Detection and Quantification of Emerging Organic Pollutants in the Umgeni and Msunduzi Rivers

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

Globally, there is a strong interest and extensive research activities on emerging organic contaminants and there is an increasing need to monitor these compounds in order to understand the risks posed to human health and the environment at large. Emerging organic contaminants consist of a wide range of organic chemicals that are typically produced as a result of various anthropogenic activities. For this study, case studies were conducted on two of Durban's key rivers; the Umgeni River and its main tributary, the Msunduzi River. Like many other rivers in South Africa, these 2 rivers are also under threat from pollution. These very same rivers also serve as one of the main sources for water supply for the surrounding population. Thus, this study is an attempt to study and monitor to some extent the levels, as well as the seasonal and spatial variation of selected organic pollutants in these rivers as they continue along their courses.

AIMS

The following were the aims of the project:

1. Understand and determine the state of the art concerning selected emerging organic pollutants in Pietermaritzburg/Durban and KwaZulu-Natal's water systems
2. To determine levels of emerging organic pollutants in selected water bodies and changes in their concentrations from source to domestic outflow
3. Development and validation of protocols for the quantification of emerging organic pollutants in natural and wastewater samples

METHODOLOGY

This study involved developing and optimising methods for the determination of the levels of selected polychlorinated biphenyl (PCB) congeners, organochlorine pesticides (OCPs), and pharmaceuticals and personal care products (PPCPs) in the samples collected along the Umgeni and Msunduzi Rivers. Using grab sampling, water and sediment samples were collected from several points along the Umgeni and Msunduzi Rivers. Samples were collected in amber glass bottles, and depending on the analyte of interest, some samples were preserved by adding sulfuric acid at the sample site. All samples were stored on ice and transported to the lab, and then stored in a 4°C fridge prior to extraction. Extractions were done using solid phase extraction or liquid-liquid extraction with the water samples, and liquid extraction using an ultrasound bath or soxhlet extraction with the sediment samples. Spiked samples were used to troubleshoot method development and ascertain the suitability of the method when used on environmental samples.

SUMMARY OF FINDINGS

The presence of selected OCPs such as HCB, HCH (lindane), aldrin, heptachlor, dieldrin, endrin, mirex and DDT with its metabolites *o,p*-DDE, *p,p*-DDE, *o,p*-DDD, *p,p*-DDD, *o,p*-DDT and *p,p*-DDT and 8 PCB congeners, PCB 28, PCB 52, PCB 77, PCB 101, PCB 105, PCB 138, PCB 153 and PCB 180 in the Umgeni and Msunduzi Rivers were investigated. Concentrations for all selected OCPs and PCBs were determined, with excellent precision and accuracy, in all the samples collected. Generally, the Msunduzi River had higher levels of total OCPs and PCBs than the Umgeni River. In general, the total average PCB level in both the Umgeni and Msunduzi River sediments were very much lower than the interim fresh water sediment quality guidelines (ISQG) and probable effect level (PEL) permitted by the Canadian quality sediment guidelines. However, the levels were higher than the lowest effect level and far less than the severe effect level (SEL) as stipulated in the guidelines. OCPs on the other hand were generally higher than the ISQG values permitted in both the Umgeni and Msunduzi Rivers and is a cause for concern. With regards to pharmaceutical compounds, the presence of 18 pharmaceuticals representing five therapeutic classes, i.e. antibiotics, antipyretics, antiepileptic, stimulants and antipsychotic drugs, along the various sampling points on the Msunduzi and Umgeni Rivers were studied. Some of the pharmaceuticals were detected in both

rivers. Wastewater samples were also analysed and found to contain the selected pharmaceutical compounds.

One other major outcome of this study was the development of a new method for analysing pharmaceuticals found in the environment using GC-MS with derivatisation, which is not the conventional method for analysing pharmaceuticals. The method was optimised and validated and the results show the presence of the selected pharmaceuticals in the range of 0.0243-8.14 ng/mL with the Umgeni Estuary being the most contaminated site. A method was also developed for the analysis of polycyclic musks, a group of personal care products, and was used to quantify selected musks using simulated matrices as well as in the environment. The preliminary results show significant levels of the selected musks in both the Umgeni and Msunduzi Rivers.

CONCLUSIONS

Overall, this study provides a good understanding of the levels of selected pollutants such as PCBs, pesticides, PPCPs and polycyclic musks in two major rivers in KwaZulu-Natal along their natural courses. This study presents data on the concentration levels from the source to the mouth and the joining point of the two rivers in different matrices such as surface water, pore water, sediment, bank soil and wastewater. Appropriate methods have been developed and have shown their applicability for the analysis of the selected pollutants in the environment of KwaZulu-Natal. This work has resulted in protocols that can be used to determine selected PCBs, OCPs, pharmaceuticals and polycyclic musks in environmental samples. The PCB levels were much higher within the Msunduzi River than the Umgeni River, which may be due to various activities along the rivers. PCBs were within acceptable limits though has the potential to become harmful due to prolonged exposure. OCPs were higher than the acceptable limits in many sites along both rivers. Selected pharmaceutical compounds were detected and quantified in both river systems using LC-MS as well as a newly developed GC-MS method. Pharmaceuticals in some wastewater treatment plants in the KwaZulu-Natal region were also determined and were found to be mostly higher than in surface waters as expected and could possibly be a source of these contaminants in the river.

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Dr T Manikum	Umgeni Water
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ACRONYMS & ABBREVIATIONS

ACE	acetaminophen
AHTN	tonalide
ASP	aspirin
BDL	below detection limit
BSTFA	N,O-bis-trimethylsilyl triflouroacetamide
CAF	caffeine
CBZ	carbamazepine
CLO	clozapine
DCM	dichloromethane
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DOC	dissolved organic carbon
EOCs	emerging organic contaminants
ERY	erythromycin
ESI	electrospray ionisation
GC-MS	gas chromatography-mass spectrometry
GPS	global positioning system
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
HHCB	galaxolide
HIV	human immune deficiency virus
HLB	hydrophobic-lipophilic balance
HMB	hexamethylbenzene
IBU	ibuprofen
IS	internal standard
KZN	KwaZulu-Natal
LC-MS	liquid chromatography-mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MALDI-TOF	matrix assisted laser desorption ionisation-time of flight
MDL	method detection limit
MET	metronidazole
MK	musk ketone
MQL	method quantification limit
MRM	multiple reaction monitoring
ND	not detected
NIST	National Institute of Standards and Technology
OCPs	organochlorine pesticides
PCBs	polychlorinated biphenyls
PNT	phenanthrene
POP	persistent organic pollutant
PPCPs	pharmaceuticals and personal care products
RSD	relative standard deviation
SIM	selective ion monitoring
SMs	synthetic musks
SMX	sulfmethoxazole
SMZ	sulfmethazine
SPE	solid phase extraction
SS	surrogate standard
TDS	total dissolved solids
TMCS	trimethylchlorosilane
TMP	trimethoprim
TOC	total organic carbon
TSS	total suspended solids
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

PUBLICATIONS AND CONFERENCE PROCEEDINGS

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- Jeremiah Oluleye, Grace Birungi and Bice Martincigh (2016) Detection of Synthetic Polycyclic Musks in Sediments of the Umgeni and Msunduzi Rivers in KwaZulu-Natal, South Africa. Pesticide 2016 conference in Santiago de compostela Spain, 4-7 October 2016 (Poster)
- Bhekumuzi Gumbi, Brenda Moodley, Grace Birungi and Patrick Ndungu (2016) Development and validation of gas chromatography mass spectrometry analytical method for determination of non-steroidal anti-inflammatory drugs in South Africa South African Chemical Institute (SACI) 2015 International Conference, Durban, South Africa, 29 Nov-4 Dec 2015 (Poster)

CHAPTER 1: **BACKGROUND**

1.1 INTRODUCTION

Emerging organic contaminants (EOCs) include a wide range of chemicals that maybe in-use in various anthropogenic activities, or are compounds that are newly introduced into a commercial process or other industrial activity (Lapworth et al. 2012, Richardson and Ternes 2014, Metcalfe et al. 2003). This general description can include compounds used in cosmetics or perfumes, which may not breakdown or decompose naturally during their transportation to the wastewater treatment plant, *via* the urban sewer network. They undergo the treatment process unaffected or partially degraded, and eventually are released into the local river. Other examples could include partially metabolised pharmaceutical products, degradation products of various man-made organic compounds, new pesticides introduced into the local market, cleaning products, or new reagents or by-products from an industrial process. The continual improvement in analytical techniques and the introduction of new technologies for the detection or identification of organic compounds, some of which are new chemicals and others which could have been present previously, has resulted in the possible detection of these compounds in aquatic environments of interest. In such a case, the concept surrounding what is an EOC needs to include both aspects of anthropogenic sources/activities, and species produced *via* unidentified environmental mechanisms or are already present in a water system under investigation (Lapworth et al. 2012, Richardson and Ternes 2014, Meffe and de Bustamante 2014). Thus, EOCs can include metabolites, pharmaceuticals, pesticides, industrial chemicals, illicit drugs, hormones, by-products from known or unknown degradation pathways, or other unclassified organic compounds.

A list of EOCs can be very extensive with some estimates at over 50, 000 compounds (Hug et al. 2014). However, for this study, a list of compounds has been selected based on those that have been identified and/or prioritised on lists from regulatory frameworks within the European Union and the United States Environmental Protection Agency (Richardson and Ternes 2014, Meffe and de Bustamante 2014). It is clear from the open literature that globally there are various extensive studies in the area of EOCs which can be viewed as a means of continuous monitoring and detection of EOCs in specific regions (Lapworth et al. 2012, Meffe and de Bustamante 2014). In addition, there is a detailed and updated review published every two years on analytical trends in the subject area (Richardson and Ternes 2014). Some of the key drivers behind the various global studies are monitoring, screening for new potential contaminants, identification of transformed products, providing possible mechanisms for the transformation of EOCs, investigating the fate of EOCs, and concern over ecotoxicological effects (Lapworth et al. 2012, Richardson and Ternes 2014, Meffe and de Bustamante 2014, Hug et al. 2014).

KwaZulu-Natal is the second most densely populated province in South Africa. As per the definition outlined above, some of the key sources of EOCs are anthropogenic activities and hence a higher populated province is expected to have higher amounts of pollution. In addition, wastewater treatment plants are increasingly found to be unable to transform various EOCs into benign by-products (Meffe and de Bustamante 2014, Hug et al. 2014). The main wastewater treatment plant in Pietermaritzburg, Darvill Wastewater Works, eventually discharges into the Msunduzi River, which then joins the Umgeni River between Nagle and Inanda Dams. Another wastewater treatment plant that eventually discharges into the Umgeni River is the Northern Wastewater Works; however, the length of the river from the discharge point to the mouth of the Umgeni River (in Blue Lagoon) is comparatively short. Together, the Msunduzi and the Umgeni Rivers are key sources of water for domestic, agricultural, and industrial use within KwaZulu-Natal. Both rivers pass through rural villages, informal settlements, industrial parks, farmland, recreational areas,

and semi-fully urbanized areas. Thus, the large population density, diverse industrial and commercial activities, and various informal undertakings along these water systems can be sources for EOCs.

Research into EOCs have shown them to be responsible for a number of illnesses such as neurological, respiratory, skin disorders, male and female infertility problems and so on (Schecter et al. 2006) In addition, pharmaceuticals and personal care products, even though present in low concentrations in environmental matrices, eventually lead to accumulation in the environment, and bacteria exposed to pharmaceuticals and personal care products (PPCPs) can result in bacterial resistance build up which then impacts on long term drug resistance. Many organic pollutants are also known to have toxicological effects and long term exposure either directly (*via* drinking untreated water) or indirectly (through consumption of fish exposed to polluted water) can lead to eventual death. Hence there is a need to qualitatively and quantitatively determine the presence and concentrations of these organic pollutants in our surrounding environments. Moreover, a vast amount of research has been conducted in many European countries and in the USA and even in other African countries but there is currently a lack of information on organic pollutants within South Africa and specifically in KwaZulu-Natal. It is for these reasons that this research study was undertaken.

1.2 PROJECT AIMS

The following were the aims of the project:

1. Understand and determine the state of the art concerning emerging organic pollutants in Durban and KwaZulu-Natal's water systems
2. To determine levels of emerging organic pollutants in selected water bodies and changes in their concentrations from source to domestic outflow
3. Development and validation of protocols for the quantification of emerging organic pollutants in surface water and wastewater samples

1.3 SCOPE AND LIMITATIONS

EOCs can include upwards of 50, 000 compounds; however this is not possible to execute in this project given the timeframe. Overall the work undertaken investigated the presence of certain selected persistent organic pollutants (as per the Stockholm Convention), pharmaceutical products, endocrine disrupting compounds, and musks in the key river systems within KwaZulu-Natal, in particular the Msunduzi and Umgeni Rivers. The classes of EOPs investigated were polychlorinated biphenyls (PCBs), pesticides, PPCPs which included antipyretics, antibiotics, antiepileptic and antipsychotic drugs and also musk ketone type compounds.

CHAPTER 2: **LITERATURE REVIEW**

2.1 INTRODUCTION

The importance of water cannot be overstated, whether one is considering the significance of water within modern civilization from a health, commercial and/or industrial perspective, or environmentally. Water for potable, agricultural, industrial, commercial or personal use must meet acceptable standards, and both water quality and the availability of water are closely linked. Some estimates have shown that the total volume of wastewater produced annually around the world exceeds the amount of fresh water within all surface rivers by a factor of six. The availability of water is limited by several factors which can include increasing population, poor water management, intentional or unintentional introduction of pollutants and/or contaminants, and unequal distribution of fresh water sources (Agunbiade and Moodley 2014, Calderon-Preciado et al. 2011, Pal et al. 2014, Wintgens et al. 2008). Water scarce countries, such as South Africa, are particularly burdened with such water issues, especially when one considers national drives to address poverty alleviation, accessibility to clean water, and other socio-economic drivers. Thus, there is a strong need for alternative water generation and management methods such as reclaiming and recycling of waste water, desalination, and underground water storage.

The use of recycled water can be challenging as it may be contaminated with pathogens and trace amounts of chemicals especially those introduced through human activities by direct point pollution or indirect ways involving atmospheric deposition and surface runoff. In addition, raw water sources, for potable water treatment plants, can be located within a riverine system used as a discharge point for wastewater treatment plants. An example of this in Kwa-Zulu Natal is the Umgeni and Inanda dam waterways (Manickum et al. 2014). The presence of several classes of manmade organic compounds in various water systems have been reviewed in recent years; specifically, groundwater (Jurado et al. 2012, Lapworth et al. 2012, Luo et al. 2014, Meffe and de Bustamante 2014, Pal et al. 2014, Pal et al. 2013, Postigo et al. 2015, Vodyanitskii et al. 2016), surface water (Haman et al. 2015, Hughes et al. 2013, Jones et al. 2015, Li 2014, Luo et al. 2014, Meffe and de Bustamante 2014, Padhye 2015, Padrón et al. 2014, Pal et al. 2014, Pal et al. 2013, Petrie et al. 2015, Postigo and Barceló 2015, Rykowska et al. 2015), and coastal regions (Jiang et al. 2014, Ksenia et al. 2016, Li et al. 2014).

The identity or classification of such compounds can be used as a marker of human activities in a particular environment. For example, the presence of compounds such as biocides, pharmaceuticals and herbicides in water systems maybe an indicator of commercial farming or sanitary/ablution practices in the area (Fernandez-Gomez et al. 2013, Robles-Molina et al. 2010, 2013), while organometallics, PCBs and PAHs may be an indicator of urban runoff from industrial parks (Cornelissen et al. 2008). Once these types of chemicals (manmade organic compounds) have entered the water environment, they may have deleterious effects on human, animal, or aquatic life. In addition, several classes of manmade organic compounds released into water bodies from households, industries and agriculture are well known toxic compounds and some of them do not easily decompose; rather they accumulate in the environment or the food chain (Gerbersdorf et al. 2015, Jurado et al. 2012, Lapworth et al. 2012, Li 2014, Luo et al. 2014, Mohapatra et al. 2016, Ncube et al. 2012, Padhye 2015, Pal et al. 2014, Petrie et al. 2015, Postigo and Barceló 2015, Tijani et al. 2016). Monitoring organic pollutants has been widely prioritized in many countries.

There is an emphasis on “traditional” organic pollutants especially persistent ones including industrial chemicals, pesticides, and disinfection by-products; however, emerging organic pollutants such as pharmaceuticals and

personal care products, hormones, illicit drugs, degradation by-products, and algal toxins or other biological based species have started to receive a significant amount of attention (Daughton et al. 1999, Gerbersdorf et al. 2015, Ncube et al. 2012, Richardson and Ternes 2005, Ternes 2007). A semi-quantitative analysis that highlights these differences is provided in Figure 2.1.

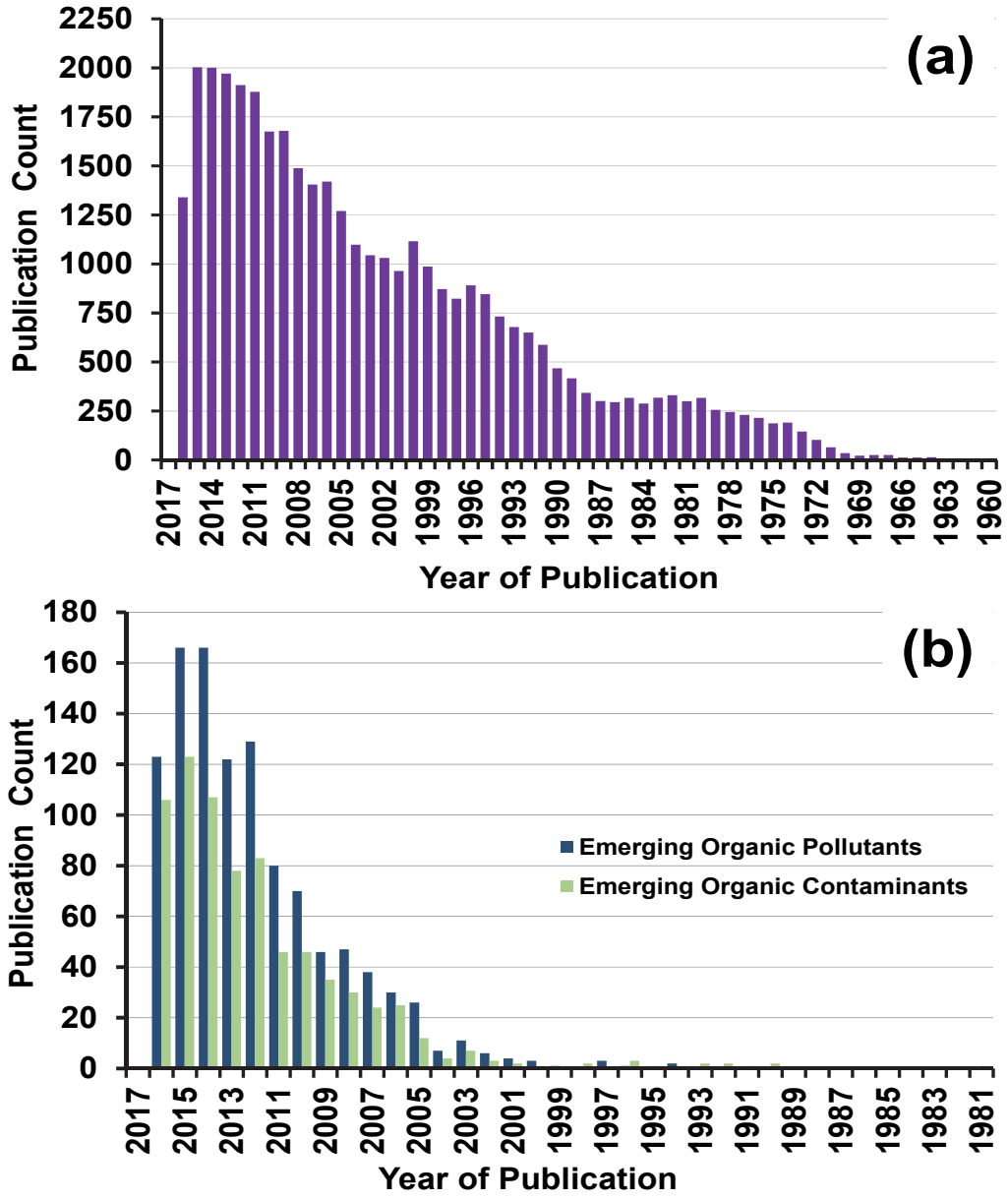


Figure 2.1 Comparison of the number of publications listed within the Scopus™ database using the search terms (a) 'Pesticide' and (b) 'Emerging organic Pollutants' vs 'Emerging Organic Contaminants'

The figure was generated by searching for the total number of publications in Scopus™ (www.scopus.com) using the search terms 'pesticide', 'emerging organic pollutants', and 'emerging organic contaminants'. For each search term the results were analysed by the subject area 'Environmental Science", and the total number of publications were 37852, 1093, and 747 for the terms pesticide', 'emerging organic pollutants', and 'emerging

organic contaminants' respectively. Finally, the numbers were analysed by year of publication and plotted in Excel™. From the data provided in Figure 2.1, the earliest publications that reported on pesticides and can be categorized within 'Environmental Science' were in the 1960's. Whereas the earliest publications dealing with emerging pollutants or contaminants were in the 1980's. Another striking difference is the time between initial reports in the database, and the years before there is a significant and consistent number of publications, in this case two arbitrary numbers are chosen, 20 and 100. With pesticide based research it took 7 and 12 years before more than 20 and 100 publications were reported per year. For emerging organic pollutants the number of years was 24 and 31, and for emerging organic contaminants it was 25 and 33. The large differences between the seemingly quicker uptake of pesticide based research compared to the emerging areas does highlight the priority traditional organics has received from the scientific community; however, these raw numbers do not take into account factors such as availability of instrumentation, funding, and a knowledge base to tackle the field of emerging pollutants or contaminants.

Despite the smaller research output surrounding emerging organic pollutants and contaminants, various bodies such as WHO (Organisation 2011), USEPA (Agency), and the European Union (Barbosa et al. 2016) have some set of guidelines or regulations for monitoring and/or removal of various organic compounds before discharging water into the environment at large. However, there is still some concern about the presence of unregulated emerging contaminants/pollutants in water systems especially those that do not appear on the priority organic pollutant list (Agency) or relevant European Union directives (Barbosa et al. 2016). The situation surrounding emerging organic pollutants and contaminants is exacerbated by the simple fact that new technologies, new product developments and new commercial/industrial processes continue to emerge on a monthly or yearly basis. This means there are potentially new and unregulated organic compounds that could be introduced into various water systems, and how such compounds may interact with one another or the environment is unknown. For example, new water treatment techniques may yield new compounds or metabolites that differ from the current halogen based disinfection by-products. Furthermore, these compounds and or their metabolites may be bioactive and their continuous introduction into the aquatic environment should be a matter of great concern.

2.2 ORGANIC COMPOUNDS AS CONTAMINANTS OF EMERGING CONCERN

2.2.1 What are emerging contaminants?

The term "emerging contaminant" can be used to refer to anthropogenic compounds that may be newly synthesised, unregulated, those currently being detected at concentration levels higher than expected or those perceived to have deleterious effects. These compounds can be classified as "emerging contaminants of concern" if they are considered potential threats to aquatic and/or human life or in cases where the risk to aquatic and/or human life, their sources, frequency of occurrences and fate has not been fully determined. In most cases very little toxicology information about these compounds is available (Boles et al. 2010, Dugas et al. 2016, Fernandez-Gomez et al. 2013, Gerbersdorf et al. 2015, Murnyak et al. 2011, Pal et al. 2014, Pal et al. 2013, Rykowska and Wasiak 2015, Ternes 2007, Tijani et al. 2016, Wintgens et al. 2008). Emerging contaminants cover a wide range of compounds with a variety of chemistries including: pharmaceuticals and personal care products (PPCPs), endocrine disruptors such as steroids and hormones; perfluorinated compounds (PFCs), plasticizers, surfactants, pesticides and herbicides; drinking water disinfection by-products (DBPs), flame retardants, industrial additives, nanomaterials, algal toxins, pesticide transformation products and drug metabolites as well as microorganisms (Boles and Wells 2010, Dugas et al. 2016, Fernandez-Gomez et al. 2013, la Farre et al. 2008, Murnyak et al. 2011, Pal et al. 2014, Pal et al. 2013, Richardson and Ternes 2005, Rykowska and Wasiak 2015, Ternes 2007, Tijani et al. 2016, Voutsa et al. 2006, Wintgens et al. 2008, Zhang et

al. 2008). These compounds have been detected in various parts of the world and often occur in mixtures (Hughes et al. 2013, Ternes 2007, Wille et al. 2011, Xu et al. 2011, Zhang et al. 2011, Zhang et al. 2008), and there has been some recent work in Africa (Agunbiade and Moodley 2014, 2016, Belhaj et al. 2015, Christiaan et al. 2015, Daso et al. 2012, Inam et al. 2015, K'Oreje et al. 2012, K'Oreje et al. 2016, Kermia et al. 2016, Ncube et al. 2012, Okbah et al. 2013, Sorensen et al. 2015, Tijani et al. 2016). However, examining the data from Figure 1 (b), the total amount of work from Africa is very limited with 31/1589 publications on 'Emerging Organic Pollutants' and 19/1098 publications on 'Emerging Organic Contaminants'.

