ancog et al., 2016). Some skepticism regarding reforestation is due to the unknown success of reforestation projects in providing ecological and socio-economic benefits, as most projects have either completely failed or provided little success mainly due to the newer trees succumbing to the pressures that resulted in its initial loss, such as reforested land being converted into agricultural land (Le et al., 2014). The Philippine's reforestation project was accountable for the planting of an estimated 1.7 million ha of forests during 1960-2002 but only half of the trees survived due to the expansion of the agricultural sector (Le et al., 2014).

The concept of reforestation is being welcomed and exercised more regularly by countries, and has resulted in a decrease in the net loss of forests globally. Between 1990-2000, 8.3 million ha of forest were removed per year compared to the 5.2 million ha lost per year during 2000-2010 (Le et al., 2014). Reforestation of agricultural lands has become increasingly prominent with the intention to sequester carbon in woody biomass and more often contribute to reduced greenhouse gas emissions (England et al., 2016). Another successful example of forest restoration can be seen in Malaysia, with tropical food crops and fruit yielding trees helping to return tropical food trees to their natural habitat thus providing satisfactory food for locals as well as strengthening the economy through food exports (Yacob et al., 2012). Different reforestation practices exist as observed in Australia, where mixed species plantations were established on small scale, tropics and subtropics were replenished with fast growing timber trees, and a combination of trees and

shrubs (Kanowski et al., 2003). An advantage of planting trees is that they sequester more atmospheric carbon compared to crops and pastures in terms of their biomass (Pan et al., 2011).

The magnitude of the impact of organic pollutants including persistent organic pollutants (POPs) on the environment and human health can be evaluated by assessing the effect of anthropogenic activities within the region of contamination (Masood et al., 2016). Persistent Organic Pollutants (POPs) are chemicals introduced into the environment through anthropogenic activities (Harrad, 2012). These pollutants are considered persistent due to their toxicity, bioaccumulation and long-range transport potential (Buccini, 2003). These chemicals accumulate in soils, sediments, and negatively affect human health through accumulation in the ecological food chain (Harrad, 2012). The United Nations Environment Programme (UNEP) Stockholm Convention on POPs was adopted in 2001 for the regulation of twelve chemicals, which include: polychlorinated biphenyls (PCBs), dioxins and a wide range of organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs) etc. (UNEP, 2011). Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants, of which sixteen among the hundreds of PAHs present in the environment, are labelled as priority pollutants. This indicates that these PAHs should be monitored regularly, as stated by the US Environmental Protection Agency (US EPA). The 16 PAHs include: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-c,d]pyrene (Sun et al., 1998).

PAHs originate from anthropogenic sources and are classified as petrogenic, which refer to crude or refined petroleum sources, and pyrogenic from fossil fuel and biomass combustion (Masood et al., 2016). Pyrogenic PAHs are identified as having four to seven fused aromatic benzene rings with high molecular weight molecules, while petrogenic PAHs are low molecular weight molecules with two to three fused aromatic benzene rings (Chiu et al., 2015). PAHs are persistent, carcinogenic, toxic and mutagenic (He et al., 2014). The growing population has contributed greatly to the rise of PAH concentrations in freshwater and marine ecosystems. This was observed in Malaysia over a period of twelve years that saw an increase of an additional 12.1 million vehicles on the roads (Keshavarzifard et al., 2014). The effect on human health caused by exposure to PAHs

is dependent on numerous factors, namely, length of exposure, concentration or toxicity of the compound, as well as any previous health illnesses and age of the person (Dudhagara et al., 2016).

Agricultural production is assisted by chemical pesticides, such as acetochlor, hexazinone and oxamyl, to improve crop protection and yield, but the benefits of these pesticides is overshadowed by concerns regarding the presence of pesticide residue in various environments (Yadav et al., 2015). Acetochlor is a chloroacetamide herbicide that biodegrades poorly in the natural environment and is commonly found in groundwater aquifers of agricultural areas (Luo et al., 2015). This chemical may accumulate in the aquatic environment if used excessively due to its low adsorption in soils (Hou et al., 2014). Hexazinone is an active herbicide utilised on sugar cane plantations as a control for weeds and woody plants for forestry plantations. This compound is capable of contaminating ground water as a result of its water solubility (Toro et al., 2015). Carbamate pesticides, such as oxamyl, which is used for crop protection, have been found in rivers as a result of their high solubility in water (280 g L^{-1}) (Osman et al., 2015). Oxamyl is a threat to humans and the environment due to their toxicological effects and excessive application (Tomlin, 2002).

1.2 Aim

The aim of this work was to determine if the reforestation process improves the quality of the surrounding soil and river water based on an investigation of the concentrations of selected organic chemical pollutants.

1.3 Objectives

1. To validate a method for the extraction, pre-concentration and analysis of PAHs and pesticides from river water, sediment, and soil and to validate the method.
2. To analyse for extracted PAHs using gas chromatography and extracted pesticides using liquid chromatography.
3. To investigate differences in organic pollutant concentrations between newer and older planting sites.

4. To investigate the presence of organic pollutants in the river water before and after the reforested site as well as within the reforested site.

1.4 Hypothesis

Organic chemical pollutants are potentially being taken up by trees resulting in the decrease in their concentration in the soil. Forests act as filters to prevent numerous pollutants from entering waterways, therefore forested areas/regions should contain higher water quality.

1.5 Research scope

The analysis of PAHs and pesticides is centered around the Buffelsdraai area in KwaZulu-Natal (KZN), South Africa. The geographical area of interest is the Buffelsdraai landfill site, which is surrounded by the reforestation site. The Reforestation Project commenced with eThekwini municipality's Environmental Planning and Climate Protection Department, in partnership with the Wildlands Conservation Trust and Durban Solid Waste to reduce the effects of climate change (Douwes et al., 2016). Soil samples were collected from the reforestation site, whilst sediment and water samples were collected from the surrounding Black and White Mhlasini Rivers. The river serves as a boundary, separating the nearby rural community from the reforestation site. Some of the reforested sites slope towards the river, and there is therefore the potential for pollutants in the soil to leach into the river. Studies involving the detection of certain PAHs and pesticides have been conducted in South Africa, but no present work has been published concerning organic pollutants within the Buffelsdraai reforestation site. This analysis will provide significant knowledge and information for the eThekwini municipality as well as research in this field of study, so as to illustrate the benefits of the reforestation project in the elimination or reduction of organic pollutants.

1.6 Outline of dissertation structure

Each chapter is centered around the discussion of reforestation and organic chemical pollutants, containing literature review, materials and methods, results and discussions, and conclusion. Chapter 1 provided the rationale for the research and the introduction for subsequent chapters.

Chapter 2 is a literature review on reforestation, organic pollutants such as PAHs and pesticides, sampling methods, different extraction methods, and instrumentation used in this study.
Chapter 3 focuses on the materials and methods used for extraction of PAHs and pesticides.
Chapter 4 details the method development and validation for the extraction of the organic pollutants, and includes the data analysis of the real samples collected from Buffelsdraai.
Chapter 5 is the final chapter integrating the work and provides conclusions to the reported work. Future work, learning and research possibilities are included in this final chapter.

1.7 References

ANCOG, R. C., FLORECE, L. M. & NICOPIOR, O. B. 2016. Fire occurrence and fire mitigation strategies in a grassland reforestation area in the Philippines. Forest Policy and Economics, 64**,** 35-45.

BUCCINI, J. 2003. The development of a global treaty on persistent organic pollutants (POPs). Persistent Organic Pollutants, 30**,** 493-493.

CHIU, T. R., NANYAN, N. F. M. & ALI, M. M. 2015. Distribution of Polycyclic Aromatic Hydrocarbons (PAHs) in surface sediments of Kapas Island, Terengganu, Malaysia. Procedia Environmental Sciences, 30**,** 162-167.

CUNNINGHAM, S., MAC NALLY, R., BAKER, P., CAVAGNARO, T., BERINGER, J., THOMSON, J. & THOMPSON, R. 2015. Balancing the environmental benefits of reforestation in agricultural regions. Perspectives in Plant Ecology, Evolution and Systematics, 17**,** 301-317.

DOUWES, E., ROUGET, M., DIEDERICHS, N., O'DONOGHUE, S., ROY, K. & ROBERTS, D. 2016. The Buffelsdraai Landfill Site Community Reforestation Project. Unasylva, 67**,** 12.

DUDHAGARA, D. R., RAJPARA, R. K., BHATT, J. K., GOSAI, H. B., SACHANIYA, B. K. & DAVE, B. P. 2016. Distribution, sources and ecological risk assessment of PAHs in historically contaminated surface sediments at Bhavnagar coast, Gujarat, India. Environmental Pollution, 213**,** 338-346.

ENGLAND, J. R., PAUL, K. I., CUNNINGHAM, S. C., MADHAVAN, D. B., BAKER, T. G., READ, Z., WILSON, B. R., CAVAGNARO, T. R., LEWIS, T. & PERRING, M. P. 2016. Previous land use and climate influence differences in soil organic carbon following reforestation of agricultural land with mixed-species plantings. Agriculture, Ecosystems & Environment, 227**,** 61-72.

HARRAD, S. 2012. Persistent Organic Pollutants: Environmental behaviour and pathways of human exposure, Springer US.

HE, X., PANG, Y., SONG, X., CHEN, B., FENG, Z. & MA, Y. 2014. Distribution, sources and ecological risk assessment of PAHs in surface sediments from Guan River Estuary, China. Marine Pollution Bulletin, 80**,** 52-58.

HOU, Y., DONG, W., WANG, F., LI, J., SHEN, W., LI, Y. & CUI, Z. 2014. Degradation of acetochlor by a bacterial consortium of Rhodococcus sp. T3-1, Delftia sp. T3-6 and Sphingobium sp. MEA3-1. Letters in Applied Microbiology, 59**,** 35-42.

KANOWSKI, J., CATTERALL, C., WARDELL-JOHNSON, G., PROCTOR, H. & REIS, T. 2003. Development of forest structure on cleared rainforest land in eastern Australia under different styles of reforestation. Forest Ecology and Management, 183**,** 265-280.

KESHAVARZIFARD, M., ZAKARIA, M. P., HWAI, T. S., MUSTAFA, S., VAEZZADEH, V., MAGAM, S. M., MASOOD, N., ALKHADHER, S. A. & ABOOTALEBI-JAHROMI, F. 2014. Baseline distributions and sources of polycyclic aromatic hydrocarbons (PAHs) in the surface sediments from the Prai and Malacca Rivers, Peninsular Malaysia. Marine Pollution Bulletin, 88**,** 366-372.

KU, H. W., TOK, C. T., SURESH, A. & ONG, B. L. 2016. “Active” hydroponic greenhouse system to kick-start and augment reforestation program through carbon sequestration–an

experimental and theoretical feasibility study. Journal of Cleaner Production, 129**,** 637-646.

LE, H. D., SMITH, C. & HERBOHN, J. 2014. What drives the success of reforestation projects in tropical developing countries? The case of the Philippines. Global Environmental Change, 24**,** 334-348.

LUO, W., GU, Q., CHEN, W., ZHU, X., DUAN, Z. & YU, X. 2015. Biodegradation of acetochlor by a newly isolated Pseudomonas strain. Applied Biochemistry and Biotechnology, 176**,** 636-644.

MARLIANA, S. N. & RÜHE, F. 2014. Post-reforestation vegetation development on abandoned highland fields in Java, Indonesia. Forest Ecology and Management, 328**,** 245-253.

MASOOD, N., ZAKARIA, M. P., HALIMOON, N., ARIS, A. Z., MAGAM, S. M., KANNAN, N., MUSTAFA, S., ALI, M. M., KESHAVARZIFARD, M. & VAEZZADEH, V. 2016. Anthropogenic waste indicators (AWIs), particularly PAHs and LABs, in Malaysian sediments: Application of aquatic environment for identifying anthropogenic pollution. Marine Pollution Bulletin, 102**,** 160-175.

OSMAN, K. A., EL-BAKARY, A. S., SABER, N. A., EL-SEBAI, A. & SALAMA, M. 2015. Removal of oxamyl in soil by different treatments. Alexandria Science Exchange, 36**,** 142.

PAN, Y., BIRDSEY, R. A., FANG, J., HOUGHTON, R., KAUPPI, P. E., KURZ, W. A., PHILLIPS, O. L., SHVIDENKO, A., LEWIS, S. L. & CANADELL, J. G. 2011. A large and persistent carbon sink in the world's forests. Science, 333**,** 988-993.

PILGRIM, C., MIKHAILOVA, E., POST, C., HAINS, J. & COX, S. 2015. Spatial and temporal analysis of lake sedimentation under reforestation. Water Science, 29**,** 93-108.

SCHIRMER, J. & BULL, L. 2014. Assessing the likelihood of widespread landholder adoption of afforestation and reforestation projects. Global Environmental Change, 24**,** 306-320.

SUN, F., LITTLEJOHN, D. & GIBSON, M. D. 1998. Ultrasonication extraction and solid phase extraction clean-up for determination of US EPA 16 priority pollutant polycyclic aromatic hydrocarbons in soils by reversed-phase liquid chromatography with ultraviolet absorption detection. Analytica Chimica Acta, 364**,** 1-11.

SUTTER, C. & PARREÑO, J. C. 2007. Does the current Clean Development Mechanism (CDM) deliver its sustainable development claim? An analysis of officially registered CDM projects. Climatic Change, 84**,** 75-90.

TOMLIN, C. 2002. The e-Pesticide Manual, Version 2.2.[CD-ROM]. The Britich Crop Protection Council, UK.

TORO, M. J. U., MARESTONI, L. D. & SOTOMAYOR, M. D. P. T. 2015. A new biomimetic sensor based on molecularly imprinted polymers for highly sensitive and selective determination of hexazinone herbicide. Sensors and Actuators B: Chemical, 208**,** 299-306.

UNEP. 2011. Stockholm Convention on Persistent Organic Pollutants. Available: http://www.pops-gmp.org/res/file/UNEP-POPS-COP_5-INF-27.pdf [Accessed 13/11/2017].

WANGPIMOOL, W., PONGPUT, K., SUKVIBOOL, C., SOMBATPANIT, S. & GASSMAN, P. W. 2013. The effect of reforestation on stream flow in Upper Nan river basin using Soil and Water Assessment Tool (SWAT) model. International Soil and Water Conservation Research, 1**,** 53-63.

YACOB, A. N., LEE, T. L. & IBRAHIM, H. B. 2012. Conservation, consolidation and economic generation of indigenous community agriculture sustainable food yielding reforestation. Procedia-Social and Behavioral Sciences, 68**,** 319-329.

YADAV, I. C., DEVI, N. L., SYED, J. H., CHENG, Z., LI, J., ZHANG, G. & JONES, K. C. 2015. Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: a comprehensive review of India. Science of the Total Environment, 511**,** 123-137.

Chapter 2: Literature Review

Persistent organic pollutants (POPs) are organic chemical pollutants introduced into the environment through natural processes such as volcanoes and forest fires but are almost entirely created through anthropogenic activities. These pollutants include industrialised chemicals such as polychlorinated biphenyls (PCBs) used in electrical equipment; organochlorinated pesticides (OCPs) such as dichlorordiphenyltrichloroethane (DDT) used for malaria control and agricultural activities; by-products such as dioxins and furans from industry; and PAHs from the petroleum industry (Speight, 2016). Of all types of pesticides used globally, the most commonly used is OCPs (40%) due to their lower cost (Jayaraj et al., 2016). By 2005, DDT's global production reached approximately 6269 metric tons, however, its sole purpose was to be used as vector control in malaria stricken Africa (Klánová et al., 2009). DDT was phased out in South Africa in 1983 but due to an observed increase in malaria cases, owing to mosquito resistance to pyrethroid products, DDT was reintroduced for malaria control but only under restricted and monitored use (Eo, 2008). PCBs are also in high demand with an estimated 1.3 million tonnes produced worldwide, contributing to higher waste production, which serves as an entry point into the environment *via* landfill site diffusion (Gioia et al., 2011). A contributing factor to PAH emissions, are forest fires which contributed to 48.4 % of total PAH emissions in South America and 24.6 % in East and South Africa (Shen, 2016). POP emissions are monitored by a global treaty led by UNEP and approved by over 150 countries, including South Africa that restricts the production of POPs in accordance with the Stockholm Convention on POPs (Zeng, 2015). This chapter will discuss PAHs and pesticides with emphasis on the selected PAH and pesticide compounds that were investigated in this study.

2.1 Polyaromatic hydrocarbons

Polyaromatic Hydrocarbons (PAHs) are included on the USEPA's list of priority pollutants as they are an environmental threat and risk to human health due to their alleged mutagenic and carcinogenic properties (Crompton, 2012). Furthermore, the degradation rate of these compounds under natural conditions is slow, with their persistence in the environment increasing with an increase in molecular weight of the PAH (Haritash and Kaushik, 2009). Although PAHs have been

detected globally, no definitive data exists for detailing the trends in concentration levels of PAHs in South African environments (Chimuka et al., 2016). PAHs may disperse away from the actual source of contamination through atmospheric long-range transport (Zhang and Tao, 2009). The use of biomass fuels for cooking and heating in developing countries results in high emissions of PAHs. PAHs contaminate the environment through industrial plant and wastewater treatment plant discharges into surface water, and leakage into soils from storage containers on hazardous waste sites (ATSDR, 1995). PAHs have also been detected in soils from areas with volcanic activity (Maisto et al., 2006).

The water solubility of PAHs depends on their molecular mass, with solubility decreasing with an increase in molecular mass (Douben, 2003). PAHs also have low vapour pressures and high melting and boiling points, as listed in Table 2.1. PAHs released into the environment degrade through photodegradation and biodegradation (Douben, 2003). Degradation products may include nitrosubstituted PAHs, which have greater carcinogenicity (Neilson, 2013). PAHs obtain nitro groups *via* chemical reactions with NO_x in ambient air or through incomplete combustion (Galceran and Moyano, 1993). Human exposure may result from inhalation of air, consumption of food or water, or contact with soil containing PAHs. Once this contaminant enters the body, it accumulates in the kidneys, liver, and fat (ATSDR, 1995). Exposure to PAHs can result in endocrine disruptions as the hydrocarbons metabolise and are excreted as PAH metabolites through bile (Pampanin and Sydnes, 2017). The epoxy metabolites formed by reactive PAHs, are partially transferred before excretion to dihydrodiol epoxides, with these derivatives binding to the bases of DNA, leading to potential cancer formation (Ruppert et al., 2013). The prenatal effects of PAH exposure on humans are unknown, however, animal studies displayed birth defects such as reproductive problems and immune system disorders (Frumkin, 2016). Studies of extensive exposure through breathing or contact *via* the skin to mixtures containing PAHs and other compounds, show development of cancer in humans (ATSDR, 1995).

Table 2.1: Physical and chemical properties for polyaromatic hydrocarbons

Compound	**Molecular formula**	**Molecular weight/ g mol^{-1}**	**Melting point/ ℃**	**Boiling point/ ℃**	**Solubility[b]/ mg L^{-1}**
Naphthalene	$C_{10}H_8$	128.7	80.26	217.9	31.7
Acenaphthylene	$C_{12}H_8$	150.2	91.80	280.0	3.93
Acenapthene	$C_{12}H_{10}$	154.2	93.40	279.0	1.93
Fluorene	$C_{13}H_{10}$	166.2	114.7	295.0	1.68-1.98
Anthracene	$C_{14}H_{10}$	178.2	215.8	339.9	0.076
Phenanthrene	$C_{14}H_{10}$	178.2	99.24	340.0	1.20
Pyrene	$C_{16}H_{10}$	202.3	150.6	404.0	0.077
Fluoranthene	$C_{16}H_{10}$	202.3	110.2	384.0	0.20-0.26
Benzo[a]anthracene	$C_{18}H_{12}$	228.3	160.5	438.0	0.010
Chrysene	$C_{18}H_{12}$	228.3	255.5	448.0	0.0028
Benzo[k]fluoranthene	$C_{20}H_{12}$	252.3	217.0	480.0	0.00076
Benzo[b]fluoranthene	$C_{20}H_{12}$	252.3	168.0	481.0	0.0012
Benzo[e]pyrene	$C_{20}H_{12}$	252.3	181.4	311.0	0.0063
Indeno[1,2,3-c,d]pyrene	$C_{22}H_{12}$	276.3	162.0	536.0[a]	0.062
Dibenz[a,h]anthracene	$C_{22}H_{14}$	278.4	269.5	524.0	0.0005
Benzo[g,h,i]perylene	$C_{22}H_{12}$	276.3	272.5	550.0	0.00026

(Mackay et al., 2006), [a](Verschueren, 2001) , [b](El-Masri, 2005)

2.1.1 Naphthalene

Naphthalene is a non-polar hydrocarbon containing two benzene rings, Figure 2.1 (Pa*via*, 2005). This hydrocarbon and its methylated derivatives are considered toxic and can be found in the water-soluble fraction of petroleum (Abo-State et al., 2018). It appears naturally as a white solid, commonly emitted from biomass burning and fumigants, and is the most volatile member of the PAH group (Jia and Batterman, 2010). It is used in the insecticide industry as a moth repellent or in the production of naphthol and halogenated naphthalene (Patnaik, 2007). It is primarily used as a raw material for the production of phthalic anhydride and in carbamate insecticides (EPA, 1999). Naphthalene accumulates in the environment through wet or dry deposition and is transported into the atmosphere through volatilisation (ATSDR, 2005). It is a potential carcinogen and may result in headaches, nausea, and throat and eye irritation upon accidental exposure (Purser et al., 2015). In humans, acute exposure through inhalation and ingestion has been reported to result in cataracts (EPA, 1999).

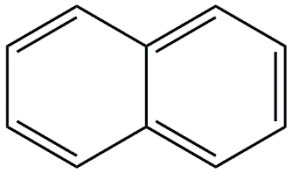


Figure 2.1: Chemical structure for naphthalene

2.1.2 Acenaphthylene

Acenaphthylene is made up of three aromatic rings, Figure 2.2, and is used in the production of plastics, pharmaceuticals, insecticides, and herbicides (Dikshith, 2016). Under typical ambient temperature, this hydrocarbon is a gas, which is capable of undergoing atmospheric gas-phase reactions with ozone (Reisen and Arey, 2002). Acenaphthylene may be removed from soil by volatilisation and biodegradation, with the latter being the least likely degradation path (Montgomery, 2010). Short-term exposure results in eye irritation, nausea, and anemia, whilst

long-term exposure causes skin allergies or chorioretinitis[1] (Cooper, 1996). There are currently no human studies that have been conducted to determine the exact level at which acenaphthylene is harmful, but it has been linked to cancer in humans (USEPA, 2001).

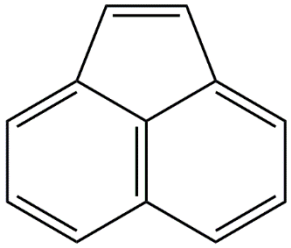


Figure 2.2: Chemical structure for acenaphthylene

2.1.3 Acenaphthene

Acenaphthene is a white crystalline solid derived from coal tar and is used as a fungicide (Cooper, 1996). It can also be formed from acenaphthylene through hydrogenation (Franck and Stadelhofer, 2012). This tricyclic aromatic hydrocarbon, Figure 2.3, is insoluble in water but dissolves in most organic solvents (Dikshith, 2016). It does not hydrolyse in water, as it contains no hydrolysable groups (Montgomery, 2007). Acenaphthene undergoes direct photolysis when exposed to sunlight and persists in the environment under anaerobic conditions (Wexler et al., 2005). It may result in skin irritation upon contact, vomiting if consumed or cause methemoglobinemia, a blood disorder (Wexler et al., 2005).

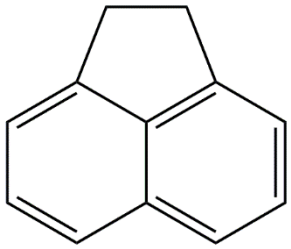


Figure 2.3: Chemical structure for acenaphthene

[1] Chorioretinitis is an inflammation that can affect an individual's vision as it targets the choroid, which is the retina lining within the eye.

2.1.4 Fluorene

Fluorene is a biphenyl molecule, Figure 2.4, with a methylene bridge and has acidic properties because of its structure (Gebhardt, 2009). It appears as a white crystal when pure and gives off fluorescence when impure (Pohanish, 2017). It does not hydrolyse, however, it can react photochemically in the atmosphere or undergo oxidation to form fluorenone after reacting with ozone (Montgomery, 2010). Fluorene is used in polyradical formation for resins and insecticides, and is formed from industrial coal gasification (Montgomery, 2007). It has long-lasting hazardous effects on the aquatic environment (Pohanish, 2017). Fluorene exposure can result in eye and skin irritation (Pohanish, 2017).

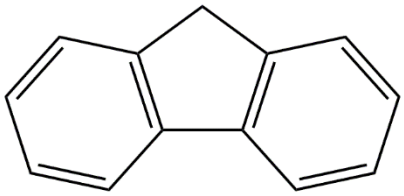


Figure 2.4: Chemical structure for fluorene

2.1.5 Phenanthrene

Phenanthrene is formed from coal combustion, coke production or wood combustion (Neilson, 2013). It is found predominantly in coal tar but also appears in crude oils and the exhausts of gasoline engines (Kostecki and Calabrese, 1993). Also, research has shown some PAH contamination of surface water and tap water by phenanthrene (D'Mello, 2003). It has a monoclinic structure and is insoluble in water (Armour, 2016). Phenanthrene appears as a colourless solid that is soluble in numerous organic solvents, namely, glacial acetic acid, benzene, ethanol, anhydrous diethyl ether, and toluene (ATSDR, 1995). This tricyclic compound, Figure 2.5, is most abundant in sediment compared to the other hydrocarbons from the PAH group (Wang, 2007). Exposure may result in skin irritation, growth depression, or hematological effects (Kostecki and Calabrese, 1993).

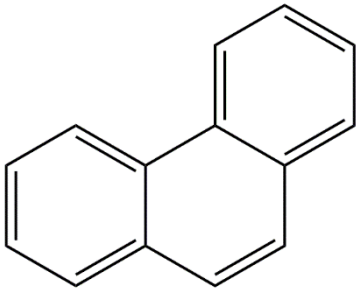


Figure 2.5: Chemical structure for phenanthrene

2.1.6 Anthracene

Anthracene, Figure 2.6, appears as white crystalline flakes but in the presence of impurities, the flakes are yellow in colour (Montgomery, 2007). The impure compound contains both phenanthrene and carbazole, which can be extracted from coal tar (Arora, 2006). Through the processes of distillation and crystallisation, the purity of this hydrocarbon can be improved in order to be used in the manufacture of mordant and reactive dyes (Weissermel and Arpe, 2003). It has a water solubility value of 0.076 mg L^{-1} and is soluble in methanol, ether, chloroform, acetone, and benzene (ATSDR, 1995). It is a skin irritant that results in nausea if inhaled or to a more severe extent, it can alter an organism's genetic structure (Vincoli, 1996). This compound may also inhibit photosynthesis in plants by restricting the plant's electron transport system (Gangolli, 2007).

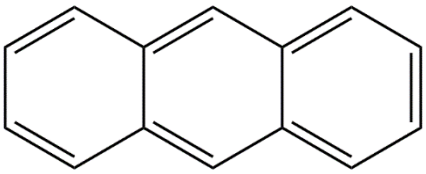


Figure 2.6: Chemical structure for anthracene

2.1.7 Fluoranthene

Fluoranthene is made up of a five-membered ring, Figure 2.7 that contains naphthalene and benzene units (Sattler, 2016). It appears as a yellow solid and is present in urban air, natural combustion, and cigarette smoke (Proctor et al., 2004). Fluoranthene is frequently found in drinking water despite PAHs having low water solubility (Harrison, 2014). It is soluble in alcohol,

acetic acid, ether, and benzene (ATSDR, 1995). As one of the most abundant aerosol PAHs in the atmosphere, degradation occurs either through photolysis or under ultraviolet light (Lens et al., 2006). Exposure to fluoranthene is increased among tobacco smokers and those in closed environments exposed to tobacco smoke (Pohanish, 2017). The World Health Organisation (WHO) determined that fluoranthene is mutagenic in bacteria but shows no carcinogenicity in animals (Harrison, 2014).

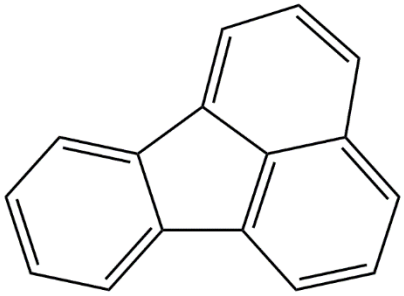


Figure 2.7: Chemical structure for fluoranthene

2.1.8 Pyrene

Pyrene, Figure 2.8, is a colourless solid that may appear yellow if recrystallised using toluene or display a blue fluorescence when recrystallised using ethanol (ATSDR, 1995). It was initially utilised in the formation of pyranthrone, which is a pyrene derivative used in the synthetic dye industry (Figueira-Duarte and Müllen, 2011). The fluorescence properties of pyrene, allow for its frequent use in the dye industry for investigations into water-soluble polymers (Figueira-Duarte and Müllen, 2011). It emits unpleasant fumes when heated (Chemicals, 2018). Degradation in the environment occurs under aerobic conditions through bacteria, however, this process is hindered by the anaerobic conditions that exist in sediment and soil (Lichtfouse et al., 2005). Human exposure may occur through consumption of food contaminated with PAHs or through absorption by the skin (Chemicals, 2018).

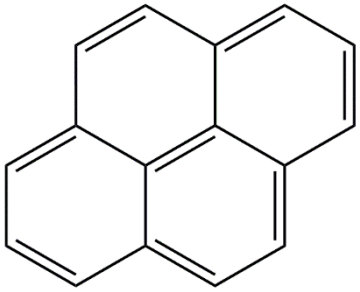


Figure 2.8: Chemical structure for pyrene

2.1.9 Benzo[a]anthracene

Benzo[a]anthracene can be either colourless or display a greenish-yellow fluorescence (Montgomery, 2010). It has a crystalline structure with four fused benzene rings, Figure 2.9. This hydrocarbon is not water soluble but is soluble in most organic solvents (Patnaik, 2007). These solvents include acetone, diethyl ether, benzene and slightly soluble in hot ethanol (ATSDR, 1995). It is found in coal tar, wood and tobacco smoke (Stellman and Office, 1998). Benzo[a]anthracene is produced *via* synthesis from naphthalene and phthalic anhydride (O'Neil, 2013). This compound is also present in asphalt products and is a product formed by petroleum combustion (Zeliger, 2011). Benzo[a]anthracene forms part of the most potent PAH carcinogens, along with benzo[a]pyrene and dibenz[a,h]anthracene (Kim et al., 2013).

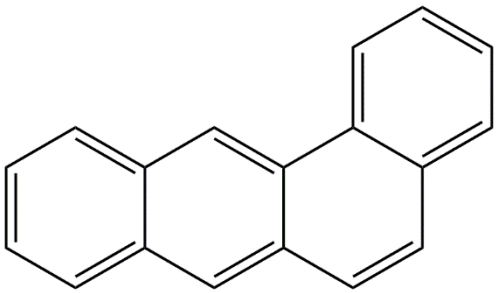


Figure 2.9: Chemical structure for benzo[a]anthracene

2.1.10 Chrysene

Chrysene is a colourless hydrocarbon that is used as a laboratory reagent (Proctor et al., 2004). It is a chemical found in creosote, which is used for wood preservation (ATSDR, 1990). Chrysene,

Figure 2.10, is released directly into the air, with combustion being the main contributor to its presence in the environment (Koren and Bisesi, 2002). Photochemical oxidation accounts for the atmospheric removal of chrysene, whilst land and water removal is *via* dry or wet deposition (Koren and Bisesi, 2002). In the environment, microbial attacks of chrysene is hindered due to its reduced bioavailability, as a result of its low water solubility (Ghevariya et al., 2011). Biodegradation of chrysene is slow, as it persists in soil or sediment with a half-life of 1000 days (Koren and Bisesi, 2002). It has tumor initiating properties but is considered as a weak carcinogen (Pryor, 2012).

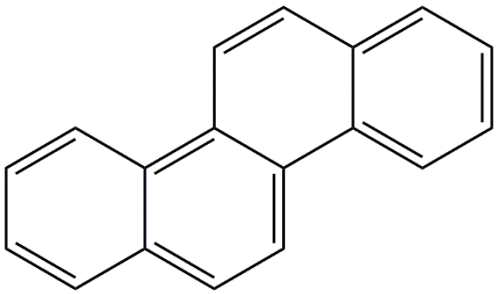


Figure 2.10: Chemical structure for chrysene

2.1.11 Benzo[b]fluoranthene

Benzo[b]fluoranthene, Figure 2.11, is not produced commercially, instead it is formed when fossil fuels or any organic matter is burned (Pohanish, 2017). It may appear as either a colourless or yellow-orange needle shaped solid. (Montgomery, 2007). It is applied in industry as an electrode binder and is a component of creosole, which is used in wood preservation (Pohanish, 2017). This compound may enter the body directly, through skin contact, inhaling polluted air containing benzo[b]fluoranthene, consumption of contaminated water, or smoking (Pohanish, 2017). Toxic fumes released upon heating of the hydrocarbon, can be inhaled resulting in genetic damage (NIOSH, 1999).

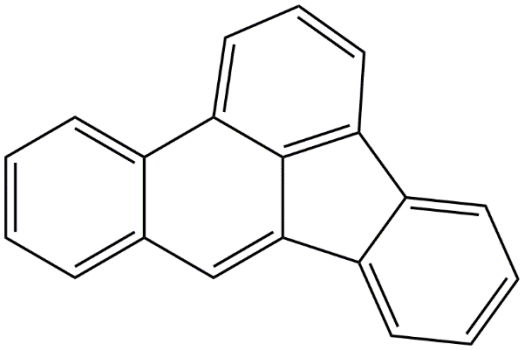


Figure 2.11: Chemical structure for benzo[b]fluoranthene

2.1.12 Benzo[k]fluoranthene

Benzo[k]fluoranthene is made up of five cyclic rings, Figure 2.12, and has a lower aqueous solubility compared to the previous PAHs (Cheremisinoff and Davletshin, 2010). It appears as a pale yellow solid with a high boiling point of 480 ℃ (ATSDR, 1995). It is formed during the combustion of gasoline or garbage, and is found in the air where it combines with dust particles (Engineers, 1996). This hydrocarbon has low mobility in soils and adsorbs to sediment (TOXNET, 2017a). It has low solubility in water, 0.00076 mg L^{-1}, and is soluble in organic solvents such as benzene, ethanol, and acetic acid (ATSDR, 1995). It is a potential human carcinogen as tumours developed in studies conducted on animal exposure to the hydrocarbon (TOXNET, 2017a).

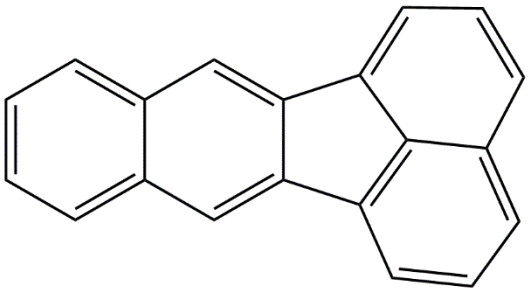


Figure 2.12: Chemical structure for benzo[k]fluoranthene

2.1.13 Benzo[e]pyrene

Benzo[e]pyrene, Figure 2.13, is a colourless crystalline solid that is insoluble in water (Patnaik, 2007). It is soluble in acetone (ATSDR, 1995). This compound is found in plant oils, grilled and smoked meat, and emissions from petroleum industries during combustion of oil and coal (TOXNET, 2017b). It is highly toxic to aquatic organisms (ECHA, 2008). Benzo[e]pyrene is not readily broken down by microorganisms and may accumulate in aquatic organisms (TOXNET, 2017b). It is considered to be a mutagen and may result in stomach tumors if consumed (Patnaik, 2007).

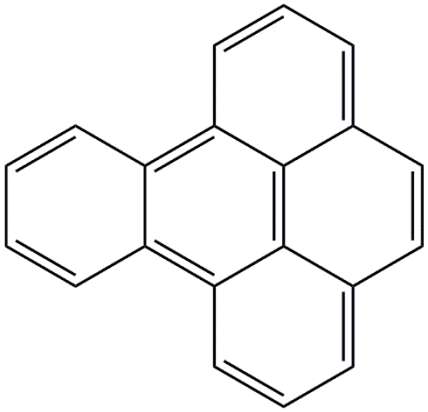


Figure 2.13: Chemical structure for benzo[e]pyrene

2.1.14 Indeno[1,2,3-c,d]pyrene

Indeno[1,2,3-c,d]pyrene, Figure 2.14, appears as a yellow solid that is slightly soluble in water and soluble in organic solvents (ATSDR, 1995). It is formed during petroleum combustion and is utilised in the dye manufacturing industry (Zeliger, 2011). It is also found in coal tar, gasoline or diesel car exhaust fumes, and petroleum asphalt (NCIt, 2017). The volatiles contained within coal tar are considered to be carcinogenic (NIOSH, 2010). When heated, it emits harsh fumes and forms hazardous products upon decomposition (Lewis, 2004).

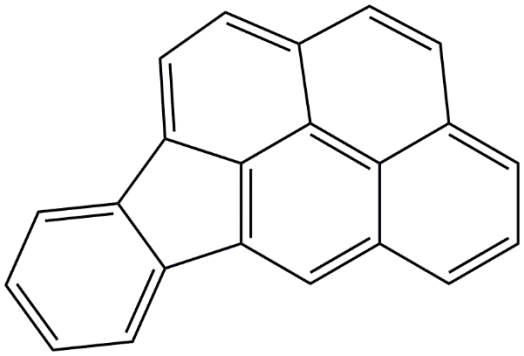


Figure 2.14: Chemical structure for indeno[1,2,3-c,d]pyrene

2.1.15 Dibenz[a,h]anthracene

Dibenz[a,h]anthracene has a crystalline structure with five benzene rings, Figure 2.15 (Pohanish, 2011). It was the first carcinogen to be synthesised (Lee, 2014). In 1930, a year after it was synthesised, Kennaway and Hieger showed that it was carcinogenic (Neilson, 2013). It is soluble in acetic acid, benzene and xylene (ATSDR, 1995). Humans may be exposed to this hydrocarbon through cigarette smoke or through the consumption of grilled or charred meat, which has shown to contain dibenz[a,h]anthracene (Chen, 2011). When tested on experimental animals, tumor's developed within the lung and blood vessels (Program, 2011).