Emerging pollutants, or an emerging organic pollutant, is a compound (or substance) that has been shown to occur in the environment but it is not included in routine monitoring programmes. In addition, emerging pollutants have been shown to have deleterious effects in the environment and/or are persistent (http://www.norman-network.net/?q=Home). Thus, one can infer that emerging organic pollutants can include various compounds that have been reported in the open literature for a number of years in terms of the occurrence, seasonal variability, effects on aquatic or terrestrial organisms, and possibly toxicological data. The two definitions given for emerging pollutants and contaminants hint at the large number of compounds that could be included as such target analytes. It has been estimated that over 300 million tonnes of chemicals are produced per year around the world (Gavrilescu et al. 2015), and that there are over seven million commercially available chemicals (Rykowska and Wasiak 2015). Furthermore, there are over 15000 new chemicals being added to the chemical abstracts database per day, hundreds-thousands of new chemicals entering and leaving commercial/industrial processes a month, and as a result, there is a high probability that there are new non-biodegradable pollutants entering the hydrosphere (Barbosa et al. 2016, Gerbersdorf et al. 2015, Kolvenbach et al. 2014, Lapworth et al. 2012, von der Ohe et al. 2011). In addition, some of these compounds are persistent in the environment, while others such as some pharmaceuticals can have a relatively short lifespan within the environment. However, they do cause harmful effects because they are continually introduced into water systems and aquatic life species are exposed to them throughout their entire life cycle (Barbosa et al. 2016, Daughton and Ternes 1999).

2.2.2 Selected classes of emerging organic pollutants

The NORMAN network (Network of reference laboratories, research centres and related organisations for monitoring of emerging environmental substances - http://www.norman-network.net/?q=Home) has a list of 1036 emerging substances (accessed August 2016), which are the most frequently discussed. Furthermore, thousands of new chemicals are frequently added to the Chemical Abstracts Database, and an untold number of chemicals from new or current processes continuously enter the environment (Barbosa et al. 2016, Gerbersdorf et al. 2015, Kolvenbach et al. 2014, Lapworth et al. 2012, von der Ohe et al. 2011). In order to try and provide valid data and valuable information of the current state of emerging organic pollutants and contaminants within the study area, the project focused on pharmaceuticals and personal care products (PPCPs), polycyclic musks, and pesticide and pesticide residues. The sections that follow provides a summary of some key literature in these various fields of research, in terms of emerging pollutants/contaminants.

2.3 SOURCES AND OCCURRENCES OF EMERGING POLLUTANTS AND CONTAMINANTS

2.3.1 Global overview

Emerging organic contaminants of concern enter the environment from various sources such as commercial farming activities, municipal treatment plants, industrial waste, raw or inadequately treated sewage discharges,

and hospital effluents (Barbosa et al. 2016, Gavrilescu et al. 2015, Lapworth et al. 2012, Li 2014, Luo et al. 2014, Manickum and John 2014, Pal et al. 2014, Tijani et al. 2016). Contaminants may also enter the environment from improper disposal of expired or unused compounds, leachates from landfills, or industrial spills (Barbosa et al. 2016, Gavrilescu et al. 2015, Lapworth et al. 2012, Li 2014, Luo et al. 2014, Manickum and John 2014, Pal et al. 2014, Tijani et al. 2016). In most developing countries the main source of emerging organic contaminants are wastewater treatment plants, human activities, illegal dumping, poor waste disposal, or open dumping of waste.

In a study by Arukwe et al. (2012), phthalates, polycyclic musks, and bisphenol A were detected in sediment samples as well as in runoff from a dumping site in Owerri Nigeria in µg/L concentrations, confirming that the dumping site was a source of emerging contaminants. Recently Sorensen et al. (2015) reported on 27 organic compounds, including various emerging contaminants, in groundwater sources in Kabwe Zambia, and found major sources of contamination were from overland or shallow lateral flow, especially in areas with poor sewage coverage and little to no household waste collection. Inam et al. (2015) found that illegal dumping sites, and landfills were sources of emerging pollutants. These studies around the African continent highlight that point sources for emerging organic contaminants and pollutants can include a variety of sources, such as, landfills wastewater treatment plants, illegal dump sites, settlement areas with poor or lack of sewage facilities, and unspecified anthropogenic activities.

In terms of diffuse or non-point sources of emerging organic contaminants, urban run-off, commercial farming, the use of manure or deactivated sludge (bio-solids from wastewater treatment plants), or leaks from the sewage system, are usually considered as the main problems (Barbosa et al. 2016, K'Oreje et al. 2016, Lapworth et al. 2012, Mohapatra et al. 2016, Petrie et al. 2015, Sorensen et al. 2015, Vodyanitskii and Yakovlev 2016). Informal settlements do not usually feature in most international reviews, but there has been some work in South Africa showing they can be a source of emerging organic contaminants and pollutants (Olujimi et al. 2012). However, the classification of informal settlements as a point-source or diffuse source is an academic debate beyond the scope of this report. Emerging contaminants are usually present in low concentrations, typically ng/L - µg/L. However, there is growing concern over the possible risk such chemicals can have on aquatic life and how the continuous release of these compounds into the environment can lead to chronic exposure. Thus these chemicals may not only affect aquatic life but may also pose a risk to human health, especially when considering the consumption of contaminated drinking water over a lifetime (Al-Odaini et al. 2010, Christiaan et al. 2015, Pal et al. 2014, Van Den Berg et al. 2006). Thus the occurrence of these chemicals in river systems that feed into potable water treatment facilities is the main concern. Groundwater sources are a growing area of R&D in this field, and we refer the reader to the references (Lapworth et al. 2012, Postigo and Barceló 2015, Vodyanitskii and Yakovlev 2016).

2.3.2 Overview of South African studies on emerging organic contaminants

Olujimi et al. (2012) analysed water samples collected 1-2 km upstream and downstream from the discharge point of 6 different wastewater treatment plants (WWTPs) around the Cape Town area. The authors targeted 17 known POPs; these included various phenols and phthalate esters which have been identified as endocrine disrupting chemicals. Seasonal variation was studied, and the authors noted that there were no similar studies done within South Africa at the time of publication. Studies done on landfill leachates have been reported in the literature, and the relevant work that has been reported in the open literature, from South Africa, have been included in the relevant sections within this report, and are summarized in Table 2.1 for ease of reference.

Agunbiade and Moodley (2016) recently reported on 8 pharmaceuticals along the Msunduzi River and found wastewater treatment plants, and illegal dumps as a source of emerging pollutants. Follow up studies along the Umgeni and Msunduzi Rivers by Matongo et al. (2015a and 2015b) found that besides wastewater plants, anthropogenic activities along the rivers were sources of emerging contaminants which was also confirmed by other researchers (David and Tammy 2010, Roll and Halden 2016). Vos et al. (2013) recently reported on the identification and quantification of 17 known persistent organic pollutants (POPs) that are listed on the Stockholm Convention regarding POPs. The 17 congeners of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), were extracted from soil and sediment samples, and were analysed using two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOF-MS), and as a comparative study they looked at using a bio-assay (H4IIE-luc). Strictly speaking this was not a multi-residue study, since they were focussed on looking for known PCDDs and PCDFs. However, the authors did identify several POPs within the samples they had gathered. It is interesting to note that authors mention the limitations regarding funding and R&D targeting emerging pollutants.

There is very limited work on pharmaceuticals, but some work on various POPs has been pursued. There is some work on pesticides in the peer-reviewed literature and it includes those found on the POP list from the Stockholm Convention. The table below has summarized some work that has looked at sediments and soil, water based samples (sludge, raw water, effluents, leachates, etc.), and has not included work using biological samples. Considering the current drought situation in South Africa, and the prevalence and increase of bore hole usage around the country, this should be an area that receives further attention in terms of research on emerging pollutants.

Table 2.1 Summary of South African studies on organic contaminants and pollutants in soil, sediment, water, and marine environments

Geographical location	Source of Samples	Pollutant / Contaminant	Sample Preparation	Sample Analysis	Number of analytes	Ref
Western Cape, KwaZulu-Natal, Mpumalanga, Gauteng	Soil & Sediment	Polychlorinated dibenzo-p-dioxins & polychlorinated dibenzofurans	Solvent extraction & GPC	GCxGC-TOF-MS & a bioassay method	17	(Vos et al. 2013)
Mpumalanga, Gauteng, Free State	Soil	Polycyclic aromatic hydrocarbons	Soxhlet extraction & Silica gel	GC-MS	15	(Okedeyi et al. 2013)
eThekwini municipality (KwaZulu-Natal)	Sediment	Polybrominated diphenyl ethers & alternative brominated flame retardants	accelerated solvent extraction, GPC & solvent exchange	Ultra-performance liquid chromatography /atmospheric pressure photoionization tandem mass spectrometry	18	(La Guardia et al. 2013)
Western Cape (Bellville, Coastal Park and Vissershok landfill sites)	Landfill leachates	Polybrominated diphenyl ethers & polybrominated biphenyls	liquid-liquid extraction & multi-layer silica column technique	GC-electron capture detector	9	(Daso et al. 2013)
Port Elizabeth, West Coast National Park, & Woody Cape	Beached Polyethylene pellets	PCB, DDT, HCH	Solvent extraction & column chromatography	GC/MS or GC-electron capture detector	22	(Ryan et al. 2012)
Jukskei River Gauteng	sediments	polybrominated diphenyl ethers	Soxhlet extraction, multi-layer silica column & solvent exchange	GC/MS or GC-electron capture detector	11	(Olukunle et al. 2012)

Cape Town area	Water 1-2 km up & down stream of WWTP's	phenols and phthalate esters (known endocrine disrupting chemicals)	SPE & derivatization	GC-MS	17	(Olujimi et al. 2012)
Marianhill Landfill site Durban	Landfill leachate	polybrominated diphenyl ethers, polybrominated biphenyls & polychlorinated biphenyls	SPE	amperometric biosensor & GC-MS	5	(Nomngongo et al. 2012)
Gauteng	Fish & water samples from canals, dams, taps	Various Industrial chemicals, Pesticides, Disinfection by-products, Polymer residues, Cyanotoxins & PPCPs	Various based on USEPA or SABS methods	GC-electron capture detector, GC-flame photometry detector, or GC-MS	100	(Ncube et al. 2012)
Cape Town	Effluent & sewage sludge	polybrominated diphenyl ethers and cogeners	liquid-liquid extraction, soxhlet extraction, GPC & multilayer silica gel column	GC-electron capture detector	19	(Daso et al. 2012)
Gauteng and Mpumalanga	Sediment	Brominated flame retardants, Alkylphenol ethoxylates	ultrasonic assisted extraction	GC-MS	11	(Chokwe et al. 2015)
Limpopo	Water and sediment	Polycyclic aromatic hydrocarbons	Liquid-liquid extraction, or ultrasonic extraction	GC-TOF-MS	16	(Edokpayi et al. 2016)
Gauteng	Sediment	Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)	Accelerated Solvent Extraction	GCxGC system with a μECD detector	17	(Rimayi et al. 2016)
Gauteng	Water, fish (carp) and sediment	Alkylphenol ethoxylates and brominated flame retardants	SPE, or ultrasonic extraction	GC-MS	15	(Chokwe et al. 2015)

Port Elizabeth Harbour	Sediment, and mussels	Polychlorinated biphenyls	liquid-liquid extraction	GC-MS	8	(Kampire et al. 2015)
Port Elizabeth	Sediment, prawns, fish and bird eggs	polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorine pesticides	Outsourced (RPS Mountainheath in the United Kingdom)	GC-MS		(Nel et al. 2015)
Thohoyandou, Limpopo	Sediment, river water, and water run-off	Polycyclic aromatic hydrocarbons	Liquid-liquid extraction, or soxhlet extraction	GC-FID	6	(Olukunle et al. 2015)
Johannesburg, Gauteng	Surface water using semi permeable membrane device passive samplers	polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorine pesticides	Dialytic extraction of analytes	GC-MS	34	(Amdany et al. 2014)
Hartbeespoort Dam,	Surface water using semi permeable membrane device passive samplers	polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorine pesticides	n-Hexane extraction	GC-MS	35	(Amdany et al. 2014)
Pretoria, Gauteng	Surface water using miniature device passive samplers	Various	GerstelTM thermal desorber system or desorption tube	GCxGC-TOFMS	95	(Naudé and Rohwer 2012)

Umgeni River, KwaZulu-Natal	Surface water	Pharmaceuticals	SPE using Supelclean™ LC-18 (1 g) and (acidic analytes) Oasis® HLB (150 mg).	Shimadzu LC-20ADXR HPLC with a diode array detector (DAD)	17	(Agunbiade and Moodley 2014)
Various Provinces	Surface water	Pharmaceuticals	SPE	3200 QTRAP hybrid triple quadrupole mass spectrometer (AB SciEx, Framingham, MA, USA) coupled to HPLC	28	(Christiaan et al. 2015)

2.4 PHARMACEUTICAL AND PERSONAL CARE PRODUCTS

Attention was drawn to PPCPs by Daughton and Ternes (1999) who raised the issue of continuous introduction of compounds with a potential for deleterious effects into water systems. These compounds include human and veterinary drugs, diagnostic agents, nutraceuticals, fragrances, sun screens and their bioactive metabolites. These compounds may be persistent or rapidly degrading compounds; however, the issue of concern is that even for those rapidly degrading compounds, the exposure effect would be similar to that of persistent pollutants because their rate of degradation is compensated by their continuous introduction into the aquatic environment (Daughton and Ternes 1999). Table 2.2 is a summary of occurrences of pharmaceuticals and personal care products (PPCPs) in water, some examples, common sample preparation methods and techniques for their analysis. As shown PPCPs exhibit a wide range of chemistries, here pharmaceuticals and synthetic polycyclic musks as examples of PPCPs are briefly reviewed.

2.4.1 Pharmaceuticals

2.4.1.1 *Sources and occurrences*

Pharmaceuticals comprise several therapeutic classes including antibiotics, hormones, analgesics and anti-inflammatory drugs, disinfection products and endocrine-disrupting compounds (Kummerer 2010). Pharmaceuticals are designed as specific bioactive compounds which interact with receptors in the human body and in animals or to target specific infectious organisms (Jelic et al. 2009, Kummerer 2010) and induce specific biological responses at low doses (Galus et al. 2013). They enter the aquatic system as biologically active parent compounds or their metabolites and often in mixtures as discharges from wastewater treatment plants where they are not effectively removed (Daughton and Ternes 1999, Kummerer 2010, Ternes 2007).

Traces of pharmaceuticals/drugs have been detected in aquatic systems including wastewater, stream waters and ground water since the early 1990's. Recently they have been detected in various parts of the world with several reports about occurrence in Europe (Spain (Dahane et al. 2013, Gracia-Lor et al. 2012, Gros et al. 2012, Jelic et al. 2011, Rodil et al. 2012, Rodriguez et al. 2003), Germany (Launay et al. 2016)), England (Lapworth et al. 2015, Wilkinson et al. 2016)), Americas (Canada (Saunders et al. 2016, Segura et al. 2011) and USA (Fairbairn et al. 2016, Ferguson et al. 2013, Maruya et al. 2016, Meador et al. 2016, Yu and Chu 2009, Yu and Wu 2012)) and Asia (China (Dai et al. 2016, Sun et al. 2016, Wang et al. 2015b, Yu and Cao 2016),Turkey (Aydin et al. 2013), India (Archana et al. 2016), Japan (Kiguchi et al. 2016) and Singapore (Xu et al. 2011, You et al. 2015)).

There are some reports about the occurrence of pharmaceuticals in aquatic systems in African countries (Agunbiade and Moodley 2014, 2016, Belhaj et al. 2015, Christiaan et al. 2015, Inam et al. 2015, K'Oreje et al. 2012, K'Oreje et al. 2016, Kermia et al. 2016, Ncube et al. 2012, Ngumba et al. 2016, Sorensen et al. 2015, Tahrani et al. 2016, Wood et al. 2015). K'Oreje et al. (2012) reported on the occurrence of 10 pharmaceuticals within the Nairobi river basin in Kenya.

Table 2.2 Examples of Classes and Common Sampling Techniques for Pharmaceuticals in Water

Class	Examples	Sampling Method	Reference
Antibiotics, antibacterial compounds	Sulfamethazine Sulfamethoxazole Trimethoprim Erythromycin Metronidazole Ciprofloxacin	Grab sampling Composite sampling Sampling Station Swing-sampling pole	(Gracia-Lor et al. 2012), (Archana et al. 2016, Aydin and Talinli 2013, Sun et al. 2016, Wang et al. 2015, Yu and Cao 2016), (Fairbairn et al. 2016), (Ferguson et al. 2013) (Maruya et al. 2016) (Meador et al. 2016)
Analgesics and anti-inflammatory drugs	Acetaminophen Ibuprofen Salicylic acid Diclofenac	Composite sampling Grab sampling Swing-sampling pole Automatic water samplers	(Gracia-Lor et al. 2012) (K'Oreje et al. 2012), (Wilkinson et al. 2016), (Fairbairn et al. 2016), (Wang et al. 2015), (Sun et al. 2016), (Dai et al. 2016), (Yu and Cao 2016), (Archana et al. 2016), (You et al. 2015) (Meador et al. 2016) (Saunders et al. 2016)
Anticonvulsant	Carbamazepine	Automatic refrigerated sampler Grab sampling ISCO automated sampler Sampling Station Swing-sampling pole Borehole	(Launay et al. 2016), (Segura et al. 2011), (Dai et al. 2016, Fairbairn et al. 2016, Kiguchi et al. 2016, Wang et al. 2015) (Jelic et al. 2011) (Lapworth et al. 2015) (Maruya et al. 2016) (Meador et al. 2016)
Stimulant	Caffeine	Automatic refrigerated	(Launay et al. 2016) (Archana et al. 2016, Aydin and Talinli 2013, Dai et al. 2016,

		sampler Grab sampling Swing-sampling pole Borehole	Fairbairn et al. 2016, Kiguchi et al. 2016, Sun et al. 2016, You et al. 2015, Yu and Cao 2016), (Lapworth et al. 2015)
Antibiotics, analgesic/anti-inflammatory, anti-epileptic drugs, antimalarials and antiretrovirals	Ibuprofen Paracetamol sulfamethoxazole zidovudine dilantin	Automatic refrigerated sampler Grab sampling Sampling Station Swing-sampling pole	(Launay et al. 2016), (K'Oreje et al. 2012), (Sun et al. 2016) (Wang et al. 2015) (Maruya et al. 2016) (Meador et al. 2016)
Antidepressants	Carbamazepine Fluoxetine Sertraline paroxetine	Robotic auto sampler Swing-sampling pole	(Togunde et al. 2012) (Meador et al. 2016)
Antifungal, Antibacterial	Irgasan	Grab Sampling	(Archana et al. 2016)
Contraceptives hormones	Ethinylestradiol Equilenin Equilin, Estrone Progesterone Testosterone	Grab Sampling Swing-sampling pole	(Wilkinson et al. 2016), (Fairbairn et al. 2016), (Yu and Cao 2016), (You et al. 2015) (Meador et al. 2016)
Anxiolytic / Antipanic/ antianxiety agent	Meprobamate	Sampling Station Swing-sampling pole Grab Sampling	(Maruya et al. 2016) (Meador et al. 2016) (Fairbairn et al. 2016)

The authors found that the antibiotics sulfamethoxazole and trimethoprim occurred at much higher concentrations (20-50 µg/L) than has been previously reported around the world. The authors also found that the levels of the anti-malarial drug sulfadoxine (0.1-2 µg/L) to be higher than what has been reported in the literature (3-43 ng/L) (K'Oreje et al. 2012).

A follow-up study by K'Oreje et al. (2016), screened for 43 pharmaceuticals from wastewater treatment plants, surface water, and ground water in two major urban areas in Kenya (Kisumu and Nairobi). The authors found 26 pharmaceuticals (amandatine, amitriptyline, carbamazepine, chloramphenicol, ciprofloxacin, diazepam, diclofenac, efavirenz, ibuprofen, indomethacin, lamivudine, levofloxacin, metronidazole, nalidixic acid, nevirapine, paracetamol, rimantadine, sulfadoxin, sulfamethoxazole, trimethoprim, venlafaxine, zidovudine, ciprofloxacin, nalidixic acid, risperidone, and sulfamethazine) in wastewater, surface water and groundwater. The most frequently detected pharmaceuticals were antiretrovirals (nevirapine and zidovudine), and antibiotics (metronidazole, sulfamethoxazole and trimethoprim). The authors also found that the levels of some of the pharmaceuticals were as high as those found at WWTPs, and concluded this was due to the presence of informal settlements.

Agunbiade and Moodley (2014) reported on the occurrence of 17 pharmaceuticals from discharges from several domestic wastewater treatment plants, and surface water and dams along the Umgeni River KwaZulu-Natal, South Africa. Nine antibiotic drugs ciprofloxacin, ampicillin, nalidixic acid, sulfamethoxazole, streptomycin, tetracycline, erythromycin, chloramphenicol, and tylosin all varied, with values between 0.21-25.6 µg/L, and depending on the antibiotic some of these values were found to be higher than what has been reported in the literature. The antipyretics studied included aspirin, ketoprofen, diclofenac, ibuprofen, and acetaminophen, and these varied between 0.8-58.7 µg/L. Other drugs of interest that were studied included atenolol, bezafibrate, and caffeine with values ranging from 0.81-60.53 µg/L. As expected the authors reported on the high levels observed before and after wastewater treatment for several of the pharmaceuticals studied, and noted the plants were able to remove between 14-94% of these EOPs.

Belhaj et al. (2015) studied the occurrence and fate of natural estrogens, estrone, 17β-estradiol, and estriol, and synthetic estrogen, 17α-ethinylestradiol in a sewage treatment plant in Tunisia. They found that 78.8% of estrone, 86.1% of 17β-estradiol, 98.5% of estriol, and 91.6% of 17α-ethinylestradiol were removed by biodegradation processes. Seasonal variations were also reported, and they found winter and spring resulted in more of estrone, and 17β-estradiol in the effluent *versus* the influent. This was attributed to the low temperature during those particular seasons.

Christiaan et al. (2015) screened water from municipal water treatment plants (potable water) from several South African cities and towns and tap water from different neighbourhoods in Bloemfontein for 700 emerging contaminants. The authors found 28 pollutants (herbicides, pharmaceuticals, industrial chemicals, antifungals, antibacterial agents, etc.), and quantified three carbamazepine, an anticonvulsant, atrazine and terbuthylazine, which are both herbicides. The pharmaceutical carbamazepine was found to vary by season within all sampling points, but was noted to be below the MCL levels as set by the US-EPA.

Inam et al. (2015) detected and quantified seven antibiotic drugs (acetamidophenol, chloramphenicol, ciprofloxacin, erythromycin, lincomycin HCl, roxythromycin, and sulfamethoxazole), three bactericides/antimicrobial agents (sulfathiazole, triclosan and triclocarban), an antiepileptic drug (carbamazepine), an analgesic drug (diclofenac sodium), a hormone (equilin), and a stimulant (caffeine) along the Ikpa River Basin. The authors looked at landfill and dumpsite leachates, surface water, storm water, and surface run-off. The antibiotics acetamidophenol, chloramphenicol, ciprofloxacin, erythromycin, lincomycin HCl, roxythromycin, and sulfamethoxazole varied from below detection limits to 59.2 ng/L. Triclosan and triclocarban

varied from 35-297 ng/L, and the other EOPs varied from below detection limit to 40 ng/L. The authors also reported on the ecological risks of the EOPs by calculating risk quotients (using measured environmental concentrations/ predicted no-effect concentrations) which were based on three different organisms, green algae, fish and invertebrates. The authors found that most of the detected compounds had a low risk to the organisms studied, and triclocarban had a high risk and triclosan posed a medium risk.

Sorensen et al. (2015) sampled 20 groundwater samples in Kabwe Zambia for EOCs. The authors screened for 1023 compounds and detected and quantified 27 organic compounds, and these included surfactants, herbicides metabolites, photo initiators, chlorination by-products, drug, insecticides, insect repellent, herbicides, flame retardants, food additives, solvents, bactericides, anti-oxidants, UV inhibitors, and plasticisers. The PPCPs that were detected were caffeine and triclosan, and these were found to be amongst the top 6 most prevalent EOCs detected.

Wood et al. (2015) sampled surface water from several sites around South Africa (dams, rivers, wastewater treatment plants, and tap water) and screened for various drugs (abacavir, didanosine, efavirenz, lamivudine, stavudine, zalcitabine, zidovudine, nevirapine, indinavir, lopinavir, ritonavir, tenofovir, xanthines) used for the treatment of HIV, as well as caffeine. Three of the target samples were ubiquitous (nevirapine, lopinavir and zidovudine), and the highest averages were found for stavudine, nevirapine and zidovudine. The authors found the drugs were present in the ng/L level.

Kermia et al. (2016) recently reported on four acidic pharmaceuticals that are usually found in drinking water, surface water, WWTP influents and effluents globally. The authors sampled various points around Algiers. The authors found that the removal efficiency for ibuprofen varied from 78.8-95%. Naproxen was not detected in the effluent of one plant, but had a 72.6% removal at another plant. Ketoprofen was also not detected at one plant, and had a -83% removal at another. Diclofenac had a 30.3 and -173.7% removal efficiency at the plants sampled. In tap water, the authors did not detect naproxen and diclofenac, and found ibuprofen and ketoprofen at 312.1 and 273 ng/L respectively. For surface water samples, naproxen, ibuprofen, and diclofenac were at 334, 372.8, and 85.2 ng/L. Ketoprofen was not detected.

Ngumba et al. (2016) analysed surface water samples along the Nairobi river basin. The authors selected sampling sites along the main rivers and tributaries that were close to informal settlements, WWTPs, and a couple of sites near recreational sites. The target analytes in their study were antiretrovirals (nevirapine, zidovudine, and lamivudine), and antibiotics (trimethoprim, sulfamethoxazole, and ciprofloxacin). For the drug sulfamethoxazole, the authors found levels that varied from < LOQ–13,800 ng/L and a median of 1800 ng/L. For trimethoprim, values were < LOQ–2650 ng/L with a median of 327 ng/L, and ciprofloxacin were < LOQ–509 ng/L with a median of 129 ng/L. The antiretrovirals lamivudine, zidovudine, and nevirapine had mean concentrations of 1000 ng/L, 660 ng/L and 769 ng/L, and the levels ranged from < LOQ–5430 ng/L, < LOQ–7680 ng/L and < LOQ–4860 ng/L respectively. Risk quotients were calculated and based on Daphnae, algae, and fish. Lamivudine was the only drug that had a negligible risk factor in the environment, all others varied from low to high for all organisms used in the assessment.

2.4.1.2 *Sampling techniques for pharmaceuticals*

Table 2.1 shows the common sampling techniques used. Literature shows that grab sampling is the most common sampling technique used for wastewater and surface water analysis (Aydin and Talinli 2013, Ferguson et al. 2013, Gracia-Lor et al. 2012, K'Oreje et al. 2012) but passive sampling using the polar organic chemical integrative sampler (POICS) (Kim et al. 2015, Roll and Halden 2016, Soderstrom et al. 2009, Zhang et al. 2008) and Chemcatcher® (Vermeirssen et al. 2009) have been reported.

2.4.1.3 *Sample clean up and pre-concentration*

The most commonly used clean up and pre-concentration technique for pharmaceuticals in water is solid phase extraction with a nonpolar sorbent but recently mixed mode sorbents have gained popularity for example hydrophilic lipophilic balance, e.g. Oasis HLB and Oasis MCX cartridges are used for multi-residue analysis (Al-Odaini et al. 2010, Andrade-Eiroa et al. 2016, Dimpe and Nomngongo 2016, Locatelli et al. 2016, Rodil et al. 2012, 2009). High throughput extraction techniques, e.g. QuEChERS for extraction of pharmaceuticals from sludge (Peysson et al. 2013) and use of new materials for SPE such as the use of multi-walled carbon nanotubes in SPE of non-steroidal anti-inflammatory drugs (NSAIDs) and β-blockers have been reported (Dahane et al. 2013).

It is very important to choose an appropriate sampling design clearly explaining when, where and how samples will be collected as well as a suitable sample handling and preservation method so as to maintain sample integrity and to reduce bias (Ni et al. 2011, Zhang and Zhang 2012). Current trends are generally towards increasing sample throughput and reduction in sample manipulation.

2.4.1.4 *Analytical techniques*

There are several multi-residue analytical methods for the monitoring of acidic drugs and their metabolites in aqueous solution that have been reported in the literature (Table 2.3). Multi-residue methods have been developed for determination of pharmaceuticals and other organic compounds for example Peysson and Vulliet (2013) developed a method for determination of 136 pharmaceuticals and hormones. Other multi-residue methods have been developed (Al-Odaini et al. 2009, Gomez et al. 2011, Robles-Molina et al. 2010, 2013, Rodil et al. 2009), however most of these methods focus on determination of parent compounds yet their transformation products also co-exist as products of human or animal metabolism or other forms of biotransformation (Aguera et al. 2013). Some recent reviews highlight the needs, strengths, and current limitations with multi-residue methods (Bletsou et al. 2015, Gosetti et al. 2016, Hernández et al. 2014, Padrón et al. 2014, Rykowska and Wasiak 2015). Table 2.3 summarizes the occurrence and analysis of various pharmaceuticals.

Table 2.3 Analytical Techniques for the Determination of Pharmaceuticals and Personal care products

Contaminants	Classes	Sample Preparation	Analytical technique	LOD (effluent ng/L or Sludge ng/g)	LOD (Surface water ng/L)	Reference
PPCPs	NSAIDs, Endocrine disruptors, Antibacterial agent	SPE (SDB-XC)	GC/MS	10-106*	ND - 107	(Boyd et al. 2003)
PPCPs **EDCs**	Antibiotics and antibacterial agents, β- blockers, Hormones, Musks	SPE (HLB)	LC/MS/MS	102-3745* <1	56-1013 <1	(Yoon et al. 2010)
PPCPs	NSAIDs, Stimulant, Antibiotic, Phthalates	SPE (Oasis HLB Extraction Cartridge)	GC/MS	171-8572*	ND - 193	(Yu and Chu 2009)
Priority pollutants, personal care products (PCPs) and other emerging contaminants	Pesticides, Synthetic fragrances, Antibacterial, antifungal agent, Antioxidant, UV filter, Flame retardant	LLE (with n-hexane)	GC/MS		4 to 66	(Gomez et al. 2009)
Multi-class emerging organic pollutants	Acidic herbicides, UV filters, Insect repellents, Organophosphorous, flame retardants, Bactericide, Pharmaceuticals and Metabolites	SPE (Oasis HLB)	LC-MS/MS		0.3-30	(Rodil et al. 2009)
Human pharmaceuticals and synthetic hormones	Anti-diabetic drugs, Antihypertensive drugs, Hypolipidemic agents,	SPE (Oasis MCX)	LC-MS/MS		0.2-281	(Al-Odaini et al. 2010)

	β2-adrenergic receptor, agonist, Antihistamine Analgesic, Sex hormones					
Pharmaceuticals	Antidepressants	C-18 thin film solid phase micro-extraction (TF-SPME)	LC-MS/MS	240-3820 *		(Togunde et al. 2012)
Multi-class priority organic pollutants	Pharmaceuticals, Herbicides, etc.	LLE (hexane)	GC-MS/MS		<3	(Robles-Molina et al. 2013)
136 pharmaceuticals and hormones (in sludge)	Analgesics, antipyretics Antiarrhythmic, Antidepressant, Antibiotics, Non-steroidal anti-inflammatory drugs	QuEChERS extraction	LC-MS(TOF)	1-2500 +		(Peysson and Vulliet 2013)
Pharmaceuticals	β- blockers, Non-steroidal anti-inflammatory drugs	SPE by packed multi-walled carbon nanotubes	LC-MS (hybrid triple quadrupole-linear ion trap-MS)		9-36 23-121	(Dahane et al. 2013)

STP – Sewage treatment plant
*Effluent
+ Sludge

Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography (GC-MS) are the most widely accepted techniques for analysis of pharmaceuticals (Richardson and Ternes 2014, Hernández et al. 2014). However, there are some alternative methods that maybe very promising for more routine monitoring in the near future; such as, photo-induced fluorescence (Hurtado-Sánchez et al. 2015), biosensors (Dmitrienko et al. 2014, Tijani et al. 2013), MALDI-TOF MS using novel nanomaterials (Wang et al. 2015), or immunoassay techniques (Richardson and Ternes 2014, Dmitrienko et al. 2014) to name a few. Samples are usually analysed by liquid chromatography (Aydin and Talinli 2013, Białk-Bielińska et al. 2016, Ferguson et al. 2013, Hernández et al. 2014, Locatelli et al. 2016, Richardson and Kimura 2016, Segura et al. 2011).

Ultra-high performance liquid chromatography (UHPLC) has been reported to provide greater resolution, higher sensitivity and a reduced total analysis time (Gracia-Lor et al. 2012, Jelic et al. 2011). Methods based on LC-MS remain as the technique of choice for analysis of polar, non-volatile and acidic analytes in aqueous solution, compared to GC-MS based methods. Gas chromatography often requires derivatization of less volatile compounds and the process is time consuming and expensive however it has been used with impressive results. Derivatization methods; such as, alkylation, acylation and silylation are often used to increase volatility, change polarity, improve selectivity and separation, increase stability of the analyte, and enhance the detectability of the target compounds with GC-MS (Hao et al. 2007, Buchberger 2011). The derivatization process is required to go to completion, and the resultant derivative must have a closely related structure which is volatile, stable and able to pass through the chromatographic column without degradation. Thus the choice of the derivatizing reagent is crucial. Early methods using diazomethanes were risky due to the explosive and carcinogenic nature of the derivatizing agent (Öllers et al. 2001). Silylation methods for pharmaceuticals have been known for decades (Blum et al. 1996); however, the adaptation of such methods for environmental samples occurred much later (Rodríguez et al. 2003), and only gained a wider acceptance more recently (Yu and Wu 2011, Helenkár et al. 2010, Bisceglia et al. 2010, Migowska et al. 2012, Kumirska et al. 2015).

Since pharmaceuticals usually occur in trace amounts in water the detector of choice is a mass spectrometer and when used in tandem offers the best sensitivity. Based on literature, some of the advantages of using GC-MS techniques for the analysis of pharmaceuticals within environmental samples include the ease of access to well-established electron-impact mass spectrometry libraries, superior sensitivity, it is not as prone as LC-MS methods to matrix effects, and even with derivatization of the target analytes it is cost effective and suitable for routine analysis (Hao et al. 2007, Buchberger 2011). A better analytical method should therefore be able to determine the parent compound as well as its transformation products. Analytical methods for determination of pharmaceuticals and their metabolites have been developed (Gros et al. 2012, Lopez-Serna et al. 2012, Santos et al. 2005, Weigel et al. 2004) and are generally applied to selected pharmaceuticals due to the cost associated with standards or require expensive techniques with high resolving power when applied to multi-class pharmaceuticals.

2.4.1.5 *Effect of exposure*

The effect of exposure to these drugs has not yet been fully determined, but it has been reported that traces of these drugs in our water supply affect aquatic, terrestrial and human life forms; for example, a mixture of commonly detected pharmaceuticals fluoxetine and clofibric acid caused significant mortality of *Daphnia species* while exposure to a mixture of antibodies caused an unpredictable change in *Daphnia* sex ratio (Flaherty et al. 2005). Chronic exposure to low concentrations of pharmaceuticals has been associated with increased stress, variations in sex steroid levels, impaired gonadal development and decreased reproduction in fish (Galus et al. 2013) a further indication of the deleterious effects of pharmaceuticals on aquatic life; moreover, some compounds may be persistent and bioaccumulate.

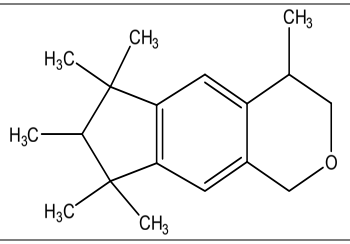
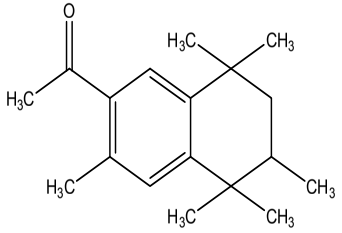
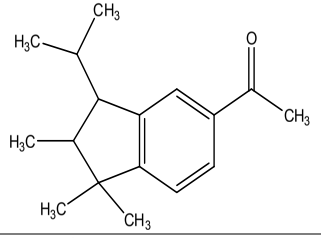
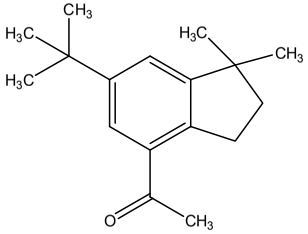
2.4.2 Synthetic polycyclic musks

2.4.2.1 *Sources and occurrences*

The most commonly used fragrances are synthetic musks which can be aromatic nitro musks, polycyclic musk compounds, macrocyclic musk compounds or alicyclic musks and are found in a wide range of personal care products such as soaps, deodorants, etc. Table 2.4 shows the most common polycyclic musks. They are found in water, sediment, aquatic life, sludge, air, solid particle, human breast milk (Daughton and Ternes 1999, Vallecillos et al. 2015). Because of their broad usage, these micro contaminants have been detected in concentrations up to several µg/L in WWTP effluents and in surface water. Their occurrence and concentration depends on the type of water source and the types of products that can be found in each area. Synthetic musks are intended for external use on the human body and are not subjected to metabolic alterations therefore, large quantities enter the environment unaltered through regular usage unlike pharmaceuticals and other persistent pollutants (Ternes et al. 2004).

Polycyclic musks are currently used in higher quantities compared to nitro musks due to concern about toxicity of the latter (Daughton and Ternes 1999). It has been demonstrated, that nitro musks can be transformed in wastewater treatment and in vertebrate physiology into the aniline transformation products (Rimkus 1999, Rimkus et al. 1999) which may be more toxic than the parent compounds. These transformation products are the main reason, why musk xylene has been withdrawn from most European markets, and thus concentrations in wastewater have correspondingly dropped by about two orders of magnitude (Kupper et al. 2006). The production of galaxolide (HHCB) and tonalide (AHTN) examples of polycyclic musks has been estimated at about 1 million pounds per year and for this reason placed on the high production volume list by the United State Environmental Protection Agency USEPA (Peck 2006). Table 2.5 is a summary of the occurrence of polycyclic usks. Macrocyclic musks are becoming more available and have advantages of a more intense smell thus less mass is needed to gain the same performance in perfumery, moreover they are more easily degradable in the environment (Kraft et al. 2001). However, their mass spectra is similar to that of natural fatty acids or their derivatives and so may easily escape attention while analysing environmental samples as well as commodities. Also their chemical properties are similar to those of natural products, so separation from these is more difficult. Presently, there are no reports on environmental samples in connection with macrocyclic musks (Bester 2009).

Table 2.4 Synthetic Polycyclic Musks in the Environment

Compound	Trade Name(s)	IUPAC Name	CAS No	Structure	Physico-chemical parameters
HHCB	Galaxolide Abbalide Pearlide	1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl -(ɣ)-2-benzopyran	1222-05-5		Mwt-258.40g Bpt-304°C Soluble-Alcohol M_{f-} $C_{18}H_{26}O$ $LogK_{ow}$- 5.9
AHTN	Tonalide Fixolide	7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahdronaphthalene	1506-02-1		Mwt-258.40g Bpt-393°C Soluble-Alcohol M_{f-} $C_{18}H_{26}O$ $LogK_{ow}$- 5.7
ATII	Traseolide	5-acetyl-1,1,2,6-tetramethyl-3-isopropylindane	68140-48-7		Mwt-258.40g Bpt-350°C Soluble-Alcohol M_{f-} $C_{18}H_{26}O$ $LogK_{ow}$- 6.3
ADBI	Celestolide Crysolide	4-acetyl-1,1-dimethyl-6-tert-butylindane	13171-00-1		Mwt-244.37g Bpt-308°C Soluble-Alcohol M_{f-} $C_{17}H_{24}O$ $LogK_{ow}$- 5.4

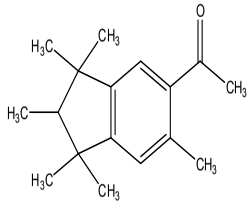
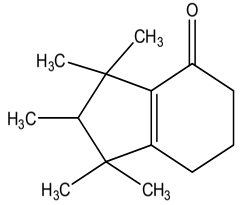
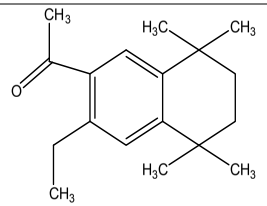
AHMI	Phantolide	6-acetyl-1,1,2,3,3,5-hexamethylindane	15323-35-0		Mwt-244.37g Bpt-393°C Soluble-Alcohol M_{f-} $C_{17}H_{24}O$ $LogK_{ow}$- 5.9
DPMI	Cashmeran	6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanon	33704-61-9		Mwt-206.33 Bpt-285°C Soluble-Alcohol M_{f-} $C_{14}H_{22}O$ $LogK_{ow}$- 5.9
AETT	Versalide	1-(3-ethyl-5,5,8,8-tetramethyl-5,6,7,8-tetrahydronaphthalen-2-yl)ethan-1-1-one	88-29-9		Mwt-258.40g Bpt-361°C Soluble-Alcohol M_{f-} $C_{18}H_{26}O$ $LogK_{ow}$- 5.7

Table 2.5 Occurrence of Polycyclic Musks in the Aquatic Environment

Polycyclic musks	WWTP ng/L Effluent	Influent	Surface Water (ng/L)	Sediment (ng/g)	Fish (ng/g)	Ref
Galaxolide HHCB	790-4443	451-1080				(Ratola et al. 2012)
Tonalide AHTN	210-1690	144-200				(Ratola et al. 2012)
Celestolide ADBI		ND-92				(Ratola et al. 2012)
Galaxolide HHCB			3.5-32.0	1.5-32.3	2.9-5.3dw* 107.9-823.3lw*	(Hu et al. 2011)
Tonalide AHTN			2.3-26.7	2.0-21.9	3.0-6.8dw* 107.1-771.7lw*	(Hu et al. 2011)
Galaxolide HHCB	476-2069	233-1432	1.40-26.2			(Ramirez et al. 2011)
Tonalide AHTN	17.7-78.7	25.4-93.6	0.34-37			(Ramirez et al. 2011)
Celestolide ADBI	3.6-35.4	<MQL-4.56	<MQL			(Ramirez et al. 2011)
Cashmeran DPMI	15.7-87.7	29.8-43.3	0.49-1.72			(Ramirez et al. 2011)
Phantolide AHMI	<MQL-25.6	<MQL-4.15	ND-0.27			(Ramirez et al. 2011)
Traesolide ATII	ND-8.1	ND	ND			(Ramirez et al. 2011)
Galaxolide HHCB			<0.05-1141			(Villa et al. 2012)
Tonalide AHTN			<0.25-23.4			(Villa et al. 2012)
Celestolide ADBI			<0.25-2.45			(Villa et al. 2012)
Galaxolide HHCB	330-2060	130-700				(Vallecillos et al. 2012)
Tonalide	<MQL-100	ND-<MQL				(Vallecillos et al. 2012)

AHTN						
Galaxolide HHCB		987-2098				(Sumner et al. 2010)
Tonalide AHTN		55-159				(Sumner et al. 2010)
Celestolide ADBI		4-13				(Sumner et al. 2010)
Phantolide AHMI		6-9				(Sumner et al. 2010)
Galaxolide HHCB			20-93	3-78		(Zhang et al. 2008)
Tonalide AHTN			8-20	2-31		(Zhang et al. 2008)
Galaxolide HHCB	11500-2050	950-2050				(Zeng et al. 2007)
Tonalide AHTN	890-3470	100-140				(Zeng et al. 2007)
Cashemeran DPMI	380-690	60-100				(Zeng et al. 2007)
Galaxolide HHCB			3.95-25.8	72.8-388	<1-125	(Reiner and Kannan 2011)
Tonalide AHTN			5.09-22.8	113-544	<1-328	(Reiner and Kannan 2011)
HHCB **AHTN**			<2-37 <1-8	<0.5-17.5 <0.5-5.7		(Lu et al. 2015)
MX **MK**			ND <2-4	ND <0.5-3.3		(Lu et al. 2015)
ADBI **AHMI**			<1-2 <1-3	<0.3-2.8 <0.3-2.9		(Lu et al. 2015)
HHCB (galaxolide®) **AHTN (tonalide®)**		530-1830 90-340	1-260 1-60			(Lange et al. 2015)
Galaxolide	2310-3490	2670-4580				(Godayol et al. 2015)

Tonalide	ND-360	ND-910			
HHCB				ND-6.3	
AHTN				ND-2.3	
HHCB			ND-2000	ND-2.7	(Lee et al. 2014)
AHTN			ND-2000	ND-1.0	
MK			ND-2000		(Lee et al. 2014)
MX			ND		
ADBI (celestolide)				ND-35 (*sl*) 85-143 (*sl*)	(Vallecillos et al. 2014)
AHMI (phantolide)				5040-7500 (*sl*) ND-127 (*sl*)	
AHTN (tonalide)				ND-1319 (*sl*) 7890-9240 (*sl*)	
ATTI (traseolide)				136-635 (*sl*) ND-341 (*sl*)	
MX (musk xylene)					
HHCB (galaxolide)					
MK (musk ketone)					
MM (musk moskene)					
DPMI	ND-32933	ND-4342			(Vallecillos et al. 2014)
(Cashmeran)	ND-44319	ND-9930			
ADBI	ND-34674	ND-3738			
(Celestolide)	ND-1741	ND-480			
AHMI	ND-3487	ND-1276			
(Phantolide)	ND-3022	ND-1748			
ATII	ND-28377	ND-4210			
(Traseolide)	ND-45091	ND-900			
HHCB	ND-49904	ND-7555			
(Galaxolide)	ND-321	ND-321			
AHTN	ND-1182	ND-221			

(Tonalide)	ND-9744	ND-4021
MX (Musk xylene)	ND-11758	ND-8939
MM (Musk moskene)	ND-4110	ND-689
MK (Musk ketone)	7.5-4119	5.95-11007
Exaltone		
Exaltolide		
Muscone		
Ambrettolide		
Musk MC4		
Musk NN		

*dw- dry weight, *lw- lipid weight, WWTP- Waste Water Treatment Plant, Ref- References, ND- Not Detected, *sl* = sludge
MQL- Method Quantification Limit

2.4.2.2 *Sample extraction and clean up*

The concentration of polycylic musks in the environment is matrix dependent with the highest amounts in wastewater treatment plant samples, sediment and fish and the least amount in surface water. The concentration in fish is greater than that in sediment due to the lipophilic nature of the polycyclic musks, so they tend to accumulate in the fish lipid. Galaxolide (HHCB) and tonalide (AHTN) are the most commonly detected polycyclic musks in the environment. Extraction and clean-up for polycyclic musk determination is matrix dependent and both classical and novel extraction techniques have been used. Classic extraction techniques such as soxhlet and soxtec (automated soxhlet extraction), ultrasound assisted extraction, use of a stirring ring and mechanical shaking have been reported.

In most of these techniques two or three consecutive extractions were performed (Zuloaga et al. 2012). Novel techniques such as focused ultrasound assisted liquid extraction, pressurized liquid extraction (PLE), pressurized hot water extraction (PHWE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), matrix solid-phase dispersion (MSPD), stir bar sorptive extraction, ultrasound-assisted emulsification micro extraction, single drop micro extraction, micro extraction by packed sorbents and hollow fibre membrane solid phase micro extraction have been reported' (Peck 2006, Peck and Hornbuckle 2006, Ratola et al. 2012, Zeng et al. 2007). Classical techniques represent high percentages of the publications. Table 2.6 is a summary of the common extraction methods used for various polycyclic musks.

Among the novel techniques, microwave assisted head space solid extraction seems to be a fast, simple, effective, low matrix effect and eco-friendly method compared to other extraction methods and therefore can be used as a green extraction technique.

Table 2.6 Extraction Methods for Polycyclic Musks in the Environment

Matrix	Extraction method	Detection	Recovery (%)	LOD/LOQ (ng/L)	Ref
Surface water/effluent	UA-DLLME (11min, 10µL CCl_4)	GC-MS-SIM	70-95	LOD 0.2 LOQ 0.6	(Yang and Ding 2012)
Wastewaters	HS-SPME (25 min)	GC-MS	75-95 HHCB 93	LOD 0.1-1.8 LOD HHCB 20	(Yang and Ding 2012)
Wastewaters	MA-LLE (60 min)	GC-MS-SIM	AHTN 93	AHTN 20	(Yang and Ding 2012)
Surface water/effluents	MA-HS-SPME (4 min)	GC-MS-SIM	64-102	LOD 0.05 LOQ <0.2	(Wu and Ding 2010)
Surface water	DLLME (8.5 min)	GC-MS-SIM	60-93	LOD 28-63 LOQ 24-227	(Yang and Ding 2012)
Various water samples	USAEME (13 min, 100µL CCl_4)	GC-MS	78-114	LOD 6-29 LOQ 20-97	(Yang and Ding 2012)
Wastewaters	MEPS-C18	LVI-GC-MS	HHCB 78 AHTN 109	LOD HHCB 42 AHTN 37	(Yang and Ding 2012)
Sludge	ASE +clean-up steps	GC-MS	HHCB 77% AHTN 69%	LOD:3 ng/g LOQ:10 ng/g	(Yang and Ding 2012)
Sediment	Soxhlet (24h+cleanup steps	GC-MS/MS	63-86%	*0.025-0.15 ng/g *0.06-5.1 ng/g	(Yang and Ding 2012)
Digested Sludge	Mechanical shaking	GC-MS	85-106	LOD 100	(Zuloaga et al. 2012)
Sewage Sludge	Soxhlet	GC-MS	76 AHTN 100HHCB	1.5 AHTN 3.1 HHCB	(Zuloaga et al. 2012)
Oysters	MA-HS-SPME Solvent free(5min)	GC-MS	80-90%	LOD:0.04 ng/g LOQ:0.1 ng/g	(Wu et al. 2012)
Marine Mammal	Soxhlet + Clean-up Step 12hrs	GC-MS	65-90%	LOD:1.0 ng/g	(Kannan et al. 2005)

*0.025-0.15 ng/g (from Lake Erie core) *0.06-5.1 ng/g (from Lake Ontario core)

HS-SPME headspace solid-phase micro extraction, MA-LLE membrane-assisted liquid-liquid extraction, MA-HS-SPME microwave-assisted head-space solid-phase, micro extraction, USAEME ultrasound-assisted emulsification-micro extraction, DLLME dispersive liquid-liquid micro extraction, MEPS micro extraction by packed sorbent, LVI large-volume injection, AS accelerated solvent extraction. SBSE stir bar sorptive extraction coated with polydimethylsiloxane, and coupled with a TD-GC-MS thermal desorption-gas chromatography-mass spectrometry system, MA-HS-SPME microwave-assisted head-space solid-phase.

2.4.2.3 *Analysis of synthetic polycyclic musks*

Analysis of synthetic polycyclic musks is often done using GC since polycyclic musks are lipophilic (Zuloaga et al. 2012). GC and LC coupled to MS and MS/MS have been used in multi-residue methods for determination of personal care products (PCPs) including polycyclic musks. Different temperature programmes are often used based on the volatility of the analytes and column chemistry. Inlet injection and detection temperature as well as the carrier gas are parameters that are considered. Devices such as

programmed temperature vaporizer (PTV) can perform large volume injection (LVI) improving the method sensitivity (Zuloaga et al. 2012). Detectors such as electron capture detector (ECD), flame ionization detector (FID), mass spectrometry (MS) and tandem MS (MS/MS) can all be used but MS and MS/MS are the most preferred techniques for lower detection limits ng/L especially when dealing with groups of similar polycyclic musks (Yang and Ding 2012).