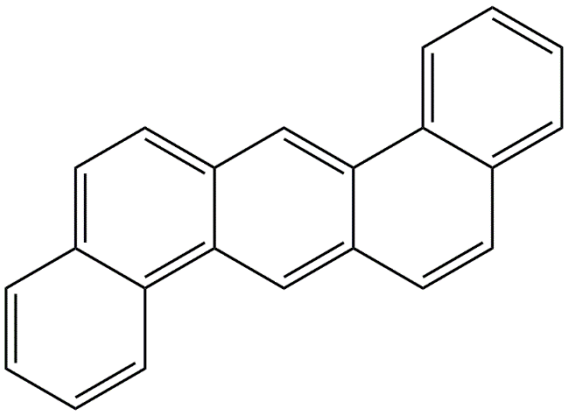


Figure 2.15: Chemical structure for dibenz[a,h]anthracene

2.1.16 Benzo[g,h,i]perylene

Benzo[g,h,i]perylene, Figure 2.16, is a colourless crystalline solid used in the production of pesticides, explosives, dyes, plastics, bile acids, and steroids (Spellman, 2016). It is soluble in acetone, benzene, and dichloromethane (ATSDR, 1995). Humans may be exposed to this compound through consumption of contaminated water, which then targets fatty tissues or organs such as the kidneys and liver (Spellman, 2016).

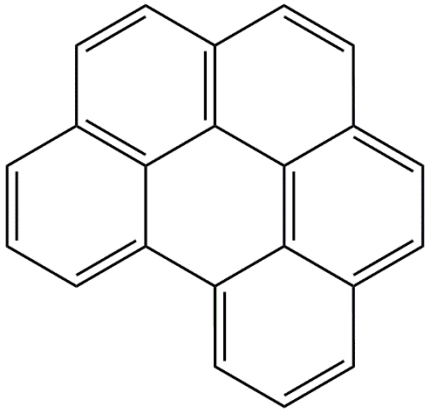


Figure 2.16: Chemical structure for benzo[g,h,i]perylene

2.2 PAH environmental guidelines

The assessment of PAH concentration levels found in the South African environment are taken from regulatory bodies, such as the US EPA or World Health Organisation (WHO), as there are no maximum allowed limits for PAHs in South Africa. The Agency for Toxic Substance and Disease Registry (ATSDR) list minimum risk levels (MRLs), which represent an estimated daily value for human exposure to hazardous substances without the result of adverse health effects such as cancer (ATSDR, 2017). Table 2.2 lists the PAHs that are included on the ATSDR's MRLs list. The World Health Organisation (WHO) regards PAH concentration levels greater than 50 ng L^{-1} in surface and coastal water as an indication of contamination, with levels as high as 10 µg L^{-1} being detected (WHO, 1998). The European commission have set a guideline limit for benzo[a]pyrene in drinking water at 0.01 µg L^{-1} (Authority, 2008).

Table 2.2: MRL's for selected PAHs (ATSDR, 2017)

ATSDR MRLs		
Compound	**Exposure**	**MRL/ mg kg^{-1} day^{-1}**
Acenaphthene	Oral	0.6
Anthracene	Oral	10
Fluoranthene	Oral	0.4
Fluorene	Oral	0.4
Naphthalene	Inhalation	0.0007 (in ppm)
	Oral	0.6

2.3 Source apportionment

Source apportionment assists in providing an indication of the possible source of PAH contamination. This analysis uses PAH ratios to determine if the contamination was from a petrogenic or pyrolytic source. However, it is possible for petrogenic and pyrolytic sources to co-exist (Soclo et al., 2000). Table 2.3 provides the PAH ratios used for this study. For the chrysene/benzo[a]anthracene ratio, if the value is <1 then the possible source of contamination is

pyrogenic but if it is >1, then it is from a petrogenic source. This same logic is applied to the other three ratios as well.

Table 2.3: Ratio to identify PAH sources (Qamar et al., 2017)

PAH Ratio	Source	
	Pyrogenic	**Petrogenic**
Chrysene/benzo[a]anthracene	<1	>1
Fluoranthene/pyrene	>1	<1
Phenanthrene/anthracene	<10	>15
LMW/HMW	<1	>1

*LMW – low molecular weight, HMW – high molecular weight

2.4 Previous research on PAHs

Research has been conducted on the occurrence of PAHs present on landfills, although currently no research has been published regarding the reduction of these organic pollutants as a result of reforestation. PAHs may originate from incineration of waste contained on landfills or the burning of sugarcane plantations before being harvested. There are no published data values for PAH concentration levels in South African landfills, however, there have been reported cases globally. The landfill in Kouroupitos, Greece, reported concentration levels for total PAHs within the landfill as 1475 µg kg^{-1} (dw), with the surrounding soil containing between 11.2-43.5 µg kg^{-1} (dw) (Chrysikou et al., 2008).

Previous research conducted at industrialised areas in South Africa have shown higher PAH concentrations compared to residential areas. Total PAHs were reported at 39 000 ng g^{-1} dw compared to 2900 ng g^{-1} as reported in the USA (Nieuwoudt et al., 2011). The province of Limpopo reported PAH concentrations between 28.7-3192.6 µg L^{-1} (Chimuka et al., 2016). In Brazil, levels of PAHs from the burning of sugarcane were compared during the harvest and no-harvest seasons, as Brazil is the world's largest producer of sugar-cane. PAH concentration levels were four times higher during the harvest season compared to the non-harvest season (de Andrade et al., 2010).

2.5 Pesticides

> *In 1971, United States of America President Nixon stated: "Pesticides have provided important benefits by protecting man from disease and increasing his ability to produce food and fiber. However, the use and misuse of pesticides has become one of the major concerns of all who are interested in a better environment" (Wheeler, 2002).*

Pesticides are chemicals used in the agricultural sector to ensure crop protection from insects or plant species that inhibit crop growth (Rathore and Nollet, 2012). Initially pesticides were produced from sulfur compounds, thereafter arsenic compounds were used for fruit and vegetable protection and eventually DDT (dichlorodiphenyltrichloroethane) was introduced with the belief that it was safe and had low toxicity (Levine, 2007). Prior to the era of synthetic pesticides, such as insecticides, fungicides, and herbicides, farmers used naturally occurring arsenic and pyrethrum, which is produced from the flowers of plants (Levine, 2007). Approximately 75% of pesticide use in the United States is for soybean, wheat, cotton, and corn protection but it is estimated that crops lost due to pests, have increased by 6% compared to the 1940s due to insects developing a pesticide resistance (Levine, 2007).

In sub-Saharan Africa, the most frequent user of pesticides is South Africa and despite the health effects associated with pesticides, over 500 active ingredients are registered for use in the agricultural sector (Dabrowski et al., 2014). These include acetochlor, hexazinone and oxamyl. Africa accounts for 3% of pesticide use in the world, of which South Africa makes up 2% (Khan and Rahman, 2017). Pesticides affect the health of humans and the environment through misuse and mishandling, especially with farmworkers exposed to these chemicals daily (Khan and Rahman, 2017). Previous research conducted on the adverse health effects of pesticides in South Africa, linked endocrine disruptions and birth defects in humans to pesticide exposure (Dabrowski et al., 2014). Agricultural workers may display acute and chronic symptoms such as eye irritation, respiratory distress, hormone disruption, and cancer due to pesticide inhalation, ingestion or through contact *via* the skin (Khan and Rahman, 2017). Although pesticides are usually associated with agricultural use, water contamination can also be attributed to urban pesticide use for

gardening, landscaping or domestic pest control (Rathore and Nollet, 2012). The degradation of pesticides present in the environment is mostly carried out by microorganisms, with the degradation products possibly being persistent and toxic (Matsumura, 2012). Degradation depends on the compounds structure, which affects its transformation, and the environment in which the pesticide is applied determines its environmental fate as pesticides are capable of undergoing chemical and photochemical degradation (Fenner et al., 2013).

2.5.1 Acetochlor

Acetochlor, Figure 2.17, is a chloroacetanilide herbicide used in the control of annual grasses and selected weeds on maize plantations (Roberts et al., 2007). At room temperature, it appears as a violet coloured oily liquid that is soluble in water and organic solvents such as acetone and toluene (Krieger, 2001). When used appropriately, this water-soluble herbicide can be used in conjunction with other pesticides (Paranjape et al., 2014). Acetochlor manufacturers formed the Acetochlor Registration Partnership (ARP) in agreement with the herbicide's registration with USEPA in 1994, which included a monitoring program to analyse acetochlor levels in lakes and reservoirs in areas that utilise acetochlor (Larson et al., 2004). This pesticide has been classified as a potential human carcinogen by the USEPA, with other health defects being renal and neurological abnormalities (Lin and Qu, 2016). The MRL value for acetochlor use in South Africa is between 0.02-0.05 mg kg^{-1}(L.P. Quinn, 2011). The acceptable daily intake for acetochlor as stated by the European Commission is 0.0036 mg kg^{-1} per day^{-1} (EU, 2018a).

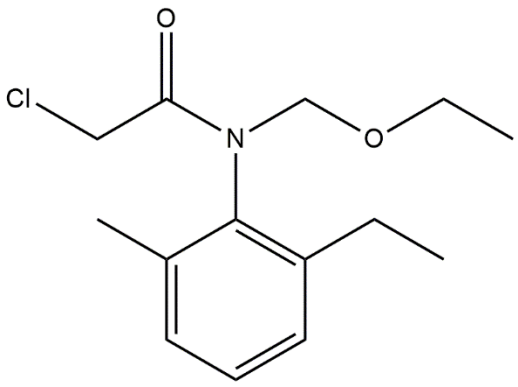


Figure 2.17: Chemical structure for acetochlor

2.5.2 Hexazinone

Hexazinone, Figure 2.18, is a herbicide that belongs to the triazine group and is responsible for inhibiting photosynthesis (Service, 2005). It is used on sugar cane plantations for selective weed control, on non-crop areas, and control against certain woody plants (Pohanish, 2014). The degradation products of hexazinone are persistent and highly mobile, allowing for them to easily contaminate groundwater (Management, 2010). This highly water-soluble herbicide (Table 2.4) is capable of photodecomposition and biodegradation under natural conditions (Service, 2005). However, it does not undergo photodegradation in surface water (Management, 2010). After being applied to soils, hexazinone can still be detected in low concentrations, even after three years (Service, 2004). This compound is reported to have a half-life in the range of 24 days-1 year (Tatum, 2004). It is weakly bound to particulates and sediment, with soil degradation carried out by micro-organisms (Management, 2010). Exposure to hexazinone may result in severe eye irritation (Service, 2006) and short-term exposure to hexazinone may result in skin irritations or permanent eye damage, with long-term exposure leading to reproductive defects (Pohanish, 2014). The MRL value recommended for hexazinone is 0.01 mg kg^{-1} (EU, 2018b).

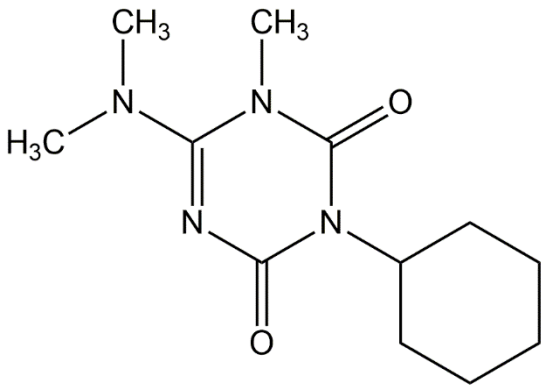


Figure 2.18: Chemical structure of hexazinone

2.5.3 Oxamyl

Oxamyl, Figure 2.19, forms part of the carbamate pesticide group. It is introduced into the environment when applied as an insecticide, or during the manufacture or storage of the pesticide (Udeh, 2004). As an insecticide, it is used on farms to protect fruits and vegetables from pests (Hayes and Laws, 2013). Oxamyl is highly water soluble as seen in Table 2.4. Primary degradation involves hydrolysis to form oxyimidothioate and nitrile, with other reactions involving N-demethylation and hydration or oxidation of nitriles to form amides and acids (Croucher et al., 2007). The presence of oxamyl in groundwater is expected to be higher than in surface water as microbial degradation is possible if aerobic and anaerobic bacteria exists (Udeh, 2004). Exposure to oxamyl may result in health effects such as headaches, nausea, and blurred vision (Kesavachandran, 2014). According to the EPA, the maximum contaminant level (MCL) which represents the maximum contaminant concentration allowed in drinking water, is 0.2 mg L^{-1} (EPA, 2012). An acceptable daily intake for oxamyl is 0.001 mg kg^{-1} day^{-1} (EU, 2018c).

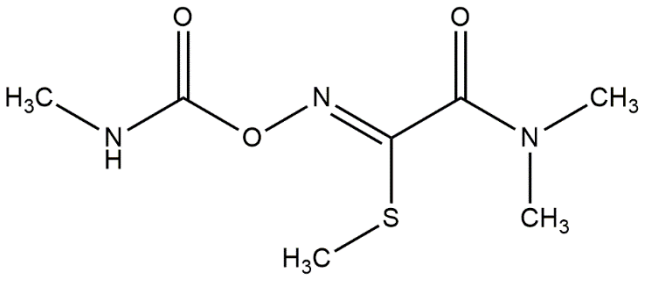


Figure 2.19: Chemical structure of oxamyl

Table 2.4: Physical and chemical properties for pesticides

Compound	Molecular formula	Molecular weight/ g mol^{-1}	Boiling point/ ℃	Melting point/ ℃	Solubility/ mg L^{-1}
Acetochlor[c]	$C_{14}H_{20}NO_2Cl$	269.77	162	-	233 @25 ℃
Hexazinone[d]	$C_{12}H_{20}N_4O_2$	252.31	-	114-115.5	29800 @20℃
Oxamyl[e]	$C_7H_{13}N_3O_3S$	219.26	-	109	282000 @25 ℃

[c](Krieger, 2001), [d](FAO, 2009), [e](Mackay et al., 2006)

2.6 Previous research of pesticides

The mismanagement of pesticides that have been stored in landfills and dump sites globally is problematic, with an estimated 50 000 tonnes of pesticides present in Africa (Weber et al., 2011). The Stockholm convention states that pesticides should not be landfilled but managed in an environmentally appropriate way and destroyed without the formation of POPs (Weber et al., 2011). USEPA stated that at least one pesticide was present in approximately 10% of community water wells and in 4% of rural water wells present in the US, which included the pesticide hexazinone (Li, 2001). River water has been monitored near sugarcane plantations, which tend to use large quantities of herbicides, as in the case of the area of Sao Paulo, Brazil (Brondi and Lanças, 2004). South Africa and many other sugarcane plantations from around the world are reliant on pesticides for crop protection. Among these are the Australian agricultural sector who have carried out such studies due to fears of pesticide effects on the Great Barrier Reef (Davis et al., 2013). Pesticide concentration levels of 13.7 µg L^{-1} were recorded in the Great Barrier Reef (Davis et al., 2013). Despite South Africa's vast agriculture sector, there is no published data regarding the presence of acetochlor, hexazinone, and oxamyl in the environment.

2.7 Extraction techniques

Analytes are often found in complex matrices such as soil, sediment, and water. An extraction technique allows for the separation and isolation of an analyte from these complex matrices, thus providing a more accurate representation of specific pollutants within a specific environment (Kealey and Haines, 2002). Extraction techniques that have been used to isolate these analytes include Soxhlet, sonication, mechanical shaking, microwave digestion, solid-phase extraction (SPE), and liquid-liquid extraction. A specific extraction technique is selected based on the complexity and nature of the compound being analysed. For example, organic compounds present in the environment may be as simple as benzene, or higher molecular weight compounds like PAHs, or compounds with more complex structures such as pesticides (Dean, 2010). Ultrasound is an extraction technique with many advantages over conventional extraction methods, such as Soxhlet extraction, as it is inexpensive, simple and easy to operate (Sun et al., 1998). Extraction methods that involve exposing samples to long periods of heating are not suited to pesticides such

as oxamyl, as it results in thermal decomposition (Caballo-López and de Castro, 2003). A disadvantage of Soxhlet extraction, super critical fluid extraction and pressurized solvent extraction are their long extraction times and in the case of Soxhlet extraction, large quantities of solvent are required (Caballo-López and de Castro, 2003). Liquid-liquid extraction (LLE) is advantageous in having high selectivity and separation efficiency (Marinsky and Marcus, 1997).

2.7.1 Ultrasonication

Ultrasonication uses ultrasonic waves to disrupt the particles of a sample to extract the required analyte. The use of ultrasonication for extraction of environmental samples can either be direct, by use of an ultrasonic probe, which is placed into the actual sample or indirectly through the use of an ultrasonic bath (Capelo-Martínez, 2009). This method provides many advantages such as better solvent penetration, uses less solvent when compared to Soxhlet extraction, the analyte is able to be extracted at lower temperatures which improves safety, and has shorter extraction times (Picó, 2013). Figure 2.20 shows an ultrasonication instrument.

Figure 2.20: An ultrasonication instrument

2.7.2 Liquid-liquid extraction (LLE)

Liquid-liquid extraction is the extraction of an aqueous sample with a water-immiscible solvent, into which the analyte of interest is extracted (Pena-Pereira et al., 2009). The sample and solvent are placed into a separatory funnel in which an aqueous layer, usually water, and an organic layer, usually dichloromethane (DCM), are formed (Anthemidis and Ioannou, 2009). The analyte transfers from the aqueous layer into the organic layer. LLE is also used in industry for salt extractions from polymer solutions such as ketone resins, and metal salt extractions from ores or wastewater (Berger, 2015).

2.8 Instrumentation

2.8.1 Gas Chromatography Mass Spectrometry (GCMS)

Since the 1950s, gas chromatography has been the most applicable method for the analysis of PAHs, with the introduction of GCMS providing a more accurate representation of the individual PAHs (Nollet and De Gelder, 2013). For environmental analysis, the combination of gas chromatography and mass spectrometry is commonly used in the identification and quantitation of organic pollutants (de Almeida Azevedo et al., 2000). It is used for the separation of compounds with low boiling points and has applications in forensics in solving criminal cases, in health care to assist in medical diagnosis, and the food industry in approving food for human consumption. An advantage of using GCMS is that it separates the components of complex matrices and provides a chromatogram of the individual compounds for qualitative analysis, and also provides quantitative information on the separated compounds (Sparkman et al., 2011). GCMS samples need to be volatile, thermally liable, and able to survive the high temperature conditions of the GC (Sparkman et al., 2011).

2.8.1.1 GCMS instrument layout

GCMS instruments comprised of many components as shown in Figure 2.21, with the GC section housing the injector port and column. The sample enters the instrument through the injector port and is transported to the column by means of an inert carrier gas, which is known as the mobile phase (Niessen, 2001). As the sample passes through the column, it interacts with the stationary phase that is coated on the column wall (Niessen, 2001). The separation of the sample into its individual components depends on its interaction with the stationary phase. This interaction is dependent on the following factors: carrier gas flow rate, boiling point of the analyte, polarity of the analyte and stationary phase, column temperature, and column length (McNair and Miller, 2011). The amount of time the sample spends on the column is represented by the retention time and is an indication of the strength of the interaction between analyte and stationary phase. With gas chromatography, selectivity can be controlled using the polarity of the stationary phase and the retention factor controlled by adjusting the temperature (Heftmann, 2004).

The choice of a suitable stationary phase is important, as it may affect separation efficiency. The most commonly used stationary phases for PAH separation are capillary columns coated with either methyl or phenyl-substituted polysiloxanes (Poster et al., 2006). An advantage of using a polysiloxane stationary phase is that it may be used at high temperatures without the adverse effect of column bleeding that would result in a low background (Poster et al., 2006). The retention time can be adjusted by selecting a suitable carrier gas flow rate. In the case of the flowrate or the column temperature being too high, the retention time will be reduced but will result in poor separation efficiency (Sparkman et al., 2011). The column length could be increased to improve separation efficiency, however, this results in longer retention times (Sparkman et al., 2011).

There are different types of detectors coupled to the GC for identification of the individual components: these include either a flame ionisation detector, electron capture detector, or mass spectrometer. The mass spectrometer is commonly used for analysis of POPs. The mass spectrometer consists of three main components: an ion source, mass analyser, and detector. The vapourised compounds that exit the GC column, enter the ionization chamber of the MS and are bombarded by electrons in order to produce both negative and positive ions which will be detected

as a signal by the instrument (Gross, 2017). The mass analyser is responsible for the sorting out of the ions, which are separated according to their mass to charge ratio (de Hoffmann and Stroobant, 2013). Within the ionization chamber, molecular ions that form will fragment due to them being energetically unstable. The separated ions are then transferred to the detector, which records the ion's relative abundance and represents it as a chromatogram showing the fragmentation pattern.

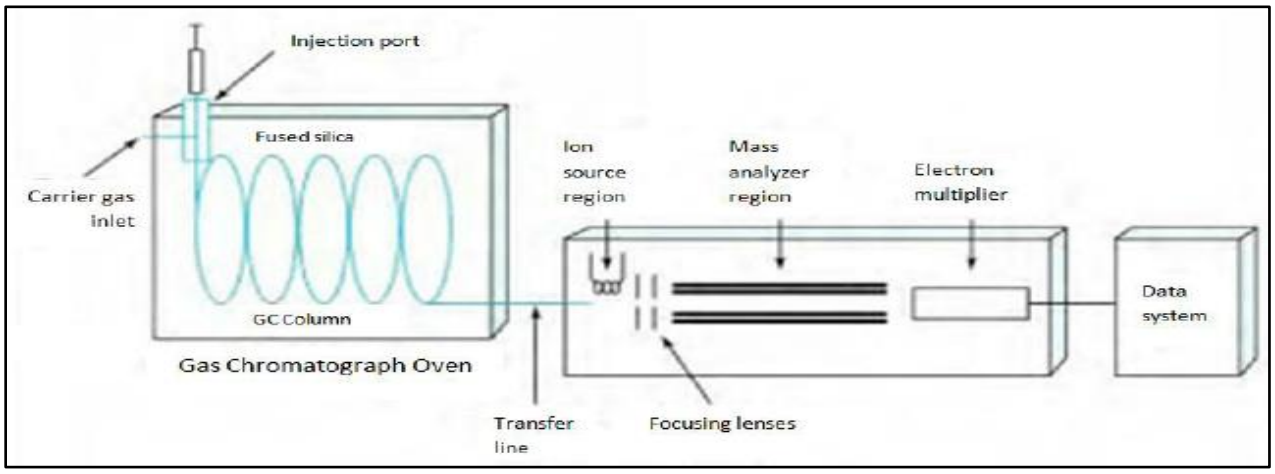


Figure 2.21: GCMS instrument layout (U.K. Mohamad Yusof, 2015)

2.8.2 Liquid Chromatography Mass Spectrometry (LCMS)

Liquid chromatography mass spectrometry (LCMS) is an analytical technique that separates a mixture into its individual components using a liquid mobile phase for the analysis of agrochemicals, food, and drugs (Ho et al., 2003). Liquid chromatography (LC) is advantageous for the separation of complex PAH mixtures, with many methods validated by USEPA for analysis of drinking water and wastewater, with LC providing greater separation efficiency (Sun et al., 1998). This method differs from the GC technique, in that the LC method uses a solid stationary phase and separation of the individual compounds is based on their polarity, whilst the GCMS method employs a liquid stationary phase and gaseous mobile phase for separation based on the boiling point of the compounds (Caballero et al., 2015). The LC column, Figure 2.22, can be either normal phase, for the analysis of relatively non-polar compounds, or reverse phase, for the analysis

of polar compounds (Polettini, 2006). LCMS allows for a simpler sample preparation as polar and non-volatile organic compounds do not need to be derivatised for analysis compared to the GCMS (Bluth, 2016).

For normal phase, the polarity of the stationary phase is higher than the mobile phase, but with the use of reverse phase chromatography, the mobile phase has a higher polarity than the stationary phase (Polettini, 2006). Solvents that may be used as a mobile phase in normal phase columns, include pentane, hexane, or DCM, with the more suitable solvents for reverse phase analysis being methanol and acetonitrile due to them being polar (Polettini, 2006).

2.8.2.1 LCMS

The LCMS method is similar to the GCMS as the sample enters the instrument through the injection port and is transported through the column by the liquid mobile phase, Figure 2.22. The separation of the sample into its individual components is not dependent on its volatility but only according to its polarity (Caballero et al., 2015). The individual components may be detected using either an ultraviolet light (UV) detector, fluorescence detector, diode array detector, electrochemical or a mass spectrometer detector (Bart, 2005). The result is a chromatogram representing the separated components. A factor that affects separation efficiency for liquid chromatography is the particle size of the stationary phase packing material (Niessen, 2006). Separation efficiency improves with reduction in particle size, however, this is limited to a particle size of 3 µm as smaller particle sizes increase backpressure (Takahashi et al., 2007).

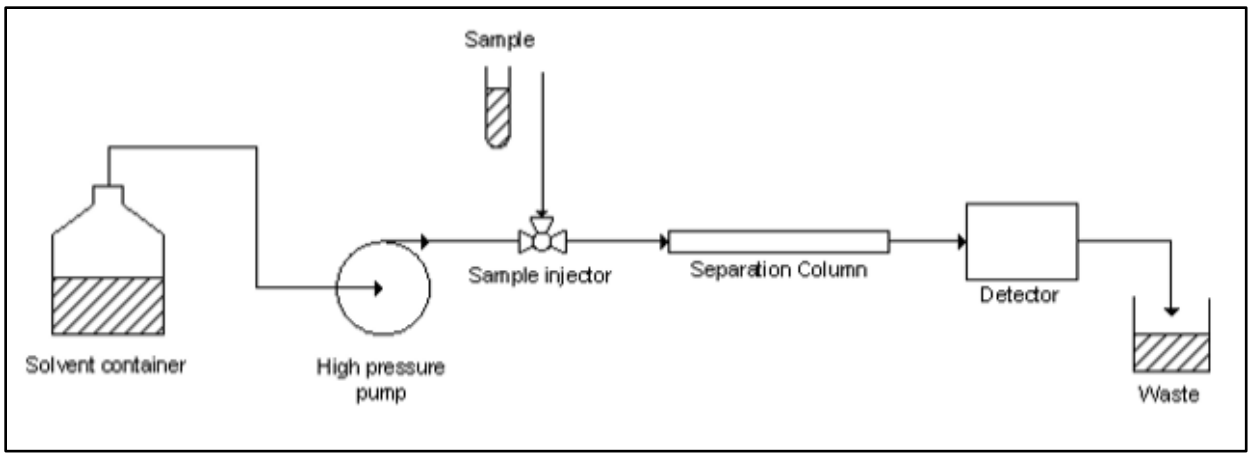


Figure 2.22: LCMS instrument layout (De Corral and Pfister, 2005).

2.9 References

ABO-STATE, M. A. M., RIAD, B. Y., BAKR, A. A. & ABDEL AZIZ, M. F. 2018. Biodegradation of naphthalene by Bordetella avium isolated from petroleum refinery wastewater in Egypt and its pathway. *Journal of Radiation Research and Applied Sciences,* 11**,** 1-9.

ANTHEMIDIS, A. N. & IOANNOU, K.-I. G. 2009. Recent developments in homogeneous and dispersive liquid–liquid extraction for inorganic elements determination. A Review. *Talanta,* 80**,** 413-421.

ARMOUR, M. A. 2016. *Hazardous Laboratory Chemicals Disposal Guide, Third Edition*, CRC Press.

ARORA, A. 2006. *Aromatic Organic Chemistry*, Discovery Publishing House.

ATSDR, U. 1990. *Toxicological Profile for Chrysene* [Online]. Available: http://www.eco-usa.net/toxics/chrysene.shtml [Accessed 20th January 2018].

ATSDR, U. 1995. Toxicological Profile for Polycyclic Aromatic Hydrocarbons. Available: https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=122&tid=25 [Accessed 12th January 2018].

ATSDR, U. 2005. *Toxicological Profile for Naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. US Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. August, 2005. Accessible via: http* [Online]. Available: https://www.atsdr.cdc.gov/toxprofiles/tp67.pdf [Accessed 15 December 2017].

ATSDR, U. 2017. Agency for Toxic Substances and Disease Registry: Minimal Risk Levels (MRLs). Available: https://www.atsdr.cdc.gov/mrls/mrllist.asp [Accessed 26th January 2018].

AUTHORITY, E. F. S. 2008. Polycyclic aromatic hydrocarbons in food 1 scientific opinion of the panel on contaminants in the food chain, adopted on 9. *EFSA J,* 724**,** 1-114.

BART, J. C. J. 2005. *Additives in Polymers: Industrial Analysis and Applications*, Wiley.

BERGER, L. R. 2015. Liquid-Liquid Extraction. Available: http://onlinelibrary.wiley.com/store/10.1002/14356007/asset/homepages/UA_LiquidLiquidExtraction.pdf?v=1&s=e3d99a3c4c66127ed5f4d07ccfe47882654dae10 [Accessed 4th November 2017].

BLUTH, M. H. 2016. *Toxicology and Drug Testing, An Issue of Clinics in Laboratory Medicine, E-Book,* Elsevier Health Sciences.

BRONDI, S. & LANÇAS, F. M. 2004. HPLC determination of pesticide residues widely employed in sugar-cane cultures in river water samples. *Journal of Liquid Chromatography & Related Technologies,* 27**,** 171-179.

CABALLERO, B., FINGLAS, P. & TOLDRA, F. 2015. *Encyclopedia of Food and Health,* Elsevier Science.

CABALLO-LÓPEZ, A. & DE CASTRO, M. L. 2003. Continuous Ultrasound-assisted extraction coupled to online filtration–solid-phase extraction–column liquid chromatography–post column derivatisation–fluorescence detection for the determination of N-methylcarbamates in soil and food. *Journal of Chromatography A,* 998**,** 51-59.

CAPELO-MARTÍNEZ, J. L. 2009. *Ultrasound in Chemistry: Analytical Applications*, Wiley.

CHEMICALS, C. 2018. *Pyrene* [Online]. Available: https://cameochemicals.noaa.gov/chemical/5163 [Accessed 24th January 2018].

CHEN, C. H. 2011. *Activation and Detoxification Enzymes: Functions and Implications*, Springer New York.

CHEREMISINOFF, N. P. & DAVLETSHIN, A. 2010. *Emergency Response Management of Offshore Oil Spills: Guidelines for Emergency Responders*, Wiley.

CHIMUKA, L., SIBIYA, P., AMDANY, R., CUKROWSKA, E. & FORBES, P. 2016. Status of PAHs in environmental compartments of South Africa: A country report. *Polycyclic Aromatic Compounds,* 36**,** 376-394.

CHRYSIKOU, L., GEMENETZIS, P., KOURAS, A., MANOLI, E., TERZI, E. & SAMARA, C. 2008. Distribution of Persistent Organic Pollutants, Polycyclic Aromatic Hydrocarbons and trace elements in soil and vegetation following a large scale landfill fire in Northern Greece. *Environment International,* 34**,** 210-225.

COOPER, A. R. 1996. *Cooper's Toxic Exposures Desk Reference with CD-ROM,* Taylor & Francis.

CROMPTON, T. R. 2012. *Organic Compounds in Soils, Sediments & Sludges: Analysis and Determination*, Taylor & Francis.

CROUCHER, L., JEWESS, P., ROBERTS, M. C., ROBERTS, T. R., HUTSON, D. H., LEE, P. W., NICHOLLS, P. H. & PLIMMER, J. R. 2007. Metabolic pathways of agrochemicals: Part 2: Insecticides and fungicides. Royal Society of Chemistry.

D'MELLO, J. P. F. 2003. *Food Safety: Contaminants and Toxins*, CABI Pub.

DABROWSKI, J. M., SHADUNG, J. M. & WEPENER, V. 2014. Prioritizing agricultural pesticides used in South Africa based on their environmental mobility and potential human health effects. *Environment International,* 62**,** 31-40.

DAVIS, A., THORBURN, P., LEWIS, S., BAINBRIDGE, Z., ATTARD, S., MILLA, R. & BRODIE, J. 2013. Environmental impacts of irrigated sugarcane production: Herbicide run-off dynamics from farms and associated drainage systems. *Agriculture, Ecosystems & Environment,* 180**,** 123-135.

DE ALMEIDA AZEVEDO, D., LACORTE, S. L., VINHAS, T., VIANA, P. & BARCELÓ, D. 2000. Monitoring of priority pesticides and other organic pollutants in river water from Portugal by Gas Chromatography–Mass Spectrometry and Liquid Chromatography–Atmospheric Pressure Chemical Ionization Mass Spectrometry. *Journal of Chromatography A,* 879**,** 13-26.

DE ANDRADE, S. J., CRISTALE, J., SILVA, F. S., ZOCOLO, G. J. & MARCHI, M. R. 2010. Contribution of sugar-cane harvesting season to atmospheric contamination by Polycyclic Aromatic Hydrocarbons (PAHs) in Araraquara city, Southeast Brazil. *Atmospheric Environment,* 44**,** 2913-2919.

DE CORRAL, J. & PFISTER, H. Hardware-accelerated 3D visualization of mass spectrometry data. Visualization, 2005. VIS 05. IEEE, 2005. IEEE, 439-446.

DE HOFFMANN, E. & STROOBANT, V. 2013. *Mass Spectrometry: Principles and Applications*, Wiley.

DEAN, J. R. 2010. *Extraction Techniques in Analytical Sciences*, Wiley.

DIKSHITH, T. S. S. 2016. *Handbook of Chemicals and Safety*, CRC Press.

DOUBEN, P. E. T. 2003. *PAHs: An Ecotoxicological Perspective*, Wiley.

ECHA. 2008. *Information on chemicals* [Online]. Available: https://www.echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database/-/discli/details/131125 [Accessed 26th January 2018].

EL-MASRI, H. 2005. *Toxicological Profile for Naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene*, Agency for Toxic Substances and Disease Registry.

ENGINEERS, U. S. A. C. O. 1996. *Green Brook Sub-basin Flood Control Plan, Updated Information Concerning a Revised Recommended Plan and Mitigation Plan, Middlesex County, Union County, Somerset County: Environmental Impact Statement*.

EO, G. 2008. *GEF Country Portfolio Evaluation: South Africa (1994–2007)*, GEF Evaluation Office.

EPA. 1999. Naphthalene. Available: https://www.epa.gov/sites/production/files/2016-09/documents/naphthalene.pdf [Accessed 10th January 2018].

EPA. 2012. *2012 Edition of the Drinking Water Standards and Health Advisories* [Online]. Available: https://www.epa.gov/sites/production/files/2015-09/documents/dwstandards2012.pdf [Accessed 29th January 2018].

EU. 2018a. *EU pesticide database* [Online]. Available: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.detail&language=EN&selectedID=913 [Accessed 03 March 2018].

EU. 2018b. *EU Pesticides Database* [Online]. Available: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.detail&language=EN&selectedID=1456 [Accessed 06 March 2018].

EU. 2018c. *Pesticide database* [Online]. Available: http://ec.europa.eu/food/plant/pesticides/eu-pesticides-

database/public/?event=activesubstance.detail&language=EN&selectedID=1645 [Accessed 03 March 2018].

FAO. 2009. FAO specifications and evaluations for agricultural pesticides. Available: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Hexazinone_2017_11_27.pdf [Accessed 12th January 2018].

FENNER, K., CANONICA, S., WACKETT, L. P. & ELSNER, M. 2013. Evaluating pesticide degradation in the environment: Blind spots and emerging opportunities. *Science,* 341**,** 752-758.

FIGUEIRA-DUARTE, T. M. & MÜLLEN, K. 2011. Pyrene-based materials for organic electronics. *Chemical Reviews,* 111**,** 7260-7314.

FRANCK, H. G. & STADELHOFER, J. W. 2012. *Industrial Aromatic Chemistry: Raw Materials · Processes · Products*, Springer Berlin Heidelberg.

FRUMKIN, H. 2016. *Environmental Health: From Global to Local*, Wiley.

GALCERAN, M. & MOYANO, E. 1993. Determination of oxygenated and nitro-substituted Polycyclic Aromatic Hydrocarbons by HPLC and Electrochemical Detection. *Talanta,* 40**,** 615-621.

GANGOLLI, S. D. 2007. The Dictionary of Substances and their Effects (DOSE): A-B. Royal Society of Chemistry.

GEBHARDT, P. 2009. *Synthesis and Characterisation of New Polymerisable Mesogens Containing Fluorene Moieties: Sonogashira Cross Coupling*, Diplom.de.

GHEVARIYA, C. M., BHATT, J. K. & DAVE, B. P. 2011. Enhanced chrysene degradation by halotolerant achromobacter xylosoxidans using response surface methodology. *Bioresource Technology,* 102**,** 9668-9674.

GIOIA, R., ECKHARDT, S., BREIVIK, K., JAWARD, F. M., PRIETO, A., NIZZETTO, L. & JONES, K. C. 2011. Evidence for major emissions of PCBs in the West African region. *Environmental Science & Technology,* 45**,** 1349-1355.

GROSS, J. H. 2017. *Mass Spectrometry: A Textbook*, Springer International Publishing.

HARITASH, A. & KAUSHIK, C. 2009. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Journal of Hazardous Materials,* 169**,** 1-15.

HARRISON, R. M. 2014. *Pollution 5th Edition: Causes, Effects and Control*, Royal Society of Chemistry.

HAYES, W. J. & LAWS, E. R. 2013. *Classes of Pesticides*, Elsevier Science.

HEFTMANN, E. 2004. *Chromatography: Fundamentals and applications of chromatography and related differential migration methods - Part A: Fundamentals and techniques*, Elsevier Science.

HO, W. F., PRICHARD, E. R., STUART, B. & CHEMISTRY, R. S. O. 2003. *High Performance Liquid Chromatography*, Royal Society of Chemistry.

JAYARAJ, R., MEGHA, P. & SREEDEV, P. 2016. Organochlorine pesticides, their toxic effects on living organisms and their fate in the environment. *Interdisciplinary Toxicology,* 9**,** 90-100.

JIA, C. & BATTERMAN, S. 2010. A critical review of naphthalene sources and exposures relevant to indoor and outdoor air. *International Journal of Environmental Research and Public Health,* 7**,** 2903-2939.

KEALEY, D. & HAINES, P. J. 2002. *BIOS Instant Notes in Analytical Chemistry*, Taylor & Francis.

KESAVACHANDRAN, C. 2014. *Health Effects of Pesticides*, Energy and Resources Institute, The Energy and Resources Institute (TERI).

KHAN, M. S. & RAHMAN, M. S. 2017. *Pesticide Residue in Foods: Sources, Management, and Control*, Springer International Publishing.

KIM, K.-H., JAHAN, S. A., KABIR, E. & BROWN, R. J. 2013. A review of airborne Polycyclic Aromatic Hydrocarbons (PAHs) and their human health effects. *Environment International,* 60**,** 71-80.

KLÁNOVÁ, J., ČUPR, P., HOLOUBEK, I., BORŮVKOVÁ, J., PŘIBYLOVÁ, P., KAREŠ, R., TOMŠEJ, T. & OCELKA, T. 2009. Monitoring of persistent organic pollutants in Africa. Part 1: Passive air sampling across the continent in 2008. *Journal of Environmental Monitoring,* 11**,** 1952-1963.

KOREN, H. & BISESI, M. S. 2002. *Handbook of Environmental Health, Fourth Edition, Volume I: Biological, Chemical, and Physical Agents of Environmentally Related Disease*, CRC Press.

KOSTECKI, P. T. & CALABRESE, E. J. 1993. *Hydrocarbon Contaminated Soils*, Taylor & Francis.

KRIEGER, R. 2001. *Handbook of Pesticide Toxicology, Two-Volume Set: Principles and Agents*, Elsevier Science.

L.P. QUINN, B. J. D. V., M. FERNANDES-WHALEY, C. ROOS, H. BOUWMAN, H. KYLIN, R. PIETERS, J. VAN DEN BERG. 2011. Pesticide use in South Africa: One of the largest importers of pesticides in Africa. Available: https://www.environment.gov.za/sites/default/files/docs/pesticide_usein_sa.pdf [Accessed 12th January 2018].

LARSON, S. J., CRAWFORD, C. G. & GILLIOM, R. J. 2004. *Development and Application of Watershed Regressions for Pesticides (WARP) for Estimating Atrazine Concentration Distributions in Streams*, US Department of the Interior, US Geological Survey.

LEE, H. 2014. *Pharmaceutical Industry Practices on Genotoxic Impurities*, CRC Press.

LENS, P., KENNES, C., CLOIREC, P. & DESHUSSES, M. 2006. *Waste Gas Treatment for Resource Recovery*, IWA Publishing.

LEVINE, M. J. 2007. *Pesticides: A Toxic Time Bomb in Our Midst*, Praeger Publishers.

LEWIS, R. J. S. 2004. Dangerous Properties of Industrial Materials. *In:* WILEY-INTERSCIENCE (ed.) 11 ed. Hoboken, NJ: Wiley & Sons.

LI, Q. 2001. Occurrence of herbicides and their degradates in Hawaii's groundwater. *Bulletin of Environmental Contamination and Toxicology,* 66**,** 653-659.

LICHTFOUSE, E., SCHWARZBAUER, J. & ROBERT, D. 2005. *Environmental Chemistry: Green Chemistry and Pollutants in Ecosystems*, Springer.

LIN, G. & QU, M. 2016. *Smart Use of State Public Health Data for Health Disparity Assessment*, CRC Press.

MACKAY, D., SHIU, W. Y., MA, K. C. & LEE, S. C. 2006. *Handbook of Physical-Chemical Properties and Environmental Fate for Organic Chemicals, Second Edition*, CRC Press.

MAISTO, G., DE NICOLA, F., IOVIENO, P., PRATI, M. V. & ALFANI, A. 2006. PAHs and trace elements in volcanic urban and natural soils. *Geoderma,* 136**,** 20-27.

MANAGEMENT, U. S. B. O. L. 2010. *Vegetation Treatments Using Herbicides on BLM Lands in Oregon: Environmental Impact Statement*.

MARINSKY, J. A. & MARCUS, Y. 1997. *Ion Exchange and Solvent Extraction: A Series of Advances*, Taylor & Francis.

MATSUMURA 2012. *Environmental Toxicology of Pesticides*, Elsevier Science.

MCNAIR, H. M. & MILLER, J. M. 2011. *Basic Gas Chromatography*, Wiley.

MONTGOMERY, J. H. 2007. *Groundwater Chemicals Desk Reference, Fourth Edition*, CRC Press.

MONTGOMERY, J. H. 2010. *Groundwater Chemicals Desk Reference, 3rd Edition*, CRC Press.

NCIT. 2017. *Indeno[1,2,3-c,d]pyrene* [Online]. Available: https://ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI_Thesaurus&ns=NCI_Thesaurus&code=C44393 [Accessed 26th January 2018].

NEILSON, A. N. 2013. *PAHs and Related Compounds: Chemistry*, Springer Berlin Heidelberg.

NIESSEN, W. M. A. 2001. *Current Practice of Gas Chromatography-Mass Spectrometry*, CRC Press.

NIESSEN, W. M. A. 2006. *Liquid Chromatography-Mass Spectrometry, Third Edition*, CRC Press.

NIEUWOUDT, C., PIETERS, R., QUINN, L. P., KYLIN, H., BORGEN, A. R. & BOUWMAN, H. 2011. Polycyclic Aromatic Hydrocarbons (PAHs) in soil and sediment from industrial, residential, and agricultural areas in central South Africa: An initial assessment. *Soil and Sediment Contamination,* 20**,** 188-204.

NIOSH. 1999. *Benzo[b]fluoranthene* [Online]. Available: https://www.cdc.gov/niosh/ipcsneng/neng0720.html [Accessed 26th January 2018].

NIOSH. 2010. *NIOSH Pocket Guide to Chemical Hazards* [Online]. Available: https://www.cdc.gov/niosh/npg/ [Accessed 26th Janaury 2018].

NOLLET, L. M. L. & DE GELDER, L. S. P. 2013. *Handbook of Water Analysis, Third Edition*, CRC Press.

O'NEIL, M. J. 2013. The Merck Index-An encyclopedia of chemicals, drugs, and biologicals. UK:Royal Society of Chemistry.

PAMPANIN, D. M. & SYDNES, M. O. 2017. *Petrogenic Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Analysis, Synthesis, Toxicity and Environmental Impact*, Bentham Science Publishers.

PARANJAPE, K., GOWARIKER, V., KRISHNAMURTHY, V. N. & GOWARIKER, S. 2014. *The Pesticide Encyclopedia*, CABI.

PATNAIK, P. 2007. *A Comprehensive Guide to the Hazardous Properties of Chemical Substances*, Wiley.

PAVIA, D. L. 2005. *Introduction to Organic Laboratory Techniques: A Small Scale Approach*, Thomson Brooks/Cole.

PENA-PEREIRA, F., LAVILLA, I. & BENDICHO, C. 2009. Miniaturized preconcentration methods based on liquid–liquid extraction and their application in inorganic ultratrace analysis and speciation: A Review. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 64**,** 1-15.

PICÓ, Y. 2013. Ultrasound-assisted extraction for food and environmental samples. *TrAC Trends in Analytical Chemistry*, 43**,** 84-99.

POHANISH, R. P. 2011. *Sittig's Handbook of Toxic and Hazardous Chemicals and Carcinogens*, Elsevier Science.

POHANISH, R. P. 2014. *Sittig's Handbook of Pesticides and Agricultural Chemicals*, Elsevier Science.

POHANISH, R. P. 2017. *Sittig's Handbook of Toxic and Hazardous Chemicals and Carcinogens*, Elsevier Science.

POLETTINI, A. 2006. *Applications of LC-MS in Toxicology*, Pharmaceutical Press.

POSTER, D. L., SCHANTZ, M. M., SANDER, L. C. & WISE, S. A. 2006. Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) in environmental samples: A critical review of Gas Chromatographic (GC) Methods. *Analytical and Bioanalytical Chemistry*, 386**,** 859-881.

PROCTOR, N. H., HUGHES, J. P. & HATHAWAY, G. J. 2004. *Proctor and Hughes' Chemical Hazards of the Workplace*, Wiley.

PROGRAM, N. T. 2011. *Report on Carcinogens (12th Ed.)*, DIANE Publishing Company.

PRYOR, W. 2012. *Free Radicals in Biology*, Elsevier Science.

PURSER, D. A., MAYNARD, R. L. & WAKEFIELD, J. C. 2015. *Toxicology, Survival and Health Hazards of Combustion Products*, Royal Society of Chemistry.

QAMAR, Z., KHAN, S., KHAN, A., AAMIR, M., NAWAB, J. & WAQAS, M. 2017. Appraisement, source apportionment and health risk of polycyclic aromatic hydrocarbons (PAHs) in vehicle-wash wastewater, Pakistan. *Science of the Total Environment*, 605**,** 106-113.

RATHORE, H. S. & NOLLET, L. M. L. 2012. *Pesticides: Evaluation of Environmental Pollution*, CRC Press.

REISEN, F. & AREY, J. 2002. Reactions of Hydroxyl Radicals and Ozone with Acenaphthene and Acenaphthylene. *Environmental Science & Technology,* 36**,** 4302-4311.

ROBERTS, M. C., CROUCHER, L., ROBERTS, T. R., HUTSON, D. H., LEE, P. W., NICHOLLS, P. H. & PLIMMER, J. R. 2007. *Metabolic Pathways of Agrochemicals: Part 1: Herbicides and Plant Growth Regulators*, Royal Society of Chemistry.

RUPPERT, H., KAPPAS, M. & IBENDORF, J. 2013. *Sustainable Bioenergy Production - An Integrated Approach*, Springer Netherlands.

SATTLER, K. D. 2016. *Carbon Nanomaterials Sourcebook: Nanoparticles, Nanocapsules, Nanofibers, Nanoporous Structures, and Nanocomposites,* CRC Press.

SERVICE, U. S. F. 2004. *Eldorado National Forest (N.F.), Silver Pearl Land Exchange: Environmental Impact Statement*.

SERVICE, U. S. F. 2005. *Carson and Santa Fe National Forests (N.F.), Invasive Plant Control Project: Environmental Impact Statement*.

SERVICE, U. S. F. 2006. *Custer National Forest (N.F.), Weed Management: Environmental Impact Statement*.

SHEN, H. 2016. *Polycyclic Aromatic Hydrocarbons: Their Global Atmospheric Emissions, Transport, and Lung Cancer Risk*, Springer Berlin Heidelberg.

SOCLO, H., GARRIGUES, P. & EWALD, M. 2000. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. *Marine pollution bulletin,* 40**,** 387-396.

SPARKMAN, O. D., PENTON, Z. & KITSON, F. G. 2011. *Gas Chromatography and Mass Spectrometry: A Practical Guide*, Elsevier Science.

SPEIGHT, J. G. 2016. Environmental organic chemistry for engineers. Elsevier Science.

SPELLMAN, F. R. 2016. *Contaminated Sediments in Freshwater Systems*, CRC Press.

STELLMAN, J. M. & OFFICE, I. L. 1998. *Encyclopaedia of Occupational Health and Safety: Guides, indexes, directory,* International Labour Office.

SUN, F., LITTLEJOHN, D. & GIBSON, M. D. 1998. Ultrasonication extraction and solid phase extraction clean-up for determination of US EPA 16 priority pollutant Polycyclic Aromatic Hydrocarbons in soils by reversed-phase liquid chromatography with ultraviolet absorption detection1. *Analytica Chimica Acta,* 364**,** 1-11.

TAKAHASHI, N., ISOBE, T., DESIDERIO, D. M. & NIBBERING, N. M. 2007. *Proteomic Biology Using LC/MS: Large Scale Analysis of Cellular Dynamics and Function*, Wiley.

TATUM, V. L. 2004. Toxicity, transport, and fate of forest herbicides. *Wildlife Society Bulletin,* 32**,** 1042-1048.

TOXNET. 2017a. *HSDB: Benzo(k)fluoranthene* [Online]. Available: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+207-08-9 [Accessed 29th January 2018].

TOXNET. 2017b. *HSDB: Benzo[e]pyrene* [Online]. Available: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+@rel+192-97-2 [Accessed 26th January 2018].

U.K. MOHAMAD YUSOF, A. C. S., N.F. M. RADZI, A.J.ISHAK, M.K. HASSAN, S.A. AHMAD, S. KHAMIS 2015. Selection of feature analysis electronic nose signals based on the correlation between gas sensor and herbal phytochemical. *Australian Journal of Basic and Applied Sciences,* 9**,** 360-367.

UDEH, P. J. 2004. *A Guide to Healthy Drinking Water: All You Need to Know about The Water You Drink*, iUniverse.

USEPA. 2001. Acenaphthylene fact sheet Available: https://archive.epa.gov/epawaste/hazard/wastemin/web/pdf/acnphthy.pdf [Accessed 24th January 2018].

VERSCHUEREN, K. 2001. *Handbook of Environmental Data on Organic Chemicals*, New York, John Wiley & Sons.

VINCOLI, J. W. 1996. *Risk Management for Hazardous Chemicals*, Taylor & Francis.

WANG, B. Y. 2007. *Environmental Biodegradation Research Focus*, Nova Science Publishers.

WEBER, R., WATSON, A., FORTER, M. & OLIAEI, F. 2011. Persistent Organic Pollutants and landfills-A review of past experiences and future challenges. *Waste Management & Research,* 29**,** 107-121.

WEISSERMEL, K. & ARPE, H. J. 2003. *Industrial Organic Chemistry*, Wiley-VCH.

WEXLER, P., ANDERSON, B., DE PEYSTER, A., GAD, S. C., HAKKINEN, P. J. B., KAMRIN, M., LOCEY, B., MEHENDALE, H. M., POPE, C. & SHUGART, L. 2005. *Encyclopedia of Toxicology*, Elsevier Science.

WHEELER, W. B. 2002. *Pesticides in Agriculture and The Environment*, CRC Press.

WHO. 1998. Polynuclear Aromatic Hydrocarbons in drinking-water. Available: http://www.who.int/water_sanitation_health/dwq/chemicals/polyaromahydrocarbons.pdf [Accessed 27/01/2018].

ZELIGER, H. 2011. *Human Toxicology of Chemical Mixtures*, Elsevier Science.

ZENG, E. Y. 2015. *Persistent Organic Pollutants (POPs): Analytical Techniques, Environmental Fate and Biological Effects*, Elsevier Science.

ZHANG, Y. & TAO, S. 2009. Global atmospheric emission inventory of Polycyclic Aromatic Hydrocarbons (PAHs) for 2004. *Atmospheric Environment,* 43**,** 812-819.

Chapter 3: Materials and Methods

3.1 Sampling sites

The study area was Buffelsdraai, which is located north-west of the main city of Durban in KwaZulu-Natal, South Africa. The location is the Buffelsdraai landfill site, situated nearby the town of Verulam, which is managed by the eThekwini municipality's department of Durban Solid Waste (DSW). This site was previously a sugarcane plantation prior to it being converted into a landfill in 2006. It covers approximately 116.2 ha, with a total of more than 7000 planted trees surrounding the landfill (Douwes et al., 2016). These trees form the buffer zone that surrounds the landfill. Buffelsdraai and Osindisweni, which are the neighbouring communities, are protected by the buffer zone from the odour and unpleasant views associated with landfills. The reforestation site, Figure 3.1, is divided into 30 blocks. These blocks represent different planting phases, which refer to the year in which the trees were planted. Due to the large scale of the reforestation area, dividing the site into separate blocks allows for better management in the preparation of land for the planting of trees, which is done in the spring and summer so as to make use of the high rainfalls. Soil samples were collected from planting phases 2 (2010/2011), 3 (2011/2012), 4 (2012/2013), 5 (2013/2014), and 6 (2014/2015). These sampling sites were selected based on the topography of the land, whether it slopes towards the surrounding rivers and also their close proximity to the river, to determine if the reforestation assists with improving water quality. The control soil sample was collected from an area on the site where no trees had been planted. Water and sediment samples were collected from both the Black (which flows through the reforestation site) and White Mhlasini Rivers, which flows alongside the reforestation site. Sampling sites along the rivers were selected upstream and downstream of the reforestation site.

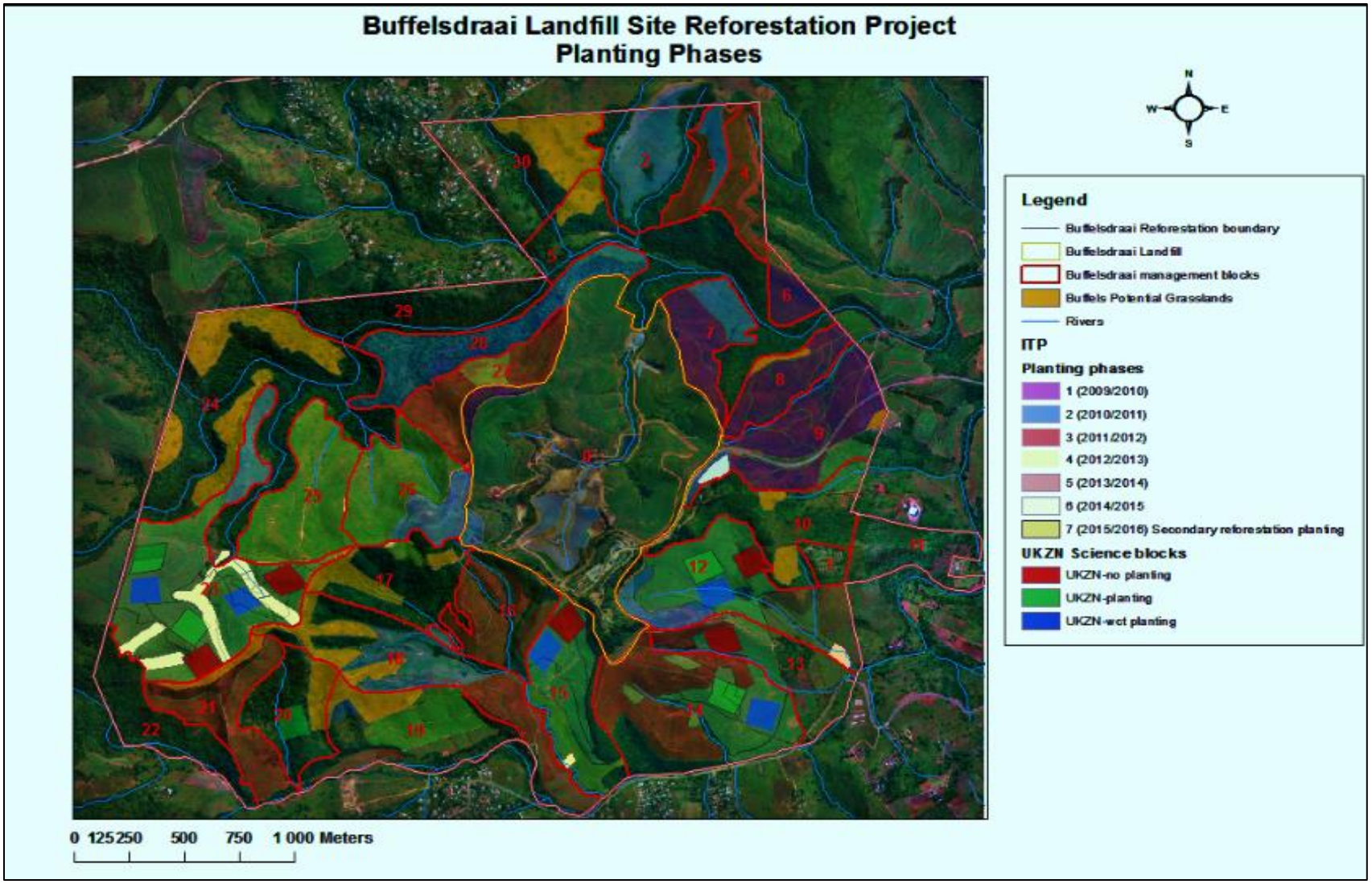


Figure 3.1: Map showing the location of the landfill site, reforestation site, surrounding rivers, and the neighbouring communities as constructed by eThekwini using the ArcMap software

Table 3.1: GPS coordinates for soil sampling sites

Site code		Coordinates		Description of sampling site
		South	East	
2010/11	T	29.610868°	30.987360°	Slopes steeply down towards the Black Mhlasini River, highly dense vegetation surrounding trees
	M	29.613012°	30.987311°	
	B	29.614567°	30.986988°	
2011/12	T	29.626470°	30.975460°	Slopes slightly towards the Black Mhlasini River, vegetation surrounding trees was less dense
	M	29.625228°	30.974799°	
	B	29.623815°	30.974055°	
2012/13	T	29.623261°	30.976415°	Slopes slightly towards the Black Mhlasini River, vegetation surrounding trees was less dense
	M	29.622728°	30.976182°	
	B	29.622139°	30.975697°	
2013/14	T	29.632495°	30.992985°	Slopes steeply towards the White Mhlasini River, highly dense vegetation surrounding trees
	M	29.633661°	30.992873°	
	B	29.634580°	30.992745°	
2014/15	T	29.634081°	30.965649°	Slopes slightly towards the White Mhlasini River, vegetation surrounding trees was less dense
	M	29.632887°	30.965466°	
	B	29.631442°	30.965355°	

*T = top, M= middle, B= bottom

Table 1 shows the GPS coordinates for the individual soil sampling sites and a description of the surrounding environment. Soils samples were collected from the top (T), middle (M), and bottom (B) section of each planting phase.

Table 3.2: GPS coordinates for water and sediment sampling sites

Site code		Coordinates		Description of sampling site
		South	East	
Black Mhlasini	BM 1	29.620069°	30.948950°	Upstream of the reforestation site
	BM 2	29.622111°	30.966015°	Before community, steep banks
	BM 3	29.620728°	30.990815°	After community, steep banks
	BM 4	29.620648°	31.004853°	Downstream of reforestation site, dense vegetation surrounding river, sand mining taking place near riverbanks.
White Mhlasini	WM 1	29.638632°	30.947723°	Steep banks with dense vegetation, upstream of reforestation site
	WM 2	29.640785°	30.969649°	Before community
	WM 3	29.633874°	30.996329°	After community
	WM 4	29.625341°	31.003808°	Downstream of reforestation site

*BM – Black Mhlasini River, WM – White Mhlasini River

3.2 Reagents and materials

All reagents and chemicals were purchased from Sigma-Aldrich® South Africa and Supelco, and were HPLC-grade. The polyaromatic hydrocarbon mixture of standards containing naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[e]pyrene, indeno[1,2,3-c,d]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene was purchased from Supelco. The internal standard acenaphthene-d10 was purchased from Supelco. The pesticide standards acetochlor, hexazinone, and oxamyl were purchased from Sigma-Aldrich® South Africa. Silica gel of high purity, Davisil grade 923, pore size 30 Å, 100-200 mesh was purchased from Sigma-Aldrich® South Africa. To activate the silica gel, it was placed in an oven at 130 ℃ for 16 hours prior to use.

3.3 Sample collection

All sample bottles were cleaned before sampling. They were washed three times with liquid detergent soap (DynaChem), rinsed with tap water followed by deionized water, and then HPLC grade acetone. Soil samples were collected from the reforestation site by scooping soil at a depth of 0-10 cm using a stainless steel spade and stored in glass bottles with the bottle lids lined with aluminium foil. Sediment samples were collected from the riverbed of the Black and White Mhlasini Rivers using a stainless steel corer, with core depths of approximately 0-15 cm. The core samples were stored in aluminium foil. River water samples were collected in 2.5 L glass amber reagent bottles. The bottles were first rinsed three times with the river water and filled with the sample until overflowing to ensure no headspace remained in the bottle. All sample bottle lids were lined with aluminium foil to prevent phthalate contamination. Table 3.1 shows the coordinates for the soil samples and Table 3.2, the coordinates for sediment and water samples. Samples were stored in ice during transportation to the laboratory. Water samples were gravity filtered before extraction. The water samples were stored in a fridge at 4 ℃ to preserve the samples from biological degradation and prevent volatilisation of analytes (Harsham, 1995). Soil and sediment samples were air dried for five days; crushed using a mortar and pestle, then sieved using

a 125 µm stainless steel sieve. The extractions of all samples were conducted within two weeks of sample collection.

3.4 Sample extraction

3.4.1 Soil and sediment

Ultrasonication extraction was used for soil and sediment. This technique was chosen as it provides higher extraction efficiencies, reduces cost and is easy to operate compared to other extraction techniques such as soxhlet extraction (Lau et al., 2010). Soil (5 g) or sediment (1 g) was weighed in a 100 mL glass beaker and was extracted three times with 25 mL of dichloromethane (DCM) at 30 ℃ for 30 minutes in an ultrasonic bath (SCIENTECH model 702). The combined extracts were filtered and concentrated to 1 mL using a rotary evaporator (Heidolph, Basis Hei-VAP).

3.4.2 Water

Liquid-liquid extraction (LLE) was used for the water samples (Wise et al., 2015). A 500 mL aliquot sample was transferred to a 1 L separating funnel and extracted three times with 30 mL DCM. With each addition, the sample was shaken vigorously with periodic venting and allowed to separate for 10 minutes before collecting the organic layer. The combined extracts were dried with anhydrous sodium sulphate (Na_2SO_4) and filtered. Extracts were concentrated to 1 mL using a rotary evaporator.

3.5 Validation of method

The extraction methods were validated using a recovery test, inter-day and intra-day analyses, and determining the limits of detection (LOD) and limits of quantitation (LOQ). The recovery test assists in determining the extraction efficiency of the extraction method. LOD values represent the lowest concentration that can be detected by the instrument and LOQ values represent the lowest concentration that can be quantified by the instrument with good accuracy and precision. Intra-day and inter-day analysis were conducted to determine the reproducibility of the analysis within a

short period of time and over a longer period of time. Soil and sediment used for method development, were collected from the reforestation site. This ensured that the sample matrix used for method development was similar to that of the actual reforestation site samples. Samples were spiked and then extracted in triplicate using the methods stated under section 3.4.

3.6 Clean-up

The clean-up of the sample extracts is an important step for the removal of interferences that would affect column efficiency and result in poor peak resolution (Manahan, 2004). The extraneous substances may also result in damage of the column and instrumentation. Silica gel clean up, EPA method 3630 C, which uses a standard column chromatography technique, was used for the clean-up of both the PAH and pesticide extracts (EPA, 1996). The silica gel was first activated on a glass tray, by loosely covering it with foil, and placing it in an oven for 16 hours at 130 ℃. The activated silica was then mixed with DCM to form a slurry and transferred into a 10 mm ID chromatographic column. Anhydrous sodium sulfate (2 cm) was added to the top of the slurry for the removal of excess water from the sample extracts. The column was pre-eluted with 40 mL pentane to remove any impurities that may be present in the column. The sample extract was transferred in cyclohexane to the column and eluted with 25 mL of pentane to remove any interferences that would inhibit extraction efficiency. This eluate was discarded. The column was then eluted with 25 mL DCM:pentane (2:3) (v/v) mixture and the eluent collected for concentration.

3.7 PAH and pesticide standards

3.7.1 PAHs

A 100 ppm working solution was prepared by transferring 0.5 mL of a 2000 ppm stock standard into a 10 mL volumetric flask. The solution was made up to the mark using acetonitrile. The flask was then shaken to homogenise the solution and stored in a fridge at 4 ℃. PAH standards were prepared from the working solution by pipetting the required volume into 1 mL GC vials and made up to the mark using acetonitrile (Table 3.3). The acenaphthene D-10 internal standard was

prepared by dissolving 10 mg of the standard in acetonitrile in a 10 mL volumetric flask and made up to the mark using acetonitrile.

3.7.1.1 Preparation of PAH working solution and standards

The standards were prepared using the dilution factor method:

$C_1V_1 = C_2V_2$……(1)

Where C = concentration, ppm

V = volume, mL

Using equation 1, the 100 ppm working solution was prepared in a 10 mL volumetric flask as follows:

(2000 ppm) * (V_1) = (100 ppm) * (10 mL)

$$V_1 = \frac{(100\ \text{ppm}) * (10\text{mL})}{(2000\ \text{ppm})} = 0.5\ \text{mL}$$

Therefore, 0.5 mL of the 2000 ppm stock solution was pipetted into the 10 mL volumetric flask. The PAH standards prepared in 1 mL GC vials from the working solution ranged from 2-10 ppm, with the volume of working solution required for each standard listed in Table 3.3. This range worked best on the instrument and provided consistent results. Samples were spiked to fall within the detection range of the instrument.

Table 3.3: Volume of the 100 ppm working solution required for the PAH standards

Concentration/ ppm	Required volume from working solution/ µL
2	20
4	40
6	60
8	80
10	100

3.7.2 Pesticides

Individual 100 ppm working solutions of each pesticide were prepared by dissolving 1 mg of each standard in acetonitrile in separate 10 mL volumetric flasks. Each flask was made up to the mark using acetonitrile. The pesticide standards, in the range 2-10 ppm, were then prepared as a mixture of the individual pesticides. As stated in Table 3.3, the same required volume of each individual pesticide working solution was transferred into a 1 mL GC vial and made up to the mark using acetonitrile.

3.8 Sample analysis

3.8.1 PAHs

A Shimadzu QP2010 SE gas chromatography mass spectrometer (GCMS) was used to determine the individual PAHs present in the samples. The parameters for the instrument are listed in Table 3.4.

Table 3.4: GCMS instrument parameters

Parameter	
Column	DB-5MS capillary (30 m length, 0.25 µm diameter, 0.25 µm film thickness)
Carrier gas	Helium
Carrier gas flowrate	0.72 mL min^{-1}
Injection mode	Splitless
Injector temperature	250 ℃
Injection volume	5 µL
Detector temperature	320 ℃
Oven temperature program	Ramped from 110 ℃ to 210 ℃ at 37 ℃ min^{-1} Ramped from 210 ℃ to 260 ℃ at 3 ℃ min^{-1} Ramped from 260 ℃ to 300 ℃ at 5 ℃ min^{-1}, held for 4.5 mins
Mass spectrometer	Quadrupole, electron ionisation (EI) Ion source temperature: 200 ℃ Detector voltage: 0 kV

3.8.2 Pesticides

A Shimadzu LCMS-2020 liquid chromatography mass spectrometer (LCMS) with a Shim-pack HPLC packed column was used to determine the individual pesticides present in the samples. The parameters for the instrument are listed in Table 3.5.

Table 3.5: LCMS instrument parameters

Parameter	
Column	3 µm C-18, 2.1 x 100 mm
Solvent	A – 50.0% H_2O B – 50.0% Acetonitrile (ACN)
Solvent flow rate	0.2 mL min^{-1}
Injection volume	5 µL
Program	0 – 3 mins (50.0% ACN) 3-7.5 mins (50.0% to 100% ACN) 7.5 – 15 mins (100% to 50.0% ACN) 15 – 25 mins (50% ACN)
Mass spectrometer	Single quadrupole, Electrospray Ionisation (ESI): • positive mode, 0 kV • Ion temperature: 350 ℃

3.9 Recovery

3.9.1 Soil and sediment recoveries

Since the samples used for method development were expected to have either no analytes present or have analytes present at concentrations below the LOD of the instrument, samples had to be spiked. The recovery test was performed by determining the percentage of analyte recovered from the extraction of a spiked sample and comparing this to the extraction of an unspiked sample. To determine the percentage recovered, the following equation was used (Hibbert, 2007):

Percentage recovery = $\frac{(\text{spiked sample result}) - (\text{unspiked sample result})}{(\text{known spike added concentration})}$ x 100…….(2)

Example: A 5 g soil sample was weighed into a beaker, spiked with 5 µg g^{-1} PAH mix standard and homogenized. Another unspiked 5 g soil sample was weighed into a separate beaker. The beakers were covered in foil and placed on a mechanical shaker for 24 hours before being extracted using the ultrasonication method stated under section 3.4. The concentration of the spiked soil sample was 5.426 µg g^{-1} and was compared to the unspiked soil sample to determine the percentage recovery using equation (2). The unspiked soil sample either had no analyte present or was below the detection limit as the concentration was 0 µg g^{-1}. The recovery analysis was performed in triplicate. The following calculation is an example showing the percentage recovery:

Percentage recovery = $\frac{(5.426\ \mu g\ g^{-1} - 0\ \mu g\ g^{-1})}{5\ \mu g\ g^{-1}}$ x 100 = 108.5 %

3.9.2 Water recoveries

Percentage recovery = $\frac{\text{response of spiked sample } \mu g\ L^{-1}}{\text{known spike added concentration } \mu g\ L^{-1}}$ x 100……..(3)

Example: A 500 mL tap water sample was spiked with 5 mg L^{-1}. The water sample was then extracted using the method stated under section 3.4. The response of the spiked sample was 4.463 mg L^{-1}. The analysis was performed in triplicate. The following calculation is an example showing the percentage recovery:

Percentage recovery = $\frac{(4.463\ mg\ L^{-1})}{5\ mg\ L^{-1}}$ x 100 = 89.3 %

3.10 Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD = 3.3 x $\frac{SD}{m}$……..(4)

$$LOQ = 10 \times \frac{SD}{m} \quad \text{.....(5)}$$

Where, SD = standard deviation

m = gradient

To determine the SD and m values, a calibration graph for each analyte was constructed, appendix C. The analysis of each analyte was conducted in triplicate with a concentration range of 2-10 ppm. Peak areas of each standard were plotted against the concentration values. For example, the equation of three graphs for an analyte were: y = 0.7345x + 0.3143, y = 0.829x-0.0092, and y = 0.853x + 0.2101. Using these equations, the standard deviation was calculated from the intercept values and the average gradient determined to be 0.8055. The LOD and LOQ values were then determined using equations 4 and 5.

$$LOD = 3.3 \times \frac{0.1551}{0.8055} = 0.327$$

$$LOQ = 10 \times \frac{0.1551}{0.8055} = 0.992$$

3.11 Inter-day and Intra-day

To ensure that the analysis of each sample is consistent, inter-day and intra-day analysis was conducted. Intra-day analysis was performed by analysing the extracted sample in replica on the same day and inter-day analysis involved the analysis of the extracted sample over consecutive days. This would assist in determining if there were any inconsistencies with the instrument.

3.12 Concentration of actual sample

3.12.1 Water samples

The individual analyte concentration present in each sample, was determined using the following equation:

$C_sV_s = C_eV_e$…….(6)

Where, C_s = concentration of analyte in the sample, ng mL^{-1}

V_s = volume of water sample, mL

C_e = concentration of analyte in the sample extract, µg mL^{-1}

V_e = volume of extract, mL

Example: Using the extraction method stated under section 3.4, a 1 mL spiked sample extract was obtained for analysis on the GCMS. The concentration of the analyte in the spiked sample was obtained by subtracting the spiked concentration amount, 5 µg mL^{-1}, from the concentration value determined for the spiked sample. Using equation 6, which takes dilution factors into account, the actual concentration of the analyte in a water sample was determined. For a sample extract concentration of 0.3612 µg mL^{-1},

$C_sV_s = C_eV_e$

$$C_s = \frac{C_eV_e}{V_s}$$

$$= \frac{(0.3612\ \mu g\ mL^{-1})\ x\ (1\ mL)}{(500\ mL)}$$

$$= 0.0007224\ \mu g\ mL^{-1}$$

Therefore, C_s = 0.0007224 µg mL^{-1} x $\frac{1000\ ng}{1\ \mu g}$ = 0.7224 ng mL^{-1}

3.12.2 Soil and sediment samples

The individual analyte concentration present in each sample, was determined using the following equation:

$C_sW_s = C_eV_e$…….(6)

Where, C_s = concentration of analyte in the sample, ng mL^{-1}

W_s = weight of the solid sample, g

C_e = concentration of analyte in the sample extract, µg mL^{-1}

V_e = volume of extract, mL

Example: Using the extraction method stated under section 3.4, the same procedure was followed as for the water samples. For a solid sample extract concentration of 0.3208 µg mL^{-1},

$C_sW_s = C_eV_e$

$$C_s = \frac{C_eV_e}{W_s}$$

$$= \frac{(0.3208\ \mu g\ mL^{-1})\ x\ (1\ mL)}{(5\ g)}$$

$$= 0.06416\ \mu g\ g^{-1}$$

Therefore, $C_s = 0.06416\ \mu g\ g^{-1} \times \frac{1000\ ng}{1\ \mu g} = 64.16\ ng\ g^{-1}$

3.13 Percentage of total individual PAH removed from soil

The percentage of total individual PAH removed was based on the concentration determined in the soil samples collected from the individual planting phases and compared to the control soil sample. This was done to determine the percentage by which the concentration had potentially decreased as a result of these pollutants possibly being taken up by the planted trees.

Example: For a total naphthalene concentration of 1.42 µg g^{-1} in the soil sample collected from the 2010/2011 planting phase and 0.574 µg g^{-1} in the control soil sample. The percentage removed is as follows:

Percentage of PAH removed = $\frac{(1.42\ \mu g\ g^{-1} - 0.574\ \mu g\ g^{-1})}{(1.42\ \mu g\ g^{-1})} * 100 = 59.6\ \%$

3.14 Quality assurance

To ensure that the analysis was uninterrupted and the data consistent, a 2 ppm standard was analysed at the beginning, middle, and end of each sample set on the GCMS and LCMS. A method blank was analysed after each analysis of PAH and pesticide extract to assess for the presence of background contamination. A deuterated internal standard, acenaphthene D-10, was used in the analysis of PAHs. For the calibration graphs, the ratio of the response of each individual PAH to the response of acenaphthene D-10, was plotted against the concentration range, 2-10 ppm. Any instrumental error, such as a decrease in sensitivity, that may have occurred during the analysis of

batch samples, was taken into account by using the ratio of the response. If an error occurred in the midst of analysis, it would affect the response of the internal standard and response of the individual analyte, but this error would be cancelled out by plotting the responses as a ratio.

3.15 References

DOUWES, E., ROUGET, M., DIEDERICHS, N., O'DONOGHUE, S., ROY, K. & ROBERTS, D. 2016. The Buffelsdraai Landfill Site Community Reforestation Project. Unasylva, 67**,** 12.

EPA. 1996. Method 3630C silica gel clean up. Available: https://www.epa.gov/sites/production/files/2015-12/documents/3630c.pdf [Accessed: 12 December 2017].

HARSHAM, K. 1995. Water sampling for pollution, Taylor & Francis, 1, 71.

HIBBERT, D. B. 2007. Quality assurance in the analytical chemistry laboratory, Oxford University Press.

LAU, E., GAN, S. & NG, H. 2010. Extraction techniques for Polycyclic Aromatic Hydrocarbons in soils. *power,* 44**,** 13-16.

MANAHAN, S. E. 2004. Environmental chemistry, Eighth Edition, Taylor & Francis, 8, 704.

WISE, S. A., SANDER, L. C. & SCHANTZ, M. M. 2015. Analytical methods for determination of Polycyclic Aromatic Hydrocarbons (PAHs)—A historical perspective on the 16 US EPA priority pollutant PAHs. Polycyclic Aromatic Compounds, 35**,** 187-247.

Chapter 4: Results and Discussion

4.1 Physical and chemical parameters

The physical and chemical parameters listed in Table 4.1 were measured at each sampling site for the Black and White Mhlasini Rivers.

Table 4.1: Physical and chemical properties measured for the Black and White Mhlasini Rivers

River	Sampling Site	Temperature/ ℃	Conductivity/ µS cm^{-1}	TDS/ mg L^{-1}	pH
Black Mhlasini (BM)	BM 1	24.0	206.4	116.1	4.28
	BM 2	22.0	258.2	144.9	4.31
	BM 3	19.8	385.5	214.3	4.31
	BM 4	24.6	662.4	257.6	3.67
White Mhlasini (WM)	WM 1	22.2	244.7	137.6	4.30
	WM 2	20.2	312.5	174.9	4.29
	WM 3	23.9	326.7	184.4	3.68
	WM 4	24.0	356.9	201.4	3.67

*TDS – Total Dissolved Solids

The water temperatures recorded, show that sampling site 3 for the Black Mhlasini River and sampling site 2 for the White Mhlasini River had low temperature values of 19.8 ℃ and 20.2 ℃ respectively. This could be due to the dense vegetation and tree cover surrounding these sections of the river, which resulted in the area being shaded away from direct sunlight. The highest conductivity value of 662.4 µS cm^{-1}, measured at site BM 4, was significantly higher than the other sampling sites. This site also had the highest recording for total dissolved solids (TDS) of 257.6 mg L^{-1}. The high values could be due to the presence of sand mining occurring within close proximity of the river, which may result in a higher concentration of dissolved organic matter in the river water. The conductivity and TDS values display an increasing trend from sampling sites 1 to sampling sites 4 for both rivers. The pH values suggest that the river water is slightly acidic. A possible contributing factor was the possible decomposition of the surrounding vegetation,

which reduces oxygen levels and increases the level of carbon dioxide in the surrounding environment (Araoye, 2009). The high levels of carbon dioxide in the river water results in the formation of carbonic acid, which contributes protons to the water leading to low pH.