2.4.3 Pesticides and pesticide residues

The original definition for emerging organic pollutants or contaminants, described in Section 2.2, does include pesticides. Pesticides are routinely monitored around the world, and their occurrence and fate in water systems is an area of concern due to the health risks, and danger they can pose to the environment in general (Tankiewicz et al. 2010). Thus from a perspective of 'potential threats to aquatic and/or human life', pesticides do fall into the category of emerging pollutants. Furthermore, with the ban or limitations on the use of organochlorine pesticides, and the increasing and widespread application of organophosphorus pesticides and organonitrogen pesticides (Tankiewicz et al. 2010), some questions arise on the stability, water and/or fat solubility, transport, and chemistry of these compounds in water ways. Another concern raised earlier in the report, is when new chemicals are introduced by industry/commercial activities, these can be considered as emerging contaminants, thus pesticides, especially new derivatives, fall into this category.

Pesticides include a wide variety of chemical compounds that are used as herbicides, insecticides, fungicides and disinfectants and more recently bio-pesticides have also been identified as those pesticides containing natural active ingredients. These all aim to control growth of unwanted weeds or the presence of pests, insects and bacterial strains that may interfere with agricultural activity or cause the spread of diseases. Chemical pesticides can be classified into organophosphates, carbamates, organochlorines and pyrethroids and the presence of various pesticides in the environment has been evaluated and monitored since the 1970s (Martínez Vidal et al. 2009, Tankiewicz et al. 2010, Van Dyk and Pletschke 2011). In 2012, Ansara-Ross et al. 2012), published a review on pesticides in water systems in South Africa. The review mined data from journal articles, theses, reports, books and book chapters, and covered a wide range of years.

2.4.3.1 *Sampling techniques for pesticides in aquatic environments*

In the analysis of pesticides, sampling of water soil and sediment generally has used the grab sampling method which focuses on sampling of the top surface matrix (Chen et al. 2011, Kuo et al. 2012, Teng et al. 2013, Toan et al. 2013, Yuan et al. 2013). Sediment core sampling has also been carried out which together with radiometric dating allows for the determination of pesticide use in the area over many decades (Janniche et al. 2010, Lin et al. 2012, Yuan et al. 2013). Pesticide monitoring requires a less intrusive method of sampling. The use of event-triggered passive sampling systems is useful in the monitoring of pesticides after particular events such as a heavy rainfall. Here bottles are attached to upright rods placed at the sampling point, above the normal level of the water. During a heavy rainfall, the water level rises and once it reaches the opening in the bottle, it is able to fill passively (Bereswill et al. 2012, Liess et al. 1996, Schulz et al. 2001).

2.4.3.2 *Analytical techniques for pesticides*

Sample preparation of water samples may involve various extractions, concentration of the analyte and clean-up steps. Liquid-liquid extraction has been widely used and generally has a subsequent clean-up step that passes the solution through a packing material such as florisil or deactivated alumina/silica gel to remove unwanted analytes (Yuan et al. 2013). Solid-phase extraction (SPE) may also use different packing materials with C18 most commonly used. Soil and sediment samples may be freeze-dried and thereafter taken through soxhlet extraction (Lin et al. 2012, Teng et al. 2013, Yuan et al. 2013). If further clean-up is

required, the extract may be subjected to either a multilayer acid/base silica column if polychlorinated biphenyls (PCBs) are to be analysed or a silica or alumina column if organochlorine pesticides (OCPs) are to be analysed (Lin et al. 2012, Teng et al. 2013). Cold extraction is another method of extraction used and is carried out by shaking the soil or sediment with a suitable solvent for a period of time and further clean-up can be carried out by passing the extract through a suitable packing material such as florisil, alumina or C18 cartridge (Kuo et al. 2012, Liess et al. 1996, Nowik et al. 2011).

More recently a combined extraction method has also been developed for trace analysis of OCPs where the sample is first subjected to pressurized hot water extraction (PHWE) followed by solid phase micro-extraction (SPME) (Concha-Graña et al. 2010). The method is almost completely automated, is environmentally friendly and uses shorter sample preparation and analysis time (Concha-Graña et al. 2010). Analysis of pesticides is usually carried out using GC-MS and if halogenated pesticides such as OCPs are to be analysed, GC-ECD has been the instrument of choice.

2.5 REVIEW OF METHODS FOR SAMPLING AND CHARACTERISATION OF EMERGING ORGANIC CONTAMINANTS

2.5.1 Sampling emerging organic pollutants/contaminants in natural water systems

Like most organic compounds, emerging organic pollutants with similar physical or chemical properties often occur in the same environment; and while individual classes can be extracted and analysed in separate steps, the process is lengthy and would require large quantities of solvents and/or reagents. The cost effective nature of simultaneous extraction and analysis of co-existing pollutants has gained interest recently because a wide range of compounds and their metabolites can be simultaneously determined providing broader knowledge about the occurrence and fate of emerging contaminants in the environment (Bletsou et al. 2015, Huntscha et al. 2012, Padrón et al. 2014). Such advantages make this method ideal for a project of this size, and thus we provide a brief review on multi-residue methods for the determination of emerging organic and priority contaminants of concern in water systems. In general, the important steps in a multi-residue method for the determination of organic contaminants/pollutants (Figure 2.2) are: sampling and sample preparation, sample pre-concentration, identification and quantification of analytes. The next section begins with an overview of sampling methods. In general, water sources are sampled and the sample is cleaned and pre-concentrated before analysis with an instrumental technique or other analytical method. The quality of the results from a particular analysis is dependent on the sampling strategy. In order to obtain accurate results, sample collection must be appropriate and the choice of a sampling design is critical. A large number of samples can provide more objective information but their collection may be expensive.

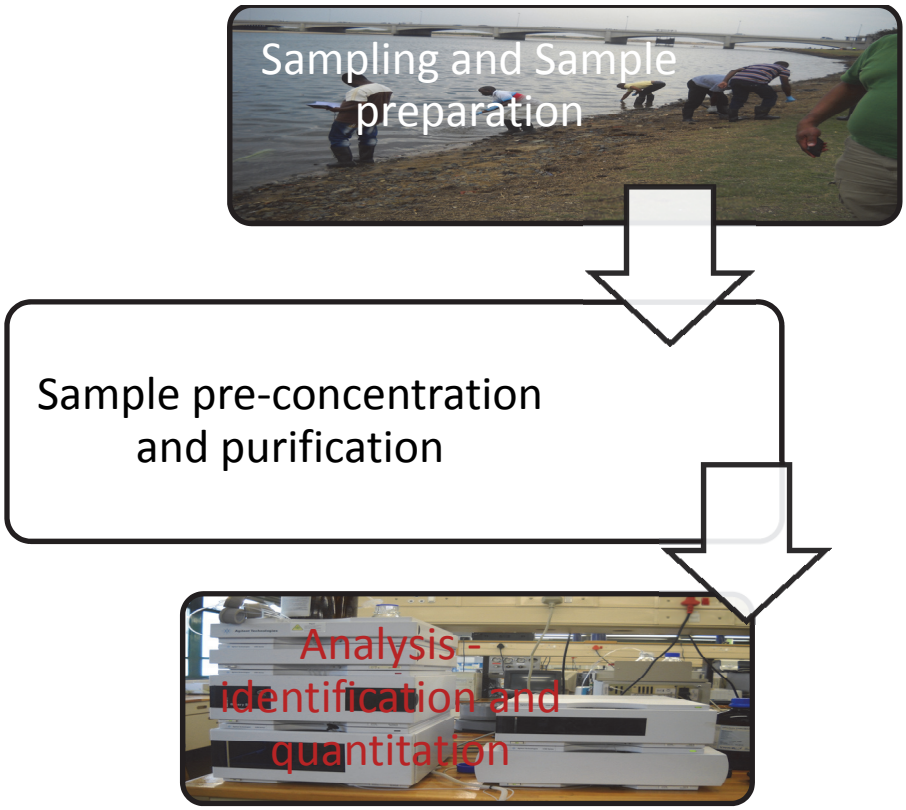


Figure 2.2: Simplified schematic on the process flow for the analysis of emerging organic pollutants in natural water

For high quality data, appropriate identification of sites and number of sites is crucial (Jones et al. 2015, Ni et al. 2011, Petrie et al. 2015, Zhang and Zhang 2012). Location, time, frequency and sample number must be judicious after identifying sources of temporal and spatial variability (Jones et al. 2015, Ni et al. 2011, Petrie et al. 2015, Zhang and Zhang 2012), in any case minimum requirements for reporting analytical data for environmental samples have been stated in the IUPAC technical report (Egli et al. 2003). In general sampling for monitoring is done over a long period of time covering all seasons while a shorter period may be required in case of a spill or in order to test for occurrence of the analytes of interest. A challenge associated with analysis of water samples is presence of analytes in trace amounts (ng/L to µg/L) (Togunde et al. 2012), which often requires pre-concentration techniques and very sensitive methods for their analysis.

2.5.1.1 *Active sampling*

Active sampling is a common approach for obtaining water samples for use in monitoring environmental concentrations of organic pollutants or contaminants of concern. A sample is obtained using pumps or is simply grabbed from a sampling site or series of sites for screening or monitoring purposes. Spot sampling has been widely applied (Calderon-Preciado et al. 2011, Jones et al. 2015, Petrie et al. 2015, Snyder 2010, Terzic et al. 2008, Voutsa et al. 2006, Yoon et al. 2010) and a grab or composite sample can be used to determine the occurrence of the pollutants. Active sampling is simple, quick and cost effective, however it only generates information about analyte concentrations at specific times, i.e. when the samples were obtained. It may not be suitable for providing information about intra-day variations unless the frequency of sampling is increased which is not only laborious but may also be expensive. Active sampling is therefore more suitable for screening tests rather than monitoring analyses. Continuous sampling offers a better approach for monitoring pollutants and reduces the uncertainty of short- and long-term concentration

variations associated with spot sampling; hence there is increasing interest in the development of passive sampling techniques.

2.5.1.2 *Passive sampling*

Passive sampling allows the average contaminant concentration over an extended period (time weighted average) to be obtained and also can be used as a pre-concentration step by using passive accumulation devices (PAD). In addition to the ease of sample handling and lower long term cost offered by passive sampling there is a possibility for detecting trace and ultra-trace concentrations due to in-situ pre-concentration on the PAD, lower solvent consumption and elimination of matrix interferences (Bueno et al. 2009, Ibrahim et al. 2013, Jones et al. 2015, Seethapathy et al. 2008). Some examples of passive sampling techniques include the use of semi-permeable membrane device (SPMD), polar organic chemical integrative sampler (POCIS) and Chemcatcher®™ (Ibrahim et al. 2013, Jones et al. 2015, Zhang et al. 2008).

Semi-permeable membrane device (SPMD) is a biomimetic method modelled on the mechanism of bioaccumulation of organic contaminants in fatty tissues of living organisms. The system is based on the attainment of an equilibrium state between the receiving phase and the environment in which the analyte is located within (Camilleri et al. 2012, Jones et al. 2015). A SPMD consists of a semi-permeable membrane surrounding a lipid layer which acts as a sorbent for analytes of interest; the most common is a low-density polyethylene (LDPE) tube filled with high purity triolein and sealed at both ends. Semipermeable membrane devices accumulate nonpolar analytes from the surrounding environment because the membrane and the receiving phase are both hydrophobic therefore the SPMD is suited to selectively accumulate a time weighted average of hydrophobic contaminants (log K_{ow} >3) from water (Camilleri et al. 2012, Jones et al. 2015, Seethapathy et al. 2008). After a pre-set time, the SPMD contents are eluted with a suitable solvent such as hexane for pesticide residues and quantified using a suitable technique.

Extraction disks with C18-bonded silica particles embedded in a PTFE disk have also been developed (van Stee et al. 2002) and work on a similar principle. In a study comparing the extraction efficiency of solid phase extraction (SPE) and SPMD, it was found that SPE was efficient for low boiling point compounds while efficiency for the high boiling range compounds was greater in the SPMD extracts. This demonstrated the complementary nature of the two extraction techniques and pointed out how both are necessary for screening a large number of organic pollutants (van Stee et al. 2002).

Polar organic chemical integrative samplers (POCIS) consist of a sorbent of specific chemistry enclosed between two micro-porous membranes usually made from polyethersulfone. They are used for sampling polar organic compounds with log K_{ow} < 3 from aquatic environments. Due to the chemistry of the sorbent POCIS can be used to sample and pre-concentrate various hydrophilic compounds including: pharmaceuticals and personal care products, pesticides, degradation products of these compounds and many other organic compounds found in water. Commercial POCIS that are available include the pharmaceutical-POCIS made up of an oasis HLB sorbent and the pesticide-POCIS which contains a polystyrene divinylbenzene combined with active carbon as the sorbent. The latter is also referred to as a "generic' POCIS that is typically made up of a mixture of three sorbents enclosed in between micro-porous membranes (Ibrahim et al. 2013, Seethapathy et al. 2008). An investigation of the uptake of pesticides and their degradation products by pharm-POCIS revealed a linear increase (r^2 > 0.7) of uptake over 15 days with an equilibrium being reached after 21 days of exposure of the device for most of the compounds over a wide range of hydrophobicity (log K_{ow}, 1.7-3.7) (Ibrahim et al. 2013). This application of the pharma-POCIS to organic pollutants demostrates potential of application to multi-residue analysis of organic compounds in water. The use of passive sampling devices was reported for pharmaceuticals and personal care products in the concentration range of 0.1 to 11 ng/L for most pharmaceuticals, up to 25 ng/L for gemfibrozil and 28 ng/L for caffeine in Lake Ontario (Helm et al. 2012). Application of an automatic sampling device triggered by rain

has been reported (Gomez et al. 2009, Xu et al. 2011) and offers additional advantages of reducing bias in sampling and obtaining a more representative sample.

Based on the mechanism of the SPMD and the POCIS, an integrated sampler can in principle be used for polar or hydrophobic compounds in water depending on the chemistry of the receiving phase. The Chemcatcher® passive sampler was developed on this principle and it can contain a C18, SDB-RPS or SDB-XC (or other chemistry) receiving phase immobilised in a disk with PTFE fibrils enclosed in a polyethersulfone, polyethylene or polypropylene membrane for use on polar organic contaminants in water (log K_{ow} 2-4) (Camilleri et al. 2012, Seethapathy et al. 2008). In the investigation of uptake kinetics of emerging pollutants including endocrine disruptors and pharmaceuticals on the polar C18 Chemcatcher® a linear accumulation (r^2 above 0.98) for 14 days peaking at 21 days (Camilleri et al. 2012) was obtained and is in agreement with other passive sampling devices such as the pharma-POCIS (Ibrahim et al. 2013).

There are some recent reports in the literature that compare various passive sampling devices and grab or discrete sampling methods (Coes et al. 2014, Kim et al. 2014, Moschet et al. 2015, Roll and Halden 2016, Vrana et al. 2016).

2.5.2 Sample preparation, purification and pre-concentration

In most cases about a one litre sample of water is collected, filtered and concentrated by a variety of pre-concentration procedures to enrich polar or nonpolar compounds. In terms of the physical-chemical processes involved, these can involve a single phase in terms of using liquids only (liquid-liquid extraction), or the method can use various phases in terms of solid-liquid interactions or even polarity differences. We summarize the various methods used for emerging organic pollutants in water samples below.

2.5.2.1 *Liquid-liquid extraction*

Liquid-liquid extraction (LLE) is well-known for having the distinct advantage of freedom from the influence of various particulates as well as elimination of the need to filter the sample. LLE has been applied for the extraction of a range of priority and emerging contaminants with recovery rates of more than 70% for most analytes (Gomez et al. 2009, Robles-Molina et al. 2013). However, the process is laborious and uses a large amount of organic solvents, which can have some implications in terms of green-chemistry or best practices concerns. Another challenge in extraction of diverse chemical compounds is the lack of a universal solvent for the elution of analytes and this problem is exacerbated in high matrix samples such as sewage effluent. Often a series of elutions have to be performed using eluents of different polarity to achieve a good recovery for all analytes, but in cases where this is not possible hexane was found to give a good recovery. Furthermore, other organic matter may be co-extracted and further attempts to remove matrix, e.g. through filtering can lead to sample loss (Robles-Molina et al. 2013). LLE therefore is usually suitable for “cleaner” samples such as river and stream water.

2.5.2.2 *Liquid micro-extraction*

Techniques or protocols that use microliter volumes of liquid (less than 100 µL or a drop) for the extraction and/or pre-concentration of analytes of interest, can be collectively termed as liquid micro-extraction (Ahmad et al. 2015, Alexovič et al. 2016, Dimpe et al. 2016, Leong et al. 2014, Spietelun et al. 2014). There are some variations in terms of how the method is implemented, and these can include the use of a single drop of solvent, the use of a hollow fibre, or the use of low or high density single or mixed solvent systems (Ahmad et al. 2015, Alexovič et al. 2016, Dimpe and Nomngongo 2016, Leong et al. 2014, Spietelun et al. 2014). The key advantages of liquid micro extraction methodologies is the reduced cost, smaller solvent volume used, smaller amount of waste produced, the methods are relatively simple to implement, short extraction times, and very high enrichment factors. However, there are some draw-backs and these are

mainly the small volumes used may result in analyte losses, solvents used may not be effective for a wide range of analytes, some solvents ideal for these methods are considered highly toxic, and loss of precision (Ahmad et al. 2015, Alexovič et al. 2016, Dimpe and Nomngongo 2016, Leong et al. 2014, Spietelun et al. 2014).

2.5.2.3 *Solid phase extraction*

Solid phase extraction (SPE) is the method of choice for pre-concentration due to the variety in the surface chemistry of the sorbents available (Andrade-Eiroa et al. 2016, Andrade-Eiroa et al. 2016). Sorbents such as C8, C18 (reverse phase), alumina, florisil (normal phase), have been used to extract analytes of interest from water. Polar SDB-XC Empore disk (Boyd et al. 2003), was used to extract pharmaceuticals and personal care products from surface and treated water. Non polar components were well recovered (> 80%) but recovery of mid polar to highly polar analytes was poor (~50-60% and 2.8-47% respectively) which probably explains why only Bisphenol A and estrone d4 were detected in the wastewater treatment plant effluent. C18 cartridges were used in extraction of surfactants from wastewater (Terzic et al. 2008) with acceptable recoveries. The use of mixed mode sorbents in multi-residue extractions has become more popular due to the wide range of polarity exhibited by emerging organic contaminants and they have been reported to result in good recoveries for multi-class residues. For example, Oasis MCX cartridge, a polymeric resin with cation exchange groups demonstrated a good recovery (~70%) for pharmaceuticals of different therapeutic classes and synthetic hormones (Al-Odaini et al. 2010). Hydrophilic-Lipophilic Balance (HLB) sorbents have been used in multi-residue extraction and their popularity is due to their unique chemistry (Andrade-Eiroa et al. 2016, Andrade-Eiroa et al. 2016, Giger 2009, Rodil et al. 2009, Terzic et al. 2008, Voutsa et al. 2006, Xu et al. 2011, Yoon et al. 2010, Zhang et al. 2008) which enables sorption and extraction of a wide range of compounds, and such systems are some of the most widely used for emerging organic pollutants and contaminants of interest (Richardson and Kimura 2016).

The use of small and handy devices has also been reported, for example, stir bar sorptive extraction (SBSE) (Dimpe and Nomngongo 2016, Nogueira 2015, Płotka-Wasylka et al. 2015) and solid-phase micro-extraction (SPME) (Dimpe and Nomngongo 2016, Gilart et al. 2014, Nogueira 2015, Płotka-Wasylka et al. 2015, Richardson and Kimura 2016) have been used in pre-concentration of PPCPs, PAHs and pesticides. The use of PDMS-coated stir-bars was reported in SBSE of priority organic pollutants. More than 75% recovery was achieved for non-polar and mid polarity compounds however the recovery was low for highly polar compounds (Gomez et al. 2011). Thin film solid phase micro-extraction (TF-SPME) was reported to provide better extraction efficiency compared to conventional SPE (Togunde et al. 2012).

2.5.2.4 *Perspectives on sample preparation, purification and pre-concentration*

Current trends are towards online sample enrichment methods before analysis including online head space extraction, large volume injection (LVI) (Gomez et al. 2009), etc. which not only reduce the total analysis time but also reduce interference and increase selectivity of the method. In LVI, a large volume (several microliters up to a few millilitres) of a filtrated or centrifuged sample is injected directly into a chromatographic column without pre-concentration. Detection limits are better due to large sample volumes used however introduction of large sample amounts may reduce method performance due to matrix effects in samples with high matrix load. On-line SPE offers sample enrichment as well as clean up reducing the matrix effects (Huntscha, et al. 2012) because of availability of sorbents with a range of chemistries. A multi-bed (multi-chemistry) SPE cartridge was used for a simultaneous, automated analysis of various pharmaceuticals, pesticides, biocides, corrosion inhibitors, an artificial sweetener and several of their transformation products in surface, ground and wastewater. More than 80% of the compounds were quantified to 10 ng/L, demonstrating good method performance in the determination of 88 polar organic compounds (Huntscha et al. 2012).

An interesting and growing area is the development of new sorbent materials that take advantage of recent developments in nanoscience and nanotechnology. In this regard, carbon nanotubes, monolithic mesoporous materials, nanofibers, and core-shell materials offer some alternative more efficient means for preparing samples. This nascent but growing area of research was recently reviewed (Dimpe and Nomngongo 2016, Liu et al. 2014).

2.5.3 Analytical techniques for the separation, analysis, identification, and quantification of emerging organic pollutants

There are a wide range of analytical techniques available for the separation, analysis, identification, and quantification of emerging organic pollutants that have been extracted and purified from an environmental matrix. Multi-residue techniques are an excellent way to detect emerging contaminants in water ways. The techniques developed look at either different forms of a specific class of compounds, or different classes of compounds. However, there are very few examples were this technique has been applied in South Africa. The study by Ncube et al. (2012) is one notable example and some of the data from that published paper is presented in Table 2.1.

In terms of identifying unknown compounds high resolution mass spectrometry is the method of choice (Bletsou et al. 2015, Hernández et al. 2014, Padrón et al. 2014, Richardson and Kimura 2016, Rykowska and Wasiak 2015). However, nuclear magnetic resonance spectroscopy can be used, and has been successfully utilized to identify various unknown organic pollutants (Gonsior et al. 2014, Masoom et al. 2015, Richardson and Kimura 2016, van Leerdam et al. 2014). In addition, there are several instrumental techniques, often used in tandem, for the separation, identification, and quantification of the analytes. In addition, several new technologies offer cheaper and easier routes to analyse for known analytes (Ballesteros-Gómez and Rubio 2011, Richardson 2011, Richardson and Ternes 2011).

2.5.3.1 *Biosensor based methods*

In general biosensors offer a cheap and relatively easy way to monitor for specific organic pollutants. These systems can be used to detect and quantify specific analytes (Díaz-González et al. 2016, Liu et al. 2013, Rao et al. 2014, Van Dyk and Pletschke 2011), and this is the main limitation when applying such systems to a project like this one. These systems have been used very effectively on known compounds (Díaz-González et al. 2016, Liu et al. 2013, Rao et al. 2014, Van Dyk and Pletschke 2011); however, when looking at unknowns, such systems are not suitable, since the design of the functional moiety within the sensor relies upon some knowledge of the structure of the analyte of interest (Ballesteros-Gómez and Rubio 2011, Richardson 2011, Richardson and Ternes 2011). But it should be noted, this would probably be one of the more prospective technologies to develop for monitoring river water for various organic pollutants. The advantages and disadvantages of enzymatic based biosensors and the role they could play in monitoring rivers for specific organic contaminants and pollutants has been reviewed recently (Díaz-González et al. 2016, Liu et al. 2014, Van Dyk and Pletschke 2011).

(Nomngongo et al. (2012), used an amperometric biosensor to detect polychlorinated biphenyls from landfill leachate samples collected from the Mariannhill Landfill outside Durban, and determined the landfill as a source of emerging pollutants. Occurrence of steroid hormones in a South African wastewater treatment plant was investigated using enzyme linked immune sorbent assay (ELISA) method with detection limits of 0.2-5 ng/L for estrone, 17-β-estradiol, 17-α-ethinylestradiol, estriol, progestrone and testestrone (Manickum and John 2014, 2015). In this study, steroid hormones occurred in both the influent and effluent of a wastewater treatment plant in high concentration (23-408 ng/L). Progesterone and testosterone were highest and estrone was the lowest percentage in the influent. While the occurrence of endocrine disrupting hormones has been widely reported in other parts of the world (Al-Odaini et al. 2010, Camilleri et al. 2012, Yoon et al. 2010, Yu and Wu 2012) this study is one of the few done in South Africa and it demonstrated the

presence of endocrine disruptors, a group of emerging contaminants of concern, in the KwaZulu-Natal water systems (Manickum and John 2014, 2015). There is therefore a need to investigate whether these and other emerging pollutants occur in other water bodies in the region especially those from which water for public use is generated. Furthermore, their transport and fate downstream must be investigated.

Truter et al. recently reported on steroid estrogen concentrations from several sampling sites along the upper Olifants River (Truter et al. 2016). Using ELISA methodologies, they determined that the concentration of 17β-estradiol and 17α-ethinylestradiol varied from 0.72-30.8 ng/L and 0.45-10.83 ng/L respectively. Although some sites had higher levels than what has been reported in the literature, they noted that this could be attributed to farm animals treated with growth promotors. In addition, they found that wastewater treatment plants were a major source of contamination. POCIS in combination with an immunochemical ELISA technique and HPLC/ MS was used in the study of distribution of sulfonamides in streams and wastewater in the Czech Republic (Cernoch et al. 2012). As expected the concentration of sulfonamides was higher in wastewater compared to streams (> 8000 ng/L SMX waste water, < 20 up to 736 ng/L in streams). In general, significant correlations were detected between ELISA results and LC/MS methods however ELISA tended to overestimate the SMX concentrations. This is probably because of ELISA's limited selectivity as it responds to all sulfonamide residues. The use of ELISA therefore serves as a screen for the presence of sulfonamide residues.