4.2 Method validation

4.2.1 PAHs

The extraction methods were validated by determining the recovery, inter-day and intra-day, LOD, and LOQ for each individual analyte. Table 4.2 shows the data for the recovery of PAHs from soil, sediment, and water. The PAHs were divided into lower molecular weight (LMW), which have less than four carbon rings, and high molecular weight (HMW), which have four or more carbon rings. The LMW hydrocarbons had slightly higher recoveries compared to the HMW hydrocarbons for all three matrices. This could be due to HMW PAHs being less volatile than LMW PAHs, as well as less soluble in water (Wild and Jones, 1995). The high volatility may allow for easier extraction of the LMW PAHs resulting in higher recoveries, as the use of sonication in this study may encourage volatilisation of these hydrocarbons. Benzo[e]pyrene had a significantly higher recovery percentage from soil, 109.30 ± 0.31%, and sediment, 110.60 ± 0.13%. Indeno[1,2,3-c,d]pyrene also had higher recoveries for soil, 80.99 ± 2.31%, and sediment, 81.02 ± 5.49%. These two PAHs had higher recoveries from soil and sediment, compared to the water recoveries as they are more hydrophobic. This was not the case for fluoranthene and pyrene, as both had better water recoveries, which could be due to these PAHs being more water soluble and thus less hydrophobic than benzo[e]pyrene and indeno[1,2,3-c,d]pyrene. The limit of detection (LOD), Table 4.3, and limit of quantitation (LOQ), Table 4.4, were determined for each of the individual PAHs using the GCMS. LOD and LOQ data assists in the determination of calibration ranges for standards and determining the correct spiking concentration needed for the samples. It determines the sensitivity of the instrument. The LOD determined for soil, sediment, and water were in the range of 0.014-0.633 µg g^{-1}, 0.005-0.247 µg g^{-1}, and 0.004-0.278 µg L^{-1}, respectively. The LOQ determined for soil, sediment, and water were in the range of 0.042-1.919 µg g^{-1}, 0.008-0.749 µg g^{-1}, and 0.011-0.842 µg L^{-1}, respectively. The R^2 values represent the linearity of the calibration graphs, which were between 0.9903-0.9981. The inter-day and intra-day data for the PAHs determined in the

soil, sediment, and water samples are listed under Table 4.5. This data assisted in determining the reproducibility of the method on the same day as well as on different days. According to the data, the method is reproducible as the intra-day and inter-day analysis showed minimal deviation of < 4%, which is acceptable as it is below the recommended 15 % (EMEA, 2010).

Table 4.2: Recovery percentage of each PAH extracted from soil, sediment, and water, N=3.

PAHs		Soil/ %	Sediment/ %	Water/ %
Low molecular weight (LMW)	Naphthalene	108.51 ± 2.31	107.78 ± 7.23	89.26 ± 3.11
	Acenaphthylene	100.67 ± 3.12	100.09 ± 3.59	101.73 ± 2.48
	Acenaphthene	91.13 ± 0.32	90.45 ± 2.68	92.11 ± 4.39
	Fluorene	93.96 ± 0.29	93.19 ± 6.18	91.01 ± 0.40
	Anthracene	84.66 ± 0.86	84.43 ± 3.63	70.81 ± 1.20
High molecular weight (HMW)	Phenanthrene	80.52 ± 0.47	81.20 ± 0.17	70.38 ± 1.25
	Pyrene	62.47 ± 2.15	62.08 ± 0.10	90.05 ± 2.21
	Fluoranthene	60.66 ± 2.74	61.98 ± 0.26	98.88 ± 1.98
	Benzo[a]anthracene	84.29 ± 1.64	83.20 ± 6.56	68.61 ± 0.17
	Chrysene	86.97 ± 2.88	87.75 ± 3.47	83.62 ± 0.12
	Benzo[k]fluoranthene	80.55 ± 1.88	79.87 ± 1.61	83.80 ± 0.10
	Benzo[b]fluoranthene	74.84 ± 2.07	75.31 ± 2.01	76.05 ± 1.19
	Benzo[e]pyrene	109.30 ± 0.31	110.60 ± 0.13	60.71 ± 4.85
	Indeno[1,2,3 – c,d]pyrene	80.99 ± 2.31	81.02 ± 5.49	62.08 ± 0.03
	Dibenz[a,h]anthracene	101.37 ± 3.42	100.39 ± 3.10	72.1 ± 1.45
	Benzo[g,h,i]perylene	98.68 ± 2.05	98.56 ± 0.18	97.44 ± 0.69

Table 4.3: LOD and R^2 values for each PAH, N=3

Compound	LOD			R^2
	Soil/ µg g^{-1}	Sediment/ µg g^{-1}	Water µg L^{-1}	
Naphthalene	0.622 ± 0.004	0.137 ± 0.003	0.273 ± 0.026	0.9963
Acenaphthylene	0.621 ± 0.020	0.113 ± 0.083	0.199 ± 0.016	0.9914
Acenaphthene	0.115 ± 0.079	0.089 ± 0.028	0.223 ± 0.029	0.9953
Fluorene	0.085 ± 0.009	0.119 ± 0.035	0.043 ± 0.013	0.9916
Anthracene	0.269 ± 0.053	0.033 ± 0.015	0.019 ± 0.006	0.9926
Phenanthrene	0.471 ± 0.061	0.110 ± 0.054	0.015 ± 0.003	0.9910
Pyrene	0.021 ± 0.003	0.005 ± 0.003	0.122 ± 0.007	0.9959
Fluoranthene	0.014 ± 0.004	0.247 ± 0.058	0.004 ± 0.003	0.9952
Benzo[a]anthracene	0.196 ± 0.081	0.157 ± 0.097	0.092 ± 0.010	0.9903
Chrysene	0.165 ± 0.020	0.177 ± 0.013	0.075 ± 0.009	0.9914
Benzo[k]fluoranthene	0.118 ± 0.054	0.056 ± 0.081	0.148 ± 0.003	0.9981
Benzo[b]fluoranthene	0.053 ± 0.007	0.013 ± 0.003	0.206 ± 0.048	0.9972
Benzo[e]pyrene	0.163 ± 0.057	0.024 ± 0.004	0.250 ± 0.024	0.9916
Indeno[1,2,3 – c,d]pyrene	0.077 ± 0.008	0.016 ± 0.007	0.151 ± 0.001	0.9971
Dibenz[a,h]anthracene	0.633 ± 0.083	0.023 ± 0.012	0.278 ± 0.054	0.9966
Benzo[g,h,i]perylene	0.039 ± 0.009	0.046 ± 0.011	0.144 ± 0.023	0.9935

Table 4.4: LOQ values for each PAH, N=3

Compound	LOQ		
	Soil/ µg g^{-1}	**Sediment/ µg g^{-1}**	**Water/ µg L^{-1}**
Naphthalene	1.886 ± 0.004	0.415 ± 0.003	0.827 ± 0.026
Acenaphthylene	1.881 ± 0.020	0.342 ± 0.083	0.604 ± 0.016
Acenaphthene	0.349 ± 0.079	0.269 ± 0.028	0.675 ± 0.029
Fluorene	0.258 ± 0.009	0.362 ± 0.035	0.131 ± 0.013
Anthracene	0.814 ± 0.053	0.101 ± 0.015	0.059 ± 0.006
Phenanthrene	1.426 ± 0.061	0.333 ± 0.054	0.047 ± 0.003
Pyrene	0.063 ± 0.003	0.008 ± 0.003	0.369 ± 0.007
Fluoranthene	0.042 ± 0.004	0.749 ± 0.058	0.011 ± 0.038
Benzo[a]anthracene	0.595 ± 0.081	0.477 ± 0.097	0.279 ± 0.010
Chrysene	0.499 ± 0.020	0.535 ± 0.013	0.229 ± 0.009
Benzo[k]fluoranthene	0.359 ± 0.054	0.170 ± 0.047	0.449 ± 0.003
Benzo[b]fluoranthene	0.159 ± 0.007	0.039 ± 0.017	0.623 ± 0.048
Benzo[e]pyrene	0.495 ± 0.057	0.072 ± 0.018	0.757 ± 0.024
Indeno[1,2,3 – c,d]pyrene	0.232 ± 0.008	0.048 ± 0.007	0.458 ± 0.001
Dibenz[a,h]anthracene	1.919 ± 0.083	0.069 ± 0.012	0.842 ± 0.054
Benzo[g,h,i]perylene	0.117 ± 0.009	0.139 ± 0.011	0.437 ± 0.023

Table 4.5: Inter-day and intra-day data for PAHs, N=3

Compound	Soil (%)		Sediment (%)		Water (%)	
	Intra-day	**Inter-day**	**Intra-day**	**Inter-day**	**Intra-day**	**Inter-day**
Naphthalene	98.53 ± 0.83	98.85 ± 1.45	112.72 ± 2.45	114.35 ± 0.81	91.37 ± 0.93	90.35 ± 3.66
Acenaphthylene	104.27 ± 2.03	101.84 ± 0.25	104.34 ± 1.15	94.67 ± 0.05	79.33 ± 1.13	75.11 ± 2.11
Acenaphthene	92.98 ± 1.01	95.88 ± 0.41	119.93 ± 1.62	114.71 ± 2.65	79.64 ± 1.42	78.12 ± 3.55
Fluorene	109.13 ± 2.11	120.04 ± 3.38	106.75 ± 1.24	101.97 ± 1.43	95.13 ± 0.58	87.95 ± 0.95
Anthracene	81.16 ± 1.52	80.01 ± 2.11	84.95 ± 0.60	85.98 ± 0.98	74.61 ± 1.04	77.73 ± 0.60
Phenanthrene	82.85 ± 1.26	83.70 ± 3.71	90.11 ± 1.28	89.23 ± 1.60	79.33 ± 1.13	84.36 ± 2.83
Pyrene	103.11 ± 1.99	108.83 ± 1.78	81.17 ± 2.68	90.59 ± 2.86	80.62 ± 0.30	88.67 ± 3.60
Fluoranthene	104.29 ± 2.76	112.53 ± 3.37	73.83 ± 1.76	81.49 ± 2.96	91.99 ± 0.78	93.18 ± 3.50
Benzo[a]anthracene	104.30 ± 1.94	107.98 ± 3.26	105.25 ± 1.96	102.95 ± 2.92	112.48 ± 1.44	113.05 ± 0.74
Chrysene	111.82 ± 2.15	115.03 ± 0.25	109.59 ± 1.88	98.24 ± 1.85	114.49 ± 1.43	107.87 ± 0.54
Benzo[k]fluoranthene	103.95 ± 1.03	108.67 ± 0.83	75.59 ± 1.35	81.99 ± 1.38	116.94 ± 1.50	108.82 ± 3.19
Benzo[b]fluoranthene	97.93 ± 1.37	90.42 ± 1.01	102.17 ± 2.28	97.13 ± 3.08	127.50 ± 1.35	118.28 ± 2.63
Benzo[e]pyrene	101.08 ± 2.76	99.40 ± 1.80	95.42 ± 1.29	92.96 ± 0.96	97.60 ± 0.45	102.20 ± 3.81
Indeno[1,2,3-c,d]pyrene	109.96 ± 1.55	113.51 ± 0.65	82.63 ± 1.74	86.50 ± 3.46	76.64 ± 2.68	78.44 ± 0.46
Dibenz[a,h]anthracene	109.55 ± 1.31	116.50 ± 1.94	90.46 ± 1.97	92.20 ± 1.95	78.13 ± 3.08	82.12 ± 3.16
Benzo[g,h,i]perylene	112.22 ± 3.01	112.16 ± 2.05	89.55 ± 1.77	89.97 ± 0.65	80.88 ± 2.71	80.66 ± 0.51

4.2.2 Pesticides

The recovery percentage of acetochlor, hexazinone, and oxamyl are listed in Table 4.6. Acetochlor had a slightly lower recovery from soil and sediment compared to the water recovery percentage of 98.1 ± 0.24%. Oxamyl had a higher recovery from soil, 112.7 ± 0.26%, and sediment, 109.3 ± 0.16% compared to 94.6 ± 0.72% recovery from water. The difference in the recovery values could be due to acetochlor being less water-soluble. Acetochlor is also less polar and would be easier to extract from water. The data listed in Table 4.7 shows the LOD values for soil, sediment, and water, which ranged from 0.109-0.419 µg g^{-1}, 0.296-0.474 µg g^{-1}, and 0.097-0.397 µg L^{-1}, respectively. The LOQ values for soil, sediment, and water ranged from 0.331-1.268 µg g^{-1}, 0.896-1.437 µg g^{-1}, and 0.293-1.204 µg L^{-1}, respectively. Table 4.8 shows the inter-day and intra-day results for oxamyl, hexazinone, and acetochlor determined in the soil, sediment, and water samples. According to the data, the method is reproducible as the recoveries showed minimal deviation of < 5%.

Table 4.6: Recovery percentage of each pesticide extracted from soil, sediment, and water in triplicate

Pesticide	**Soil (%)**	**Sediment (%)**	**Water (%)**
Acetochlor	83.2 ± 0.25	83.1 ± 0.28	98.1 ± 0.24
Hexazinone	97.7 ± 0.21	99.7 ± 0.27	95.3 ± 0.40
Oxamyl	112.7 ± 0.26	109.3 ± 0.16	94.6 ± 0.72

Table 4.7: LOD, LOQ and R^2 values for the individual pesticides, N=3

Compound	LOD			LOQ			R^2
	Soil/ µg g^{-1}	Sediment/ µg g^{-1}	Water/ µg L^{-1}	Soil/ µg g^{-1}	Sediment/ µg g^{-1}	Water/ µg L^{-1}	
Acetochlor	0.419 ± 0.077	0.459 ± 0.178	0.397 ± 0.017	1.268 ± 0.077	1.392 ± 0.178	1.204 ± 0.017	0.9979
Hexazinone	0.234 ± 0.071	0.296 ± 0.090	0.097 ± 0.045	0.710 ± 0.071	0.896 ± 0.090	0.293 ± 0.045	0.9941
Oxamyl	0.109 ± 0.33	0.474 ± 0.063	0.133 ± 0.04	0.331 ± 0.033	1.437 ± 0.063	0.403 ± 0.04	0.9963

Table 4.8: Inter-day and intra-day data for pesticides, N=3

Compound	Soil/ %		Sediment/ %		Water/ %	
	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day	Intra-day
Acetochlor	88.18 ± 1.47	88.16 ± 0.93	87.77 ± 4.54	88.77 ± 1.61	97.15 ± 0.49	97.69 ± 1.01
Hexazinone	78.47 ± 0.45	79.17 ± 0.86	79.39 ± 1.42	79.05 ± 1.31	82.16 ± 3.79	80.74 ± 1.07
Oxamyl	101.9 ± 0.93	103.21 ± 0.93	100.72 ± 1.23	103.27 ± 2.51	81.14 ± 4.05	81.29 ± 0.30

4.3 Results for analysis of soil, sediment, and river water samples

4.3.1 PAHs

4.3.1.1 Soil

Soil samples were analysed from six different planting phases within the reforestation zone. These included samples from five planting phases (2010/2011, 2011/2012, 2012/2013, 2013/2014, and 2014/2015) and a control soil sample. The control sample was collected from within the reforestation zone, on a site where no trees had been planted. Table 4.9 shows the concentration of the sixteen PAHs determined from extraction of the soil samples from each planting phase. The results presented in Tables 4.9 – 4.22 are the results after subtracting the spiked concentration. Hence the concentrations are lower than the LOD and LOQ results as they were present in only trace quantities. That was the reason why the samples were spiked to bring their concentrations above the LOD and LOQ so that the analytes can be detected and quantified. No traces of acenaphthylene, acenaphthene, fluorene, anthracene, or phenanthrene were found in the soil samples as these analytes were either not present in the soil or were present in concentrations that were below the limit of detection for the instrument. Soil samples collected from planting phase 2010/2011 had the lowest total PAH concentration of 4.258 ± 0.069 µg g^{-1}. The total PAH concentrations gradually increased from the earliest planting phase (2010/2011) to the most recent phase (2014/2015). The highest concentration, 8.390 ± 0.116 µg g^{-1}, of PAHs was determined in the control samples, where no trees were planted. Napthalene was the most dominant PAH. A possible source of PAH contamination in the environment could be from nearby sugarcane burning taking place on the neighbouring farms. Figure 4.1 illustrates the concentrations of PAHs at the different planting phases and the control sample. A possible explanation for the increase in PAH concentrations at the different planting phases, is that the trees planted in these sections of the reforestation site, have been potentially taking up the PAHs over the years. This has resulted in the soil at the earlier planting phases having a lower concentration of PAHs. The concentration of PAHs determined in the soil samples collected from the 2010/2011 planting phase, were approximately 50% less than the PAH concentrations determined in the control sample. This is a possible indication of the positive effect of reforestation on the concentration of PAHs. Previous research conducted on

industrial sites in China (Danshuikeng, DSK and Xiqiaoshan, XQS) showed that PAHs can be absorbed by trees (Kuang et al., 2011). Currently, there are 95 different species planted at the Buffelsdraai reforestation site. Other studies have been conducted which indicated that organic pollutants are being slightly taken up by specific parts of the tree such as the roots and leaves, but no research has been published detailing the uptake ability of the specific species planted at Buffelsdraai (Simonich and Hites, 1995). Currently, Canadian soil guideline values only exist for naphthalene and benzo[a]pyrene. Therefore, in order to show if the naphthalene concentrations obtained in this study were above the guideline, naphthalene was plotted against the different planting phases (Figure 4.2). The highest concentration of naphthalene was determined in the control soil sample; however, all sampling sites in which napthalene was detected, were above the recommended naphthalene guideline value of 0.1 µg g^{-1}, as illustrated by the threshold line (CCME, 2008). The total concentration of naphthalene varied amongst the different planting phases. This could be due to naphthalene having the lowest molecular mass and it is the most mobile in relation to other PAHs (Agency, 2005). Table 4.9 also gives an indication of the effect of reforestation on the total concentration of the individual PAHs. The concentration of chrysene was much lower in the earlier planting sites as this hydrocarbon has been possibly taken up by the trees over the years. This slight decrease in the earlier planting sites was also noted for fluoranthene, benzo[k]fluoranthene and indeno[1,2,3-c,d]pyrene. Table 4.10 displays the percentage of PAHs that have been potentially taken up by the trees compared to the reference soil samples. The earlier planting phases showed better removal of pyrene, fluoranthene, benzo[k]fluoranthene and indeno[1,2,3-c,d]pyrene compared to the later planting phases. This could be due to the earlier planted trees having had more time to take up these pollutants, which shows the positive long-term effect of reforestation. Benzo[g,h,i]perylene were the least removed PAHs. This could be due to these pollutants being more persistent due to their higher molecular mass and their high hydrophobicity which results in greater interaction with the soil. This then makes it difficult for them to be removed from the soil.

Table 4.9: Concentration values for PAHs in soil, N=3

RT/ min	Compound	PAH Concentration/ µg g^{-1}					
		Planting phase					Control
		2010/2011	2011/2012	2012/2013	2013/2014	2014/2015	
4.390	Naphthalene	0.574 ± 0.052	0.607 ± 0.009	0.820 ± 0.017	0.563 ± 0.023	0.772 ± 0.034	1.420 ± 0.012
6.930	Acenaphthylene	-	-	-	-	-	-
7.255	Acenaphthene	-	-	-	-	-	-
8.295	Fluorene	-	-	-	-	-	-
10.495	Anthracene	-	-	-	-	-	-
10.625	Phenanthrene	-	-	-	-	-	-
13.655	Pyrene	0.094± 0.009	0.088 ± 0.009	0.184 ± 0.016	0.199 ± 0.006	0.186 ± 0.005	0.331 ± 0.024
14.260	Fluoranthene	0.392 ± 0.051	0.434 ± 0.009	0.362 ± 0.020	0.652 ± 0.038	1.024 ± 0.048	1.001 ± 0.002
17.740	Benzo[a]anthracene	0.385 ± 0.046	0.375 ± 0.049	0.620 ± 0.016	0.478 ± 0.028	0.694 ± 0.005	0.771 ± 0.011
17.840	Chrysene	0.578 ± 0.030	0.594 ± 0.030	0.381 ± 0.028	0.368 ± 0.039	1.011 ± 0.022	0.721 ± 0.022
20.715	Benzo[k]fluoranthene	0.167 ± 0.010	0.193 ± 0.007	0.202 ± 0.012	0.350 ± 0.040	0.492 ± 0.079	0.578 ± 0.041
20.785	Benzo[b]fluoranthene	0.389 ± 0.022	0.477 ± 0.028	0.420 ± 0.038	0.451 ± 0.011	0.450 ± 0.035	0.708 ± 0.020
21.525	Benzo[e]pyrene	0.381 ± 0.016	0.260 ± 0.019	0.322 ± 0.044	0.384 ± 0.037	-	0.595 ± 0.011
24.475	Indeno[1,2,3 - c,d]pyrene	0.335 ± 0.038	0.357 ± 0.055	0.469 ± 0.033	0.656 ± 0.020	0.726 ± 0.014	0.837 ± 0.015
24.560	Dibenz[a,h]anthracene	0.420 ± 0.082	0.489 ± 0.008	0.549 ± 0.028	0.598 ± 0.031	0.538 ± 0.027	0.630 ± 0.022
25.275	Benzo[g,h,i]perylene	0.544 ± 0.019	0.671 ± 0.019	0.552 ± 0.035	0.602 ± 0.016	0.533 ± 0.033	0.603 ± 0.023
Σ PAHs		4.258 ± 0.069	4.840 ± 0.068	4.882 ± 0.066	5.776 ± 0.048	6.426 ± 0.190	8.390 ± 0.116

"-" Implies that the concentration was below the level of detection of the instrument or was not present in the sample.

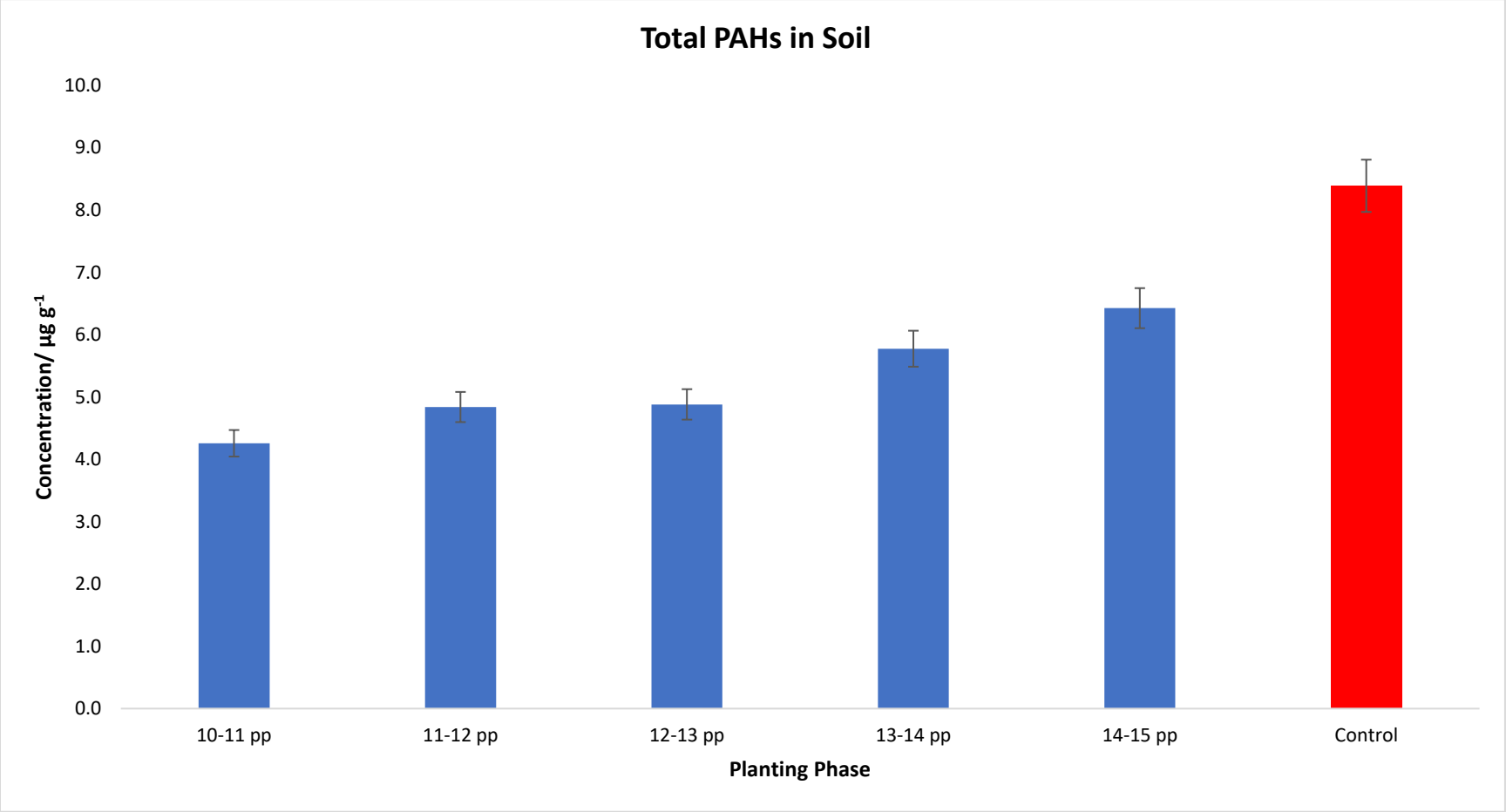


Figure 4.1: Total PAH concentrations in the soil samples for the different planting phases and the control sample, N=3

Table 4.10: Percentage removal of PAHs compared to the reference soil sample.

Compound	Percentage of PAH removed (%)				
	Planting phase				
	2010/2011	2011/2012	2012/2013	2013/2014	2014/2015
Naphthalene	59.6	57.3	42.2	60.4	45.6
Acenaphthylene	-	-	-	-	-
Acenaphthene	-	-	-	-	-
Fluorene	-	-	-	-	-
Anthracene	-	-	-	-	-
Phenanthrene	-	-	-	-	-
Pyrene	71.7	73.4	44.6	40.0	44.0
Fluoranthene	60.8	56.7	63.8	34.9	-
Benzo[a]anthracene	50.1	51.3	19.5	38.0	9.9
Chrysene	19.8	17.6	47.1	49.0	-
Benzo[k]fluoranthene	71.1	66.7	65.0	39.4	15.0
Benzo[b]fluoranthene	45.1	32.6	40.6	36.2	36.4
Benzo[e]pyrene	36.0	56.4	46.0	35.5	-
Indeno[1,2,3 - c,d]pyrene	60.0	57.4	43.9	21.6	13.3
Dibenz[a,h]anthracene	33.4	22.5	12.9	5.1	14.6
Benzo[g,h,i]perylene	9.9	-	8.5	0.2	11.7

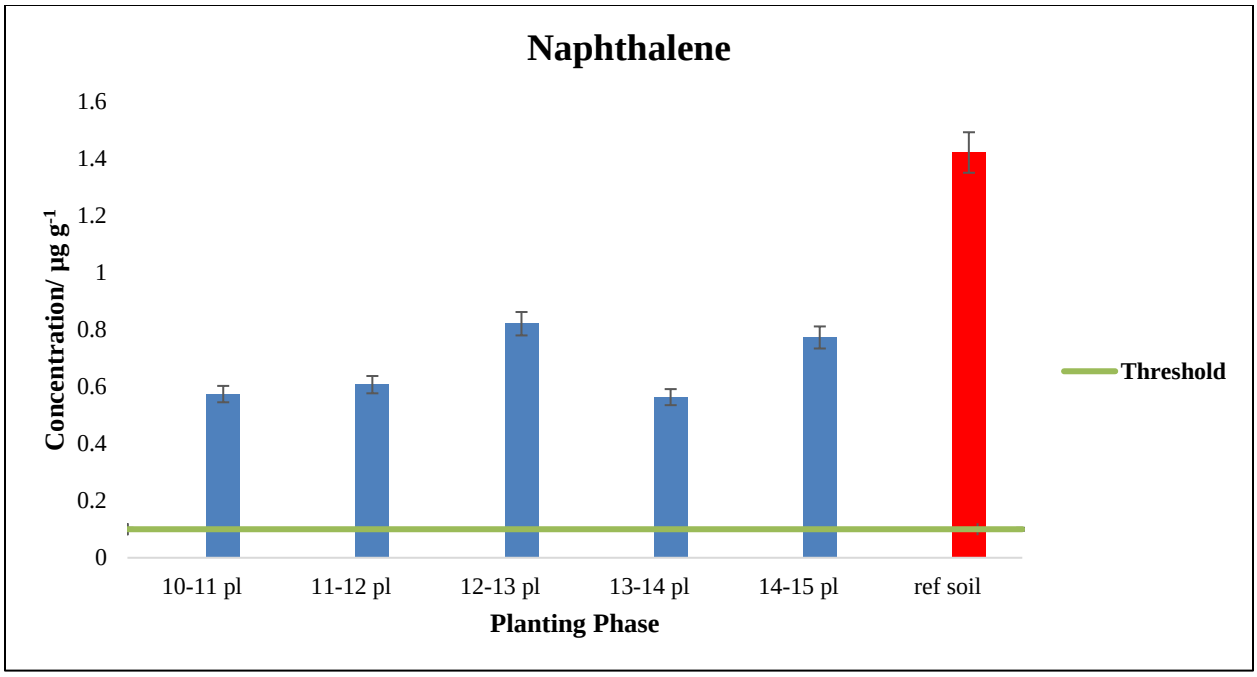


Figure 4.2: The total concentration of naphthalene for each planting phase, N=3

4.3.1.2 Sediment

The PAH concentrations determined in the sediment samples collected from the Black Mhlasini River are shown in Table 4.11. The highest concentration of total PAHs, 13.900 ± 0.351 µg g^{-1}, was determined at sampling site BM 4, which was located downstream of the reforestation site. This could have resulted from sand mining occurring within close proximity of the river. This site also had the highest TDS value, which suggested that the water is polluted. This high level could also be due to the accumulation of organic pollutants in sediment as it leached into the river water over the years either from the surrounding reforestation site, neighbouring community or from the landfill. During sand mining, the sediment close to the banks of the river is displaced and enters the river. This means that more sediment particles would be available for PAHs to adsorb onto. Hence the reason for the high concentrations of PAHs at this site. Sampling site BM 1, located upstream of the reforestation site, had a high concentration of PAHs, 11.014 ± 0.087 µg g^{-1}. This site was used as a control for the Black Mhlasini River, to illustrate the positive effect of reforestation in reducing the concentration of PAHs, when comparing areas where no trees were planted. Naphthalene was present in the highest concentration, 2.781 ± 0.021 µg g^{-1}. These high concentrations were observed throughout the different types of sample matrices analysed. A possible explanation could be the accumulation of this hydrocarbon over the years from the burning of sugarcane. This is further discussed under section 4.4.2, where the source apportionment data suggests that the common source of PAH contamination was from pyrolytic sources. This would have increased the concentration of naphthalene in the soil and possibly the surrounding environment. The remaining sites, BM 2 and BM 3, were located within the boundary of the reforestation site. Sampling site BM 2 was located before the neighbouring Osindisweni community and alongside planting phase 2010/2011. This site had the lowest total PAH concentration, 2.210 ± 0.248 µg g^{-1}, which could be a representation of the positive effect of reforestation, as fewer organic pollutants were able to enter the river as a result of these pollutants being taken up by the trees. This method is known as phytotransformation, a form of phytoremediation, which is used in the removal of organic pollutants through the use of trees or plants (Glick, 2003). Previous research showed that phytoremediation was more effective than bioremediation in the removal of PAHs which were more strongly bound to soil particles, however, a combination of these techniques proved to be most effective (Huang et al., 2004). There was an

increase in PAH concentration in the sediment samples collected from site BM 3, located after the Osindisweni community. This increase could be due to community influence, as domestic activities such as the burning of waste near the riverbanks, could be contributing to the levels of PAHs in the sediment samples. Another contributing factor could be leaching of leachate from the nearby storage tanks on the landfill site, which is located nearby sampling site BM 3. From the four different sampling sites on the Black Mhlasini River, the lowest PAH concentrations were recorded within the reforestation boundary, which suggests that much of the PAHs have possibly been taken up by the trees.

Table 4.11: PAH concentrations in sediment samples from the Black Mhlasini River, N=3

Compound	**PAH Concentration/ µg g^{-1}**			
	BM 1	**BM 2**	**BM 3**	**BM 4**
Naphthalene	2.781 ± 0.021	0.618 ± 0.022	0.498 ± 0.022	1.438 ± 0.015
Acenaphthylene	-	-	-	-
Acenaphthene	-	-	-	-
Fluorene	-	-	-	0.122 ± 0.038
Anthracene	-	-	-	0.357 ± 0.029
Phenanthrene	-	-	0.016 ± 0.054	0.323 ± 0.036
Pyrene	0.852 ± 0.091	0.048 ± 0.005	0.633 ± 0.036	1.608 ± 0.026
Fluoranthene	1.251 ± 0.0293	0.057 ± 0.015	2.597 ± 0.042	2.163 ± 0.031
Benzo[a]anthracene	1.393 ± 0.046	0.280 ± 0.032	1.204 ± 0.011	2.314 ± 0.018
Chrysene	1.377 ± 0.014	0.228 ± 0.016	0.903 ± 0.023	2.364 ± 0.029
Benzo[k]fluoranthene	0.645 ± 0.066	0.037 ± 0.049	0.426 ± 0.031	0.497 ± 0.085
Benzo[b]fluoranthene	0.632 ± 0.013	0.405 ± 0.027	0.864 ± 0.044	0.896 ± 0.039
Benzo[e]pyrene	0.579 ± 0.033	0.345 ± 0.022	0.829 ± 0.008	0.409 ± 0.072
Indeno[1,2,3 - c,d]pyrene	0.519 ± 0.024	0.067 ± 0.010	0.653 ± 0.051	0.523 ± 0.028
Dibenz[a,h]anthracene	0.477 ± 0.066	0.126 ± 0.028	0.571 ± 0.045	0.422 ± 0.047
Benzo[g,h,i]perylene	0.509 ± 0.066	-	0.526 ± 0.022	0.463 ± 0.035
Σ PAHs	11.014 ± 0.087	2.210 ± 0.248	9.720 ± 0.227	13.900 ± 0.351

The concentration of each individual PAH determined in the sediment samples from the White Mhlasini River, are shown in Table 4.12. Sampling site WM 1, located upstream of the reforestation site, had the highest total PAH concentration, 11.496 ± 0.118 μg g^{-1}. This sampling site is located outside of the reforestation zone, where no trees have been planted thus there is possibly little to no removal of organic pollutants, so high PAH concentrations would be expected. In addition, depending on the wind direction, PAHs present in the polluted air from the nearby industrial area, located south of the reforestation zone, may be settling along the White Mhlasini River. The lowest total concentration of PAHs was determined at sampling site WM 2, 4.329 ± 0.206 μg g^{-1}. This site is located close to planting phase 2011/2012, which could be having a positive effect on the concentration of PAHs entering the river, as these pollutants are possibly taken up by the planted trees allowing less PAHs to make their way down to the river. As seen from the sediment data for the Black Mhlasini River of Table 4.11, the total PAH concentrations also increased for sampling site WM 3, located after the neighbouring Buffelsdraai community. This sampling site is also located alongside the reforestation site where no trees had been planted, and this section of the reforestation site is classified as grasslands. An explanation for the increase could be due to most of the organic pollutants that have settled onto the surrounding reforestation site, being washed into the river during rainfall as a result of no trees being present in this section of the site. Thus, there is no uptake of the PAHs, which eventually make their way into the river. The sediment samples collected from the White Mhlasini River had no acenaphthene or fluorene, as it was either not present in the sediment samples or were below the LOD of the GCMS. On the other hand, the fluoranthene and pyrene concentrations were significantly higher at some sites with concentrations of 2.069 ± 0.025 μg g^{-1} and 2.138 ± 0.006 μg g^{-1} for pyrene and fluoranthene, respectively at WM 1. Previous research comparing the PAH emissions of sugarcane during harvest season and non-harvest season, showed a rapid increase in the concentration of fluoranthene and pyrene compared to the other PAHs during sugarcane burning (Mugica-Alvarez et al., 2015). This could be a reason for the high concentrations of fluoranthene and pyrene present in the sediment samples as the current reforestation site was previously a sugarcane farm. Fluoranthene and pyrene could have formed during the sugarcane harvest season when crops are burnt, washed down into the White Mhlasini River and accumulated in river sediment over the years. Analysis of soil and sediment samples in South Africa have shown low concentration ranges for acenaphthylene, acenaphthene, and fluorene, while fluoranthene and pyrene were the most

prominent due to industrial activity and influence from the informal settlement (Chimuka et al., 2016). Benzo[e]pyrene had an unusually high concentration, 2.836 ± 0.010 µg g^{-1}, at sampling site WM 3. This contributed to the vastly higher total concentration shown in Figure 4.3. This site was closest to the neighbouring community, so the high concentration could have resulted from burning of organic matter near the riverbanks. Since PAHs tend to accumulate in sediment due to their hydrophobicity, these pollutants would have settled into the sediment over time (Meador et al., 1995). Figure 4.4 shows the total fluoranthene concentration determined in the sediment samples collected from the Black Mhlasini (BM) and White Mhlasini (WM) Rivers. The threshold line represents the recommended guideline value of 0.1 µg g^{-1} (CCME, 2008). Only sampling site BM 2 was below this value, which shows that this analyte is possibly being taken up by the trees at this sampling site instead of leaching into the river. Sampling site WM 1 had the highest total fluoranthene concentration for the White Mhlasini River. The possible reason for the high level could be that there are no planted trees near the site to remove the fluoranthene. Sediment guideline values also exist for naphthalene, 0.14 µg g^{-1}, benzo[a]anthracene, 0.36 µg g^{-1}, and benzo[e]pyrene, 2.7 µg g^{-1}. These guideline values are shown in Appendix D. Benzo[e]pyrene concentrations determined in the sediment samples, were mostly below the guideline value except for site WM 3 due to possible influence from the neighbouring community.