2.5.3.2 *Instrument-based methods for identification and quantitation*

Gas chromatography (GC) and liquid chromatography (LC) are the most common separation methods used in analysis of emerging and priority organic compounds, and the use of capillary electrophoresis (CE) has also been reported (Ballesteros-Gómez and Rubio 2011, Giger 2009, Richardson 2011, Richardson and Ternes 2011).

Gas chromatography

While GC is a good technique, it is usually applied to nonpolar and mid polar volatile compounds unless a derivatisation step is included (Caban et al. 2014, Jiang et al. 2014). There have been some recent reviews on the use of GC-MS for the determination of emerging organic pollutants/contaminants within environmental matrices (Białk-Bielińska et al. 2016, Locatelli et al. 2016, Vallecillos et al. 2015). The use of GC-MS for determination of various organic pollutants has been reported. Pesticides, pharmaceuticals, personal care products, phenolic oestrogens, antioxidants and disinfection by-products, were detected in water and river water in µg/L concentrations (Arukwe et al. 2012, Białk-Bielińska et al. 2016, Calderon-Preciado et al. 2011, Locatelli et al. 2016, Richardson 2011, Richardson and Kimura 2016, Richardson and Ternes 2011, Vallecillos et al. 2015). GC methods for priority and emerging pollutants in water and river water samples with good detection limits (4 to 66 ng/L) and precision (Gomez et al. 2009) for pesticides and pharmaceuticals in wastewater have also been reported (Robles-Molina et al. 2013). Terzic et al. (2008) determined synthetic polycyclic musks, organic flame retardants, pesticides, herbicides and their metabolites using different GC-MS methods. All these compounds were in ng/L to µg/L concentration in wastewater. Organic priority pollutants in sewage treatment plant effluents were determined using gas chromatography high-resolution mass spectrometry in Spain (Robles-Molina et al. 2010). Multi-class organic contaminants in wastewater samples were also determined using gas chromatography coupled to triple quadrupole mass spectrometry. The use of MRM enabled determination of priority organic compounds at low ng/L range (below 3 ng/L) (Robles-Molina et al. 2013).

Finally, we note that there have been some recent publications that report on the use of new derivatization agents for use in GC-MS methods for the determination of pharmaceutical residues in drinking water (Caban et al. 2015). In addition, new methods that validate and quantify emerging contaminants using GC-MS methodologies have recently been reported (Zhang et al. 2016). These few examples are indicative of the

continuing interest in GC-MS for emerging pollutants/contaminants, and may provide a means for developing economies to monitor indicative compounds using simpler instrumentation.

Liquid chromatography

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is a very sensitive technique for multi-residue determinations. It has been used to determine 23 pharmaceuticals and synthetic hormones from different therapeutic classes in different types of water with good limits of detection (0.2-281 ng/L) (Al-Odaini et al. 2010), pharmaceuticals (µg/L) and surfactants (mg/L) (Terzic et al. 2008). The use of LC-MS/MS for determination of emerging organic pollutants in wastewater, surface and tap water has been reported with wastewater containing relatively high concentrations of the analytes; for example ibuprofen a non-steroidal anti-inflammatory drug was found in concentrations of up to 10 µg/L (Rodil et al. 2009). As shown here, tandem mass spectrometry offers better detection limits and is therefore more suitable for multi-residue determination of trace organic contaminants of concern.

A recent review by Gosetti et al. (2016), highlights the critical role high resolution mass spectrometers coupled to liquid chromatography have had in non-target analysis of new organic compounds in environmental samples. This is also supported by the biennial review done by Richardson and Kimura (2016). In the review by Gosetti et al. (2016), an introduction to the limits and benefits of various low resolution systems such as hybrid triple quadrupole/ion trap, tridimensional ion trap linear ion trap, and the triple quadrupole mass analysers (coupled to an LC system) is provided. In addition, details on the advantages and limitations of high resolution systems such as time of flight (TOF), hybrid quadrupole/time-of-flight, fourier transform ion cyclotron resonance, Orbitrap, and the hybrid ion trap/orbitrap are contrasted. Besides cost of acquisition and running the various systems, the suitability of interfacing the various instruments with UPLC (Ultra high pressure liquid chromatography), advances in ion fragmentation experiments, accuracy and sensitivity, and use of data mining and analysis of data months to years after running a sample is discussed.

Another comprehensive and recent review in the literature was by Leendert et al. (2015), on the use of HPLC and UPLC systems coupled to high resolution mass spectrometers (TOF or Orbitrap systems) for multi-residue analysis of organic pollutants in water environments. The review includes information on 35 validated methods for the quantification of emerging pollutants and 21 screening methods. The biennial review by Richardson and Kimura (2016) summarizes recent trends in emerging pollutant research with water and highlights recent developments concerning instrumentation, such as LC-MS, as well as new organic micro-pollutants identified or trending in the literature. Finally, an excellent tutorial review on validating LC-MS methods is provided by Kruve et al. (2015) parts I and II.

Capillary electrophoresis (CE)

A CE method with a contactless conductivity detector was developed for determination of a mixture of pharmaceuticals containing acidic, basic and neutral drugs. For this class of emerging pollutants limits of detection ranging from 61 to 1676 µg/L were obtained (Quek et al. 2008). This limit of detection is much higher than those typically obtained using LC/MS or GC/MS however capillary electrophoresis has the advantages of simplicity, shorter analysis time and low solvent consumption. The use of pre-concentration techniques to improve detection limit in CE has been reported. These methods may be off line but online methods are preferred due the advantage of reducing total analysis time. Transient isotachophoresis (t-ITP) together with field enhancement was employed to improve the detection limit in the simultaneous separation and determination of oestrogens to achieve detection limits of 0.07-0.11 nmol/L (Li et al. 2013) demonstrating the possibility of application of this method to water samples where concentrations of such pollutants is usually trace. Oestrogens were detected in raw water samples at water works in nmol/L concentration.

2.5.3.3 *Onsite methods*

On-site analysis is suitable when there is a need for rapid identification or determination of pollutants, for example, in cases of accidental spills so as to take the necessary remedial action to protect the environment. Compared to the conventional sampling and transfer of sample to laboratory for analysis, all measurements are done on site using portable equipment/set ups. Brogat et al. (2013) developed a rapid, field based detection method for micro-pollutants in water. The method uses an automatic multiple solid phase extraction step (MSPE) followed by direct or indirect UV spectrometry (MSPE/UV). The method exhibited detection limits of 5 µg/L to 40 µg/L for the emerging contaminants that were tested. This demonstrates potential for method application in rapid screening after accidental spills.

2.6 SUMMARY

Current trends in analytical method development are towards development of rapid techniques with high resolving power, lower solvent consumption and minimizing sample manipulation. Developments in LC-tandem MS have greatly improved detection limits and identification of multiple residues in complex matrices. TOF, QqTOF and Orbitrap have proved very useful in structure determination and identification of compounds. Continuous monitoring is needed to determine environmental concentrations, transport and fate of emerging pollutants in the environment and to understand potential toxicity of mixtures; some compounds such as polycyclic musks have the ability to bioaccumulate to high levels but also chronic toxicity studies need to be conducted to understand potential effects and risks of long term exposure to low concentrations of these analytes in the environment. There is a need to develop rapid analytical methods, which are able to determine the transformation products of these compounds, as well as simple, low-cost, effective and eco-friendly sample treatment methods.

CHAPTER 3: OPTIMISATION AND VALIDATION OF METHODS FOR DETERMINATION OF SELECTED EMERGING ORGANIC CONTAMINANTS

3.1 INTRODUCTION

Water pollution by organic contaminants/pollutants has been an issue of environmental concern for a while now however there has been increasing concern about persistent organic pollutants, and emerging organic contaminants such as pharmaceuticals, personal care products, hormones and other endocrine disrupting compounds which have been detected in various water bodies in recent years (Lapworth et al. 2012, Richardson and Ternes 2014, Meffe and de Bustamante 2014, Löffler and Ternes 2003). The concern about possible undesirable effects to humans and/or aquatic life as a result of exposure to water contaminated by these compounds necessitates determination of these compounds in key water bodies. Emerging contaminants have been detected in various parts of the world including countries on the African continent and now research in South Africa is also starting to generate data regarding organic pollutants. The consumption of certain products (pharmaceuticals based on impact data, (2013) is quite high in South Africa, moreover some lifestyle practices demonstrated here such as informal living settlements and poor waste disposal may lead to emerging contaminants accessing our water systems. Therefore, determination of emerging compounds as well as regular monitoring is important. This section describes the optimization and validation of methods for determination of selected EOCs in samples collected from the Umgeni and Msunduzi Rivers and surrounding catchment.

3.2 DESCRIPTION OF STUDY SITES

3.2.1 The Umgeni River

The Umgeni River is one of the major rivers in KwaZulu-Natal, spanning a distance of 225 km from the source to the mouth with a total surface area of 4416 km^2 (Groundspeak, 2013). It has it source in the Drakensberg mountains meandering through various rural, urban, agricultural and industrial areas. There are four main dams along the path of this river and a number of tributaries join it adding to the pollution load along with the Northern Wastewater Treatment plant which empties its treated water back into the Umgeni River close to the mouth. In addition, there are a number of informal settlements along the Umgeni River which may also contribute to the pollution in the river. Thus the various activities along this river make it an interesting site for investigating the qualitative and quantitative presence of organic pollutants. Figure 3.1 shows some photographs taken during the sampling trips to highlight the range of activities.

3.2.2 The Msunduzi River

The Msunduzi River in Pietermaritzburg is one of the main tributaries of the Umgeni River and hence was chosen as a suitable river to investigate in order to determine if its pollution contributes to the Umgeni River. The Msunduzi River passes through highly industrialized areas, receives runoff from rural communities and the municipality along its course as well as agricultural areas and treated discharge from the Darvill Wastewater Treatment Plant, which all contribute to the levels of organic pollution in the river. Figure 3.2 shows some of the surrounding areas of the Msunduzi River.

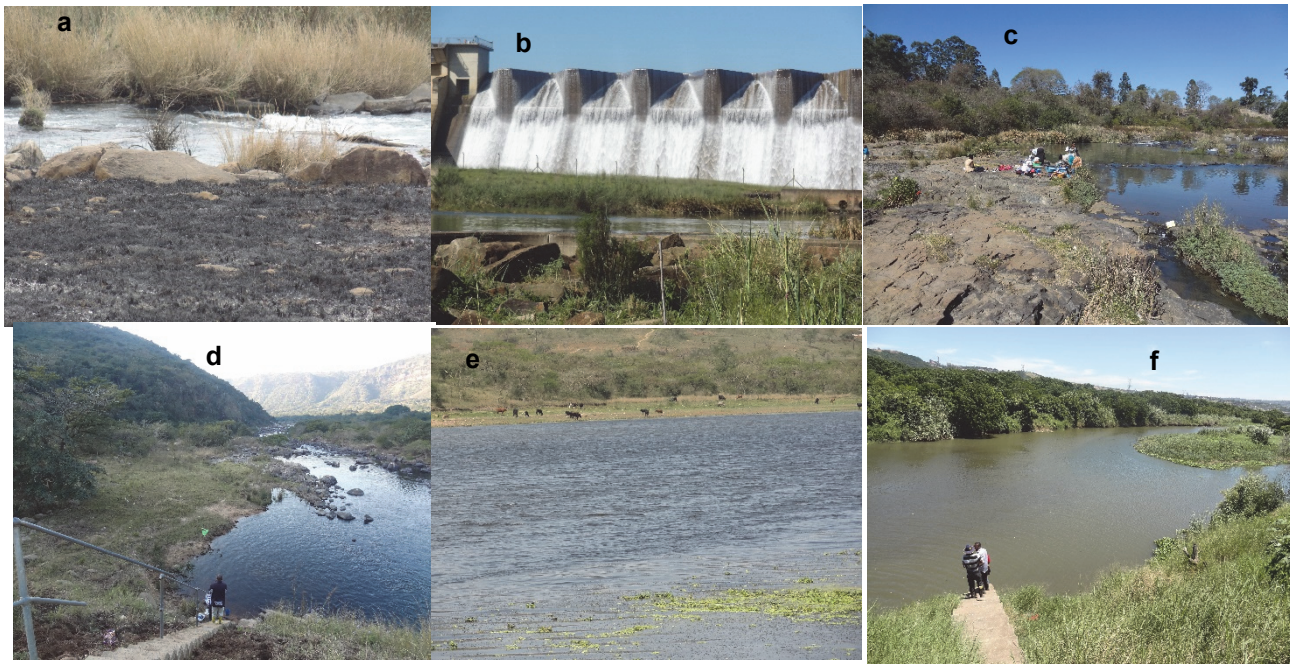


Figure 3.1 Sites along the Umgeni River showing the various activites (a) Midmar dam inlet showing the burning of grass along the banks of the dam, (b) Midmar dam outlet, (c) women washing clothes at the top of the Howick Falls site, (d) Inanda dam outlet, (e) cattle grazing on the banks of the Nagel dam and (f) joining of the Umhlangane River to the Umgeni River at the exit pipe of the Northern Wastewater Treatment Plant effluent into the Umgeni River

Figure 3.2 Sites along the Msunduzi River (a) near Henley dam in a semi-rural area, (b) Camps Drift with the weir in the background and (c) Msunduzi town showing animals grazing on the banks of the river

3.3 SAMPLING

3.3.1 Sampling technique

In this study, grab sampling was used entirely throughout the project. An overview on practical considerations regarding sampling is given in the references (David and Tammy 2010, Gerbersdorf et al. 2015), and we refer the reader to our earlier comments on spot sampling and the references (Calderon-Preciado et al. 2011, Jones et al. 2015, Petrie et al. 2015, Snyder 2010, Terzic et al. 2008, Voutsa et al. 2006, Yoon et al. 2010).

3.3.2 Sampling sites

Initially, the sample sites were selected based on information gathered using the open literature, various reports, books, thesis and maps (Google maps), which is the typical background research one does before embarking on a sampling campaign. During the actual sampling trips made, sample sites changed due to factors that couldn't be accounted for during planning; such as accessibility, and cultural misunderstandings. Accessibility factors included the geographical location of the pre-chosen sites, either there were no obvious foot paths or animal trails that led to the river, or that part of the river may have been within a ravine or some other type of geographical feature made it difficult to access the river. Also, crocodile attacks or sightings are rare along the Msunduzi and Umgeni Rivers, but as an added precaution the number of isolated sampling sites were limited. Cultural misunderstandings in this case simply means some locations were either close too or within villages or hamlets and the local community were extremely weary of why we wanted to take the water from 'their' river. Personnel safety was also taken into account during the actual sampling trips which included the use of proper protective equipment and supplies (waders, gum boats, gloves, disinfectant, etc.), use of a 4-wheel drive vehicle (bakkie/pick-up truck), and GPS enabled device with updated maps and software.

3.3.2.1 *Umgeni River sampling points*

Fifteen sampling sites were chosen along the Umgeni River based on activities in the area around them such as agricultural activities, industrial activities or residential area and some sites were in an area where there was a combination of all these activities. These sampling stations include 12 sites chosen along the river from the source in Midmar dam to the mouth where the river empties into the Indian Ocean at Blue Lagoon; as well as 3 sites around the Northern Wastewater Treatment Works which empties its treated water into the Umgeni River (Figure 3.3). Apart from these activities, sites were also chosen based on their accessibility in order to be able to collect a desired representative sample (Table 3.1).

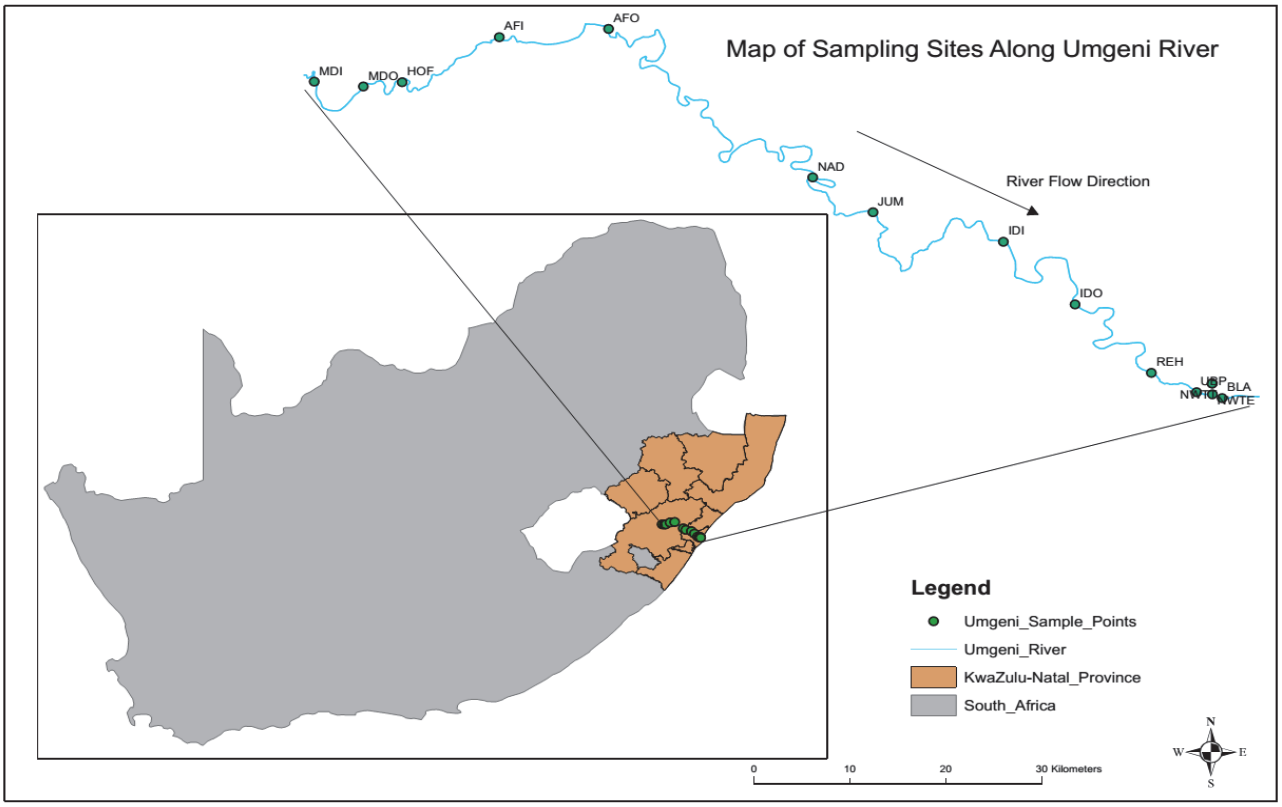


Figure 3.3 Map showing the position of the selected sampling sites along the Umgeni River in Durban, South Africa.

Table 3.1 Coordinates of the sampling sites and the physical parameters of the Umgeni River sampling sites during winter 2013

No	Sampling site	Temp. (°C)	Water Temp. (°C)	pH	Conductivity (µs/cm)	TDS (mg/L)	Coordinates	
							South	East
1	Midmar dam inlet (MDI)	12.3	11.6	5.54	83.7	49	29 29′ 16.05"S	30 09' 23.10"E
2	Midmar dam outlet (MDO)	12.3	13.2	5.69	75.5	44	29 29' 34.02"S	30 12' 09.13"E
3	Howick Falls (HOF)	17.8	13.8	5.99	89.7	53	29 29' 18.18"S	30 14' 19.70"E
4	Albert Falls inlet (AFI)	18.6	13.5	5.78	111.5	65	29 26' 31.94"S	30 19 47.10"E
5	Albert Falls outlet (AFO)	19.2	15.4	6.04	93.8	55	29 26' 01.81"S	30 25' 55.76"E
6	Nagle dam (NAD)	18.4	15.4	5.00	114.0	66	29 35' 08.42"S	30 37' 23.94"E
7	Joining point Umgeni-Msunduzi Rivers (JUM)	15.6	15.7	5.56	367.0	214	29 37' 16.61"S	30 40' 46.59"E
8	Inanda dam inlet (IDI	17.2	16.6	4.98	278.0	160	29 39' 05.20"S	30 48' 06.24"E
9	Inanda dam outlet (IDO)	15.1	15.9	4.53	257.0	149	29 42' 55.74"S	30 52' 07.69"E
10	Reservoir Hills (REH)	21.4	17.9	5.63	305.0	176	29 47' 08.05"S	30 56' 25.51"E
11	Umgeni business park (UBP)	21.4	17.6	4.90	334.0	194	29 48' 19.05"S	30 58' 58.08"E
12	Northern wastewater treatment works influent (NWTI)	22.8	21.9	4.70	970.0	568	29 48' 27.01"S	30 59' 51.05"E
13	Northern wastewater treatment works after treatment (NWTT)	19.8	19.9	4.64	1238	719	29 47' 47.02"S	30 59' 51.01"E
14	Northern wastewater treatment works effluent (NWTE)	21.0	19.8	4.94	674	392	29 47' 47.08"S	30 59' 50.06"E
15	Blue Lagoon (BLA)	21.4	20.0	5.12	-	-	29 48' 41.03"S	31 02' 12.05"E

3.3.2.2 *Msunduzi River sampling points*

Bearing these characteristics in mind we purposively selected ten sampling sites along the Msunduzi River in order to represent various anthropogenic activities such as domestic, industrial, agricultural and municipal activities taking place along the river as shown in Figure 3.4. Coordinates of the sampling sites and the physical parameters of the Msunduzi River sampling sites are shown in Table 3.2.

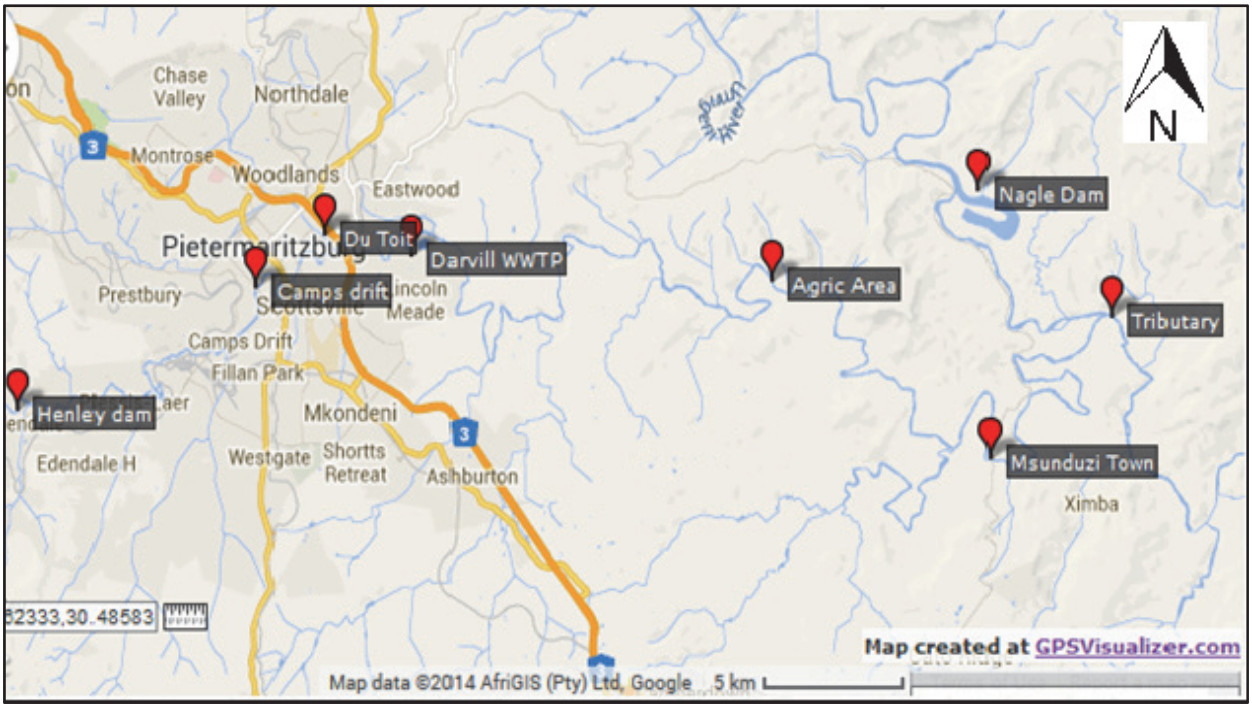


Figure 3.4 Map showing the sampling points along the Msunduzi River Catchment, Pietermaritzburg, South Africa

Table 3.2 Coordinates of the sampling sites and the physical parameters of the Msunduzi River sampling sites during winter 2013

No.	Sampling Site	Temp (°C)	Water Temp. (°C)	pH	Coordinates	
					South	East
1	Henley dam (HND)	21	14	6.21	29° 38'51"S	30° 17'32"E
2	Camps Drift (CMD)	16	21	5.15	29° 36'47"S	30° 22'36"E
3	Du Toit (DUT)	23	15	7.20	29° 35'52"S	30° 24'01"E
4	Darvill WWT inlet (WWT 1)	25	19	5.31	29° 36'15"S	30° 25'52"E
5	Darvill WWT effluent (WWT 2)	24	19	5.54	29° 36'15"S	30° 25'52"E
6	Darvill WWT discharge (WWT 3)	*	*	*	29° 36'15"S	30° 25'52"E
7	Agricultural area (AGA)	19	14	5.12	29° 36'40"S	30° 33'32"E
8	Msunduzi Town (MST)	20	15	4.77	29° 39'40"S	30° 38'10"E
9	Nagle dam (NGD)	18	15	5.56	29° 37'08"S	30° 09'30"E
10	Umgeni/Msunduzi joining point (UMJ)	15	15	5.07	29° 37'16"S	30° 40'46"E

*= Samples were not collected due to major renovation at that particular sampling point during the sampling period.

3.3.3 Sample collection

Soil, sediment and water samples were collected along the course of both rivers from 2013 to 2014 during the autumn, winter, spring and summer sampling seasons.

3.3.3.1 *Water samples*

Water samples were collected in 2.5 L amber Winchester bottles previously washed with hot water and detergent and rinsed three times respectively with deionised water and sulfuric acid. At the site, the bottles were rinsed 3 times with the river water to be sampled. The bottles were filled to overflowing leaving no headspace. Bottle caps were lined with aluminium foil to prevent contamination with phthalates and plasticisers from the lids. All the samples were kept in a cooler box containing ice and transported to the lab. A 1 mL aliquot of H_2SO_4 (50%) was transferred to each water sample for the purpose of preservation and the samples were stored in a fridge at 4°C until extraction.

3.3.3.2 *Sediment samples*

Sediment samples were collected using a grab sampler and were stored in glass bottles with the caps lined with aluminium foil. The bottles were then kept in a coolant box containing ice and were transported to the laboratory and stored in the fridge at 4°C. For the analysis of pore water, sediment samples were subsequently centrifuged to extract its pore water which was also acidified and kept in a fridge until extraction. After removal of pore water, the sediment was immediately transferred to a piece of foil for air drying.

3.3.3.3 *Soil samples*

Soil samples were collected from the banks of the Umgeni River close to the water edge at each sampling site. A metal spade or auger was used to transfer soil samples to glass bottles. The bottle caps were lined with aluminium foil and the closed samples were kept in a cooler box containing ice before being transported to the laboratory. At the lab, the soil samples were immediately transferred onto aluminium foil and exposed to air at room temperature for drying. The dried soil and sediment samples were sieved with a 75 µm, 300 µm, and 450 µm stainless steel sieves and stored in the fridge prior to extraction. All extracts and solid samples were store at 4°C and were extracted within a week of sample collection.

3.4 SELECTION OF EMERGING ORGANIC CONTAMINANTS

This study only focused on the determination of polychlorinated biphenyls (PCB) congeners, organochlorine pesticides (OCPs), pharmaceuticals and personal care products (PPCPs), including musk type compounds.

3.4.1 Pharmaceuticals and personal care products (PPCPs)

PPCPs are widely used and have been detected in various water bodies world-wide, however there is little information regarding their presence in South African water bodies. They are regarded as chemicals of emerging concern because of health risks associated with their exposure both to aquatic life and humans when they reach drinking water (Kümmerer 2010, 2009, Deblonde et al. 2011). The specific types of PPCPs found in water sources can differ between countries or regions depending on social, cultural, technological and agricultural factors. Urban and rural areas may exhibit important differences in the occurrence and concentrations of these chemicals as a result of different usage patterns. The physical and chemical characteristics of source waters can also affect the occurrence levels of pharmaceuticals by

influencing their natural degradation (Dehghani et al. 2011). Eleven pharmaceuticals were selected for analysis and monitoring using liquid chromatography-mass spectrometry (LC-MS). These were caffeine (CAF), acetaminophen (ACE), trimethoprim (TMP), sulfamethoxazole (SMX), erythromycin (ERY), clozapine (CLO), carbamazepine (CBZ), sulfamethazine (SMZ), aspirin (ASP), metronidazole (MET), and ibuprofen (IBU) (Figure 3.5).

In addition, a new method was developed for the analysis of derivatised pharmaceuticals using gas chromatography (GC-MS) (Figure 3.6). The pharmaceuticals analysed with the GC-MS method were salicylic acid, acetyl salicylic acid, nalidixic acid, ibuprofen, phenacetin, naproxen, meclofenamic, ketoprofen and diclofenac (Figure 3.6).

The selected pharmaceutical drugs of various therapeutic classes were mainly chosen because of their persistent detection in water bodies world-wide as seen from the literature, high annual consumption in South Africa, and their reported adverse effects. The annual consumption of drugs was taken from the database of South Africa (SA) ImpactRx Data Management Ltd whose main aim is to report the usage of drugs for the pharmaceutical industry (ImpactRx).

Personal care products (polycyclic musks), namely, galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK) (Figure 3.7) were also investigated as these are considered emerging pollutants and limited studies show they have the potential to be endocrine disruptors as well as cause various illnesses in humans and aquatic life over prolonged exposure. These products are found in perfumes, fabric softeners, and other personal care products and make their way into rivers and aquatic bodies from poor sewage systems, informal settlements along the river and even from wastewater treatment plants whose processes are not designed to remove these musks. There is also limited information on their presence in the environment and therefore were pollutants that were considered suitable for monitoring.

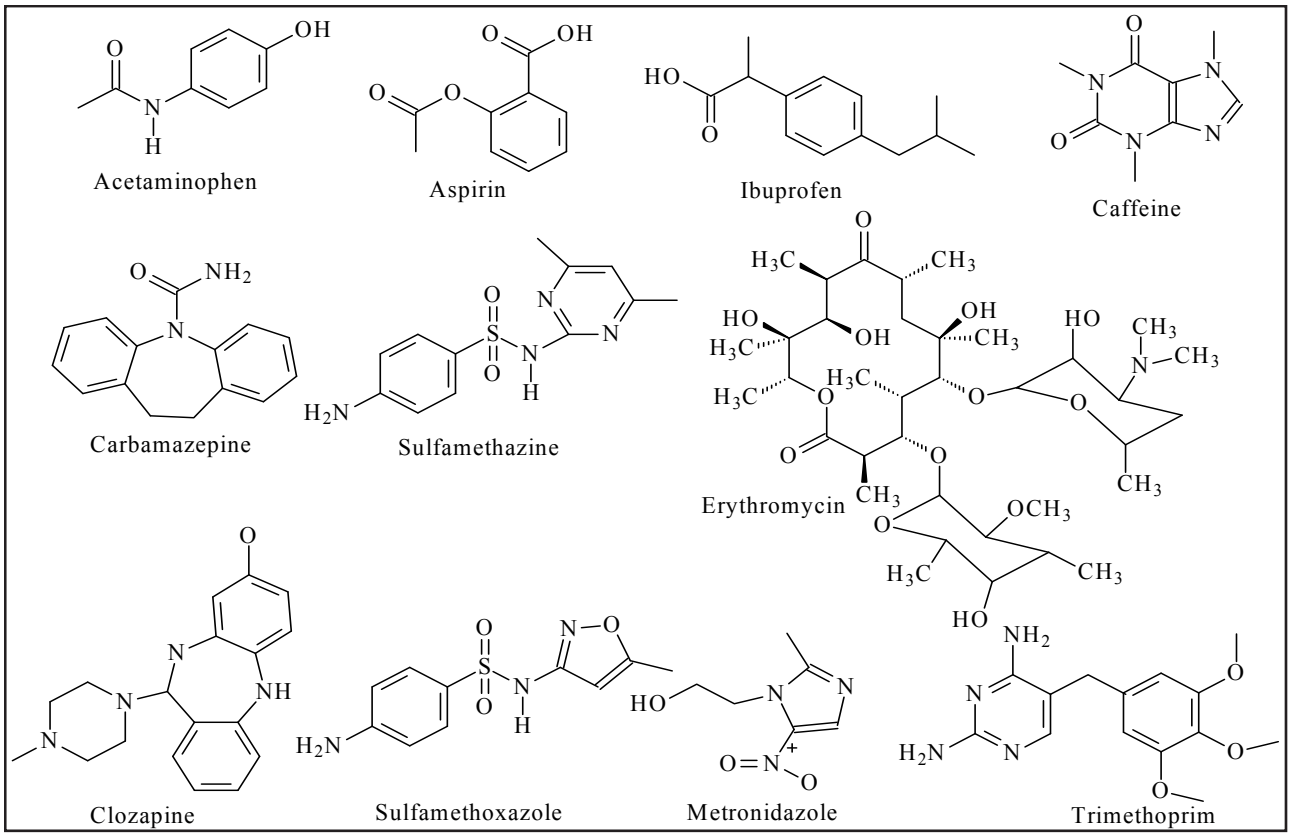


Figure 3.5 Chemical structures of selected PPCPs analysed using LC-MS

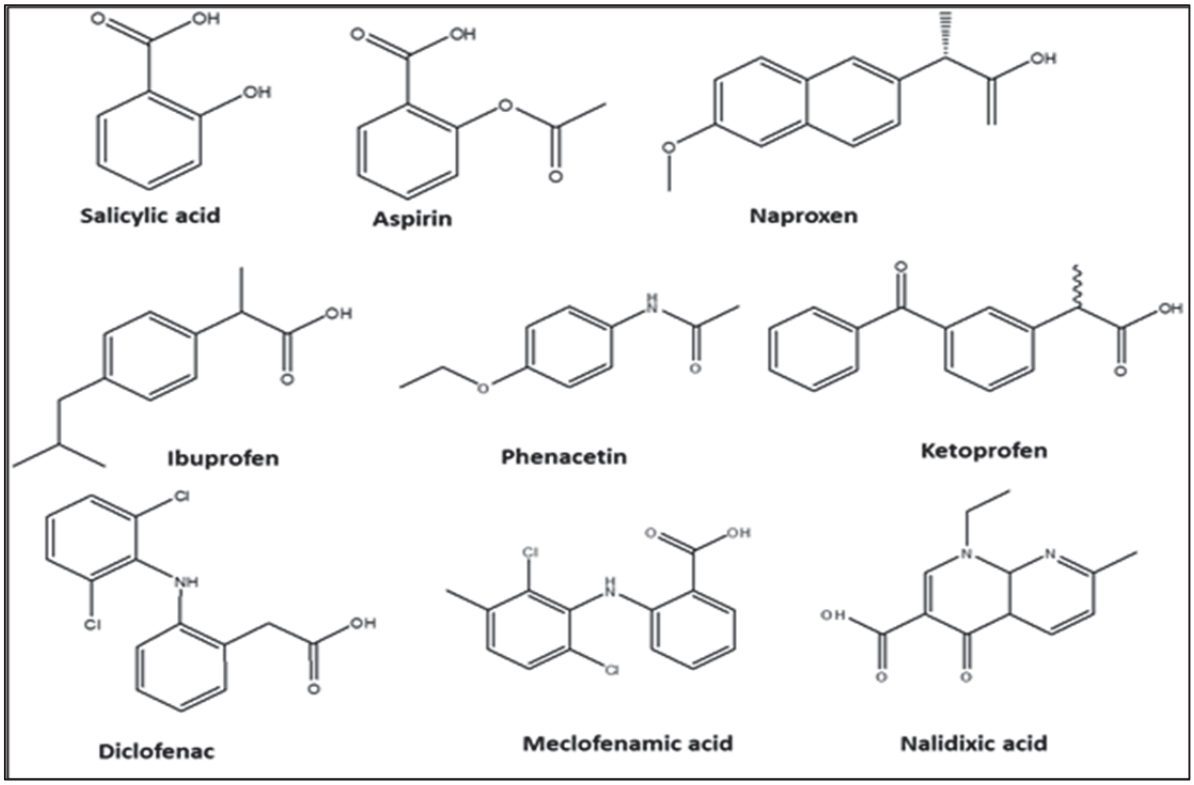


Figure 3.6 Chemical structures of the selected PPCPs analysed using GC-MS

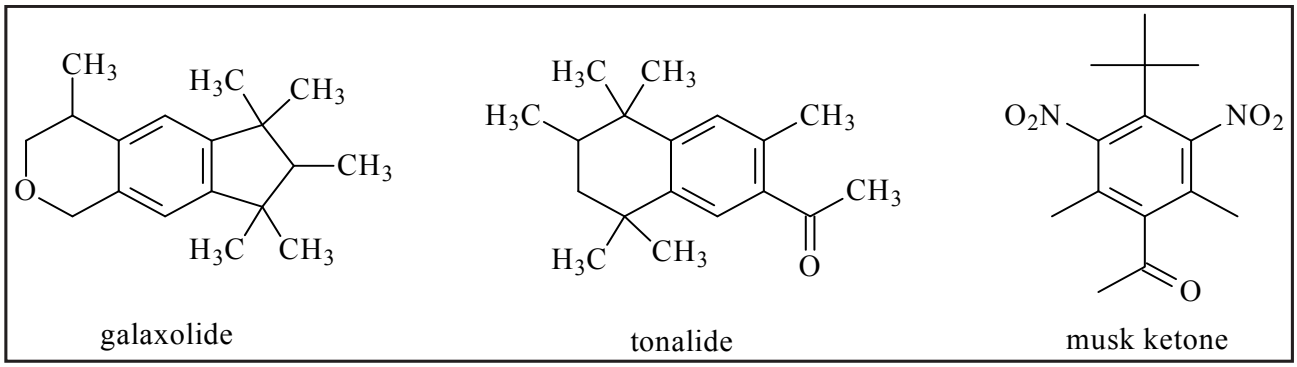


Figure 3.7 Chemical structures of selected musk compounds

3.4.2 Organochlorine pesticides (OCPs),

OCPs are chemicals designed to combat, prevent, or control the attack of various pests and vectors on agricultural crops, domestic animals and human beings. They are toxic organic chemical agents that are intentionally released into the environment to alleviate the spread of pests and vector diseases. Even though they have been banned in many countries around the world they are still present in the environment due to their resistance to chemical, photochemical and biochemical degradation. In addition, they are used in a restricted manor in some countries such as South Africa for the control of malaria. In this study, 13 OCPs, namely hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), heptachlor, aldrin, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, endrin, dieldrin and mirex were selected. These OCPs were chosen because they are recommended by the EPA and the Stockholm Convention for analysis and monitoring of environmental organic pollution (UNEP, 2005a). Apart from HCH and HCB, all the other selected OCPs are included in the dirty dozen (UNEP, 2001). Figure 3.8 shows the structures of the OCPs studied.

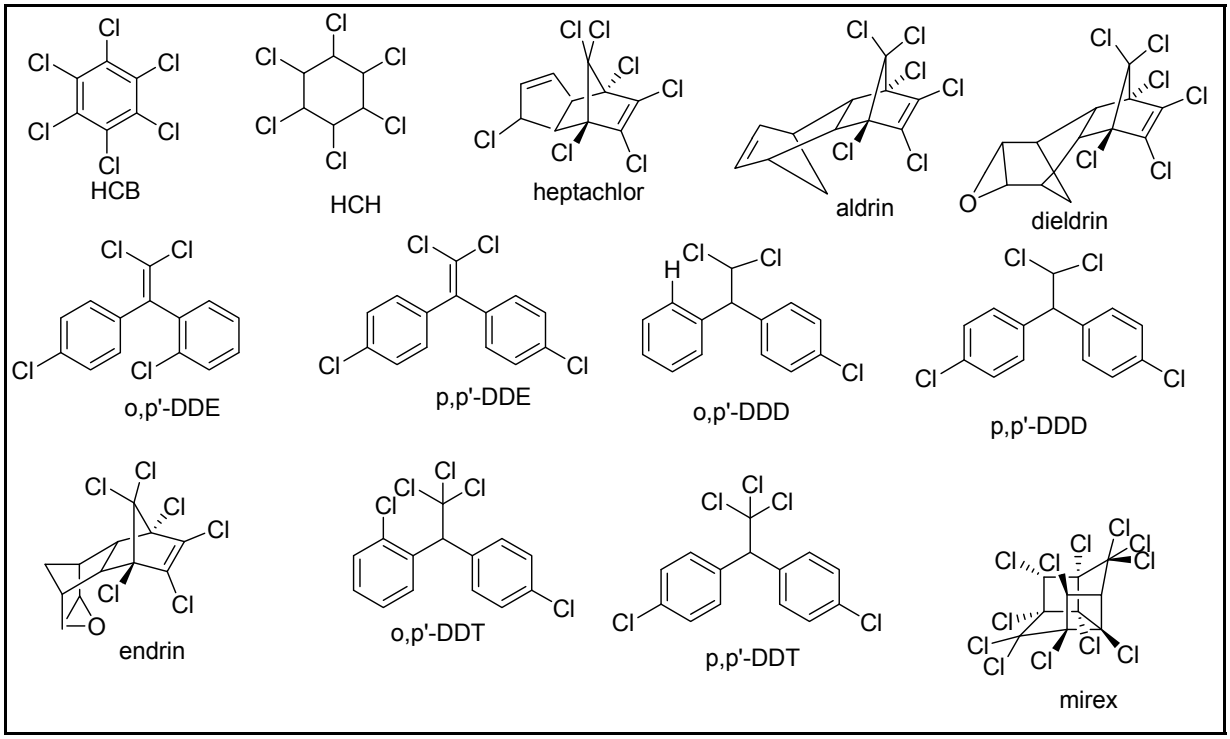


Figure 3.8 Chemical structures of selected OCPs studied

3.4.3 Polychlorinated biphenyls (PCBs)

PCBs are traditionally widely used in transformers, capacitors, hydraulic fluids and as plasticizers in paints, plastics and sealants. These compounds are on the priority list of organic pollutants because even though their production has reduced or even been stopped in some countries due to their environmental impact, they are persistent pollutants and are known to bioaccumulate in the environment from their previous usage. For this study, 8 PCBs; PCB 28, PCB 52, PCB 77, PCB 101, PCB 105, PCB 138, PCB 153 and PCB 180 were selected. With the exception of PCB 77 and PCB 105, these PCBs were selected because they are indicator PCBs and are recommended by the European Union for assessing PCB pollution (EC, 1999, EFSA, 2010). PCB 77 and PCB 105 were included because they are among the most toxic dioxin-like PCBs and are recommended by the World Health Organization (WHO) for monitoring (Moysich, 2015, WHO, 2003b). Figure 3.9 shows the structures of the PCBs studied.

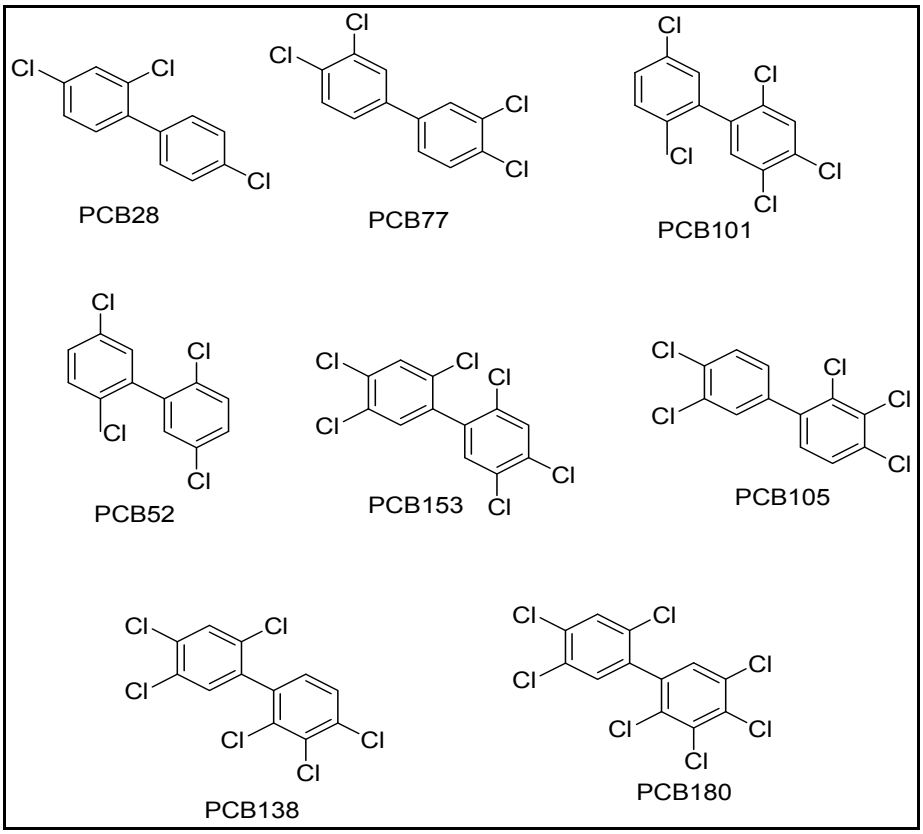


Figure 3.9 Chemical structures of selected PCBs studied

3.5 REAGENTS AND PREPARATION OF STANDARD SOLUTIONS

This section outlines all reagents used, and preparation methods adapted or used for the preparation of the standard solutions used. Where necessary, equations or methods of statistical analysis is provided.

3.5.1 Materials

All reagents and chemicals were of HPLC- grade. Eight PCB standards and 13 OCPs were purchased from Sigma Aldrich™ South Africa. The PCBs were PCB 28, PCB 52, PCB 77, PCB 101, PCB 105, PCB 138, PCB 153 and PCB 180, and the OCPs were HCB, HCH (lindane), aldrin, heptachlor, dieldrin, endrin, mirex and DDT with its metabolites *o,p*-DDE, *p,p*-DDE, *o,p*-DDD, *p,p*-DDD, *o,p*-DDT and *p,p*-DDT. High purity anhydrous sodium sulfate Gold line (CP) with CAS NO 7757-82-6 (Sigma Aldrich™ South Africa) was used as a desiccant throughout the study, while florisil and silica gel, grade 634, of 100-200 mesh (0.063-0.2 mm) size was used to prepare a florisil column as well as basic and acidic silica gel columns prepared in the laboratory for the clean-up of the extracts prior to analyses. Reagent grade sulfuric acid (specific gravity 1.84, and purchased from Sigma Aldrich™ South Africa) was used. All glassware were baked in the oven at about 130°C prior to use and the cotton wool was soaked in acetone and activated in the oven over night at 130°C prior to use.

The pharmaceutical standards used were acetaminophen, ibuprofen, aspirin, erythromycin, sulfamethazine sulfamethoxazole, trimethoprim, carbamazepine, caffeine, salicylic acid, nalidixic acid, phenacetin, naproxen, meclofenamic, ketoprofen and diclofenac. All standards were purchased from (Sigma Aldrich™, South Africa). Metronidazole and clozapine tablets were used as standards and were purchased over the counter. All water used was ultrapure (Elix Millipore Water system). Acetic acid, ammonium solution, methanol,

acetonitrile, acetone, and ethyl acetate were HPLC grade, and were purchased from Sigma Aldrich. Galaxolide (HHCB), tonalide (AHTN), musk ketone (MK), phenanthrene (PNT – used as a surrogate standard), and hexamethylbenzene (HMB – used as an internal standard) were all purchased from Sigma Aldrich, South Africa.

3.5.2 Standards and preparation of standard solutions

All standards of PPCPs including musks, OCPs and PCBs were made up in HPLC-grade solvents in appropriate concentrations for their specific calibration graphs. For samples that dissolved in water, ultrapure water was used.

3.5.2.1 *PCB and OCP stock solutions*

PCB and OCP stock solutions of 200 mg/L of individual standards were prepared by dissolving about 0.02 g of the individual PCB and OCP standards in 100 mL of n-hexane and stored in the refrigerator at 4°C. The PCB and OCP lower working concentrations were prepared by appropriate dilution in n-hexane giving the following six calibration concentrations: 0.25; 0.5; 1; 2, 4 and 8 µg/mL.

3.5.2.2 *PPCP stock solutions*

For the pharmaceutical standards stock solutions (1000 mg/L) of the standards (excluding metronidazole and clozapine tablets) were prepared by adding 0.0100 g of the standard into 10 mL volumetric flask and then adding a 50:50 (v/v) methanol and ultrapure water solution. Once the material had completely dissolved, the solution of methanol and ultrapure water was added up to the calibration mark and the stock solutions were then stored in a fridge at 4°C until further use. Standard mixtures of 1-100 µg/L, were prepared daily by appropriate dilution of the stock solutions using methanol. The stock solutions were renewed monthly to eliminate the effect of their instability.

3.5.2.3 *Stock solution for synthetic musks*

A stock solution containing all three synthetic musks (SMs) and the surrogate standard was prepared in HPLC grade ethyl acetate. Calibration curves for the musks and the surrogate standard were developed by using 7 standard concentrations except for musk ketone for which 6 concentrations were used (0.05, 0.1, 0.5, 1, 2, 2.5, 5 µg/mL). The internal standard HMB (10 µL, 0.1 µg/L) was added to all calibration standards.

3.6 OPTIMISATION AND VALIDATION OF METHODS FOR THE DETERMINATION OF PHARMACEUTICALS USING GC-MS

3.6.1 Sample extraction procedure for pharmaceuticals

Optimization of the extraction procedure for GC-MS analysis is described in the following sections.

3.6.1.1 *Optimisation of the solid phase extraction procedure*

The solid phase extraction step was optimized by varying pH, flow rate of extraction, elution solvent, reconstitution solvent and sorbents independently. In each study 1 L of double-distilled water was spiked with a mixture of the target analytes and surrogate standards, previously prepared in methanol, to give a final concentration of 1 ng/mL. Each study was done in triplicate enabling estimation of repeatability. Before

extraction, water samples were adjusted to three different pH values, pH 2, pH 7 and pH 9 with HCl (4 mol/L) and NaOH (2 mol/L). Oasis HLB was chosen as the adsorbent, because this type of cartridge has been most widely used in micro-extraction of pharmaceutical compounds in aqueous solution (Matongo et al. 2015a, Matongo et al. 2015b, Hernández et al. 2014, Hao et al. 2007, Buchberger 2011). Results obtained showed that pH 2 favoured the extraction of acidic drugs, these results were similar to what has been reported in the literature. Ethyl acetate was chosen as the elution solvent because it has been reported to work well in micro-extraction of pharmaceuticals which then undergoes silylation, and ethyl acetate can be easily removed by using a stream of dry nitrogen. Methanol, acetone/ethyl acetate (1:1) and gradient elution (acetone 4 mL, ethyl acetate 4 mL and methanol 1 mL) were studied separately for the elution of acidic drugs at pH 2 using oasis HLB. For all of the tests, the total volume of elution solvents was 9 mL. The mixture of acetone/ethyl acetate (1:1) showed the highest recoveries.