Table 4.12: PAH concentrations in sediment samples from White Mhlasini River, N=3

Compound	PAH Concentration/ µg g^{-1}			
	WM 1	**WM 2**	**WM 3**	**WM 4**
Naphthalene	1.015 ± 0.029	0.543 ± 0.012	0.832 ± 0.075	0.781 ± 0.031
Acenaphthylene	-	-	-	-
Acenaphthene	-	-	-	-
Fluorene	-	-	-	-
Anthracene	0.232 ± 0.065	-	0.046 ± 0.065	0.248 ± 0.007
Phenanthrene	0.185 ± 0.065	-	0.003 ± 0.065	0.232 ± 0.013
Pyrene	2.069 ± 0.025	0.424 ± 0.013	0.676 ± 0.015	0.729 ± 0.042
Fluoranthene	2.138 ± 0.006	0.818 ± 0.003	1.520 ± 0.022	1.245 ± 0.019
Benzo[a]anthracene	1.148 ± 0.028	0.484 ± 0.034	0.699 ± 0.031	0.715 ± 0.009
Chrysene	0.759 ± 0.051	0.419 ± 0.034	0.786 ± 0.023	0.382 ± 0.049
Benzo[k]fluoranthene	0.841 ± 0.061	0.109 ± 0.044	0.453 ± 0.043	0.405 ± 0.029
Benzo[b]fluoranthene	0.785 ± 0.042	0.398 ± 0.010	0.526 ± 0.018	0.554 ± 0.071
Benzo[e]pyrene	0.488 ± 0.029	0.442 ± 0.034	2.836 ± 0.010	1.449 ± 0.032
Indeno[1,2,3 - c,d]pyrene	0.624 ± 0.065	0.130 ± 0.027	0.492 ± 0.031	0.366 ± 0.019
Dibenz[a,h]anthracene	0.658 ± 0.019	0.373 ± 0.041	0.496 ± 0.009	0.435 ± 0.028
Benzo[g,h,i]perylene	0.553 ± 0.014	0.188 ± 0.019	0.477 ± 0.032	0.329 ± 0.064
Σ PAHs	11.496 ± 0.118	4.329 ± 0.206	9.841 ± 0.232	7.869 ± 0.247

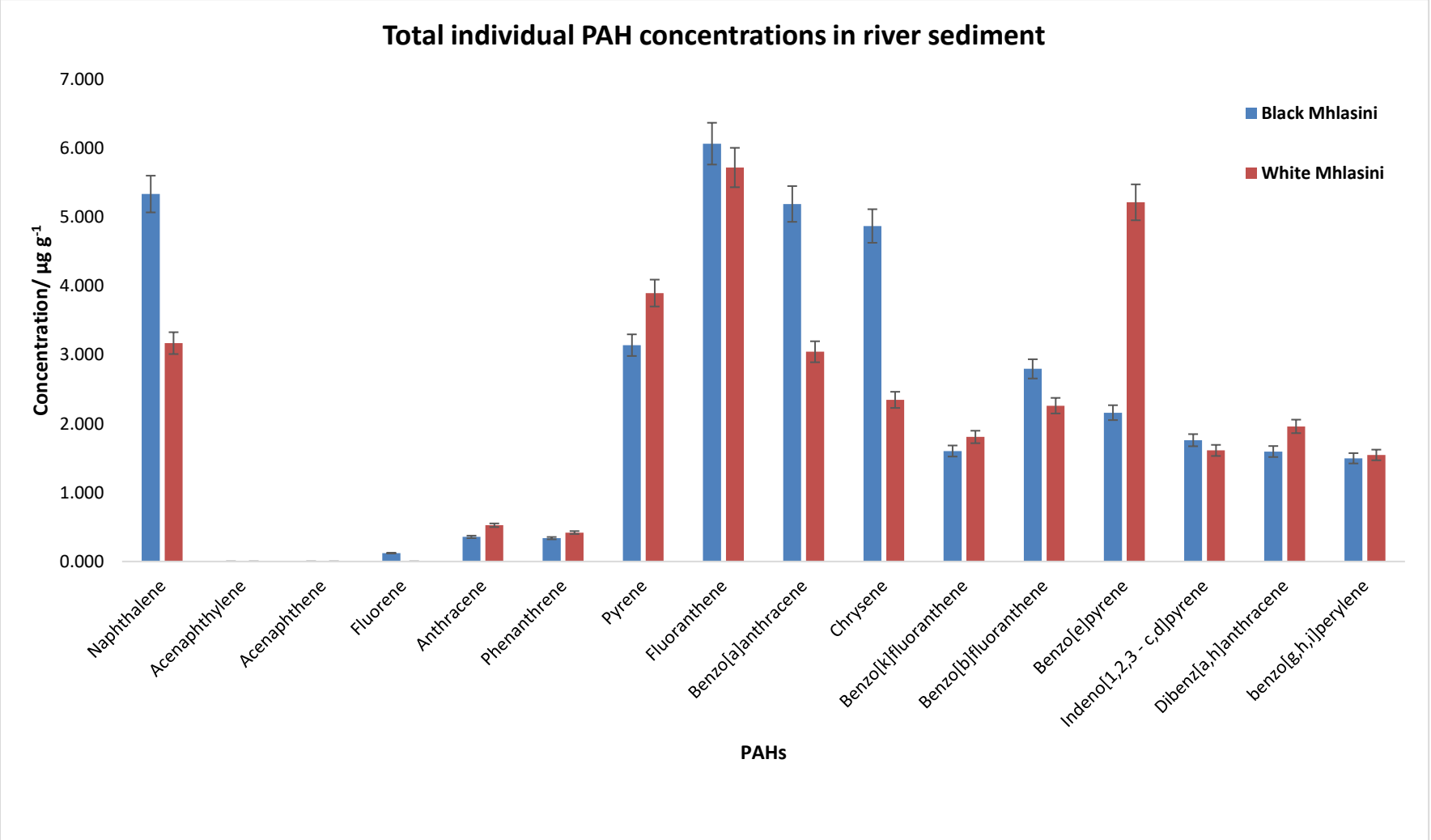


Figure 4.3: The individual total PAH concentrations found in the Black and White Mhlasini Rivers, N=3

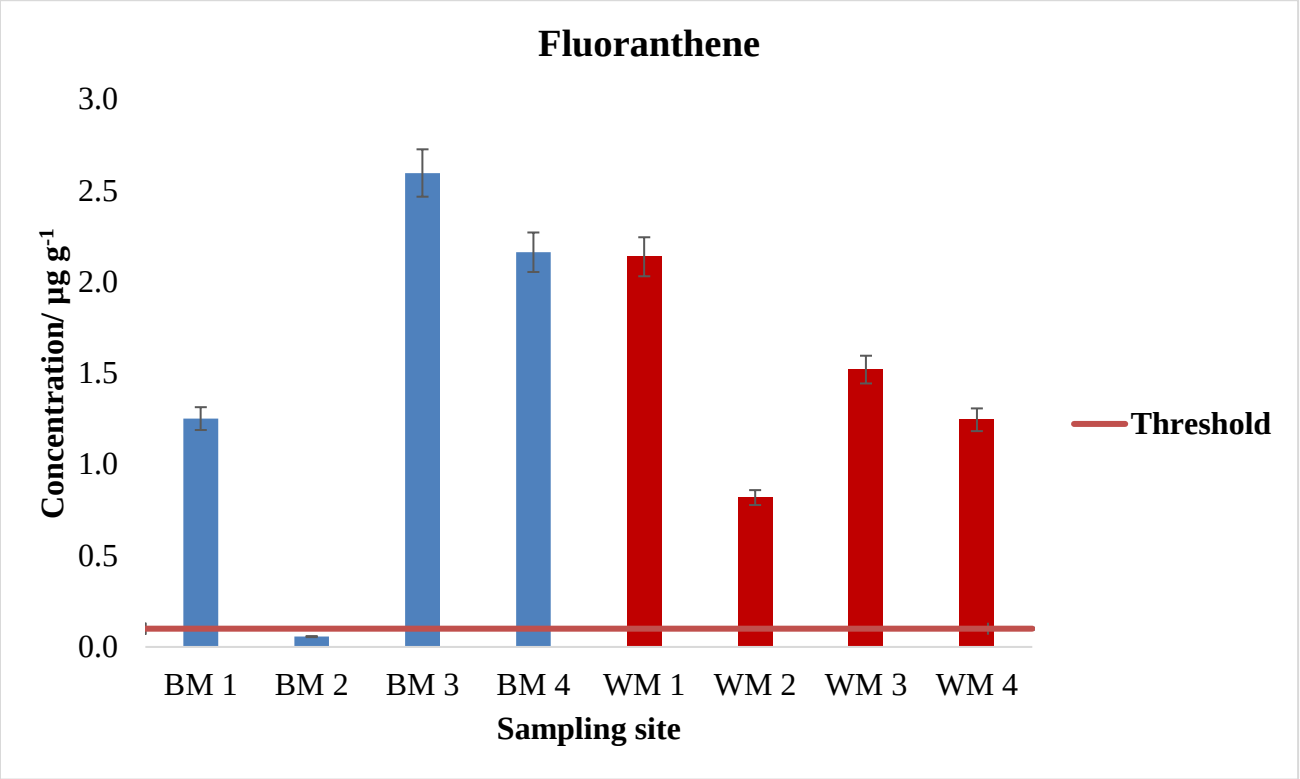


Figure 4.4: Total fluoranthene concentration for each sediment sampling site, N=3

4.3.1.3 Water

The concentration of the individual PAHs determined in the water samples collected from the Black Mhlasini River, are shown in Table 4.13. The total PAH concentrations were much lower compared to the concentrations determined in the sediment samples. This was expected as PAHs are generally hydrophobic and due to their non-polar structure, mobility is inhibited once they are partitioned to the sediment (Abdel-Shafy and Mansour, 2016). However, traces of PAHs are still found in water samples as these pollutants, especially the LMW PAHs, are soluble in water. This also explains the high concentration values for naphthalene in water, which has a much higher solubility value, 31.7 mg L^{-1}, compared to the other PAHs as listed in chapter 2. Sampling site BM 2 had the lowest total concentration of PAHs, 6.360 ± 0.906 ng L^{-1}, as only benzo[a]anthracene, chrysene, benzo[k]fluoranthene, benzo[b]fluoranthene, and benzo[e]pyrene were detected in the river water sample. This could also be evidence of the positive effect reforestation has on improving the quality of the river water, as sampling site BM 2 was situated alongside planting phase 2010/2011. The highest total PAH concentrations were determined at sampling sites BM 1, 85.468 ± 0.865 ng L^{-1}, which is located outside the reforestation site boundary and therefore has no trees planted which would result in uptake of the PAHs. According to Figure 4.5, of all the analytes that were detected and quantified, fluorene had the lowest concentration for both the Black and White Mhlasini River.

Table 4.13: PAH concentrations in river water samples from Black Mhlasini River, N=3

Compound	PAH Concentration/ ng L^{-1}			
	BM 1	**BM 2**	**BM 3**	**BM 4**
Naphthalene	25.936 ± 0.033	-	13.408 ± 0.003	18.651 ± 0.036
Acenaphthylene	-	-	-	0.994 ± 0.025
Acenaphthene	-	-	-	-
Fluorene	0.699 ± 0.022	-	-	-
Anthracene	-	-	-	-
Phenanthrene	1.267 ± 0.049	-	-	-
Pyrene	8.423 ± 0.012	-	-	-
Fluoranthene	6.267 ± 0.013	-	-	0.628 ± 0.078
Benzo[a]anthracene	4.024 ± 0.012	0.546 ± 0.012	3.140 ± 0.099	2.942 ± 0.005
Chrysene	2.814 ± 0.062	3.464 ± 0.062	4.656 ± 0.003	2.176 ± 0.040
Benzo[k]fluoranthene	6.379 ± 0.055	0.120 ± 0.055	5.264 ± 0.049	4.632 ± 0.033
Benzo[b]fluoranthene	3.938 ± 0.097	2.077 ± 0.097	5.843 ± 0.049	5.153 ± 0.002
Benzo[e]pyrene	4.157 ± 0.056	0.154 ± 0.056	2.582 ± 0.026	0.849 ± 0.061
Indeno[1,2,3 - c,d]pyrene	7.167 ± 0.008	-	3.537 ± 0.034	1.344 ± 0.019
Dibenz[a,h]anthracene	7.436 ± 0.022	-	0.588 ± 0.007	-
Benzo[g,h,i]perylene	6.962 ± 0.018	-	0.096 ± 0.007	0.673 ± 0.034
Σ PAHs	85.468 ± 0.865	6.360 ± 0.906	39.114 ± 0.548	38.041 ± 0.117

The concentration of the individual PAHs determined in the water samples collected from the White Mhlasini River, are shown in Table 4.14. The total PAH concentrations in the White Mhlasini River were much higher than the concentrations determined for the Black Mhlasini River. The difference in the total PAH concentrations between the rivers, could be due to a greater community influence on the White Mhlasini River, as the neighbouring Buffelsdraai community is situated much closer to this river. This allows for easier access to the river for use in either agricultural activities, such as the watering of crops or domestic activities, and for the disposal of waste. Figure 4.5 shows a comparison between the concentrations of the total PAHs between the rivers. The White Mhlasini River had a higher total concentration for majority of the hydrocarbons. The analysis of all the samples collected from the Buffelsdraai reforestation site showed that

naphthalene was consistently present in high concentrations. This could have resulted from the accumulation of PAHs over the years through the burning of sugarcane during the harvest season as was reported in Florida, USA that showed naphthalene to have the highest emissions from sugarcane burning (Hall et al., 2012). Figure 4.5, which displays the total PAH concentrations in the river water samples, shows that naphthalene had the highest concentration. This could also be due to naphthalene being the least hydrophobic PAH and the most soluble PAH in this study. Previous research conducted on the Baiertang and Macao water columns in South China, showed that naphthalene occupied 84 and 96 %, respectively of the total PAHs present in the water samples (Luo et al., 2004). Other studies also showed concentration levels in the range of 0.03-9.1 μg L^{-1} detected in the Gao-ping River, Taiwan (Doong and Lin, 2004). As seen in Figure 4.3, there was a greater presence of the HMW hydrocarbons compared to the LMW hydrocarbons, which was also evident for the river water samples. This was possibly due to aerobic biodegradation occurring at a much slower rate for HMW hydrocarbons (McCutcheon and Schnoor, 2004). The World Health Organisation (WHO) provides a combined guideline value for fluoranthene, pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-c,d]pyrene. According to WHO, sampling sites BM 1 and WM 3 are above the 50 ng L^{-1}, guideline value (WHO, 1998). This was due to site BM 1 being located in an area where no trees are planted to take up these pollutants, and there is a greater community influence at sampling site WM 3. Figure 4.6, shows that the total PAHs were mostly present in sediment, 72.78 %, and as a result of PAHs being highly hydrophobic, there were only 0.14 % of total PAHs found in the river water samples. The high percentage of PAHs present in sediment and soil, compared to water, is as a result of them being lipophilic. The high fluoranthene concentrations determined at sampling sites WM 1 and 3, could be due to fluoranthene being the most abundant aerosol PAH and also being frequently present in drinking water as stated under section 2.1.7 (Lens et al., 2006).

Table 4.14: PAH concentrations in river water samples from White Mhlasini river, N=3

Compound	PAH Concentration/ ng L^{-1}			
	WM 1	**WM 2**	**WM 3**	**WM 4**
Naphthalene	16.307 ± 0.096	7.539 ± 0.039	12.17 ± 0.096	9.460 ± 0.088
Acenaphthylene	5.329 ± 0.054	0.294 ± 0.026	1.728 ± 0.025	2.845 ± 0.038
Acenaphthene	-	-	-	-
Fluorene	0.558 ± 0.094	-	-	-
Anthracene	-	-	-	-
Phenanthrene	-	-	-	-
Pyrene	0.334 ± 0.073	-	-	-
Fluoranthene	4.719 ± 0.089	0.964 ± 0.094	10.10 ± 0.018	-
Benzo[a]anthracene	6.050 ± 0.074	5.296 ± 0.006	4.556 ± 0.060	2.896 ± 0.094
Chrysene	4.739 ± 0.061	5.435 ± 0.038	5.319 ± 0.042	6.752 ± 0.027
Benzo[k]fluoranthene	3.046 ± 0.047	6.522 ± 0.081	6.184 ± 0.087	-
Benzo[b]fluoranthene	5.846 ± 0.006	4.793 ± 0.081	6.169 ± 0.084	6.409 ± 0.001
Benzo[e]pyrene	5.070 ± 0.041	7.569 ± 0.006	4.446 ± 0.042	0.387 ± 0.088
Indeno[1,2,3 - c,d]pyrene	5.440 ± 0.010	5.317 ± 0.044	6.302 ± 0.015	8.464 ± 0.019
Dibenz[a,h]anthracene	5.633 ± 0.004	6.032 ± 0.03	7.604 ± 0.011	3.789 ± 0.047
benzo[g,h,i]perylene	5.688 ± 0.028	4.678 ± 0.028	10.042 ± 0.038	0.246 ± 0.084
Σ PAHs	68.757 ± 0.579	54.438 ± 0.668	74.085 ± 0.925	41.248 ± 0.299

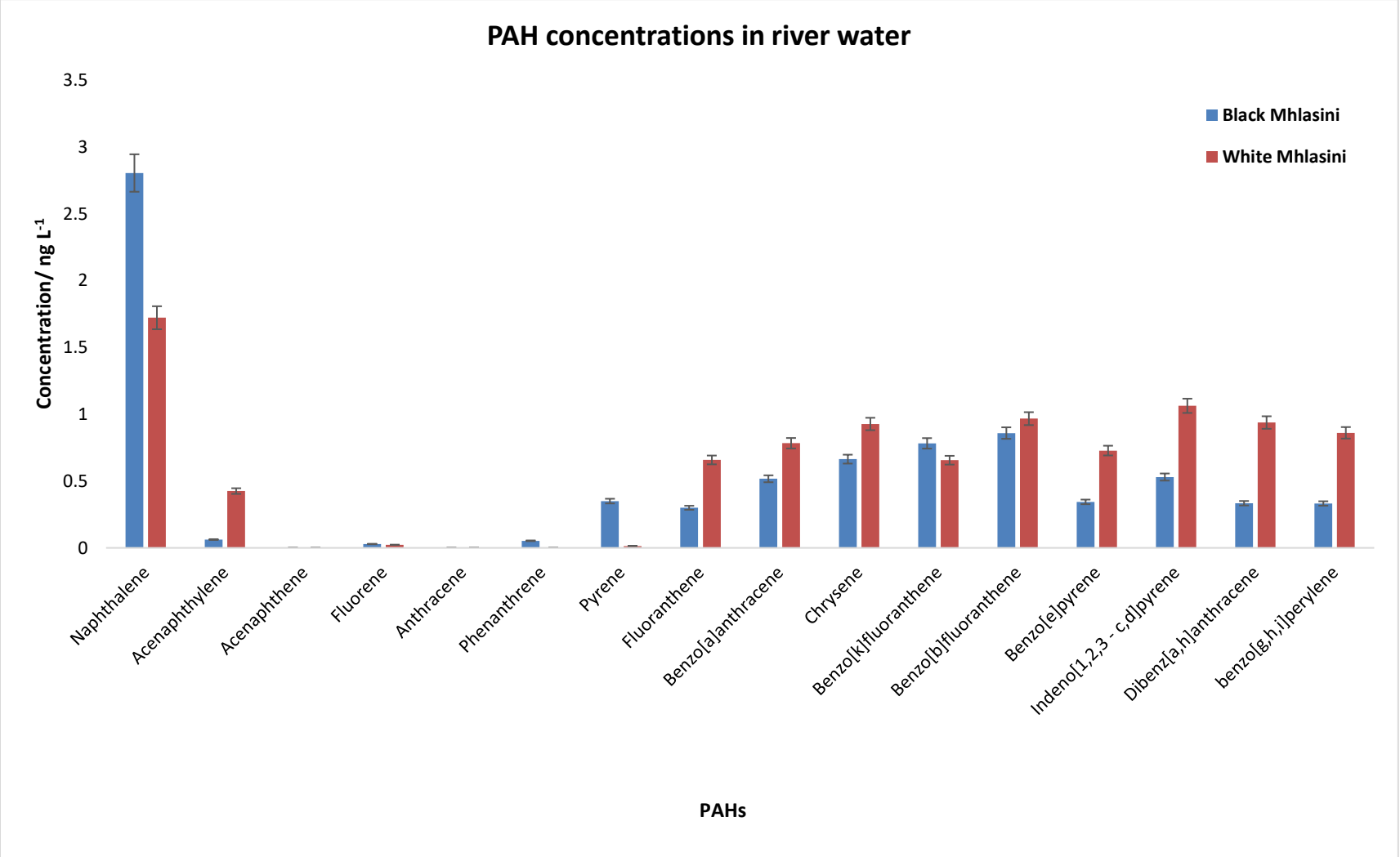


Figure 4.5: The individual total PAH concentration compared to each other for both the Black and White Mhlasini Rivers, N=3

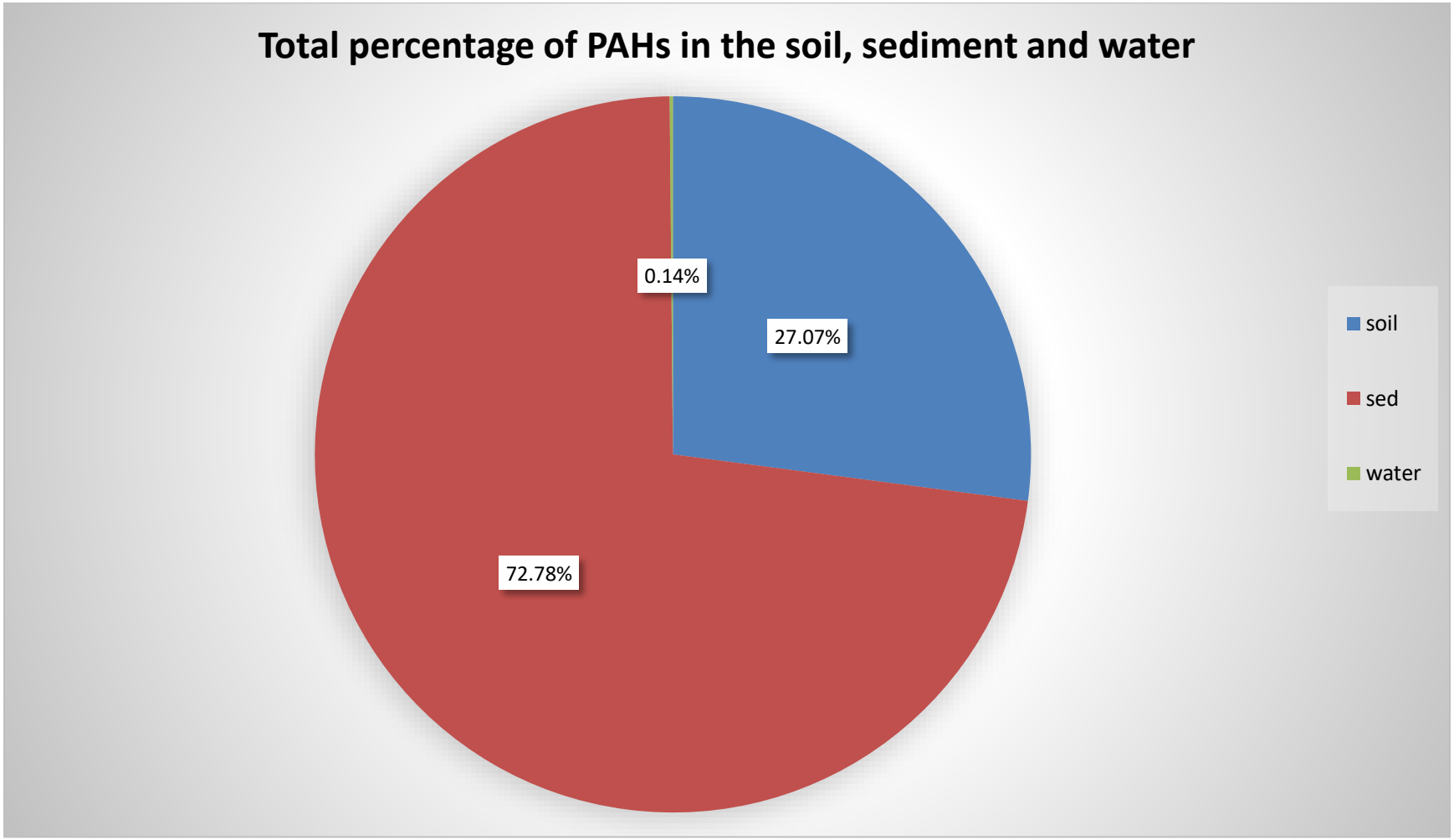


Figure 4.6: The distribution of total PAHs in soil, sediment and water, N=3

4.3.1.4 Source apportionment for PAHs

Tables 4.15, 4.16 and 4.17 provide information regarding the potential source of the PAH contamination in the three matrices. It is difficult to determine the origin of the source of contamination in sediment samples as it is possible to have a combination of petrogenic and pyrolytic sources (Soclo et al., 2000). The first ratio between LMW/HMW (<1) PAHs showed that the PAHs detected in the soil, sediment and water samples originated from pyrolytic sources, which refers to the burning of fossil fuel and biomass. Sampling site BM 4 (Table 4.17), suggests that there are also petrogenic sources in the water at this particular site as there is a slightly higher concentration of LMW PAHs. As stated before, at site BM 4, sand mining is currently taking place alongside the river. The use of heavy-duty industrial equipment, which utilise diesel, could be contributing to the emissions of petrogenic PAHs, which could make their way into the river water. Other possible petrogenic contributions may occur from the exhaust fumes of the waste disposal vehicles constantly entering the site to transport waste to the landfill site. This particular site is close to the main entrance of the reforestation site as well as close to the main road that runs alongside the river. Thus, this site is exposed to vehicles travelling on the road, as well as every vehicle that enters the reforestation zone passing through this site. There is therefore high exposure of this site to exhaust fumes. The phenanthrene/anthracene ratio (<10) could not be calculated for all sites as these PAHs were either not present or were below the detection limit of the instrument. However, for sites BM 4 and WM 2-4, for which the ratio could be calculated, the results indicated that the main source of contamination originated from pyrolytic sources. The fluoranthene/pyrene ratio (>1) also shows that PAH contamination originated from pyrolytic sources. This could have originated from the burning of sugarcane during the harvest season when this area was a sugarcane farm. This ratio provides a much more specific understanding of the possible source of contamination compared to the LMW/HMW ratio as it gives a clearer indication of the specific HMWs that contribute to PAHs. Planting phases 2010-2012 and 2014/2015 has chrysene/benzo[a]anthracene ratio values >1 which means the main source of contamination was from petrogenic sources. This could have resulted from the constant movement of waste disposal vehicles on the site resulting in vehicle exhaust fumes depositing these pollutants on the soil. The chrysene/benzo[a]anthracene ratio found in the river water samples revealed the source of contamination to mostly be from petrogenic sources. This could have resulted from constant motor

vehicle activity alongside the reforestation area as the nearby roads are close to the banks of the rivers. Table 4.17 shows a high fluoranthene/pyrene ratio, 14.14, which is due to the high fluoranthene concentration at water sampling site WM1. The high concentration was possibly due to fluoranthene being the most abundant aerosol PAH present in the atmosphere as stated under section 2.1.7 (Lens et al., 2006).

Table 4.15: PAH ratio indicating the source for each soil sampling site, N=3

Ratio	**Planting Phase**				
	2010/11	**2011/12**	**2012/13**	**2013/14**	**2014/15**
LMW/HMW	0.16	0.23	0.20	0.19	0.14
Phenanthrene/ anthracene	-	-	-	-	-
Fluoranthene/ pyrene	4.19	4.92	1.97	3.28	5.52
Chrysene/Benzo[a]anthracene	1.50	1.58	0.61	0.77	1.46

Table 4.16: PAH ratio indicating the source for each sediment sampling site, N=3

Ratio	**Black Mhlasini River**				**White Mhlasini River**			
	BM 1	**BM 2**	**BM 3**	**BM 4**	**WM 1**	**WM 2**	**WM 3**	**WM 4**
LMW/HMW	0.39	0.34	0.05	0.16	0.14	0.12	0.10	0.15
Phenanthrene/ anthracene	-	-	-	0.90	-	0.80	0.06	0.94
Fluoranthene/ pyrene	1.19	1.47	4.10	1.34	1.93	1.03	2.25	1.71
Chrysene/Benzo[a]anthracene	0.82	0.99	0.75	1.02	0.87	0.66	1.13	0.54

Table 4.17: PAH ratio indicating the source for each water sampling site, N=3

Ratio	Black Mhlasini River				White Mhlasini River			
	BM 1	**BM 2**	**BM 3**	**BM 4**	**WM 1**	**WM 2**	**WM 3**	**WM 4**
LMW/HMW	-	0.45	0.52	1.07	0.48	0.03	0.23	0.28
Phenanthrene/ anthracene	-	-	-	-	-	-	-	-
Fluoranthene/ pyrene	-	0.74	-	-	14.14	-	-	-
Chrysene/Benzo[a]anthracene	6.35	0.70	1.48	0.74	0.78	1.03	1.17	2.33

4.3.2 Pesticides

4.3.2.1 Soil

The concentration of oxamyl, hexazinone, and acetochlor determined in the soil samples from each planting phase and the control sample, are listed in Table 4.18. The total pesticide concentrations for planting phases 2010/2011 to 2013/2014 were in the range 1.324 ± 0.063 to 1.742 ± 0.058 µg g^{-1}, respectively. The control sample had the highest concentration of total pesticides, 2.115 ± 0.097 µg g^{-1}, as no trees were planted to assist in the uptake of these pesticides as seen in Figure 4.7. Here again, the total relative concentration of pesticides found in the area where the trees were planted later were slightly higher than those obtained in the area with earlier planted trees. This could mean that the trees were possibly able to take up pesticides. Research conducted on the sugarcane belt in Kenya, revealed hexazinone concentrations of 8.25 µg g^{-1} and still showed traces of hexazinone five years later after its usage had stopped (Muendo et al., 2012). However, it is not definitely clear that the planted trees are taking up these pesticides as their half-lives may also influence the concentration of pesticides detected in the samples. Oxamyl has a half-life of between 6-12 months (Osman et al., 2015) and hexazinone's half-life is 90 days (Tu et al., 2001). The degradation of the pesticides may be playing a role in the low concentrations.

Table 4.18: Concentration of pesticides in soil, N=3

RT /min	Compound	Pesticide Concentration/ µg g^{-1}					
		Planting phase					
		2010/2011	2011/2012	2012/2013	2013/2014	2014/2015	Control
2.092	Oxamyl	0.365 ± 0.064	0.465 ± 0.082	0.532 ± 0.017	0.680 ± 0.045	0.643 ± 0.044	0.742 ± 0.030
2.807	Hexazinone	0.396 ± 0.076	0.582 ± 0.076	0.681 ± 0.042	0.612 ± 0.057	0.799 ± 0.020	0.864 ± 0.059
9.422	Acetochlor	0.563 ± 0.057	0.224 ± 0.040	0.140 ± 0.005	0.280 ± 0.072	0.300 ± 0.045	0.509 ± 0.076
Σ Pesticides		1.324 ± 0.063	1.271 ± 0.054	1.353 ± 0.041	1.571 ± 0.056	1.742 ± 0.058	2.115 ± 0.097

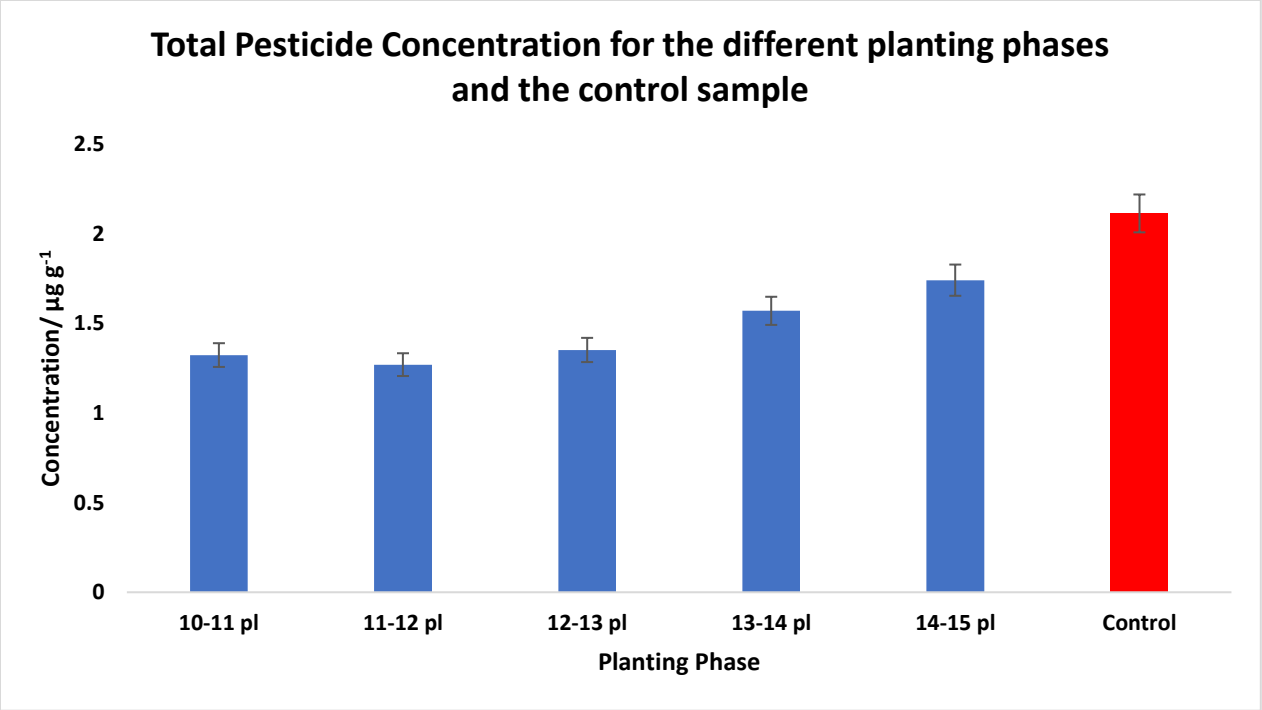


Figure 4.7: The total pesticide concentration in the soil samples from the different planting phases, N=3

4.3.2.2 Sediment

Table 4.19 shows the concentration of the pesticides determined in the sediment samples collected from the Black Mhlasini River. The total pesticide concentration was shown to be much higher outside of the reforestation boundary compared to the samples collected from within the reforestation boundary. Sampling site BM 1 had the highest concentration of pesticides for the Black Mhlasini River, 1.175 ± 0.063 µg g^{-1}. A possible explanation for the low total pesticide concentration at sampling site BM 2, is that this site is in close proximity to the 2010/2011 planting phase. Thus, most of the PAHs are potentially being taken up by the trees, which prevents these pollutants from entering the waterways as it was also observed in water samples (Table 4.21). A similar trend was observed for the sediment samples collected from the White Mhlasini River, Table 4.20, as the total pesticide concentration decreased moving downstream of the reforestation site. Sampling site WM 1 had the highest total pesticide concentration for the White Mhlasini River, 0.514 ± 0.011 µg g^{-1}. The reason for the highest concentrations at BM 1 and WM 1 sites could be due to these sampling sites being located outside of the reforestation boundary, where no reforestation has taken place. As the river flows downstream, it passes both through (Black Mhlasini) and alongside (White Mhlasini) the reforestation site, which is possibly responsible for the decrease in the concentration of pesticides entering the river due to uptake by the planted trees. Hexazinone had the highest concentration in sediment compared to oxamyl as it has a much lower water solubility value compared to oxamyl and thus prefers to partition to the organic matter in the sediment.

Table 4.19: Concentration of pesticides in sediment samples from Black Mhlasini River, N=3

Compound	**Pesticide Concentration/ µg g^{-1}**			
	BM 1	BM 2	BM 3	BM 4
Oxamyl	0.401 ± 0.052	0.181 ± 0.061	0.095 ± 0.041	0.467 ± 0.038
Hexazinone	0.431 ± 0.043	0.289 ± 0.022	0.333 ± 0.031	0.368 ± 0.055
Acetochlor	0.343 ± 0.034	-	0.167 ± 0.052	0.189 ± 0.034
Σ Pesticides	1.175 ± 0.063	0.469 ± 0.025	0.595 ± 0.035	1.024 ± 0.052

Table 4.20: Concentration of pesticides in sediment samples from White Mhlasini River, N=3

Compound	**Pesticide Concentration/ µg g^{-1}**			
	WM 1	WM 2	WM 3	WM 4
Oxamyl	0.229 ± 0.029	0.072 ± 0.024	0.123 ± 0.073	0.184 ± 0.001
Hexazinone	0.285 ± 0.035	0.126 ± 0.047	0.251 ± 0.061	0.319 ± 0.057
Acetochlor	-	-	0.040 ± 0.015	-
Σ Pesticides	0.514 ± 0.011	0.197 ± 0.003	0.414 ± 0.032	0.503 ± 0.029

4.3.2.3 Water

Tables 4.21 and 4.22 show the pesticide concentrations determined from the river water samples collected from the Black and White Mhlasini Rivers. The total pesticide concentration showed the same trend as observed for the sediment samples. The furthest points upstream of the reforestation site, BM 1 and WM 1, had the highest total pesticide concentrations, 12.950 ± 0.0752 ng L^{-1} and 4.644 ± 0.007 ng L^{-1}, respectively. Oxamyl had the highest concentration, 8.278 ± 0.081 ng L^{-1}, for the Black Mhlasini River and was consistently present in all water samples. This could be due to the high water solubility of oxamyl, 282000 mg L^{-1}, compared to hexazinone and acetochlor, Figure 4.8. The Black Mhlasini River had a higher concentration of pesticides as it was more exposed to the nearby Inanda farm, located north-west of the landfill, with a potential of pesticides leaching into the river. There was no concentration of acetochlor determined in the White Mhlasini river water sample, which could be due to it being below the detection limit for the instrument or as a result of its extremely low solubility in water, 233 mg L^{-1}. Figure 4.9 displays the distribution of the total pesticides in the three different matrix types. A greater percentage of pesticides was found in the soil samples, 55.11 % and least amount in the water samples, 0.38 %.