Two other different adsorbents were studied in order to ascertain whether the oasis HLB could be replaced with cheaper or more efficient cartridges. The two other types of cartridges were cyno, and an environmental C18 cartridge. Each were compared to the oasis HLB, and this was done at optimum pH and using the best elution solvent. The results are presented in Figure 3.10

Water samples were spiked and resulted in a 1 ng/mL final concentration of the solution. Recoveries ranged from 0 to 140% (for cyno) and 0-160% (for environmental C18). Also the blank runs on the GC-MS with the cyno and environmental C18 samples had some extra peaks, and these were shown to directly interfere with the integration of the target analyte peaks. Hence, the recoveries with these cartridges were not acceptable as per the guidelines followed. Oasis HLB cartridges had recoveries that ranged from 60-120% for all target acidic drugs. In addition, salicylic, nalidixic and naproxen were not recovered by the cyno cartridge. Environmental C18 cartridges did show high recoveries for ibuprofen and ketoprofen. Finally, after all of the optimization experiments, oasis HLB cartridge was selected for the extraction of the target drugs at pH 2, with a flow rate between 4-10 mL/min, and final elution with 9 mL (1:1, v/v) mixture of acetone and ethyl acetate.

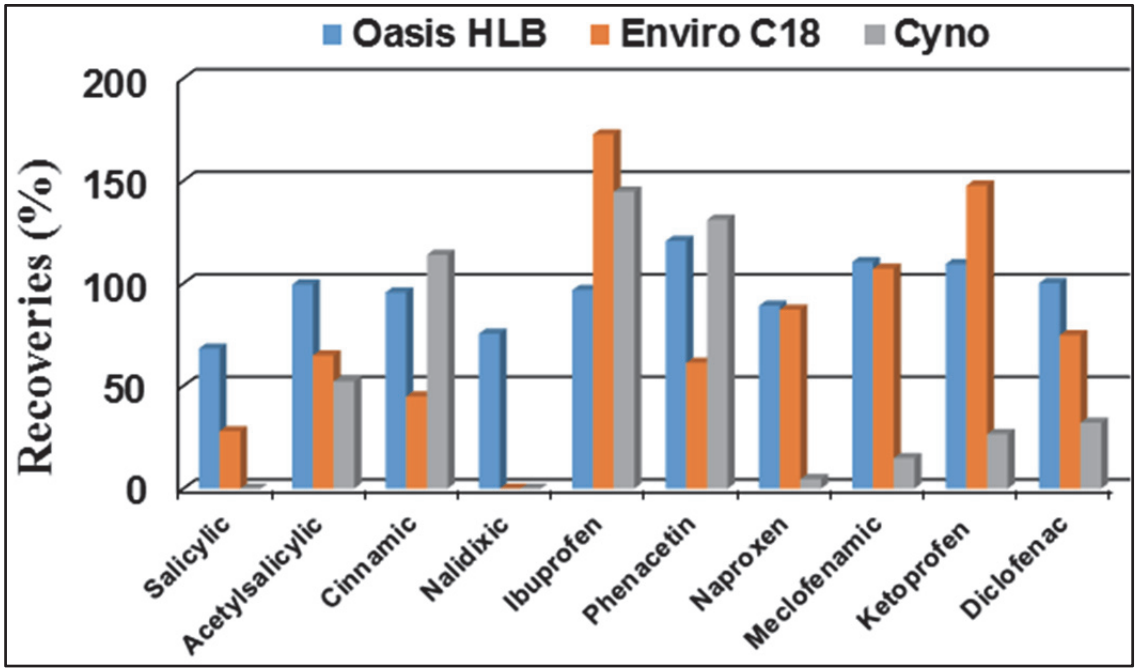


Figure 3.10 Extraction recovery obtained at different sorbents with pH 2 and 9 mL of acetone/ethyl acetate

3.6.1.2 *Extraction from water samples*

Before processing, river water samples were filtered through a 0.45 µm glass fiber (Millipore) to remove particulate materials. For a particular sample, 1.0 L was mixed with a solution of cinnamic acid in acetonitrile (10 mg/L) as a surrogate standard to give a final concentration of 100 ng/L of cinnamic acid. The solution was then acidified to pH ≤ 2 with HCl. The water sample was then passed through an oasis HLB SPE cartridge that had been previously conditioned with 8 mL methanol and 10 mL of distilled water (pH ≤ 2). The water sample was percolated through the cartridge at a flow-rate between 3 and 6 mL/min (flow rate was controlled with a vacuum). After the 1 L sample of water had passed through the cartridge, the cartridge was left under vacuum for 30 min to air dry and then a gentle stream of nitrogen was applied for 5 min. The analytes were eluted off the cartridge with 8 mL of acetone/ethyl acetate in a ratio of 1:1 and 1 mL of acetonitrile at a flow-rate between 0.5 and 1 mL/min. The eluted analytes from the SPE cartridge, containing the target compounds, were then mixed with 10 µL of a solution of chlorobenzoic acid in acetonitrile (10 mg/L), which was used as an internal standard. The mixture was dried under a gentle stream of nitrogen.

The sample was then derivatized by adding 100 µL of N,O-bis (trimethylsilyl) triflouroacetamide (BSTFA, 99% purchased from Sigma-Aldrich) and 10 µL trimethylsilyl, in a GC sample vial that was sealed with caps containing Teflon lined septa. The derivatization process was taken to completion by placing the vials in a 70°C water bath for 30 min. The derivatized sample was partially dried under nitrogen and finally re-dissolved in 1 mL acetonitrile before injection into the GC-MS.

3.6.2 The derivatisation method for GC-MS analysis of pharmaceuticals

Usually, the key obstacle in the analysis of various pharmaceutical analytes by GC-MS methodologies is the derivatization step (Helenkár et al. 2010), therefore the initial work in this study was optimising the derivatization of the target analytes.

3.6.2.1 *Optimization of the derivatization method by varying solvent, temperature and time*

A solution of 10 mg/L containing all of the target analytes, the internal and surrogate standards was prepared and used to optimize temperature, time, solvent, GC-MS conditions (retention time and fragmentation pattern) and concentration of N,O-bis (trimethylsilyl) triflouroacetamide (BSTFA). Figure 3.11 shows the chemical structures of the internal standards and derivatizing reagents used.

An independent injector standard 4,4'-di-tertbutylbiphenyl of 10 mg/L was prepared separately and auto-injected into the GC-MS 5 times to evaluate stability of the entire instrument. 4,4'-Di-tertbutylbiphenyl does not require any derivatizing reagent to be analysed by GC-MS, and the peak area obtained from each of the 5 injections was constant with a relative standard deviation of less than 2%. Thus the instrument was considered suitable for further optimization of derivatization parameters.

Several stock solutions were prepared using methanol because most acidic compounds dissolve completely in protic solvents. During derivatization in methanol, it was determined that a labile hydrogen atom from the methanol molecule was competing with an active hydrogen from the analyte for silylation, which resulted in the incomplete formation of silyl derivatives. In some cases, methanol formed esters with the analytes, which resulted in additional peaks (impurities) and affected the integration of analyte peaks. Hence methanol was removed completely under a gentle stream of nitrogen. The analyte residues were reconstituted in a solution of BSTFA + 10%TMSC, since it has been shown in literature that this mixture readily dissolves a large variety of organic substances, and it can be used to derivatize the analytes without addition of extra solvents (Kumirska et al. 2013). However, in many cases a solvent is needed after silylation for sample dilution to a desired solution concentration prior to GC-MS analysis, so acetonitrile was used as diluent.

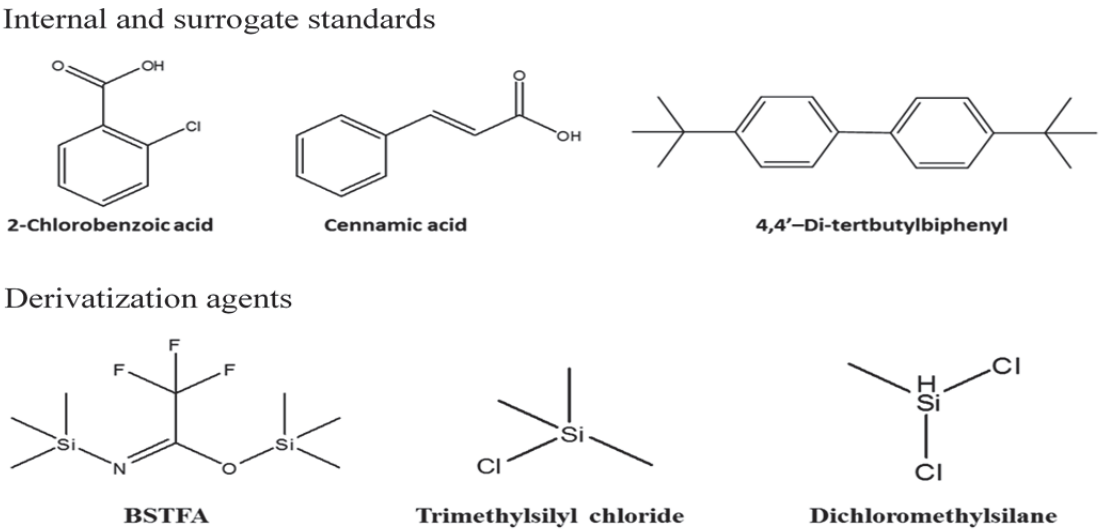


Figure 3.11 Chemical structures of the internal standards and derivatizing reagents used

Heating the solution after addition of the silylation reagents is necessary for complete derivatization of the analytes, hence the temperature was varied from 30ºC to 100ºC (at 10ºC intervals) to find the optimum condition. The reaction time was also varied from 10 min to 50 min (at 10 min intervals). In both cases only one parameter was investigated at a time, while the other was kept constant. The peak area of the analytes was then plotted against each parameter, and the optimum condition was taken as the point where the peak area no longer increased. The results obtained showed that there was no significant difference in the effectiveness of silyl derivative formation at 70ºC to 100ºC and using a reaction time longer than 30 minutes. The optimum conditions are presented in Table 3.3.

Table 3.3 Optimum conditions that were determined and then used to derivatize the target analytes

Parameters	Optimum Conditions
Derivatization solvent	Silylation reagent
Diluent	Acetonitrile
Temperature	70ºC
Time	30 minutes
BSTFA + 10%TMCS	100 µL

3.6.2.2 *Effect of the derivatizing reagent (BSTFA) on the GC-MS response*

The amount of the derivatizing reagent is important in a quantification analysis, because it should always be in excess. However, special attention is needed to prevent the silylation reagents becoming impurities in the final chromatogram, since such peaks are often mistaken for analyte peaks. Also, excessive silylation agents may damage the GC column (Kumirska et al. 2013). The amount needed for derivatization was optimized, by varying the volume of BSTFA + 10% TMCS, and the results of the GC-MS response (peak area) are presented in Figure 3.12. From Figure 3.12, there was no significant difference in the peak area when the volume of the silyl derivatizing reagent was between 100 µL and 120 µL. Given that the investigated solution of analytes was 10 mg/L, it is highly unlikely for the environmental concentration of targeted analytes to reach such levels. From these results, a 100 µL volume of BSTFA + 10% TMCS was used as the optimum amount needed for derivatization of target analytes in the environmental samples. For all standards and samples, a gentle stream of nitrogen was used to remove any excess silylation reagent followed by dilution with acetonitrile to 1 mL prior to GC-MS analysis.

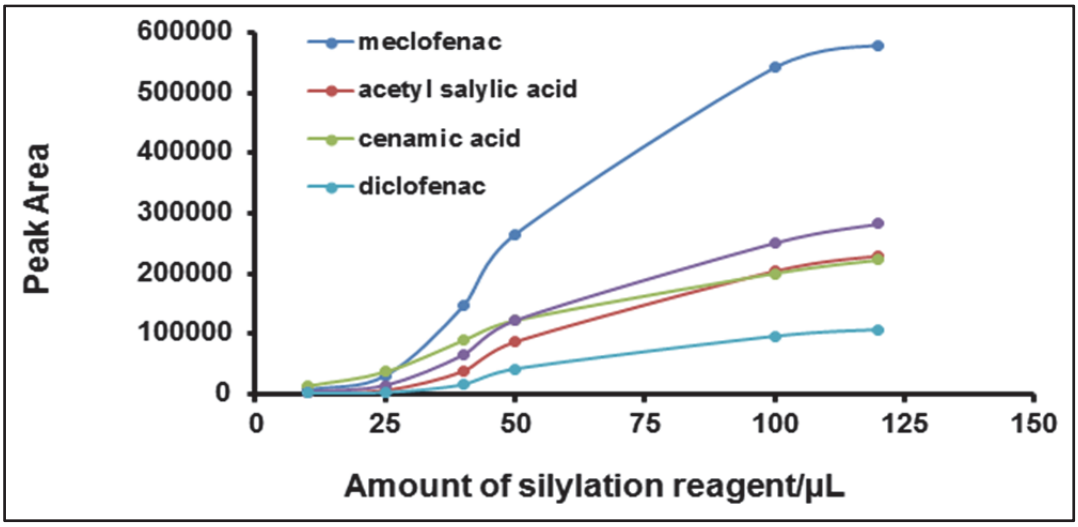


Figure 3.12 GC-MS peak area response versus the volume of silylation reagents used to derivatize selected analytes

3.6.3 Methodology for analysis of pharmaceuticals using GC-MS

The derivatised PPCP samples were analysed on a Shimadzu GC-MS (GC-MS-QP2010SE). The initial column oven temperature was 70°C, the injection port temperature was kept at 250°C and 2 µL of the samples were auto-injected in splitless mode. The carrier gas was helium at a constant flow rate of 8.0 mL/min and 61.5 kPa pressure. The oven temperature was kept at 70°C for 1 min, then the temperature was increased at 30°C/min to 190°C (held for 1 min), followed by a 15°C/min ramp to 230°C (held for 3 min) and finally to 270°C (at 30°C/min) which was held for 1 min. The transfer line was set at 280°C and the ion source at 200°C. Electron energy for the filament was set at 70.0 kV. The ITD setting were as follows: mass range 50-850 *m/z* (full scan only) with start time of 4 min and end time of 14 min. For quantification of analytes, the GC-MS was operated in SIM mode.

3.6.4 Validation of the GC-MS method for PPCPs

The developed method was validated taking into account and using internationally accepted guidelines for single laboratory validation of method of analysis (Peters et al. 2007, Thompson et al. 2002, Trullols et al. 2004, Rozet et al. 2007). The method was validated in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), intra- and inter-day precision, and recoveries. All validated parameters were calculated using the peak area ratio of the target analyte to the internal standard (IS). Recoveries were described in section 3.7.3.1. To determine the linearity of the method, a minimum of six different concentrations were analysed, in triplicate, over a wide range of concentrations (1-8000 ng/mL). Linearity was established within 5-5000 ng/mL for all silylated derivatives and correlation coefficients (R^2) were higher than 0.99 for all analytes which are presented in Table 3.4. To determine the LOD, LOQ, inter-day and intra-day precision of the entire method, five different independent solutions were prepared in triplicate. This was done by spiking environmental samples (1.0 L from Midmar dam) to various final concentrations that ranged from 0.05-5 ng/mL. These solutions were extracted, derivatised and analysed with GC-MS in SIM ion mode, under optimum conditions. The LOD and LOQ of the target analytes are presented in Table 3.4 and values were in a similar range with those reported in the literature for GC-MS and LC-MS techniques (Hao et al. 2007, Helenkár et al. 2010, Lolić et al. 2015, Lin et al. 2005). Also, the results were better than those reported in the literature for HLPC methods (Agunbiade and Moodley 2014, Madikizela et al. 2014). The intra-day and inter-day precision were evaluated at three concentration levels (low, medium and high) in triplicate. The results presented in Table 3.4 show that the developed method is suitable for the analysis of environmental samples.

Table 3.4 Linearity, LOD, LOQ and intra- and inter-day precision for various pharmaceuticals using GC-MS

Acidic analytes	*m/z*	Therapeutic class	Linear range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	R^2	Intra-day precision %RSD			Inter-day precision %RSD		
							Low*	Med*	High*	Low*	Med*	High*
Salicylic acid	138	Antipyretic	5-5000	0.041	0.135	0.991	1.16	3.63	3.25	5.52	11.98	0.06
Acetylsalicylic acid	180	Antipyretic	5-5000	0.285	0.950	0.999	0.08	4.02	2.95	1.11	8.26	0.50
Cinnamic acid	148	Antibiotic	5-5000	0.117	0.390	0.999	0.12	3.15	2.07	14.74	9.80	2.42
Nalidixic acid	232	Antibiotic	5-500	0.186	0.620	0.997	2.19	11.58	20.00	11.23	4.56	1.71
Ibuprofen	206	Antipyretic	5-5000	0.143	0.477	0.999	1.04	3.52	1.35	8.20	10.59	0.97
Phenacetin	179	Antiyretic	100-5000	0.345	1.151	0.994	20.00	20.00	9.75	7.10	2.86	6.10
Naproxen	230	Antipyretic	5-5000	0.075	0.248	0.999	8.66	1.88	4.42	8.21	14.17	4.04
Meclofenac	296	Antipyretic	5-5000	0.082	0.272	0.994	3.79	1.03	3.25	6.39	8.40	1.76
Ketoprofen	254	Antipyretic	50-5000	0.004	0.013	0.993	6.27	2.80	6.64	6.47	14.47	8.49
Diclofenac	296	Antipyretic	5-5000	0.484	1.614	0.999	6.68	3.63	4.39	6.13	11.73	2.88

* Low = 0.1 ng/mL, Med = 2 ng/mL, High = 5 ng/mL

3.7 OPTIMISATION AND VALIDATION OF METHODS FOR THE DETERMINATION OF PHARMACEUTICALS USING LC-MS

3.7.1 Sample extraction and clean-up for LC-MS

The methods presented in this sub-section are specific to the LC-MS analysis of PPCPs. Part of the work has been published in peer-reviewed journals.

3.7.1.1 *Optimization of solid phase extraction method*

The solid phase extraction method for PPCPs is crucial for all extractions from sediment, wastewater, and surface waters, thus it was the initial method optimized with the pharmaceutical analytes. Due to the wide range of the target analytes with different physical-chemical characteristics, it was a challenge to extract all the target analytes in a single-step with good method performance. Therefore, optimization of the single-step was required. The effect of sample pH on recovery of target analytes in tap-water samples was investigated at three different pH values of 4.20, 5.00 and 8.28 and the results are presented in Figure 3.13.

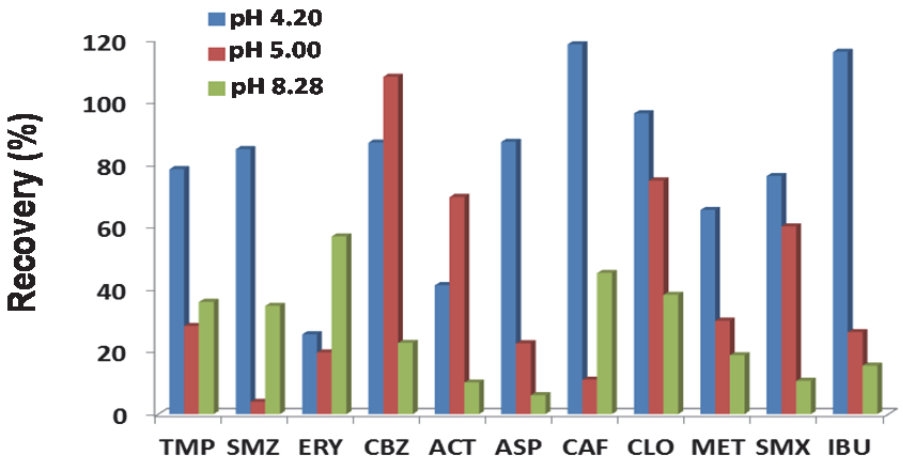


Figure 3.13 Recovery of the selected analytes from Oasis HLB SPE cartridges using tap water at different pH values. (TMP: trimethoprim; SMZ: sulfamethazine; ERY: erythromycin; CBZ: carbamazepine; ACT: acetaminophen; ASP: aspirin; CAF: caffeine; CLO: clozapine; MET: metronidazole; SMX: sulfamethoxazole; IBU: ibuprofen

Tap water was used as a model matrix for water samples but later the method was optimised for surface and wastewater. The pH meter used was 827 pH Lab Metrohm Swiss. The recovery experiments were as described for PCBs and OCPs. From the results (Figure 3.13) extraction at pH 4.20 showed the best results for all analytes and therefore was used as the optimised pH for extraction.

3.7.1.2 *Extraction of pharmaceuticals from surface water*

Surface water extraction was done by optimizing the method reported by Babić et al. (2006). The solid phase material, hydrophobic-lipophilic balance (HLB) from oasis (60 mg, 3cc), was conditioned with 5 mL methanol and equilibrated with 5 mL water adjusted to pH 4.20 with acetic acid. A 300 mL aliquot of the surface water sample of interest was loaded onto the cartridge after adjustment of the sample pH to 4.20 with either acetic acid or ammonium solution. The flow rate of the sample through the cartridge was set to 4 mL/min. Subsequently, the solid phase was dried completely by vacuum for 30 min and the analytes were

eluted ten times with 1 mL of methanol and five times with 1 mL of acetone using a flow rate of 2 mL/min. Elutes were evaporated to dryness under vacuum and re-dissolved with 1 mL of methanol for injection into the LC-MS.

3.7.1.3 *Extraction of pharmaceuticals from wastewater*

The solid phase material HLB Oasis (60 mg, 3cc) was conditioned with 5 mL methanol and equilibrated with 5 mL ultrapure water adjusted to pH 4 with acetic acid. A 100 mL aliquot of the wastewater was loaded onto the cartridge after adjustment of the pH to 4 with either acetic acid or ammonium solution. The flow rate was 4 mL/min. Subsequently, the solid phase was dried completely by vacuum for 30 minutes and analytes were then eluted ten times with 1 mL of methanol and five times 1 mL of acetone at a flow rate of 2 mL/min. The eluent was then evaporated to dryness under vacuum and re-dissolved with 1 mL of methanol for injection into the LC-MS.

3.7.1.4 *Extraction of pharmaceuticals from sediments*

The sediments were partially-dried at room temperature, thoroughly homogenized, and sieved using a 2-mm opening sieve, and then stored in a refrigerator. Sediment extraction was done by adjusting and optimizing the method reported by Löffler and Ternes (2003). The sediments (50 g) were extracted successively in an ultrasonic bath. The steps involved mixing the sediment with 50 mL of HPLC grade solvent, thoroughly shaking by hand, ultrasonicating for 15 minutes at 35°C, centrifuging for 7 minutes, and then filtering through 1.2 µm Whatman™ filter paper. The sequence followed was two extractions with 50 mL of methanol, one extraction with 50 mL of acetone, and one extraction with 50 mL of ethyl acetate. All of the recovered solvents, containing the extracts, were combined and the solvents evaporated using a rotary evaporator at 60°C and 150-200 mbar. The obtained sediment extracts were then diluted with 200 mL of double distilled water. Additionally, the flasks used for rotary evaporation were rinsed using 3 mL of methanol which was then combined with double distilled water. Afterwards, a solid phase extraction (SPE) was performed using HLB Oasis (60 mg, 3cc) SPE cartridges as described above.

3.7.2 Methodology for analysis of pharmaceuticals using LC-MS

The determination of the selected analytes was performed using HPLC Agilent 1200 series equipped with an automatic injector coupled with an 1100 series MSD Trap mass spectrometer using electrospray ionization (ESI) interface from Agilent Technologies. A Zorbax C18 (100 x 2.1 mm i.d 3.5 µm particle size) column was used for separation and quantification of the target analytes. Ions were acquired in the multiple reaction monitoring (MRM) mode with a dwell time of 7.0 ms. Acquisition parameters are shown in Table 3.5. This method was used for the identification and quantification of the 11 target analytes in the environmental samples using a single injection. All of the eleven target analytes were separated using a gradient method and the following mobile phase composition, 0.1% acetic acid in ultrapure water (Mobile Phase A), and 100% acetonitrile (Mobile Phase B).

Table 3.5 Acquisition parameters for the MS detection of the selected pharmaceuticals

Acquisition Parameters					
Mass Range Mode	Normal	Trap Drive	44.9	Scan Begin	100 *m/z*
Ion Polarity	Positive/Negative	Skim 1	40.8 Volt	Scan End	800 *m/z*
Ion Source Type	ESI	Skim 2	6.0 volt	Averages	7 Spectra
Dry Temp	350°C	Octopole RF Amplitude	150.0 Vpp	Max. Ac Time	200000 us
Nebulizer	50 psi	Capillary Exit	116.7 Volt	1CC Target	30000
Dry Gas	10 L/min	Cap Exit Offset	75.9 Volt	Charge Control	0n

The gradient program started with 5% Mobile phase B ramped to 90% mobile phase B in 25 minutes and then back to 5% mobile phase B in 2 minutes and stabilized for 5 minutes. The flow rate was 0.25 mL/min. The column temperature was kept at 35°C. An injection volume of 10 µL was used for all analyses. The MS analyses were performed in the positive electrospray ionization ESI (+) and negative electrospray ionization ESI (-). Aspirin and ibuprofen were detected in the negative ionization mode.

3.7.3 Method validation

3.7.3.1 *Recovery experiments with PPCPs, MDL and MQL for sediment and water analysis using LC-MS*

A method similar to the one reported by Löffler and Ternes (2003) was used for sediment recovery experiments. Samples were frozen (50 g) to inactivate microorganisms and any enzymatic activity. Samples were then spiked with the analytes at 800 ng concentrations and were shaken thoroughly to obtain an even distribution of the analytes. The samples were then kept overnight (~14 h), at ambient conditions, to allow for partitioning of the analytes to the sediment. The recoveries for sediments were calculated by comparing the analyte concentration of samples spiked prior to and after extraction. For surface water and wastewater recoveries, tap water was spiked with the analytes to be recovered and the same extraction procedure as described in section 3.6.1.2 was carried out. A comparison of recoveries obtained with tap water and sediment are presented in Figure 3.14.