Table 4.21: Concentration of pesticides in water collected from Black Mhlasini River, N=3

Compound	Pesticide Concentration/ ng L^{-1}			
	BM 1	**BM 2**	**BM 3**	**BM 4**
Oxamyl	6.553 ± 0.057	0.792 ± 0.023	8.278 ± 0.081	5.180 ± 0.064
Hexazinone	6.397 ± 0.081	-	2.132 ± 0.066	5.920 ± 0.008
Acetochlor	-	-	-	0.905 ± 0.081
Σ Pesticides	12.950 ± 0.0752	0.792 ± 0.0852	10.411 ± 0.176	12.005 ± 0.138

Table 4.22: Concentration of pesticides in water collected from White Mhlasini River, N=3

Compound	Pesticide Concentration/ ng L^{-1}			
	WM 1	**WM 2**	**WM 3**	**WM 4**
Oxamyl	2.747 ± 0.075	1.382 ± 0.040	1.746 ± 0.011	1.041 ± 0.009
Hexazinone	1.897 ± 0.053	2.262 ± 0.010	1.700 ± 0.083	1.253 ± 0.015
Acetochlor	-	-	-	-
Σ Pesticides	4.644 ± 0.007	3.644 ± 0.060	3.446 ± 0.096	2.294 ± 0.067

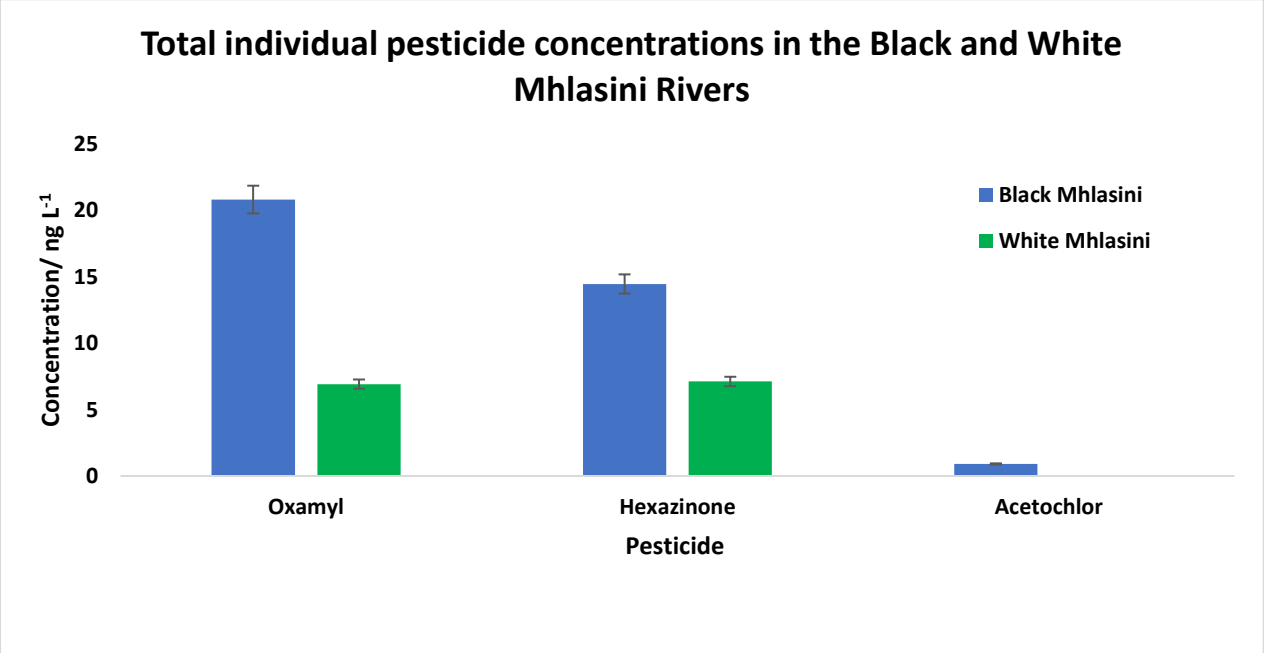


Figure 4.8: Total individual pesticide concentrations in the river water samples, N=3

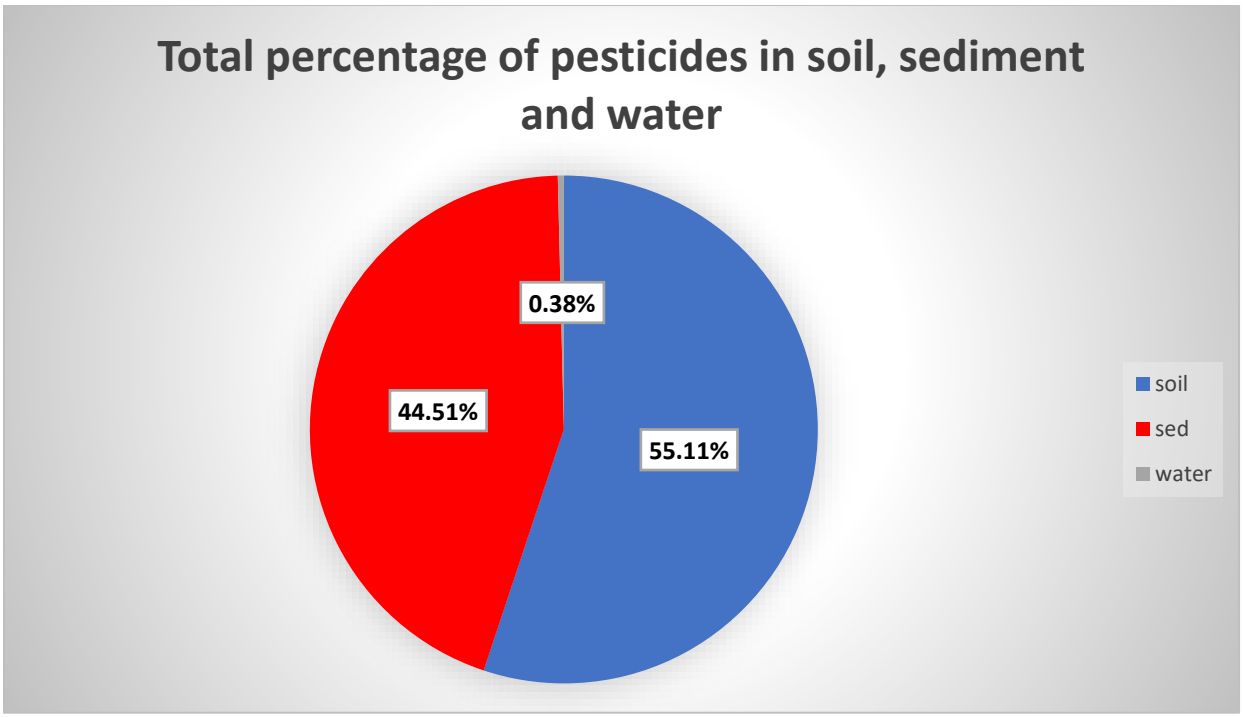


Figure 4.9: The distribution of total pesticides in soil, sediment and water, N=3

4.4 References

ABDEL-SHAFY, H. I. & MANSOUR, M. S. 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum,* 25**,** 107-123.

AGENCY, E. 2005. *Soil Guideline Values for Naphthalene Contamination* [Online]. Available: https://www.jiscmail.ac.uk/cgi-bin/wa.exe?A3=ind0812&L=CONTAMINATED-LAND-STRATEGIES&E=base64&P=1209727&B=------_%3D_NextPart_001_01C95AB3.61E989D1&T=application%2Foctet-stream;%20name=%22Napthalene_SGV%2019_%20for%20review%20071105.pdf%22&N=Napthalene_SGV%2019_%20for%20review%20071105.pdf&attachment=q [Accessed 12 September 2018].

ARAOYE, P. 2009. The seasonal variation of pH and dissolved oxygen (DO2) concentration in Asa lake Ilorin, Nigeria. *International Journal of Physical Sciences,* 4**,** 271-274.

CCME. 2008. *Canadian Soil Quality Guidelines for Carcinogenic and other Polycyclic Aromatic Hydrocarbons* [Online]. Available: https://www.ccme.ca/files/Resources/supporting_scientific_documents/pah_soqg_ssd_1401.pdf [Accessed 12 September 2018].

CHIMUKA, L., SIBIYA, P., AMDANY, R., CUKROWSKA, E. & FORBES, P. 2016. Status of PAHs in environmental compartments of South Africa: a country report. *Polycyclic Aromatic Compounds,* 36**,** 376-394.

DOONG, R.-A. & LIN, Y.-T. 2004. Characterization and distribution of polycyclic aromatic hydrocarbon contaminations in surface sediment and water from Gao-ping River, Taiwan. *Water Research,* 38**,** 1733-1744.

EMEA. 2010. *Guideline for Good Clinical Practice - European Medicines Agency* [Online]. Available: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500040499.pdf [Accessed 12 September 2018].

GLICK, B. R. 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnology Advances,* 21**,** 383-393.

HALL, D., WU, C.-Y., HSU, Y.-M., STORMER, J., ENGLING, G., CAPETO, K., WANG, J., BROWN, S., LI, H.-W. & YU, K.-M. 2012. PAHs, carbonyls, VOCs and PM2. 5 emission factors for pre-harvest burning of Florida sugarcane. *Atmospheric Environment,* 55**,** 164-172.

HUANG, X.-D., EL-ALAWI, Y., PENROSE, D. M., GLICK, B. R. & GREENBERG, B. M. 2004. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. *Environmental Pollution,* 130**,** 465-476.

KUANG, Y.-W., ZHOU, G.-Y., WEN, D.-Z., LI, J. & SUN, F.-F. 2011. Analysis of polycyclic aromatic hydrocarbons in tree-rings of Masson pine (Pinus massoniana L.) from two industrial sites in the Pearl River Delta, south China. *Journal of Environmental Monitoring,* 13**,** 2630-2637.

LENS, P., KENNES, C., CLOIREC, P. & DESHUSSES, M. 2006. *Waste Gas Treatment for Resource Recovery,* IWA Publishing.

LUO, X., MAI, B., YANG, Q., FU, J., SHENG, G. & WANG, Z. 2004. Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides in water columns from the Pearl River and the Macao harbor in the Pearl River Delta in South China. *Marine Pollution Bulletin,* 48**,** 1102-1115.

MCCUTCHEON, S. C. & SCHNOOR, J. L. 2004. *Phytoremediation: Transformation and Control of Contaminants*, Wiley.

MEADOR, J., STEIN, J., REICHERT, W. & VARANASI, U. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Reviews of Environmental Contamination and Toxicology.* Springer.

MUENDO, B. M., LALAH, J. O. & GETENGA, Z. M. 2012. Behavior of pesticide residues in agricultural soil and adjacent River Kuywa sediment and water samples from Nzoia sugarcane belt in Kenya. *The Environmentalist,* 32**,** 433-444.

MUGICA-ALVAREZ, V., SANTIAGO-DE LA ROSA, N., FIGUEROA-LARA, J., FLORES-RODRÍGUEZ, J., TORRES-RODRÍGUEZ, M. & MAGAÑA-REYES, M. 2015. Emissions of PAHs derived from sugarcane burning and processing in Chiapas and Morelos México. *Science of the Total Environment,* 527**,** 474-482.

OSMAN, K. A., EL-BAKARY, A. S., SABER, N. A., EL-SEBAI, A. & SALAMA, M. 2015. Removal of oxamyl in soil by different treatments. *ALEXANDRIA SCIENCE EXCHANGE,* 36**,** 142.

SIMONICH, S. L. & HITES, R. A. 1995. Organic pollutant accumulation in vegetation. *Environmental Science & Technology,* 29**,** 2905-2914.

SOCLO, H., GARRIGUES, P. & EWALD, M. 2000. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. *Marine Pollution Bulletin,* 40**,** 387-396.

TU, M., HURD, C., RANDALL, J. M. & TEAM, W. I. S. 2001. *Weed Control Methods Handbook: Tools and Techniques for Use in Natural Areas*, Wildland Invasive Species Team, the Nature Conservancy.

WHO. 1998. Polynuclear Aromatic Hydrocarbons in drinking-water. Available: http://www.who.int/water_sanitation_health/dwq/chemicals/polyaromahydrocarbons.pdf [Accessed 27/01/2018].

WILD, S. R. & JONES, K. C. 1995. Polynuclear aromatic hydrocarbons in the United Kingdom environment: a preliminary source inventory and budget. *Environmental pollution,* 88**,** 91-108.

Chapter 5: Conclusion and recommendations

5.1 Conclusion

This study investigated the effect the reforestation process had on the quality of the surrounding soil and river water based on the concentrations of selected organic chemical pollutants. An ultrasonication method was validated for the extraction of soil and sediment, and the LLE method for the water samples. All samples were spiked with a known concentration of the specific analyte to bring the concentrations of the analytes within the range of the instrument detection limit. The LMW hydrocarbons had slightly higher recoveries compared to the HMW hydrocarbons due to them being more volatile. PAH recoveries on the GCMS ranged between 60-110%, and pesticide recoveries on the LCMS were between 83-113%. Other validation methods included LOD, LOQ, and inter-day and intra-day analysis. The PAH LOD values were between 0.014-0.633 µg g^{-1} for soil, 0.005-0.247 µg g^{-1} for sediment, and 0.004-0.278 µg L^{-1} for water. The pesticide LOD values were between 0.109-0.419 µg g^{-1} for soil, 0.296-0.474 µg g^{-1} for sediment, and 0.097-0.397 µg L^{-1} for water. PAH LOQ values ranged between 0.042-1.919 µg g^{-1} for soil, 0.008-.749 µg g^{-1} for sediment, and 0.011-0.842 µg L^{-1} for water. Pesticide LOQ values ranged between 0.331-1.268 µg g^{-1} for soil, 0.896-1.437 µg g^{-1} for sediment, and 0.293-1.204 µg L^{-1} for water.

The actual soil samples were collected from the reforestation site located at the Buffelsdraai landfill, and the river water and sediment were collected from the surrounding Black and White Mhlasini Rivers. Soil samples showed a decreasing trend in the total PAH concentrations determined from planting phases, 2010/2011 to 2014/15. The highest total PAH concentration was determined from the control sample, which was expected as no trees have been planted in that area to take up the PAHs. The effect the trees potentially have on the uptake of PAHs, was also evident in sediment collected from sampling sites BM 1 and WM 1 as these sites were outside the reforestation boundary. These sites had higher total PAHs concentration compared to the sites, which were located within the reforestation boundary. Sampling site BM 4, also located outside the reforestation boundary, had the highest PAHs concentration. This site could also have been affected by sand mining taking place in close proximity to the river. From the three types of

matrices sampled, the total PAH contamination was highest in the sediment samples due to PAHs being hydrophobic. The source apportionment analysis assisted in identifying whether the PAHs originated from petrogenic or pyrolytic sources. For this study, most of the PAH contamination originated from pyrolytic sources, which is the burning of biomass.

Similar trends were observed for the pesticide analysis, which showed a decreasing trend in total pesticide concentration over the different planting phases for the soil samples. The total pesticide concentrations in the water and sediment samples collected from within the reforestation boundary, were slightly lower compared to sites BM 1 and WM 1 which could mean that these pesticides were potentially absorbed by the planted trees. Oxamyl had the highest concentration in the water samples as a result of it being highly water soluble.

The outcome of this study suggests that the reforestation project currently taking place at the Buffelsdraai Landfill is potentially contributing to the reduction of organic chemical pollutants, specifically pesticides that were previously used on the sugarcane farm as well as PAHs that possibly formed during the burning of the sugarcane. This allows for an improvement in the quality of the surrounding soil and river water, which benefits both the neighbouring communities and eThekwini with future planning of restoration projects.

5.2 Challenges

Throughout the research, many challenges were encountered. These include:

- Cost constraints, which restricted the number of samples collected. With the analysis of more samples, an improved representation of the reforestation project could have been displayed.
- Constant maintenance on required instrumentation delayed the analysis as samples cannot be stored for long periods of time, so this caused a delay in the collection of samples. The unavailability of certain extraction techniques, such as super critical fluid extraction and pressurized solvent extraction, caused a restriction on choice of experimental procedures. A possible advantage could be an improved extraction efficiency with the availability of those techniques

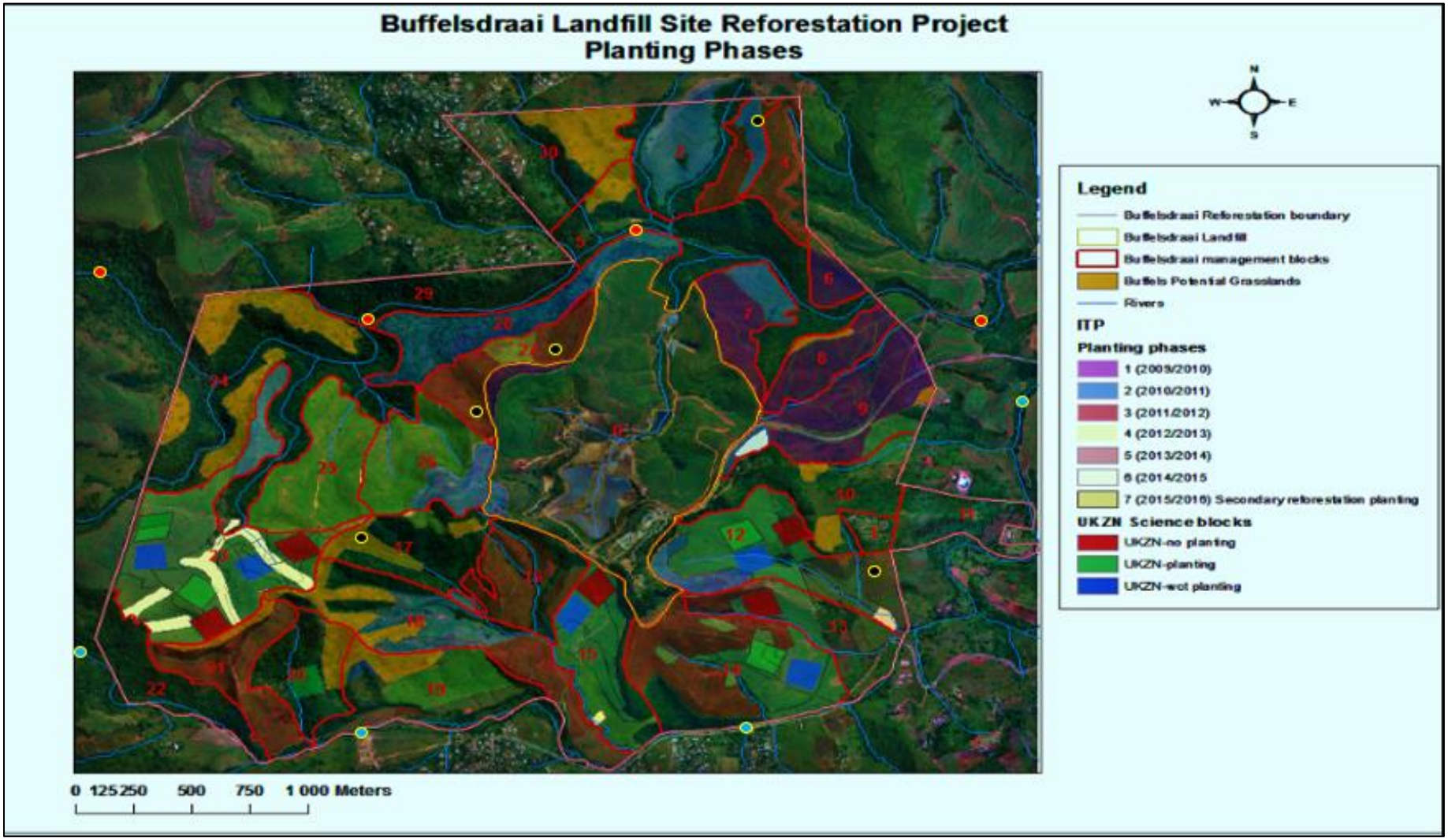
5.3 Future work

To fully understand the positive effect that reforestation has on reducing organic chemical pollutants, further work is required to provide more in-depth evidence on how reforestation assists in reducing PAHs and pesticides in the natural environment. These studies may include:

- Collecting samples of the planted trees, such as leaves, roots and bark, in order to determine the concentrations of PAHs and pesticides taken up by the trees. This will also help to determine which part of the tree takes up these pollutants.
- Assess the root of uptake by the tree planted at the Buffelsdraai reforestation site and possibly determine which species is preferable for the uptake of the different type of pollutants.
- Analyse air samples to assess the concentration of organic pollutants within the reforested area compared to outside the reforested area.
- Analyse leachate samples from the Buffelsdraai landfill to determine if any contamination of the surrounding soil and water originates from the leachate storage tanks.
- Possibly develop a mechanism to illustrate the environmental fate of the various PAHs and pesticides in soil, sediment, and water.

Appendices

Appendix A: Sampling area map displaying the sampling site locations.



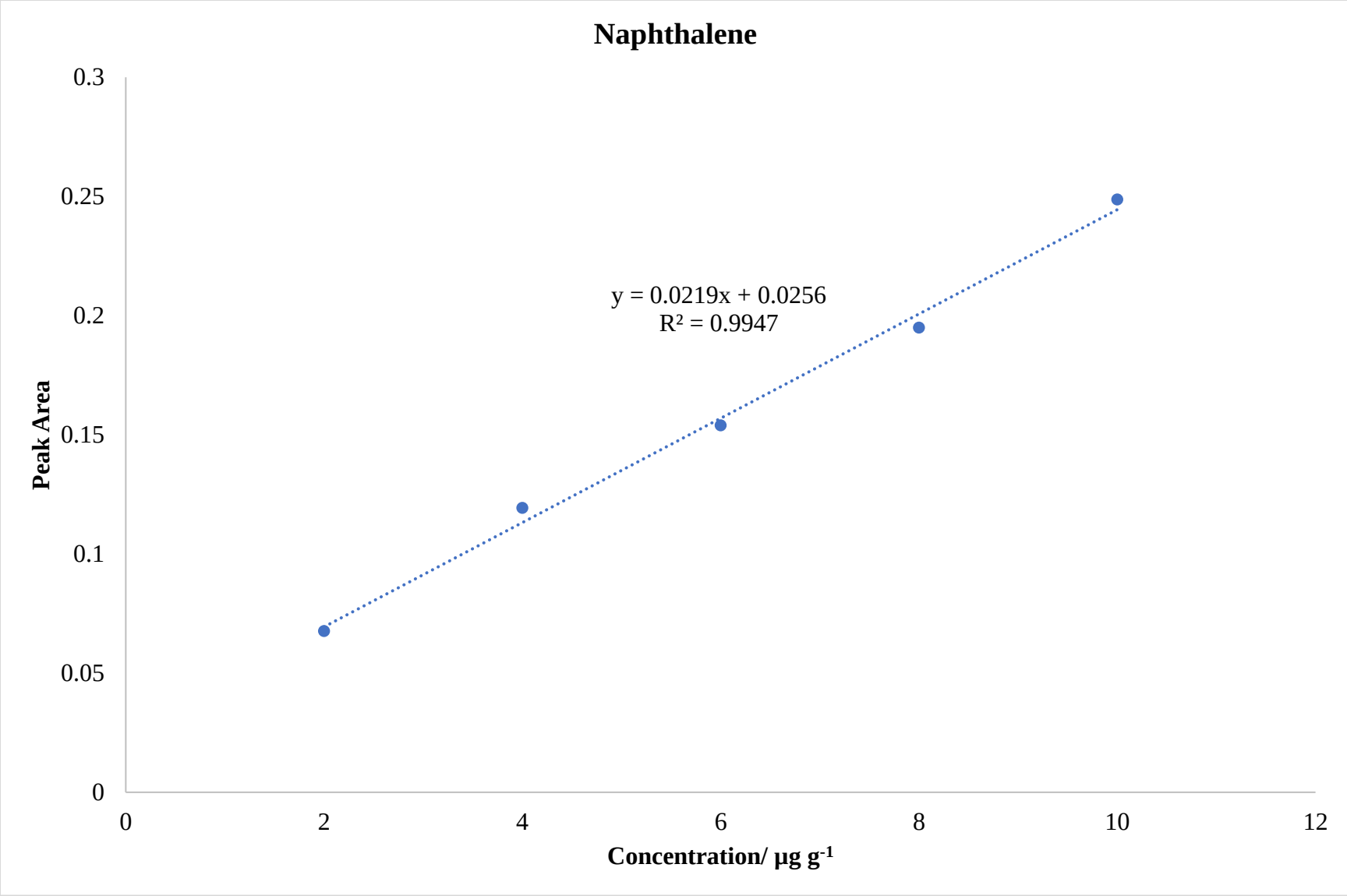
Appendix A.1: Map displaying the sampling locations, black dots are the soil sampling sites, red and blue dots are the water and sediment sampling sites on the Black and White Mhlasini Rivers, respectively

Appendix B: Plants species at the Buffelsdraai reforestation site.

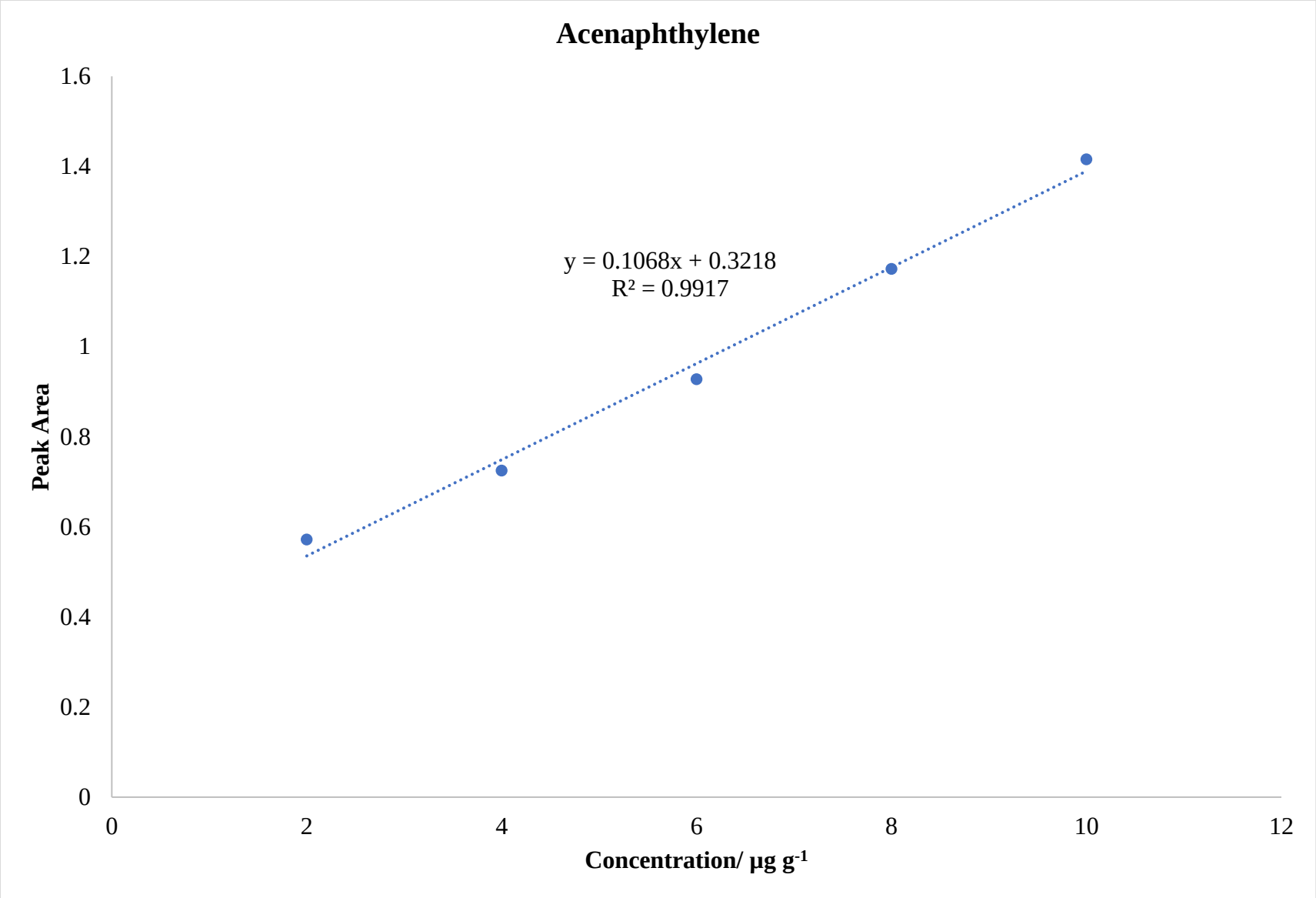
Appendix B.1: Different species of trees planted at the Buffelsdraai Reforestation site

Species Planted		
Acacia ataxacantha	*Combretum molle*	*Loxostylis alata*
Acacia caffra	*Commiphora woodii*	*Maesa alnifolia*
Acacia natalitia	*Cordia caffra*	*Millettia grandis*
Acacia nilotica	*Crassula ovata*	*Pappea capensis*
Acacia robusta	*Croton sylvaticus*	*Pavetta lanceolata*
Acokanthera oblongifolia	*Cussonia spicata*	*Phoenix reclinata*
Adiantum capillus-veneris	*Dalbergia obovata*	*Protorhus longifolia*
Agapanthus africanus	*Deinbollia oblongifolia*	*Rauvolfia caffra*
Agapanthus praecox	*Dietes grandiflora*	*Rotheca myricoides*
Albizia adianthifolia	*Diospyros dichrophylla*	*Schotia brachypetala*
Aloe arborescens	*Diospyros rotundifolia*	*Sclerocarya birrea*
Aloe barberae	*Dombeya tiliacea*	*Sclerocroton integerrimus*
Aloe ferox	*Ehretia rigida*	*Searsia chirindensis*
Aloe maculate	*Ekebergia capensis*	*Searsia pentheri*
Antidesma venosum	*Encephalartos natalensis*	*Searsia pyroides*
Apodytes dimidiata	*Erythrina lysistemon*	*Sansevieria trifasciata*
Baphia racemosa	*Euclea daphnoides*	*Spathodea campanulata*
Bauhinia tomentosa	*Eugenia uniflora*	*Spirostachys africana*
Brachylaena discolor	*Euphorbia ingens*	*Strelitzia nicolai*
Bridelia micrantha	*Euphorbia tirucalli*	*Strychnos spinosa*
Buddleja saligna	*Ficus lutea*	*Syzygium cordatum*
Calodendrum capense	*Ficus natalensis*	*Tabernaemontana ventricosa*
Calpurnia aurea	*Ficus polita*	*Tecomaria capensis*
Canthium ciliatum	*Ficus sur*	*Tetradenia riparia*
Canthium inerme	*Grewia occidentalis*	*Trema orientalis*
Carissa bispinosa	*Halleria lucida*	*Tricalysia capensis*
Cassipourea gummiflua	*Harpephyllum caffrum*	*Trichilia dregeana*
Celtis Africana	*Heteropyxis natalensis*	*Trimeria grandifolia*
Chaetacme aristata	*Hippobromus pauciflorus*	*Vangueria infausta*
Chrysophyllum viridifolium	*Kalanchoe beharensis*	*Zanthoxylum capense*
Clerodendrum glabrum	*Kraussia floribunda*	*Ziziphus mucronata*
Combretum kraussii	*Landolphia kirkii*	

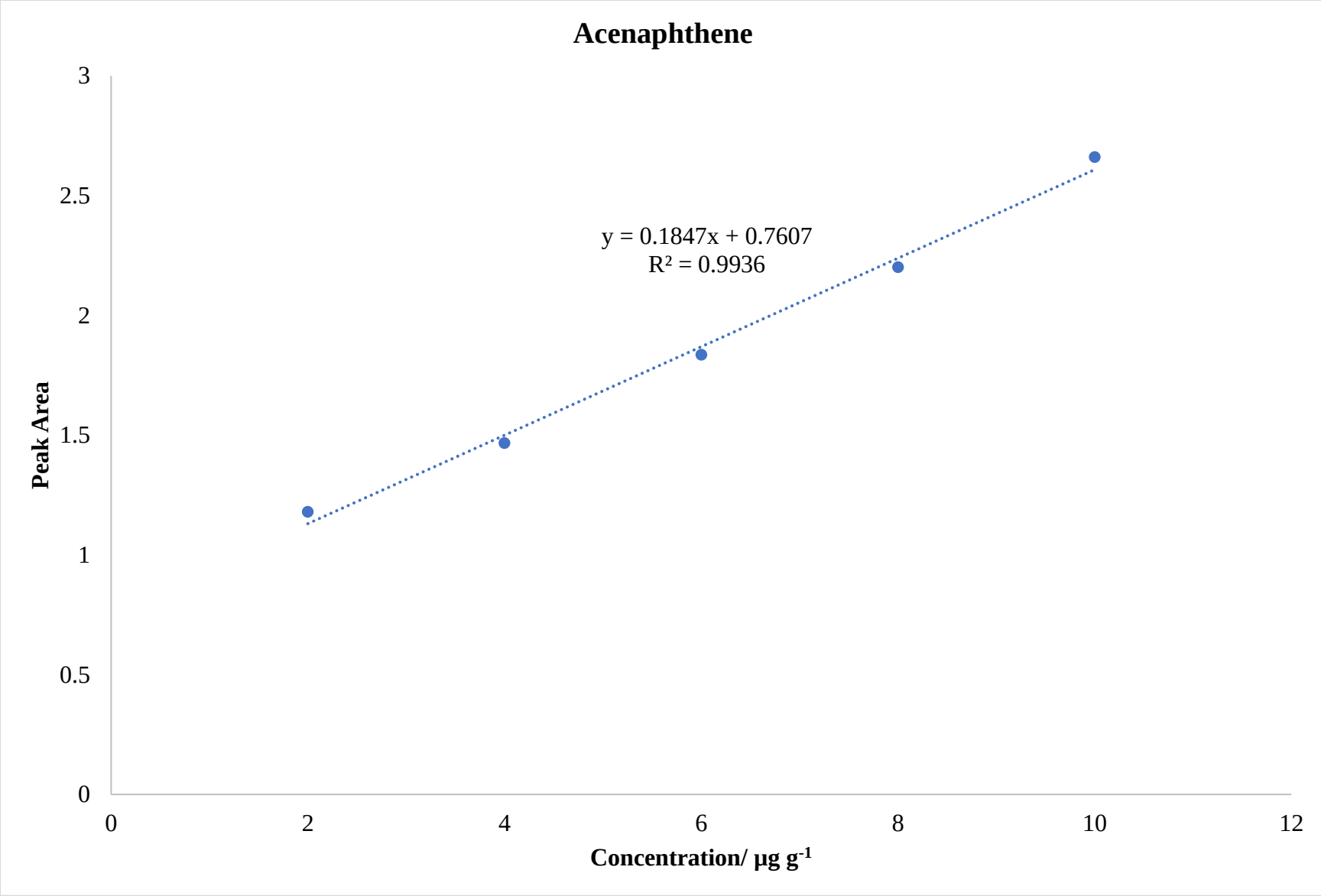
Appendix C: Calibration graphs



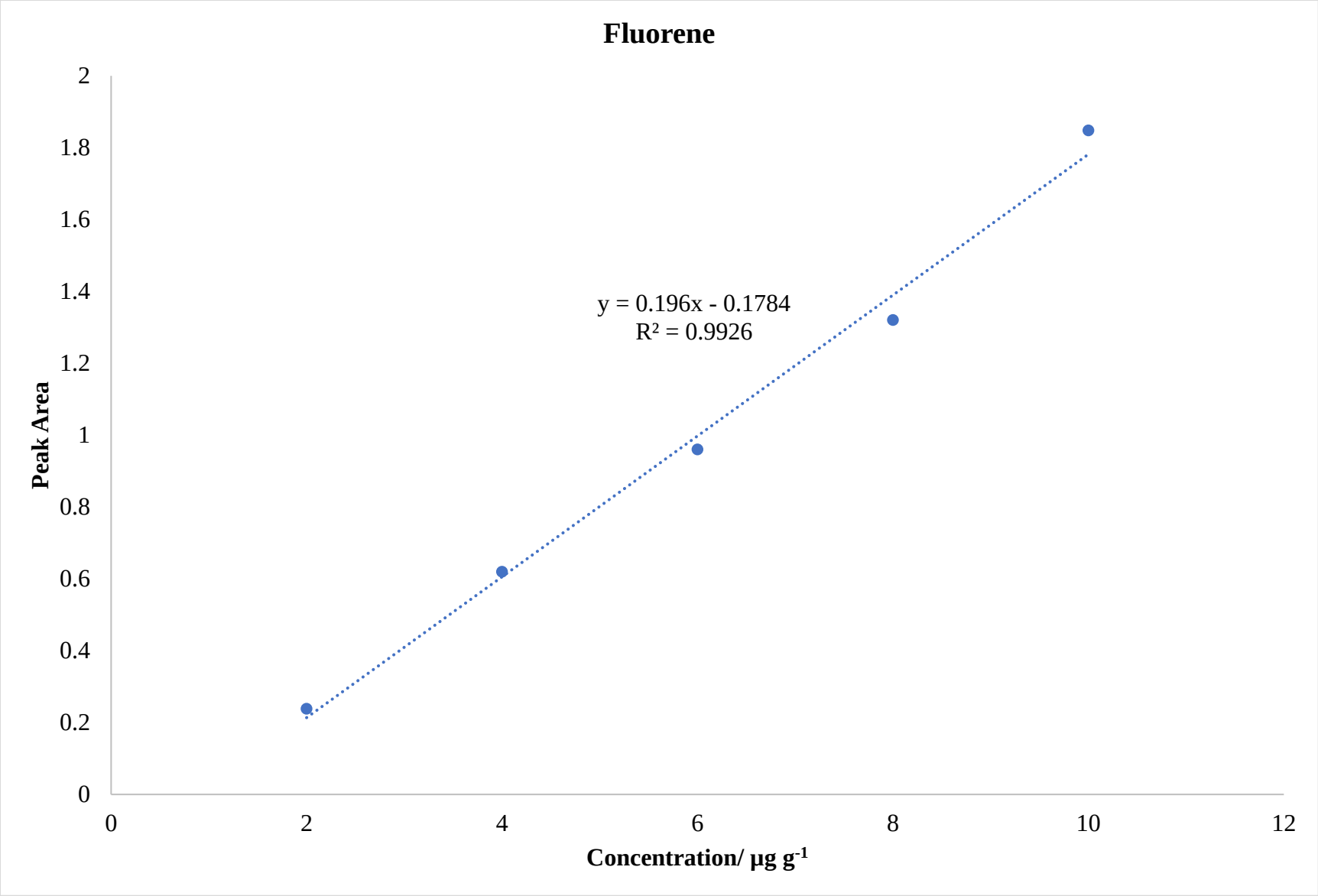
Appendix C.1: Calibration graph for naphthalene



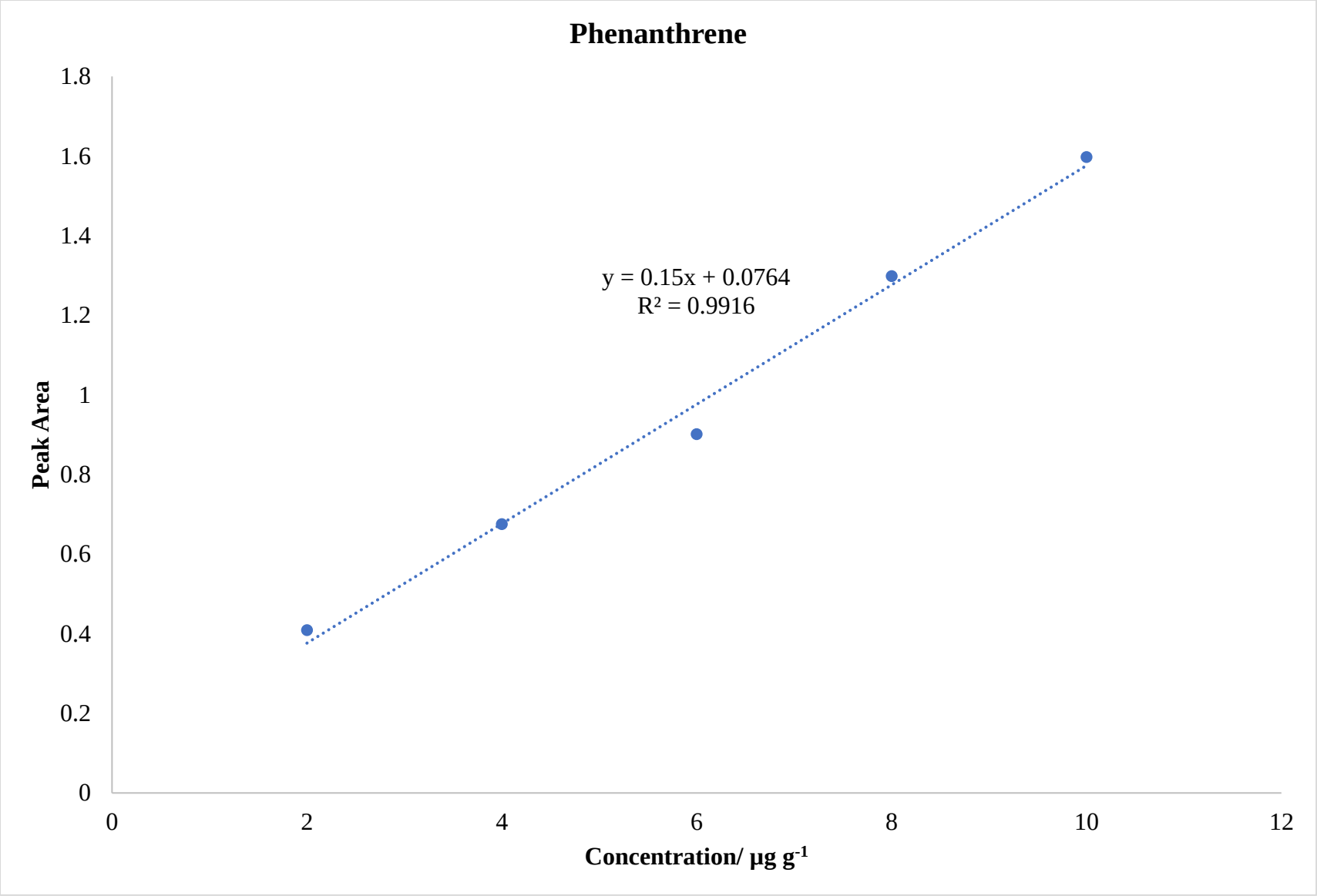
Appendix C.2: Calibration graph for acenaphthylene



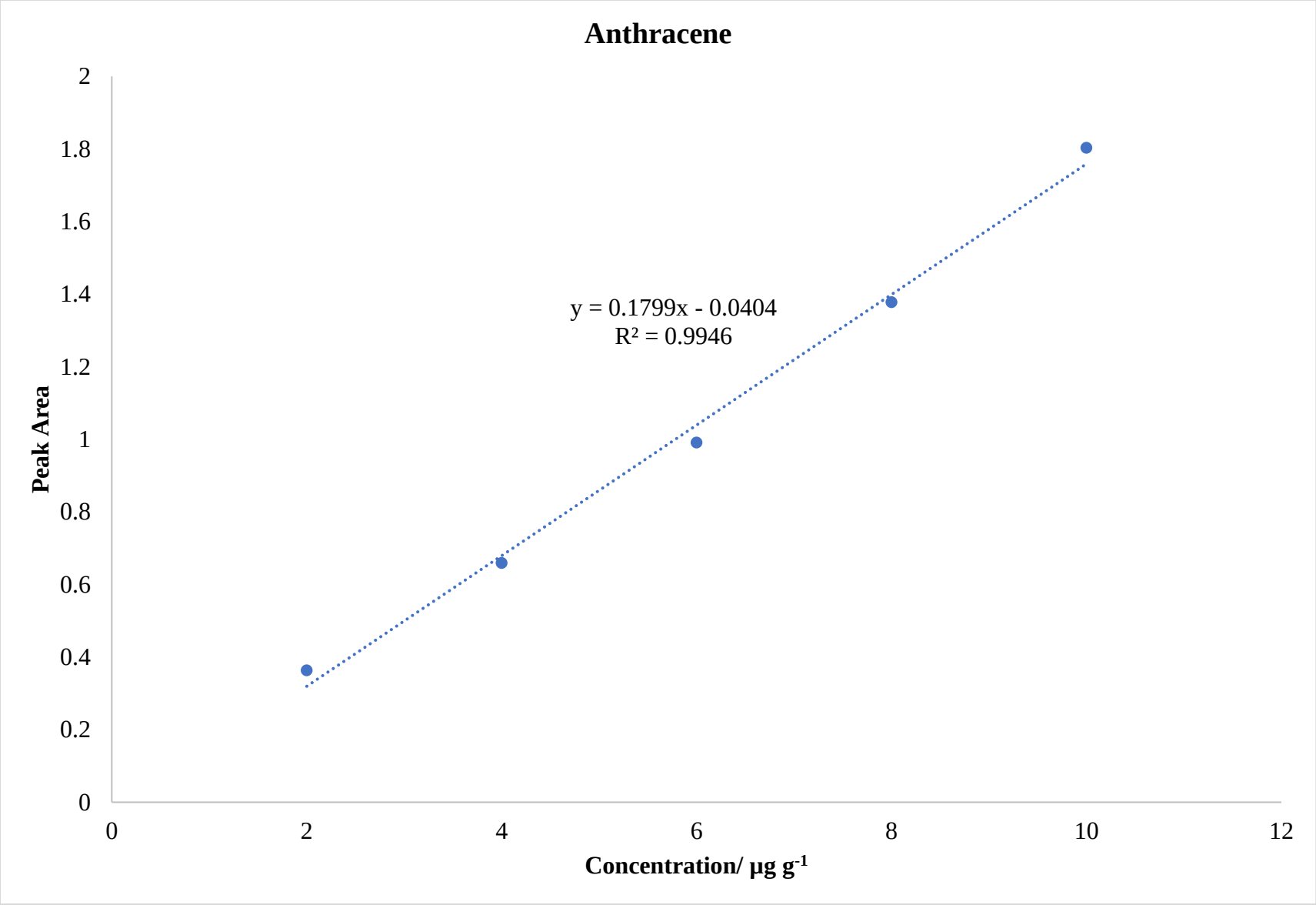
Appendix C.3: Calibration graph for acenaphthene



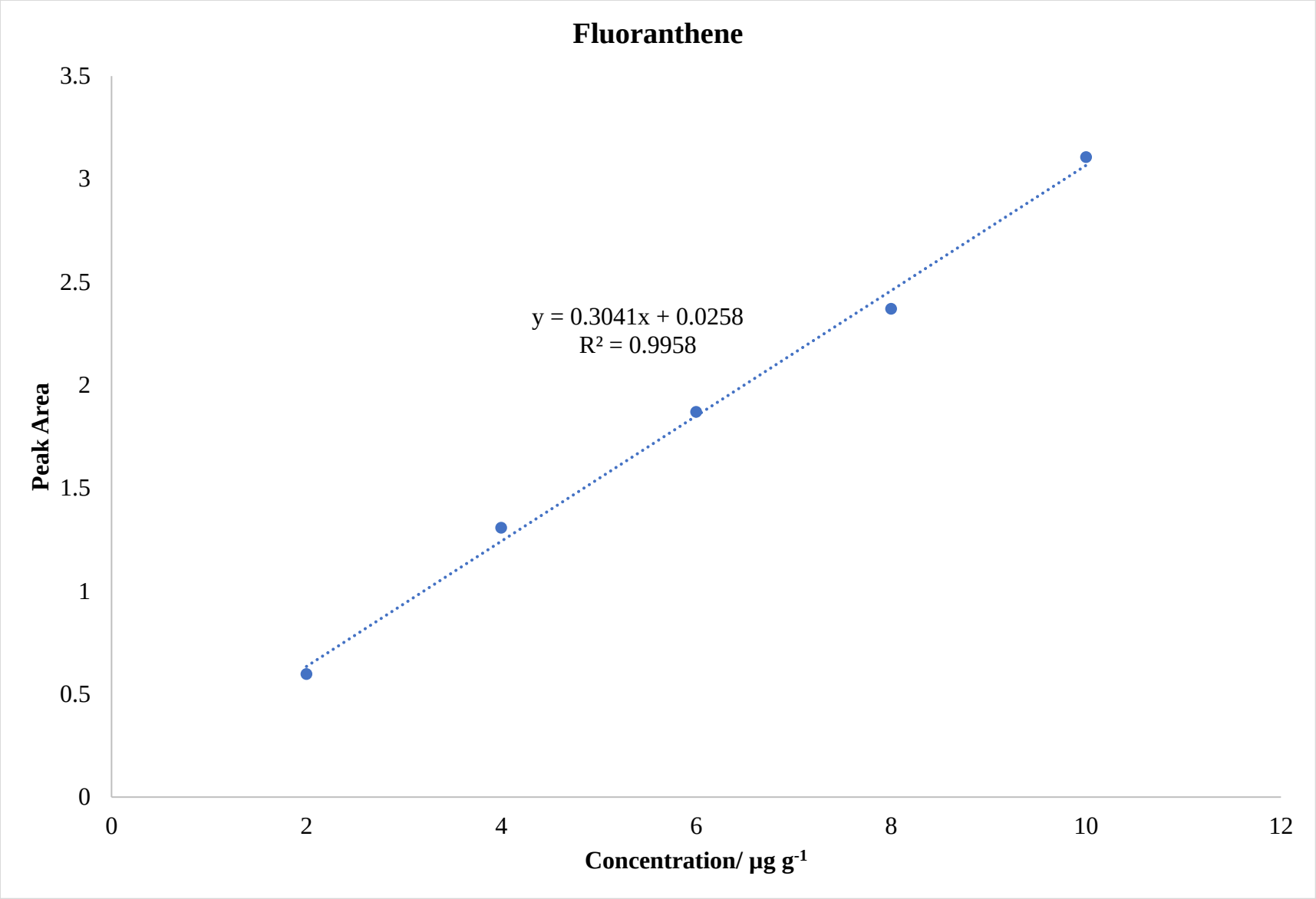
Appendix C.4: Calibration graph for fluorene



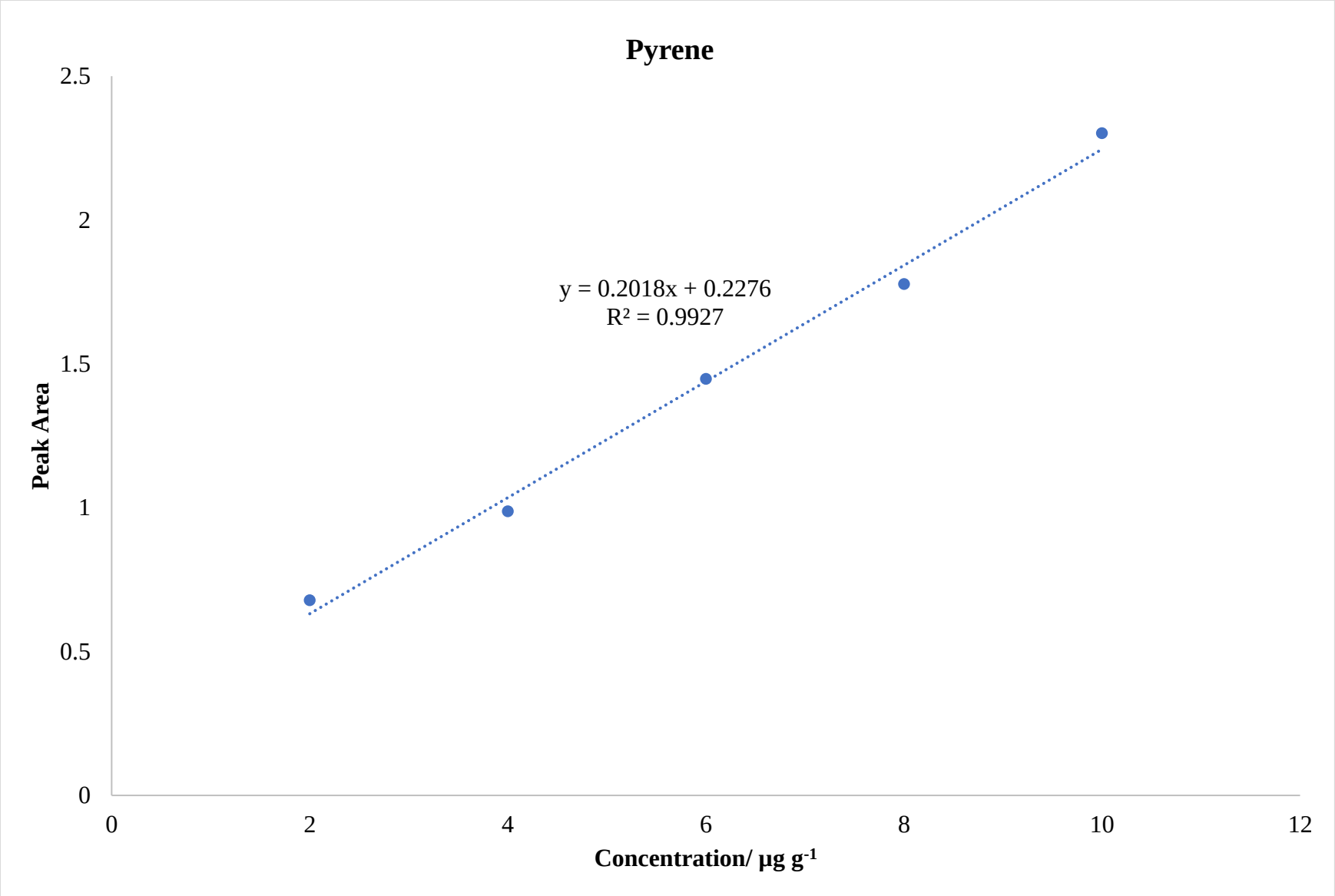
Appendix C.5: Calibration graph for phenanthrene



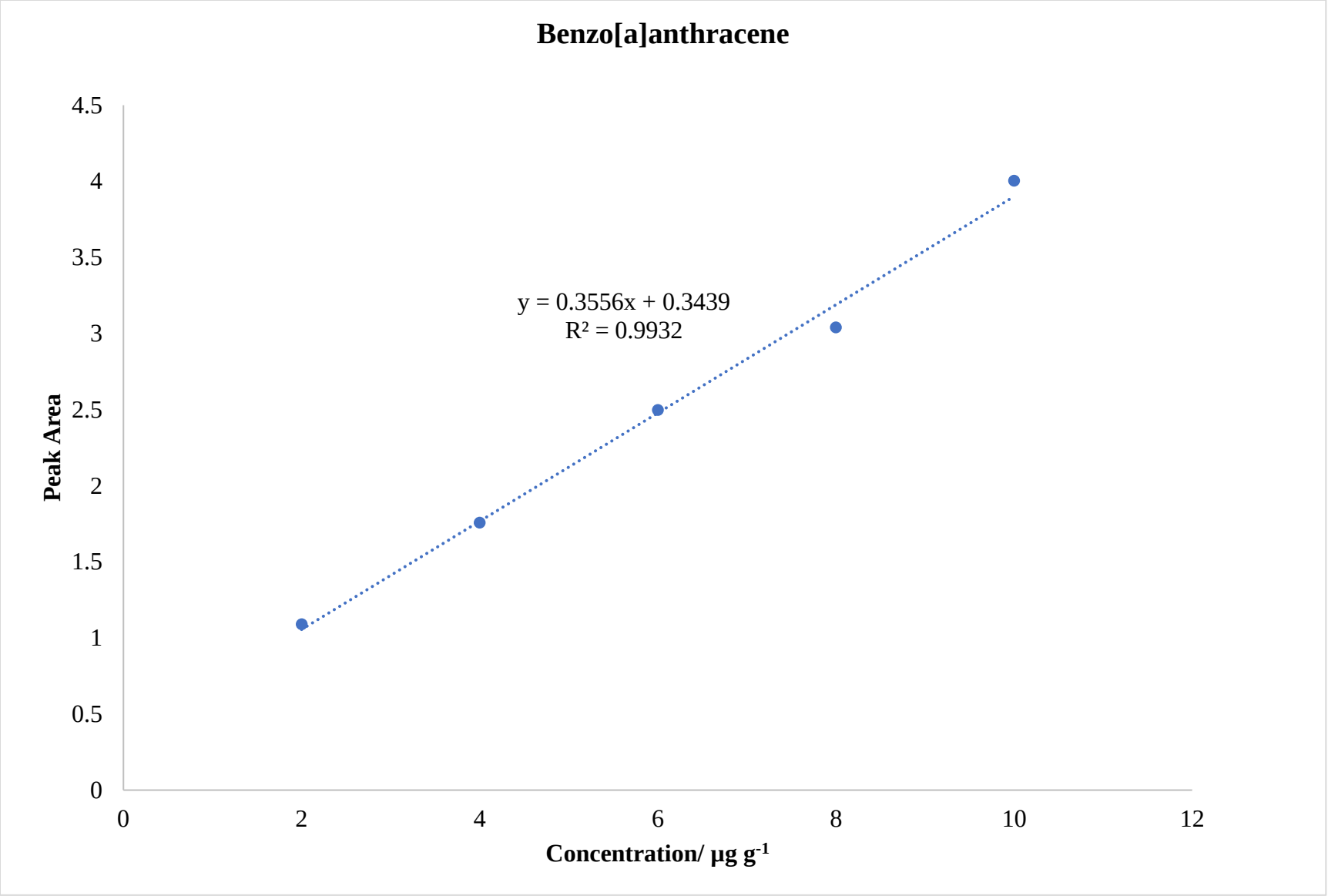
Appendix C.6: Calibration graph for anthracene



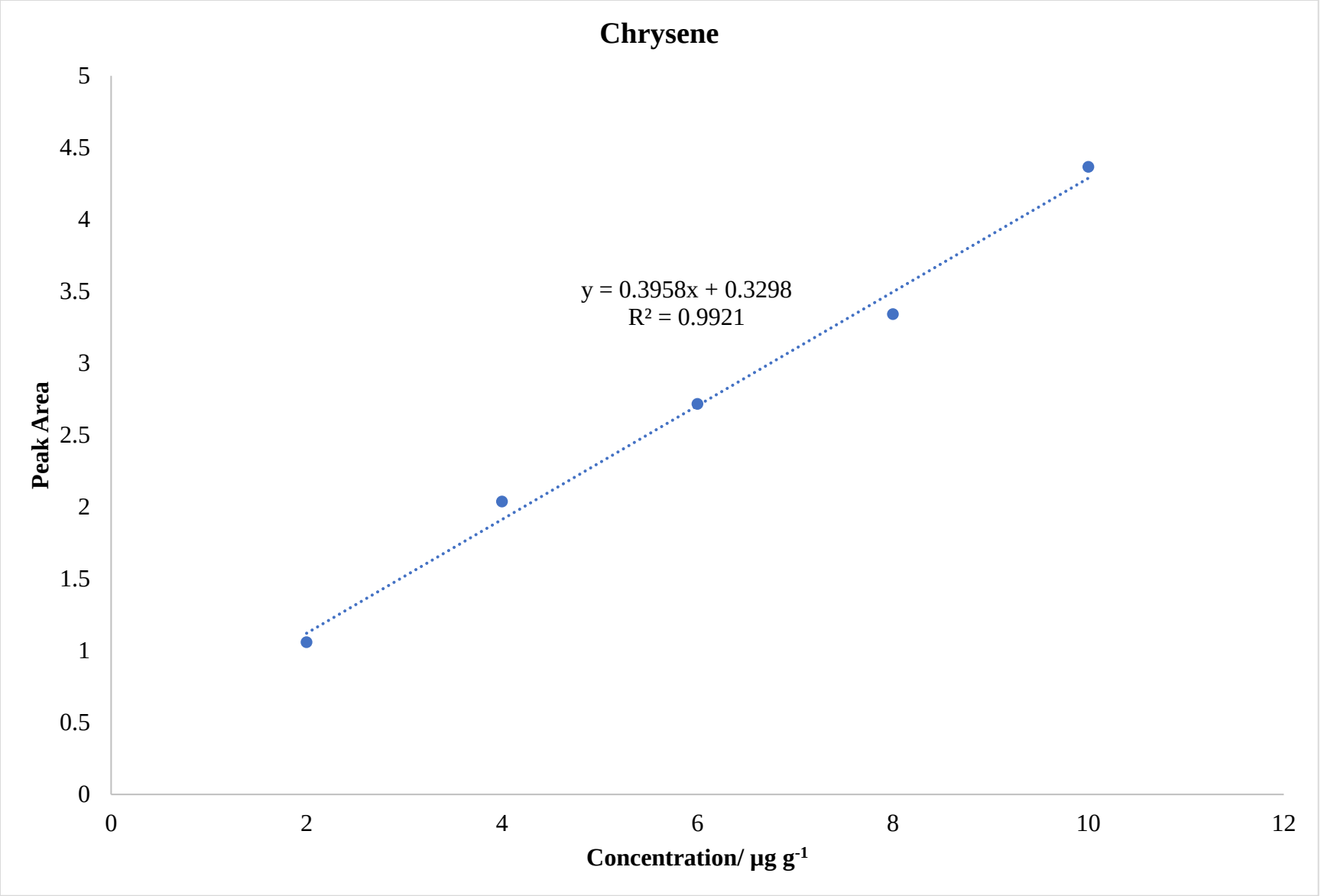
Appendix C.7: Calibration graph for fluoranthene



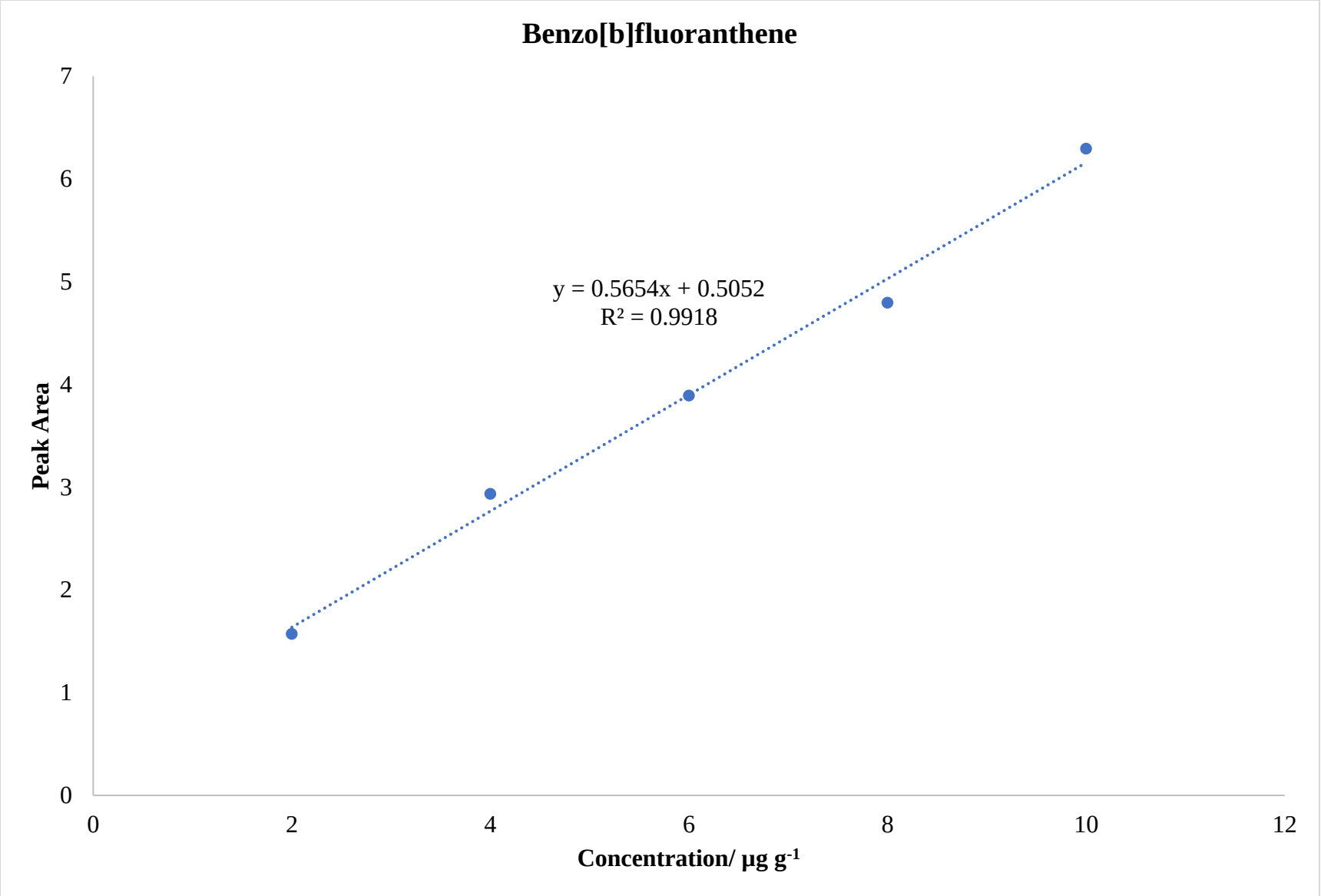
Appendix C.8: Calibration graph for pyrene



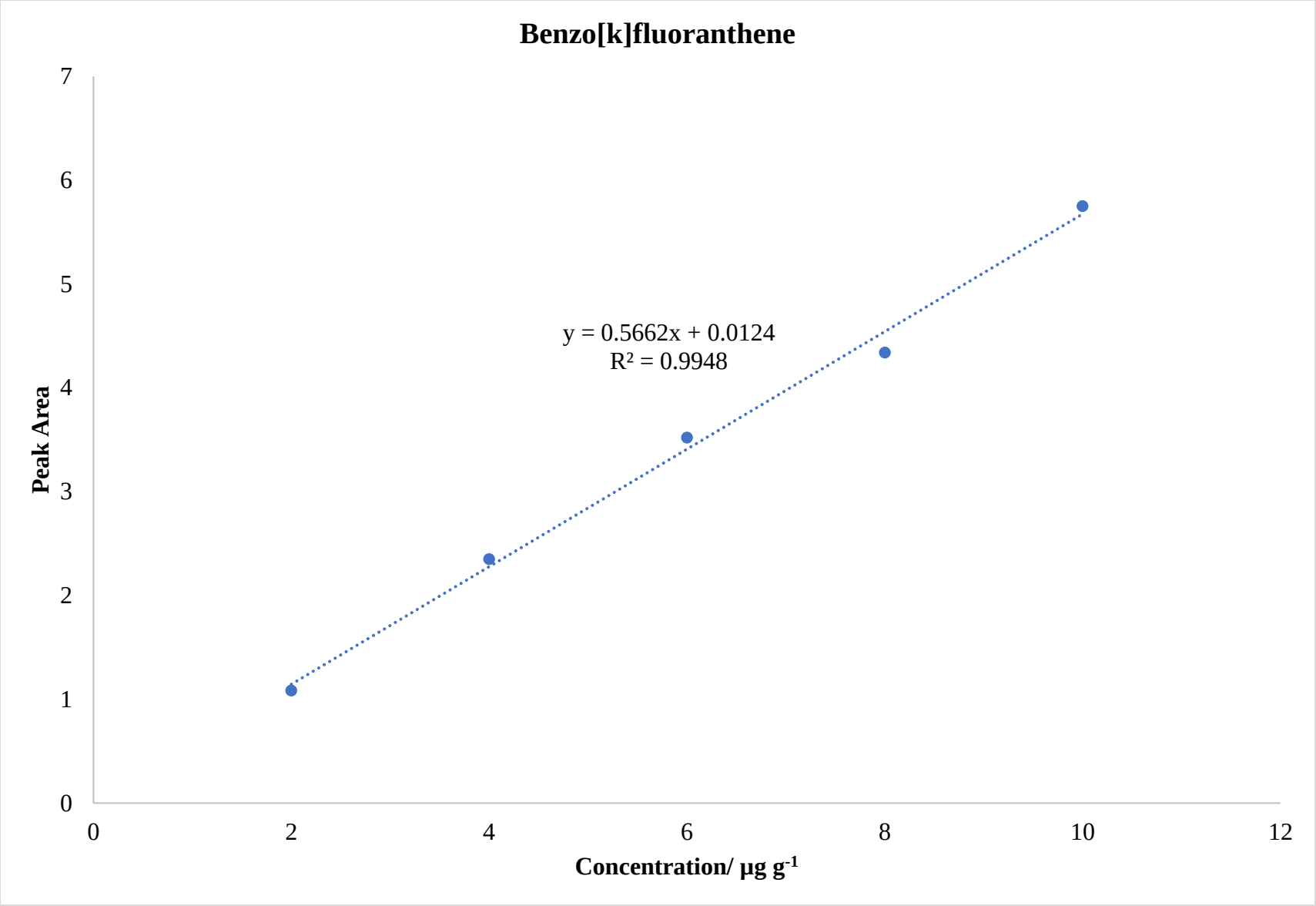
Appendix C.9: Calibration graph for benzo[a]anthracene



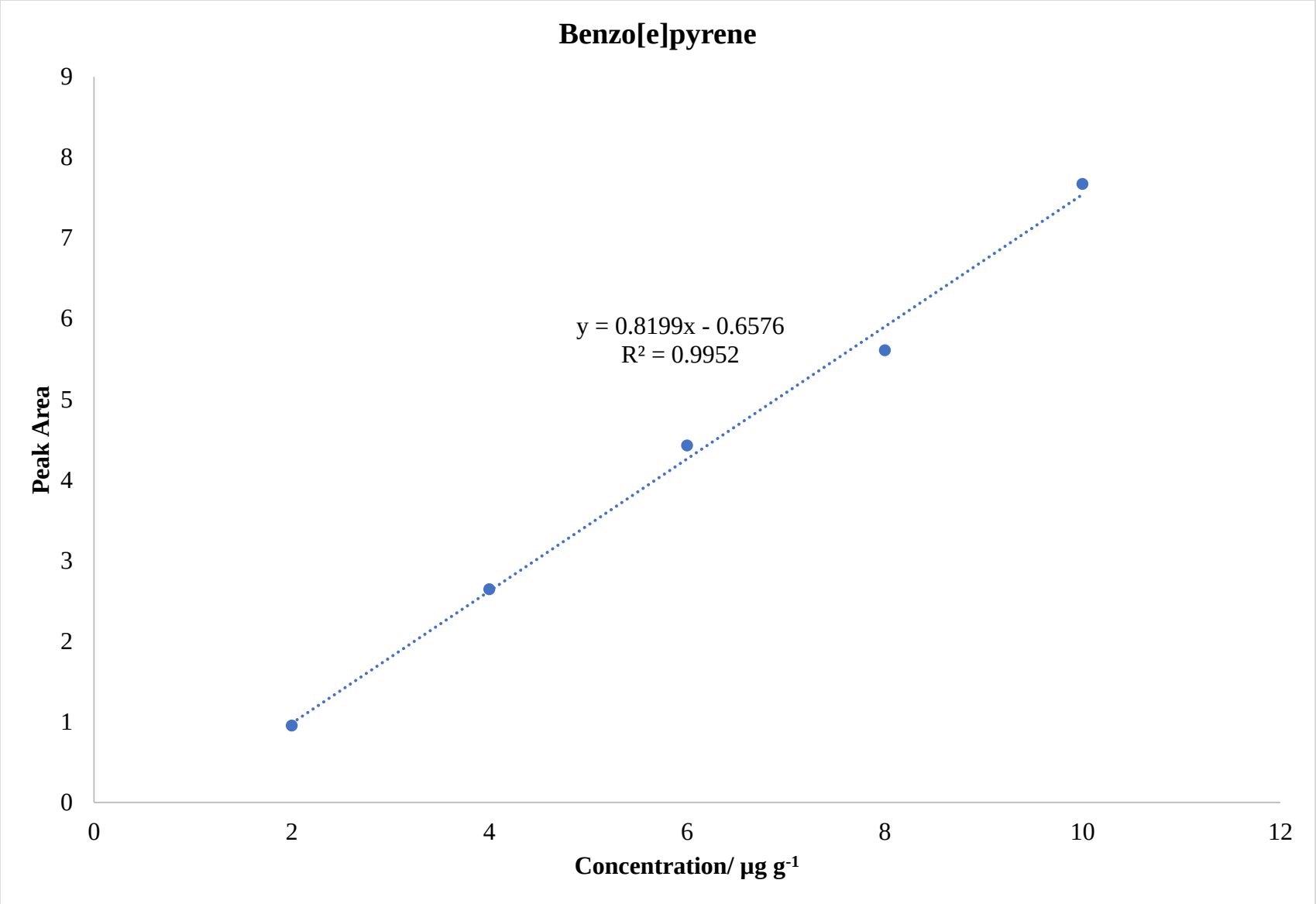
Appendix C.10: Calibration graph for chrysene



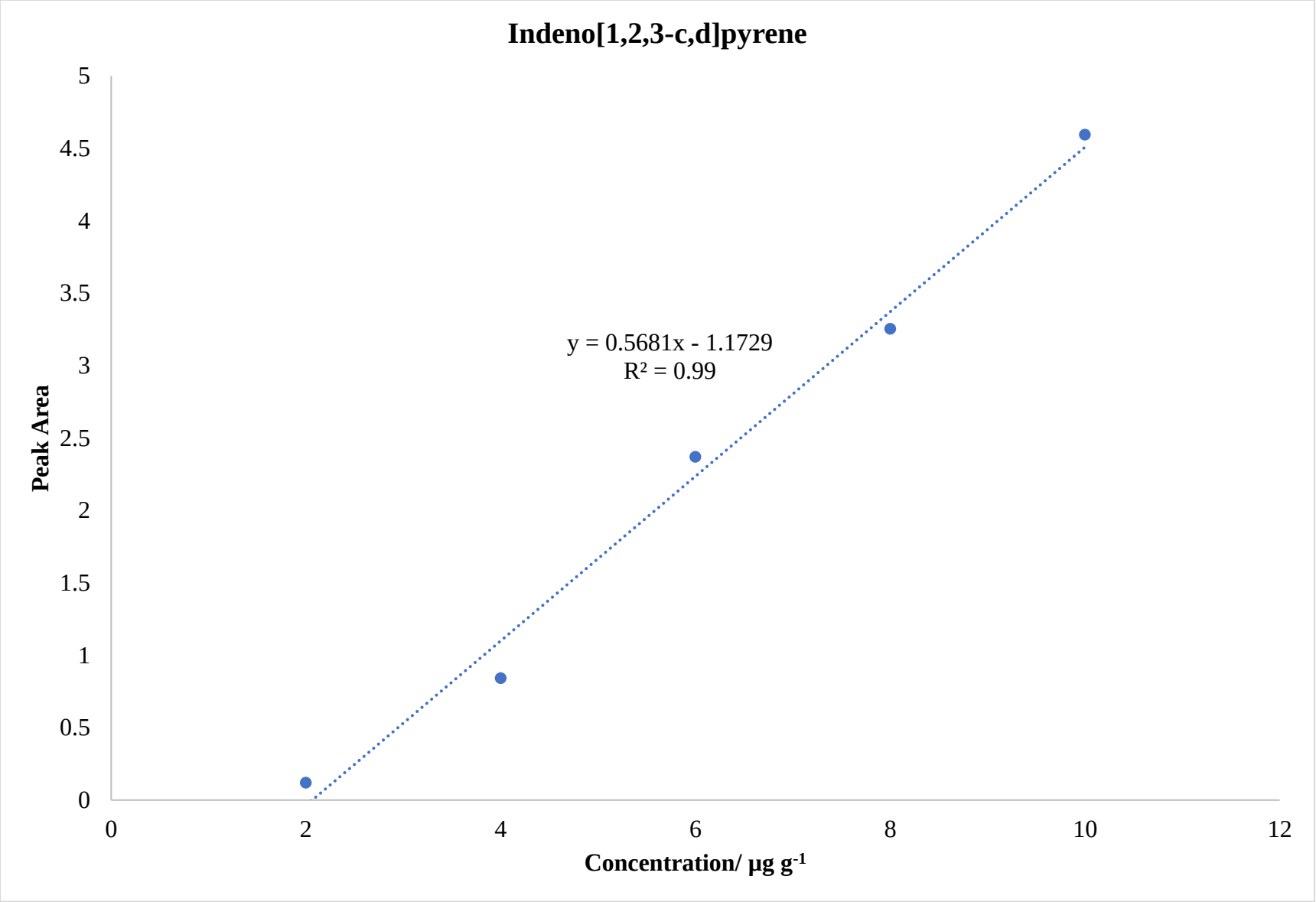
Appendix C.11: Calibration graph for benzo[b]fluoranthene



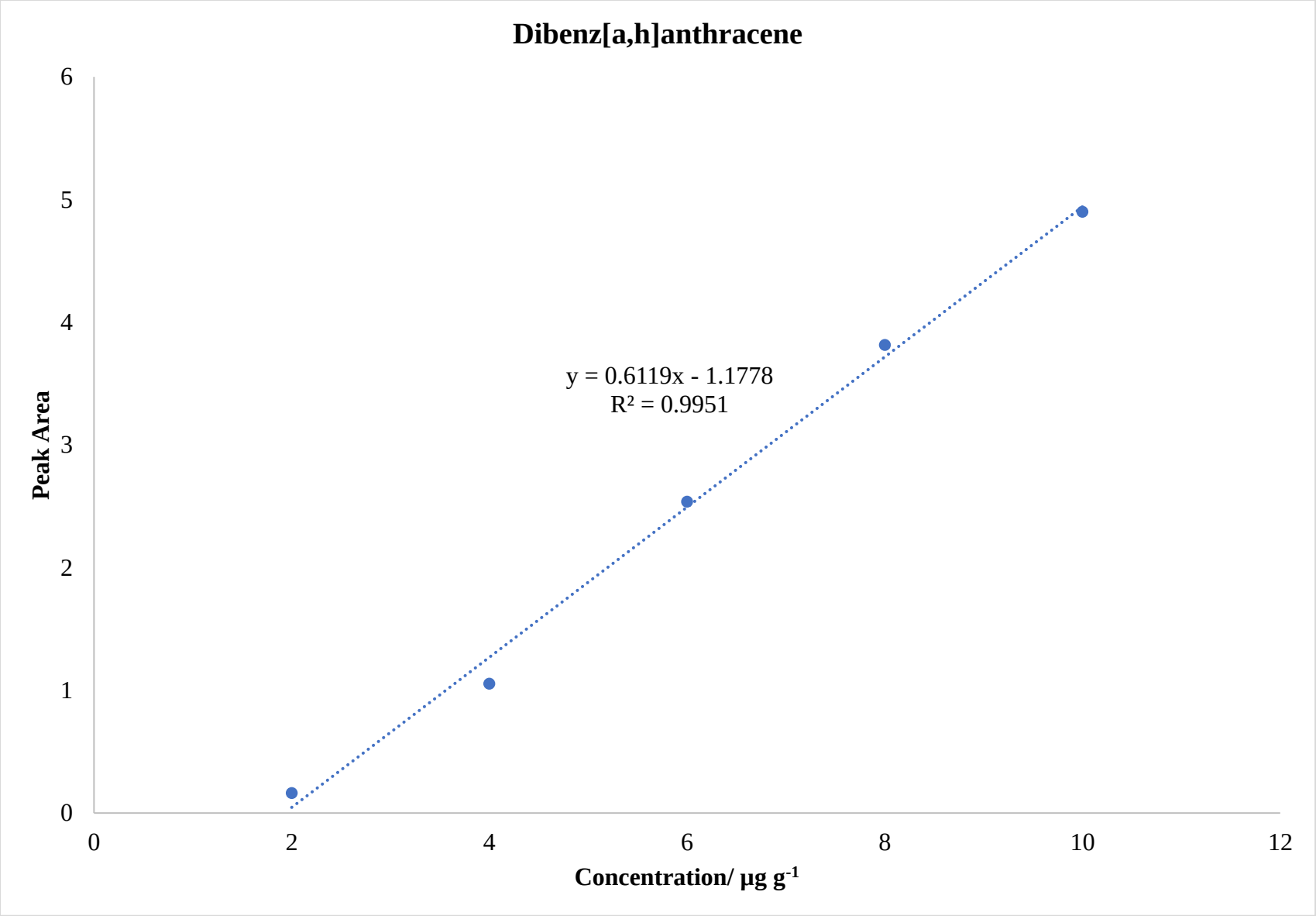
Appendix C.12: Calibration graph for benzo[k]fluoranthene



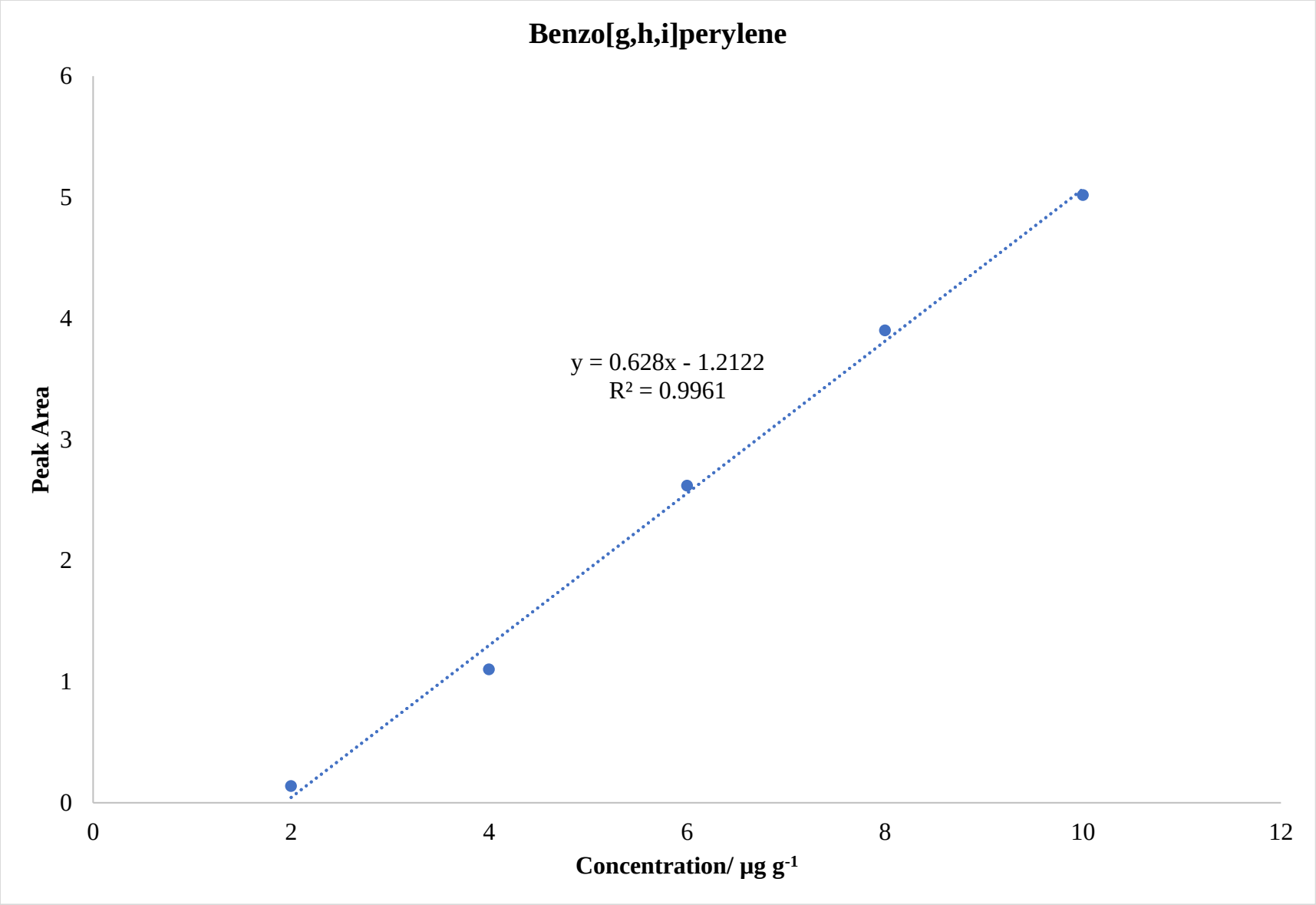
Appendix C.13: Calibration graph for benzo[e]pyrene



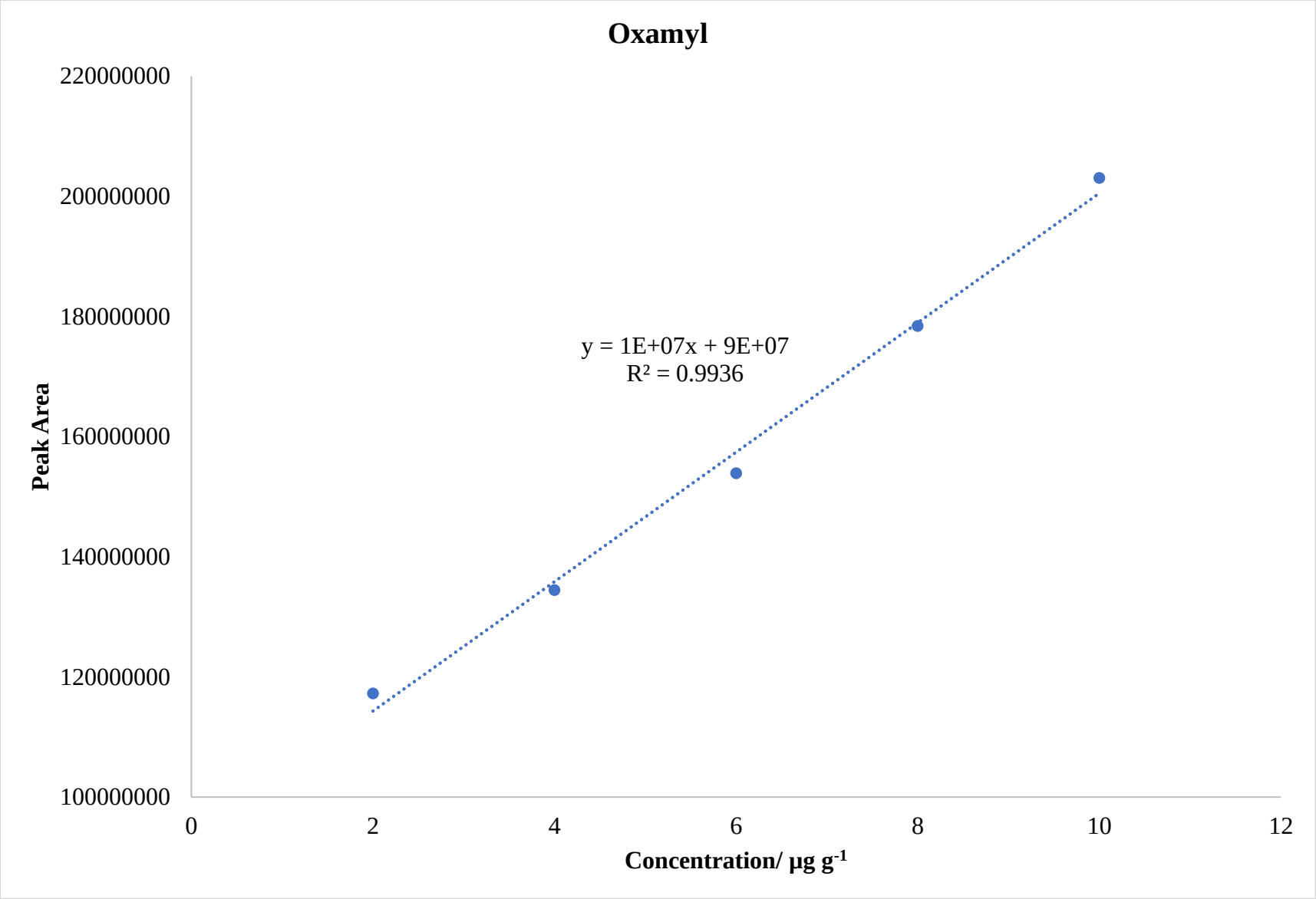
Appendix C.14: Calibration graph for indeno[1,2,3-c,d]pyrene



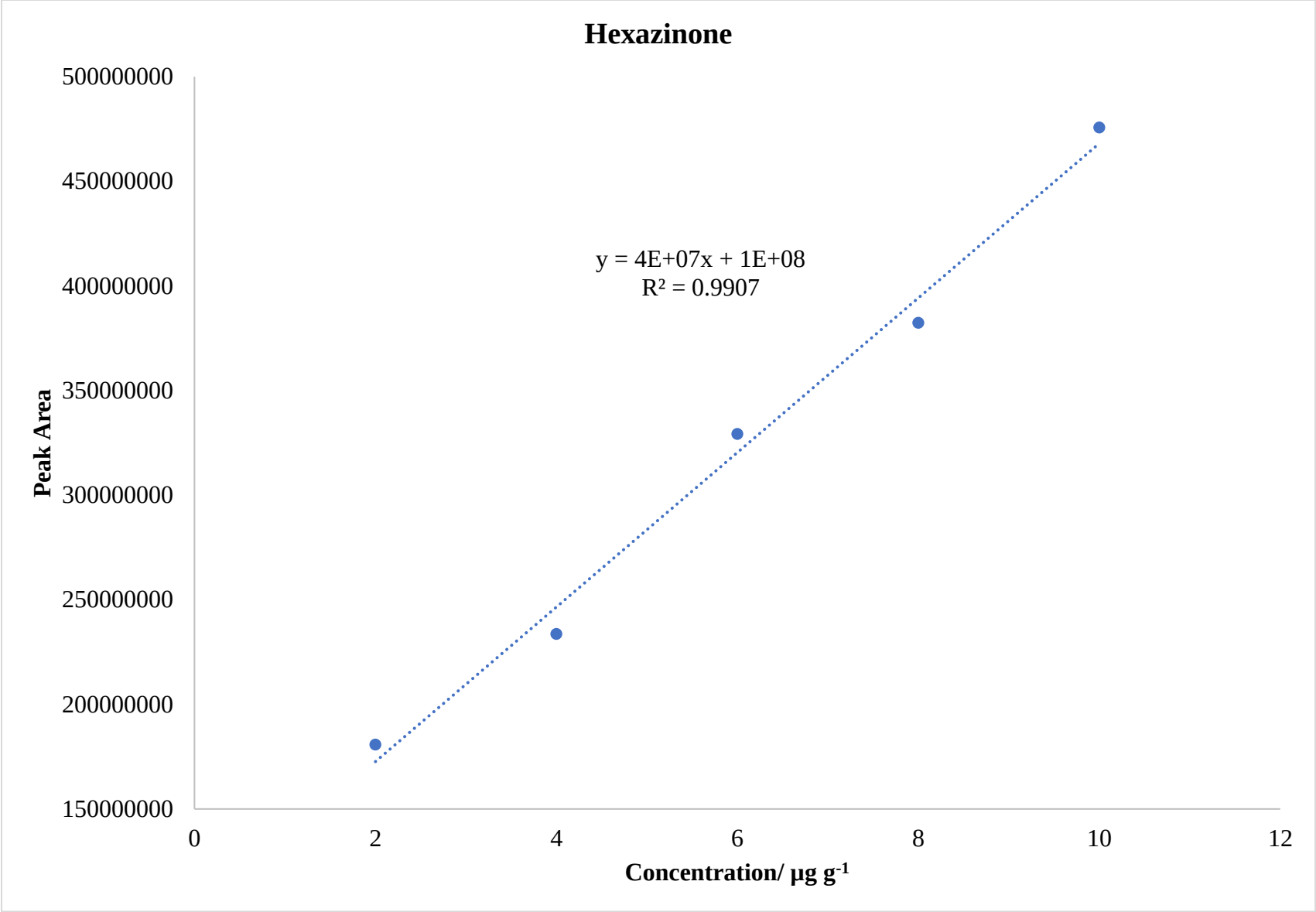
Appendix C.15: Calibration graph for dibenz[a,h]anthracene



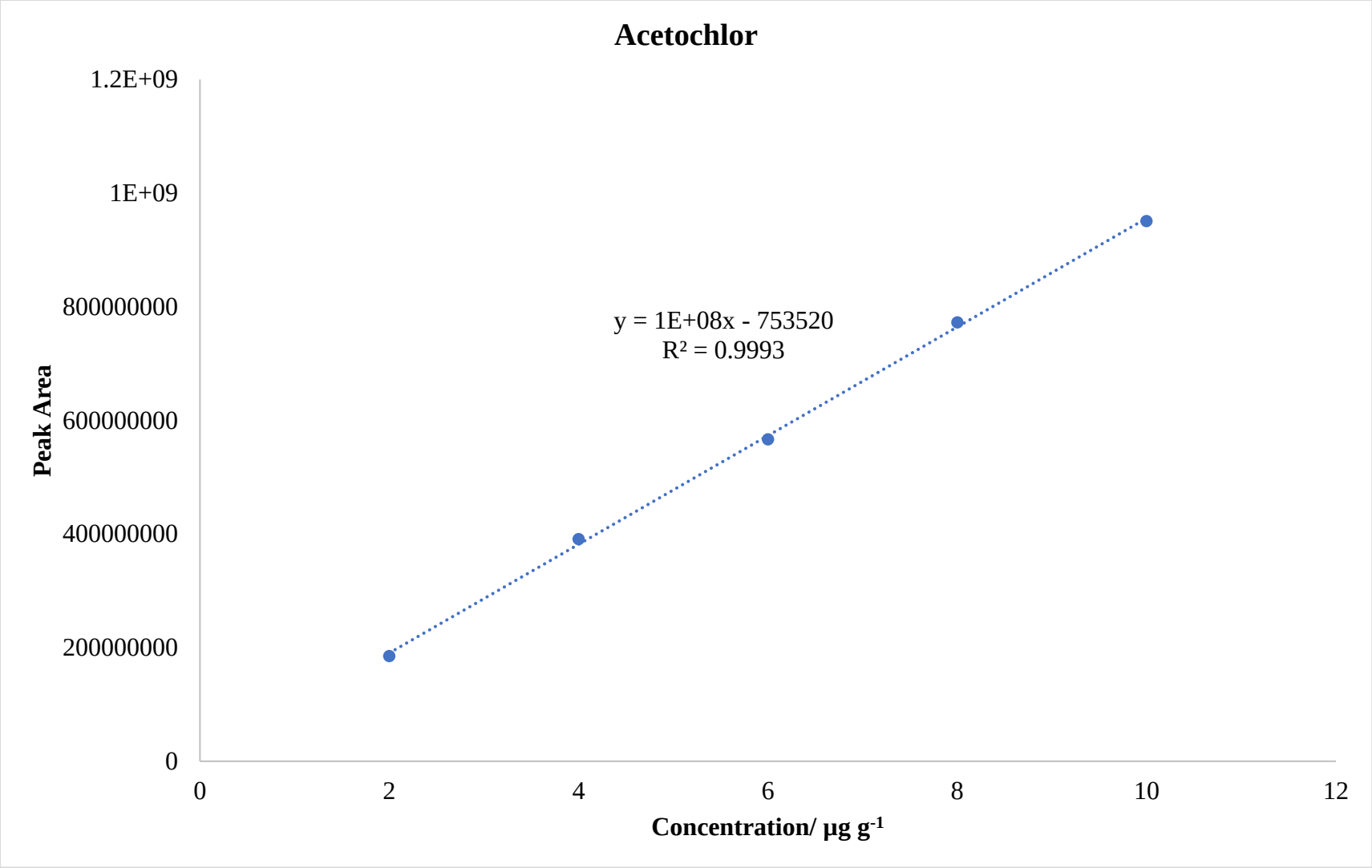
Appendix C.16: Calibration graph for benzo[g,h,i]perylene



Appendix C.17: Calibration graph for oxamyl

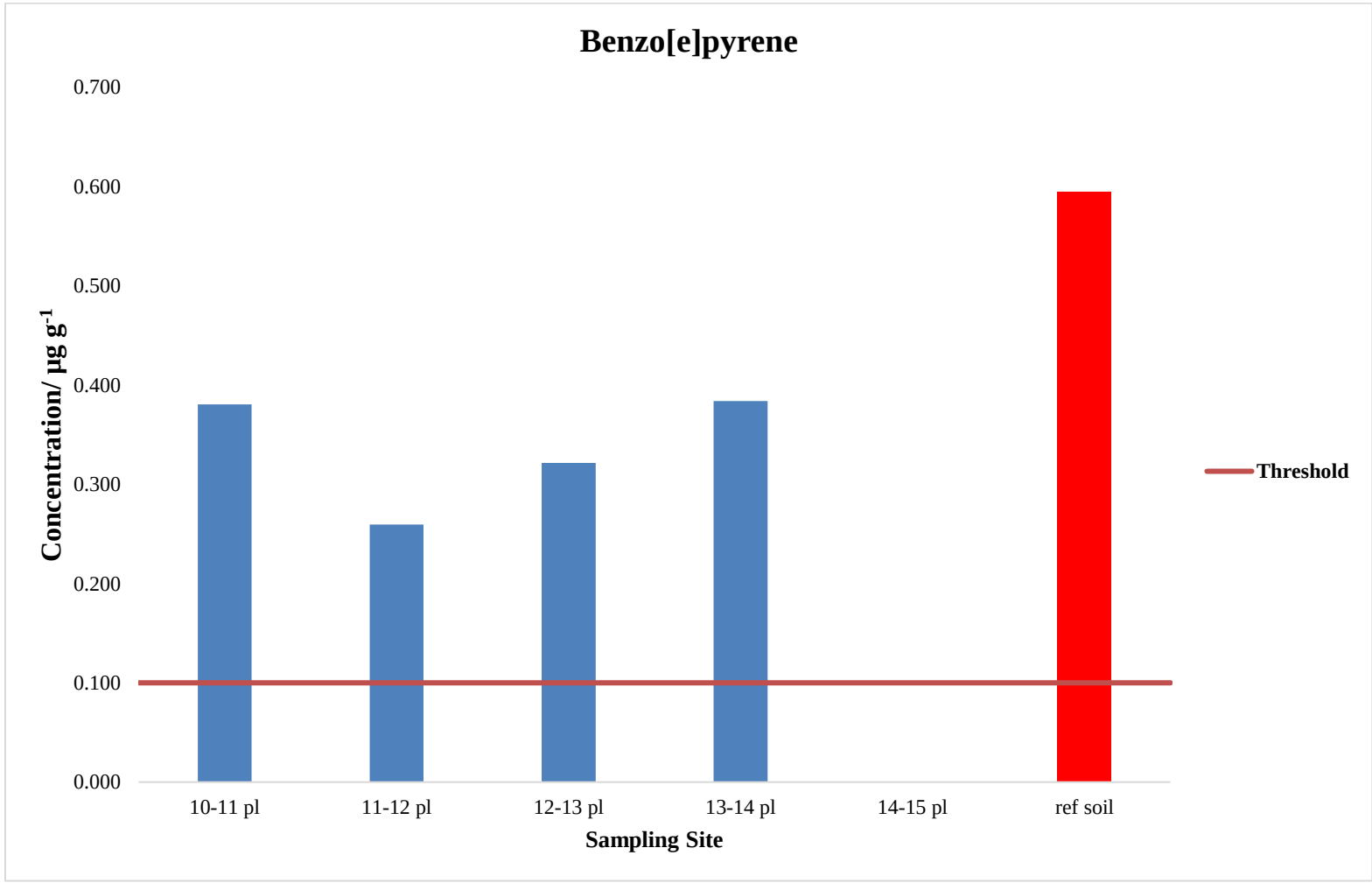


Appendix C.16: Calibration graph for hexazinone

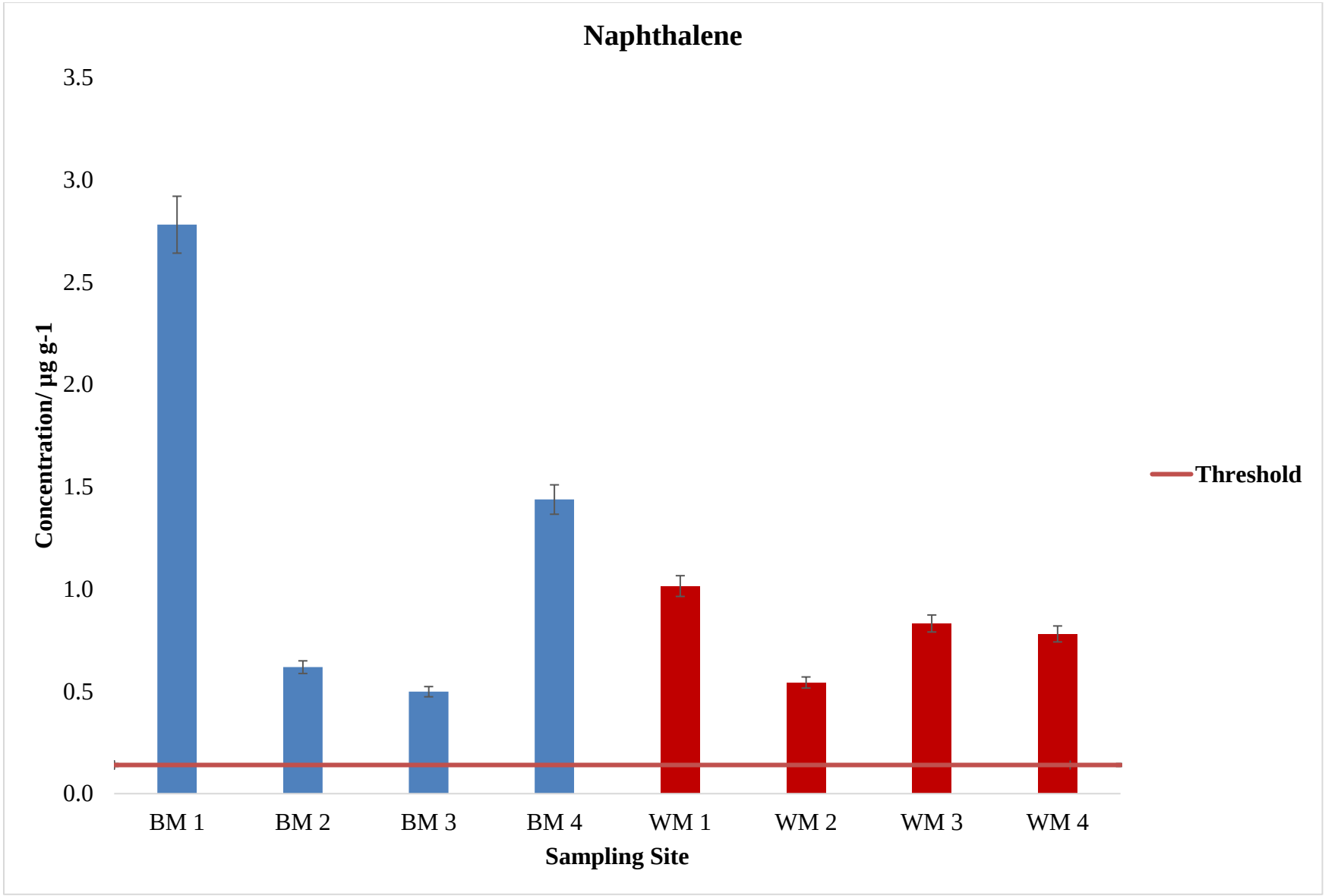


Appendix C.16: Calibration graph for acetochlor

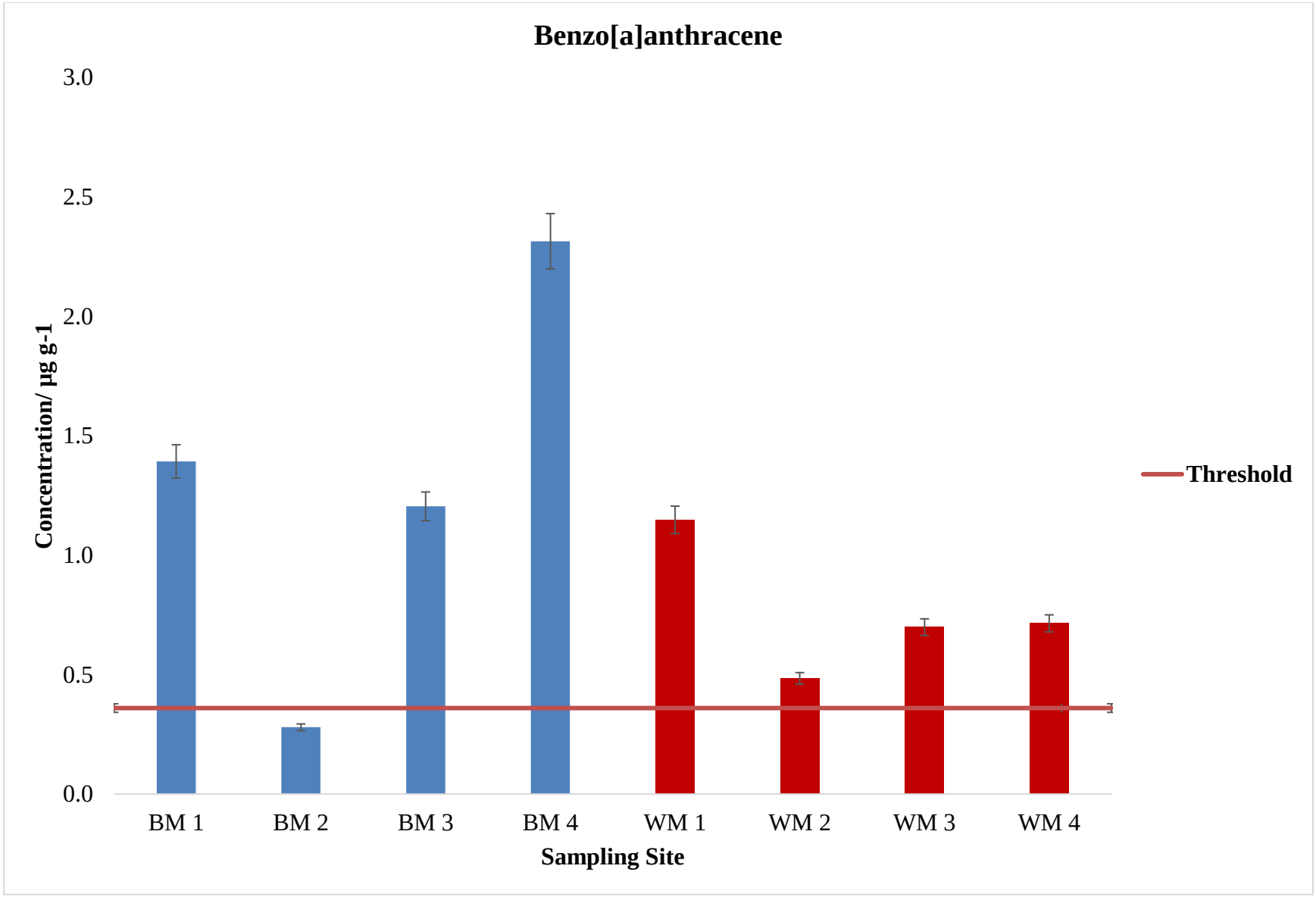
Appendix D: Guideline values for selected PAHs



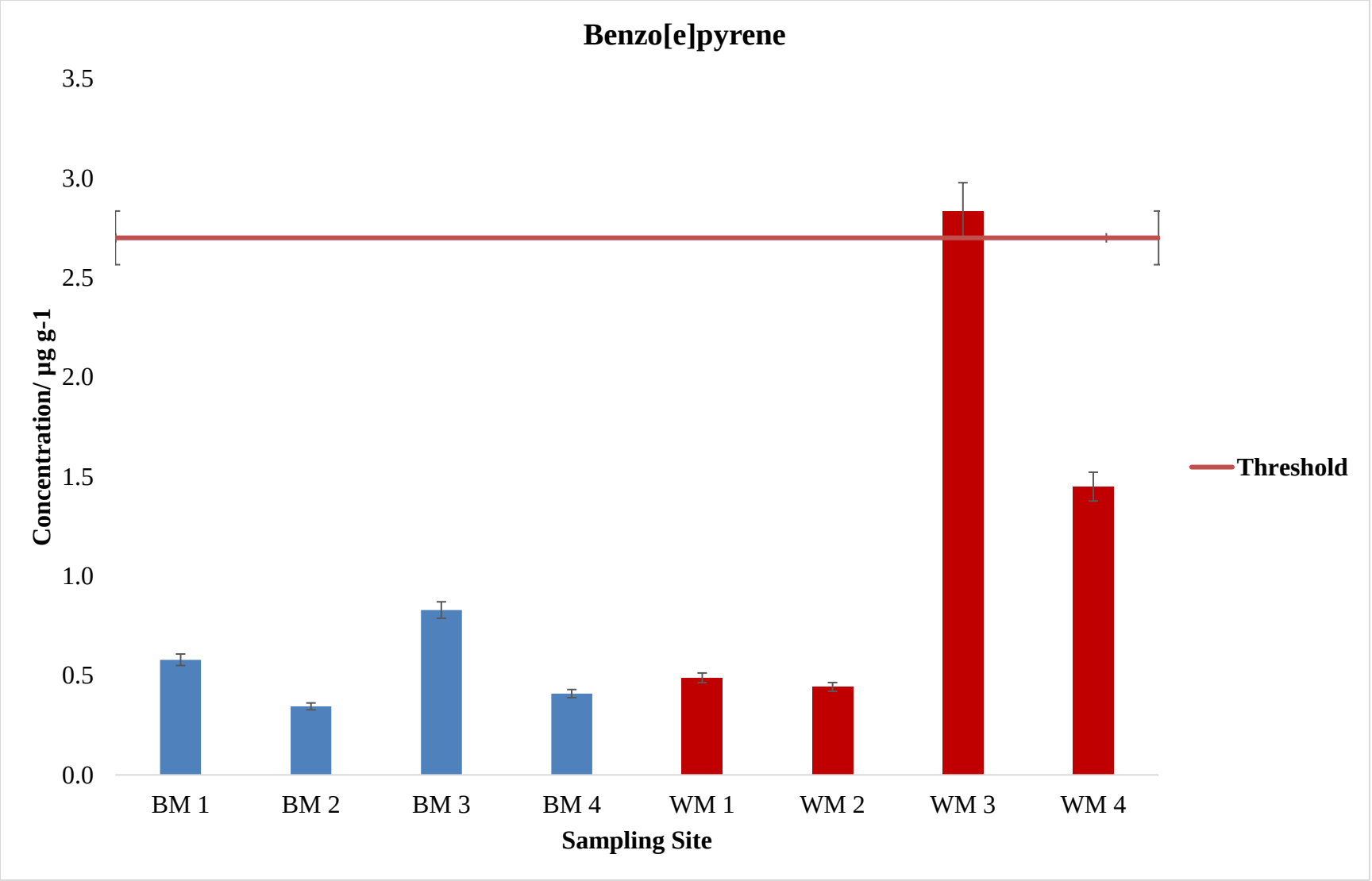
Appendix D.1: Total Benzo[e]pyrene concentration in soil with the guideline value shown



Appendix D.2: Total naphthalene concentration in sediment with the guideline value shown

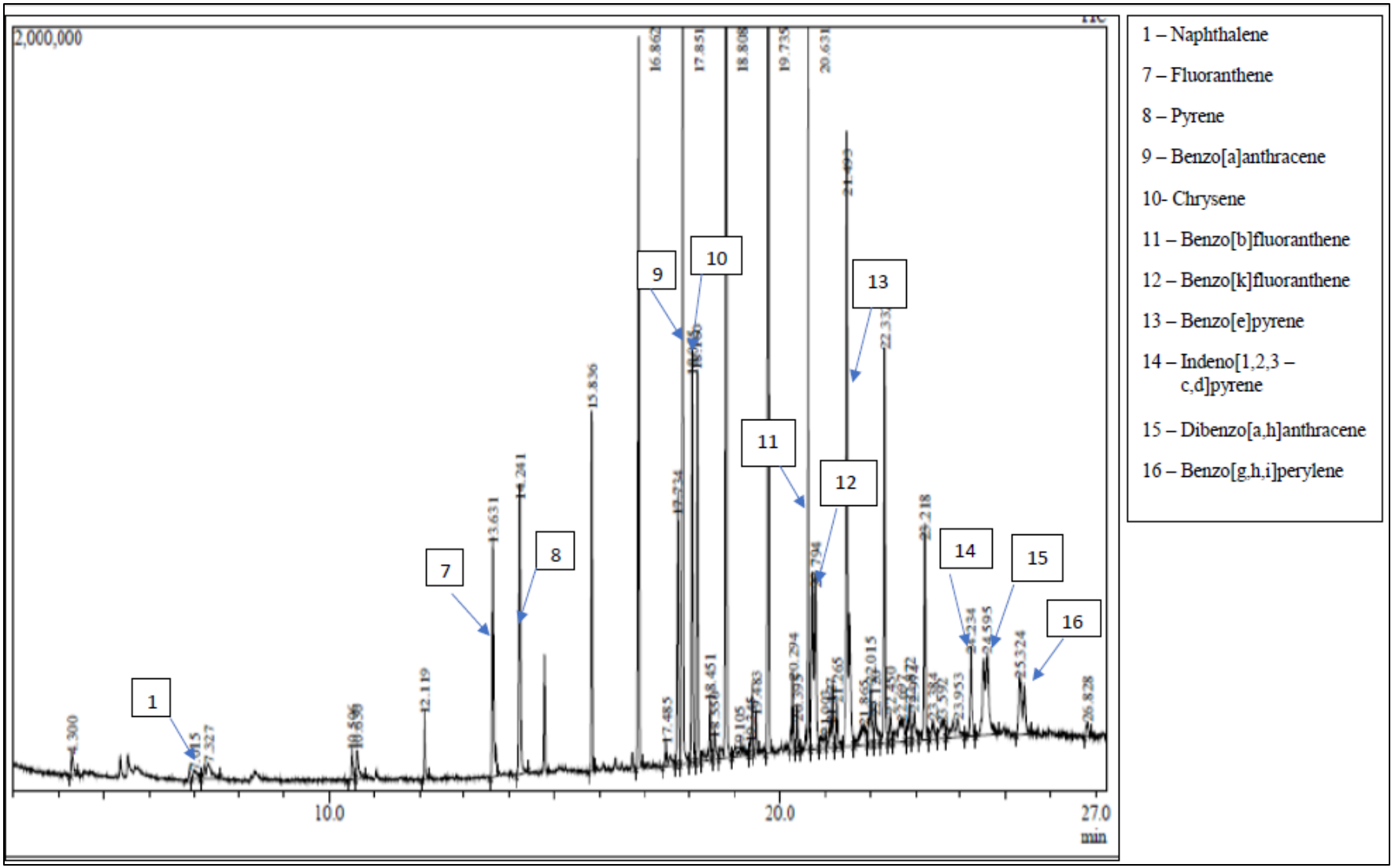


Appendix D.3: Total benzo[a]anthracene concentration in sediment with the guideline value shown

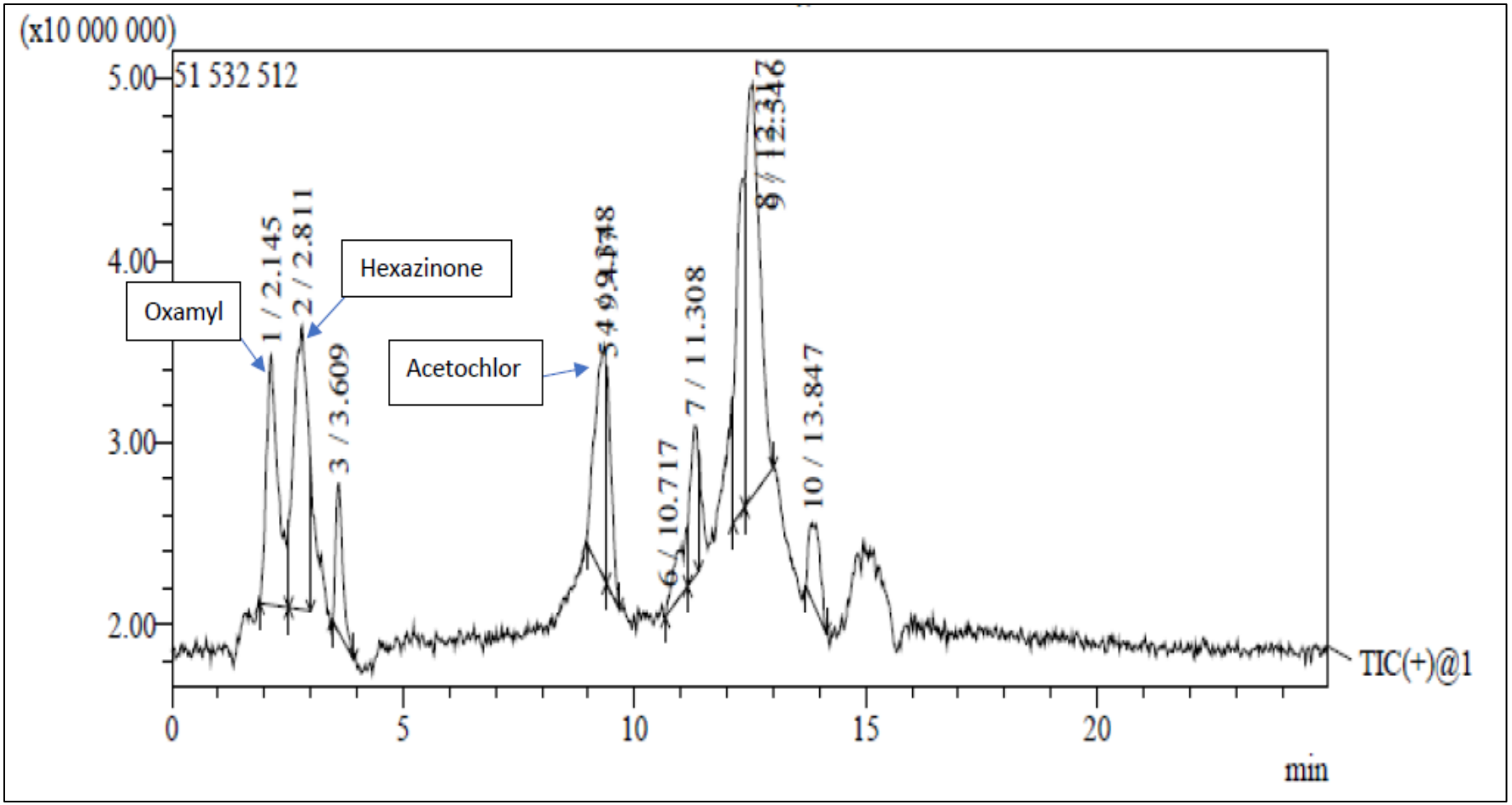


Appendix D.4: Total benzo[e]pyrene concentration in sediment with the guideline value shown

Appendix E: PAH and Pesticide chromatogram



Appendix E.1: Chromatogram showing the PAHs detected in the 2010-2011 soil sample after the clean-up.



Appendix E.2: Chromatogram showing the pesticides detected in the 2010-2011 soil sample after the clean-up.