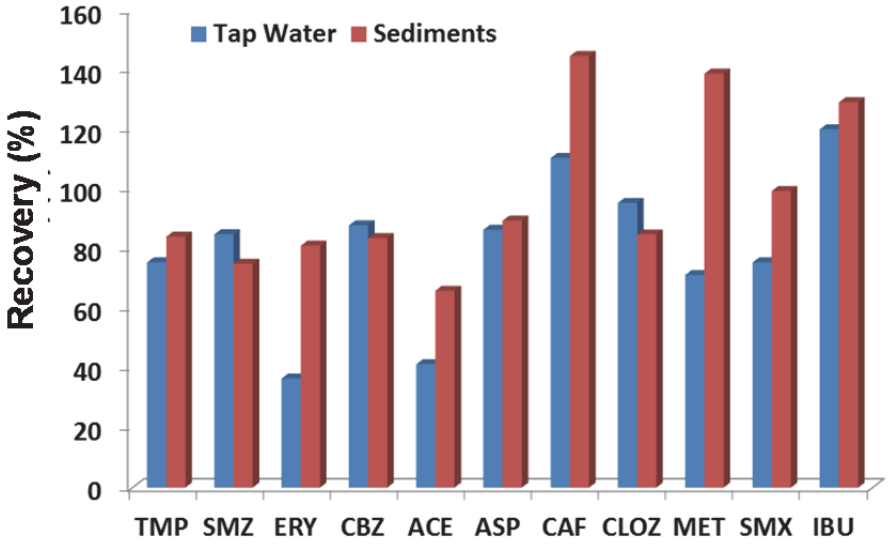


Figure 3.14 A comparison of water and sediment recoveries at the optimum pH of 4.2

Recoveries ranged from 71.20 to 144.57% for most of the analytes with relative standard deviations of 0.46 to 14.12% except for erythromycin, acetaminophen and metronidazole where the recoveries were <70%, but still suitable for the detection of pharmaceutical residues in complex matrices. The recovery expressed with R.S.D for sediments and tap water are presented in Table 3.6. The performance characteristics of the SPE-HPLC method were established by validation procedures with spiked water samples. Linearity, linear range, method detection limits (MDL) and method quantification limits (MQL) were studied (Table 3.7). For qualitative purposes, the method was evaluated by taking into account the precision in the retention factor (tR-value) and selectivity. A high repeatability in the retention was obtained with R.S.D. values lower than 1.5%. Intraday repeatability was expressed as relative standard deviation (R.S.D.). The results of the intra-day precision experiments are summarized in Table 3.6. Satisfactory results were achieved for all pharmaceuticals with R.S.D.s lower than 14.12% indicating high measurement repeatability of the SPE-HPLC method.

Table 3.6 Tap water and sediment recoveries ± Relative standard deviations

	Sediment	**Tap Water**
Standard	% Recoveries ± RSD(n=3)	% Recoveries ± RSD(n=3)
TMP	84.03±3.18	78.42±6.74
SMZ	74.95±7.33	84.83±14.12
ERY	81.01±7.93	25.53±0.15
CBZ	83.55±0.46	86.95±6.23
ACE	65.92±2.41	41.26±12.38
ASP	88.02±2.53	87.18±2.34
CAF	144.57±9.45	118.37±0.50
CLOZ	84.80±2.84	96.276±3.22
MET	138.64±9.09	65.35±9.20
SMX	99.34±0.74	76.23±8.83
IBU	129.05±1.57	116±4.10

External calibration over a range of 0.1-100 ng/mL was used. Five point calibration curves were plotted with all having R^2 > 0.99. For calibration curves the analyte concentrations were plotted *versus* the corresponding analyte peak areas. Blanks were also prepared as a quality control tool, but not included in the regression analysis. Limits of detection were calculated using a signal to noise ratio of 3 whereas limits of quantification were calculated using a signal to noise ratio of 10. The results obtained are shown in Table 3.7 Limits of detection were in the range of 0.0001-0.293 ng/mL for surface water, 0.008-0.887 ng/mL for wastewater and 0.016-1.755 ng/g for sediment, whereas limits of quantification where in the range of 0.0004-0.975 ng/mL for surface water, 0.273-2.923 ng/mL for wastewater and 0.546-5.848 ng/g for sediment depending on the pharmaceutical. This is in agreement with literature reports (Babić et al. 2006, Topuz et al. 2014).

Table 3.7 Calibration data and method validation parameters for LC-MS analysis of pharmaceuticals

					ng/mL	Wastewater		Surface Water		Sediments		
Analytes	***m/z***	**Therapeutic class**	**Calibration Equation**	**R_t /min**	**Linear Range**	**ng/mL MDL**	**ng/mL MQL**	**ng/mL MDL**	**ng/mL MQL**	**ng/g MDL**	**ng/g MQL**	**R^2**
ACE	151	Antipyretic	$y=12.42 \times 10^{6} x-3.48 \times 10^{6}$	3.5	0.1000-100	0.0081	0.2733	0.0027	0.0911	0.0162	0.5466	0.9930
ASP	179	Antipyretic	$y=17.63 \times 10^{3} x+1.41 \times 10^{4}$	2.3	2.0000-100	0.8772	2.9238	0.2924	0.9746	1.7544	5.8476	0.9956
CAF	195	Stimulant	$y=7.00 \times 10^{6} x+9.84 \times 10^{5}$	7.2	0.3473-100	0.3120	1.0419	0.1040	0.3473	0.624	2.0838	0.9973
CBZ	237	Anti-epileptic	$y=5.54 \times 10^{7} x-2.19 \times 10^{6}$	14.3	0.2970-100	0.2676	0.891	0.0892	0.2970	0.5352	1.782	0.9960
CLO	327	Anti-psychotic	$y=22.70 \times 10^{6} x-1.91 \times 10^{7}$	12.6	0.4436-100	0.3993	1.3308	0.1331	0.4436	0.7986	2.6616	0.9931
ERY	734	Antibiotic	$y=6.22 \times 10^{8} x+7.86 \times 10^{6}$	12.2	0.0004-100	0.0003	0.0012	0.0001	0.0004	0.0006	0.0024	0.9913
MET	172	Antibiotic	$y=5.22 \times 10^{5} x+5.18 \times 10^{5}$	4.4	0.9619-100	0.8658	2.8857	0.2886	0.9619	1.7316	5.7714	0.9931
IBU	205	Antipyretic	$y=1.00 \times 10^{6} x+1.13 \times 10^{5}$	8.8	0.2710-100	0.2442	0.813	0.0814	0.2710	0.4884	1.6260	0.9928
SMX	254	Antibiotic	$y=7.70 \times 10^{6} x+8.63 \times 10^{6}$	11.7	0.4135-100	0.3720	1.2405	0.1240	0.4135	0.7440	2.4810	0.9944
SMZ	279	Antibiotic	$y=3.93 \times 10^{7} x+1.66 \times 10^{7}$	9.1	0.2271-100	0.2043	0.6813	0.0681	0.2271	0.4086	1.3626	0.9907
TMP	291	Antibiotic	$y=250.01 \times 10^{6} x+7.88 \times 10^{6}$	7.8	0.5000-100	0.1230	0.4110	0.0410	0.1370	0.2460	0.822	0.9952

3.8 OPTIMISATION AND VALIDATION OF METHODS FOR THE DETERMINATION OF MUSKS AND MUSK KETONES

3.8.1 Sample extraction and clean-up of musks and musk ketones

The method for sample extraction and clean-up of musks and musk ketones was developed by carrying out extractions using varying solvents as well as solvent combinations and determining their recoveries. The time allowed for curing was also tested to determine if it contributed to errors in recovery. The following describes the method development carried out to obtain the optimised conditions for recovery.

3.8.1.1 *The effect of curing time on recovery*

Sodium sulfate anhydrous was spiked and cured for over 2 weeks, then subjected to the extraction protocol and GC-MS analysis. The percentage recovered was calculated and the results are shown in Table 3.8. The results show recoveries of 65-110% except for phenanthrene (surrogate) with a low percentage recovery of < 22%.

Table 3.8 Percentage recoveries (mean ± SD, n = 3) of the spiked anhydrous sodium sulfate cured for two (2) weeks extracted with 1:1 hexane / acetone under the same condition and concentration of analyte and surrogate standard

Analyte	% Sample 1	% Sample 2	% Sample 3
Phenanthrene	21.79 ± 0.02	15.29 ± 0.01	16.40 ± 0.00
Galaxolide	69.45 ± 0.02	65.59 ± 0.03	67.42 ± 0.02
Tonalide	86.02 ± 0.03	81.48 ± 0.03	80.93 ± 0.03
Musk ketone	108.22 ± 0.21	110.33 ± 0.08	102.52 ± 0.01

This was repeated but with a simulated sediment sample (anhydrous sodium sulfate) that had been cured for 24 hrs and the results obtained are presented in Table 3.9. The recovery for all the standards were larger than the values obtained with the 2 week cured samples, and the increment observed with the surrogate standard phenanthrene increased to 71%. These two results indicate there might be loss due to disintegration of the surrogate standard over a period of time.

Table 3.9 Percentage recoveries (mean ± SD, n = 3) of the spiked anhydrous sodium sulfate cured for 24 hrs extracted with 1:1 hexane / acetone under the same condition and concentration of analyte and surrogate standard

Analyte	% Sample 1	% Sample 2	% Sample 3
Phenanthrene	71.11 ± 0.04	52.93 ± 0.07	67.98 ± 0.04
Galaxolide	87.07 ± 0.08	71.01 ± 0.08	83.11 ± 0.01
Tonalide	110.81 ± 0.05	94.12 ± 0.10	105.27 ± 0.03
Musk ketone	286.78 ± 0.45	231.69 ± 0.40	244.13 ±0.75

3.8.1.2 *The effect of solvent combinations on recovery*

Extraction with hexane:dichloromethane solvent mixture

The effect of extracting solvent was tested. The initial solvent mixture of hexane and acetone (1:1) was compared with hexane and dichloromethane (1:1) as the extraction solvent. The extraction protocol used was similar to the one used in previous sections. Table 3.10 presents the results for the recovery using 1:1

hexane: dichloromethane as the extraction solvent on spiked anhydrous sodium sulfate. The percentage recoveries with hexane and acetone (Table 3.9) were better than that of the hexane: dichloromethane solvent extraction mixture, especially when comparing the values for phenanthrene.

Table 3.10 Percentage recoveries (mean ± SD, n = 3) of the spiked anhydrous sodium sulfate cured for 24 hrs extracted with 1:1 hexane / dichloromethane under the same condition and concentration of analyte and surrogate standard

Analyte	% Sample 1	% Sample 2	% Sample 3
Phenanthrene	38.83 ± 0.06	38.90 ± 0.07	50.60 ± 0.03
Galaxolide	60.75 ± 0.07	60.88 ± 0.09	77.00 ± 0.08
Tonalide	84.03 ± 0.11	86.25 ± 0.11	94.83 ± 0.09
Musk ketone	289.38 ± 0.47	228.77 ± 0.74	230.91±0.37

Extraction with hexane:acetone 1:1 and ethyl acetate solvent mixture

The extraction procedure was tried on acid washed sand using two different extraction solvent mediums of hexane/acetone 1:1 and ethyl acetate. The acid washed sand was used since it is a closer matrix match to the environmental sample which will contain carbon, silica, iron oxides, and various other materials. After analysis, it was discovered as shown in Table 3.9 and Table 3.11 that the percentage recovered from acid washed sand is lower than the percentage recovered when anhydrous sodium sulfate was used as a representative matrix. The reason might be the matrix effect which will be more in favour of the anhydrous sodium sulphate. The recovery on the acid washed sand spiked and cured for 24 hrs using 1:1 hexane: acetone extraction solvent is presented in Table 3.11.

Table 3.11 Percentage recoveries (mean ± SD, n = 3) of the spiked acid wash sand cured for 24 hrs extracted with 1:1 hexane / acetone under the same condition and concentration of analyte and surrogate standard

Analyte	% Sample 1	% Sample 2	% Sample 3
Phenanthrene	61.29 ± 0.05	51.85 ± 0.07	44.64 ± 0.04
Galaxolide	73.29 ± 0.02	64.11 ± 0.03	59.14 ± 0.04
Tonalide	80.52 ± 0.07	93.21 ± 0.09	86.81 ± 0.02
Musk ketone	315.16 ± 0.80	317.61 ± 0.90	342.58 ± 0.20

Table 3.12 presents the results for the recovery values for the spiked acid washed sand cured for 24 hrs, and extracted using ethyl acetate. The recovery values with the 1:1 hexane:acetone solvent was higher (see Table 3.9) than the results obtained with ethyl acetate (Table 3.12). The percentage recovered with the musk ketone is > 120% for all extraction solvents on the spiked acid washed sand cured for 24 hrs. A value of 120% is the maximum acceptable value for the recovery of such compounds. Troubleshooting the method was done by initially repeating the experiment with the spiked acid washed sand and blank acid washed sand (n = 3 for both). After analysis of the spiked and blank acid washed sand (Table 3.13), the 3 musk's (phenanthrene, galaxolide, and tonalide) were not detected in the blank. The blank was observed to have a peak close to the retention time of the musk ketone, thus the blank was subtracted from the spiked acid washed sand. However, the value for the percentage recovered was still higher than the acceptable value of 120%; specifically, from 108-153 (Table 3.13).

Table 3.12 Percentage recoveries (mean ± SD, n = 3) of the spiked acid wash sand cured for 24 hrs extracted with ethyl acetate under the same condition and concentration of analyte and surrogate standard.

Analyte	% Sample 1	% Sample 2	% Sample 3
Phenanthrene	55.51 ± 0.02	46.17 ± 0.02	43.38 ± 0.05
Galaxolide	54.02 ± 0.01	43.92 ± 0.04	43.38 ± 0.04
Tonalide	67.50 ± 0.02	62.08 ± 0.04	60.37 ± 0.06
Musk ketone	125.07 ± 0.16	129.75 ± 0.41	117.5 ± 0.51

Further troubleshooting steps that were attempted included aqua regia washing of all glassware, running the solvents as blanks, and separate runs with the 4 µg/mL stock solution (used to spike various samples). The results did not show any differences between the new values and original values. The cotton wool, sodium sulfate, silica gel and acid washed sand were then checked to ascertain if these materials were the source of the contamination for the musk ketone. Samples of each material were subjected to the extraction procedure using 1: 1 hexane: acetone. The only material that had a peak similar to the musk ketone was the cotton wool. The extraction was repeated with various samples of cotton wool obtained from other research and teaching labs at UKZN and all samples displayed a peak close to the retention time of the musk ketone. The cotton wool was then replaced with glass wool. The proposed extraction method to be used in extracting the sediment sample was further simulated on anhydrous sodium sulfate mixed with wet acid washed sand (8 mL of tap water was added). Three (3) samples of 30 g wet acid washed sand were weighed out and spiked with 133.20 ng/g (1 mL of 4 µg/mL) of the composite standards and then mixed with 30 g each of anhydrous sodium sulfate, cured for 24 hrs after which the samples were subjected to the extraction procedure described earlier.

Table 3.13 Percentage recoveries (mean ± SD, n = 3) of the spiked acid wash sand cured for 24 hrs extracted with 1:1 hexane / acetone and the blanks (B*). Phenanthrene, galaxolide, and tonalide were not detected in the blank

Analyte	% Sample 1	% Sample 2	% Sample 3
Phenanthrene	42.34 ± 0.10	44.47 ± 0.06	53.71 ± 0.02
Galaxolide	56.20 ± 0.05	59.54 ± 0.03	66.29 ± 0.02
Tonalide	74.28 ± 0.08	75.14 ± 0.03	85.31 ± 0.02
Musk ketone	223.71 ± 0.40	178.24 ± 0.50	205.85 ± 0.22
Musk ketone (B*)	69.7 ± 0.31	70.03 ± 0.16	56.32 ± 0.21
Musk ketone (Difference)	153.93 ± 0.21	108.20 ± 0.27	149.52 ± 0.26

In this wet extraction, glass wool was used instead of cotton wool and the results obtained shows the addition to the amount of musk ketone observed in cotton wool is absent when glass wool is used in the clean-up process. The recoveries are shown in Table 3.14. From these results, the following sediment extraction and pre-treatment method was adopted for real samples.

Table 3.14 Percentage recoveries using m/z absolute intensity (mean ± SD, n = 3) of the spiked wet acid washed sand mixed with anhydrous sodium sulphate cured for 24 hrs extracted with 1:1 hexane / acetone

Analyte	% Sample 1	% Sample 2	% Sample 3
Phenanthrene	26.90 ± 6.90	19.06 ± 5.97	19.25 ± 4.44
Galaxolide	43.73 ± 3.18	28.68 ± 3.05	27.93 ± 9.83
Tonalide	46.60 ± 1.10	37.00 ± 2.83	40.99 ± 7.04
Musk Ketone	58.75 ± 1.79	63.49 ± 2.65	62.69 ± 5.37

3.8.2 Methodology for analysis of musks and musk ketones using GC-MS

The method for analyzing the polycyclic musks was developed on a gas chromatography-mass spectrometry (GC-MS) using a Shimadzu GCMS-QP2010 Ultra/GC-MS-QP 2010SE. The analytes were separated on a capillary column inert cap 5 ms/sil with dimensions of 30 m × 0.25 mm i.d., × 0.25 µm with helium as the carrier gas at a flow rate of 1.00 mL/min. The GC oven temperature program was held at 80°C for 1 min, then increased at 30°C/min until 170°C, then increased at 2.5°C/min to 190°C and finally increased at 25°C/min to a final temperature of 270°C and held for 1 min. Two microliter (2 µL) injections of the standards were injected in splitless mode at an injection port temperature of 250°C, and 230°C and 290°C ion source temperature and interphase temperature respectively. The MS ionisation was carried out with electron ionization mode (70 eV) and full scan (*m/z* 50-400 amu, 0.30 scan/s) or SIM mode.

3.8.3 Validation of GC-MS method for musks and musk ketones analysis

The LODs, LOQs, inter and intra-day analysis were determined for the developed method of analysis and are presented below.

3.8.3.1 *LODs and LOQs for polycyclic musk analysis*

Seven concentration levels of the compounds galaxolide (HHCB), tonalide (AHTN) and phenanthrene (PNT) ranging from 0.05-5 µg/mL, and six concentration levels of musk ketone ranging from 0.10-5 µg/mL were prepared. Each reference standard contained 1 µg/mL hexamethyl benzene as an internal standard. The ratios of the peak areas of the standards to that of the internal standard were plotted against the corresponding concentration levels. Each solution was injected three times and the averages were used to obtain the calibration graph. This method was used to get the regression equations as shown in Table 3.15. The LOD and LOQ were in the range of 0.02-0.04 µg/mL and 0.07-0.16 µg/mL, respectively for the compounds.

Table 3.15 Linearity, LOD and LOQ of the three polycyclic musks and the surrogate standard (mean ± SD n=3)

Analyte	R_t /min	Calibration equation	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	R^2
Hexamethyl benzene (IS)	6.439 ± 0.05					
Phenanthrene	11.199 ± 0.02	Y = 1.1160x	0.05-5	0.0217	0.0725	0.9995
Galaxolide	12.139 ± 0.01	Y = 0.3942x	0.05-5	0.0280	0.0932	0.9983
Tonalide	12.287 ± 0.02	y = 0.6384x	0.05-5	0.0226	0.0755	0.9988
Musk ketone	13.783 ± 0.00	Y = 0.3108x	0.10-5	0.0470	0.1550	0.9981

B- Regression plot method (LOD = 3 × standard error/slope and LOQ = 10 × standard error/slope from the regression plot) (Thomsen et al. 2003). IS - Internal Standard, SS - Surrogate Standard

3.8.3.2 *Precision and accuracy with GC-MS analysis of musk ketones*

Precision and accuracy were investigated on an intra-day and inter-day basis for retention time, peak area ratio and concentration for recovery. The mean, standard deviation, and % R.S.D were calculated for each investigation. The results obtained are shown in Table 3.16 for precision of n = 4 for inter-day and n = 3 for intra-day and indicate a good precision with a low SD < 0.02 and RSD < 6% for both the retention time and the peak area ratio of the compounds. Intra-day variability was in the range of 0.02-0.04% for retention time, 0.13-1.63% for peak area ratio while the inter-day variability is seen to be 0.08-0.23% for retention time and 1.28-5.63% for peak area ratio.

Table 3.16 Result of intra-day and inter-day variability for retention time and peak area ratio of polycyclic musks

Analyte	Intra-day retention time	RSD (%)	Intra-day peak area ratio	RSD (%)	Inter-day retention time	RSD (%)	Inter-day peak area ratio	RSD (%)
Hexamethyl benzene	6.406 ± 0.00	0.04			6.391 ± 0.02	0.23		
Phenanthrene	11.296 ± 0.00	0.03	8.594 ± 0.03	0.34	11.271 ± 0.02	0.15	7.695 ± 0.18	2.32
Galaxolide	12.216 ± 0.00	0.02	3.018 ± 0.05	1.62	12.175 ± 0.01	0.09	2.736 ± 0.15	5.63
Tonalide	12.369 ± 0.00	0.03	5.323 ± 0.01	0.13	12.344 ± 0.02	0.17	4.848 ± 0.06	1.28
Musk ketone	13.842 ± 0.00	0.02	2.884 ± 0.02	0.58	13.829 ± 0.01	0.08	2.532 ± 0.05	2.01

For intra-day Mean ± SD & R.S.D. n=3
For inter-day Mean ± SD & R.S.D. n=4
R.S.D. (%) = (S.D./mean) × 100

Accuracy of the method was investigated by means of recovery from spiked anhydrous sodium sulfate cured for 24 hrs and the results are summarized in Table 3.17, with n = 3 for both the intra-day and inter-day values. The % RSD observed were all < 4% except for one value with musk ketone having a value of 13.45% for inter-day, which is lower than the acceptable limit of 15% for both the intra-day and inter-day.

Table 3.17 Result of intra-day and inter-day variability using spiked anhydrous sodium sulfate cured for 24 hrs (for intra-day & inter-day Mean ± SD & R.S.D. n = 3)

Analyte 4 µg/mL	Intra-day Concentration (µg/mL)	RSD (%)	Percentage recovered	Inter-day Concentration (µg/mL)	RSD (%)	Percentage recovered
Phenanthrene	2.845 ± 0.05	1.57	71.11	2.803 ± 0.05	1.65	70.07
Galaxolide	3.483 ± 0.08	2.38	87.07	2.934 ± 0.08	2.79	73.35
Tonalide	4.43 ± 0.08	1.25	110.81	3.692 ± 0.07	1.79	92.31
Musk ketone	11.471 ± 0.45	3.91	286.78	8.026 ± 1.08	13.45	200.64

R.S.D. (%) = (S.D./mean) × 100

Similar studies were done on the spiked acid washed sand and the results are presented in Table 3.18. The % RSD were all below 8% and the percent recovery values varied from 58-343%.

Table 3.18 Result of intra-day and inter-day variability from the spiked acid wash sand cured for 24 hrs (for intra-day & inter-day Mean ± SD & R.S.D. n = 3)

Analyte 4 µg/mL	Intra-day Concentration (µg/mL)	RSD (%)	Percentage recovered	Inter-day Concentration (µg/mL)	RSD (%)	Percentage recovered
Phenanthrene	2.452 ± 0.05	2.2	61.29	2.331 ± 0.14	5.89	58.26
Galaxolide	2.932 ± 0.02	0.8	73.29	2.482 ± 0.13	5.04	62.05
Tonalide	3.73 ± 0.09	2.4	93.21	3.484 ± 0.15	7.28	87.11
Musk ketone	13.703 ± 0.15	1.1	343.28	9.045 ± 0.61	6.72	226.12

R.S.D. (%) = (S.D./mean) × 100

3.9 OPTIMISATION AND VALIDATION OF METHODS FOR THE DETERMINATION OF OCPs AND PCBs

The methods adapted and developed for the analysis of OCPs and PCBs in water, pore water and sediment samples are presented in this section.

3.9.1 Sample extraction and clean-up of OCPs and PCBs

Sample extraction and clean-up is important in analysis of organic pollutants as it serves to concentrate the analyte of interest whilst at the same time removing unwanted analytes that may mask or interfere with the analytes of interest. For the samples collected from the Umgeni and Msunduzi Rivers, different types of extraction and clean-up procedures adapted from literature were used.

3.9.1.1 *Umgeni River water samples*

After trying a number of cartridges to extract water samples with a vacuum manifold, using different solvent systems for conditioning, loading, washing and eluting, the results obtained were compared to those obtained using liquid-liquid extraction with HPLC grade dichloromethane (HPLC-DCM). The latter method was preferred over solid phase extraction. The water samples were then treated as follows:

The water samples were extracted using liquid-liquid extraction (EPA method 3510) (EPA 1996a). A 1 L sample of water was transferred to a 2 L separating funnel and extracted with an aliquot of 50 mL of DCM. This process was repeated 6 times for each sample with fresh aliquots of DCM to increase recovery. All extracts were then combined and transferred into a round-bottom flask and concentrated with a rotary evaporator (Heidolph Instruments GmbH & Co.kG) to approximately 5 mL. For the clean-up process, they were transferred to a florisil (activated at 130°C for 12 hours) column containing 5 g of anhydrous sodium sulfate on top, and eluted sequentially with aliquots of 5 mL of hexane: DCM (94:6), (85:15), (50:50) and DCM (100%) (modified EPA method 3620-C) (EPA 2007). The increasing polarity of hexane-DCM solvent system allowed elution of different PCBs and OCPs having different polarity indexes and solubilities with respect to hexane and DCM. This could not be achieved using hexane (polarity index = 0.0) or DCM (polarity index = 3.1) alone. All fractions were then combined and concentrated with a rotary evaporator and finally air-dried. Their masses were recorded using an analytical balance and thereafter stored in the fridge at 4°C until analysis.

3.9.1.2 *Umgeni River sediment and soil samples*

A number of extraction methods for solids were initially attempted in order to identify the best method. These included the use of an orbital shaker, sonication, soxhlet extraction and with different solvents and solvent systems. The clean-up methods used comprised of silica gel, alumina and florisil and in all of them the drying agent was Na_2SO_4. Finally, the best recoveries were obtained using soxhlet extraction with toluene and cleaning up with florisil. This was chosen as the method of choice for sediment and soil samples. The samples were then treated as follows:

After sampling, the sediment samples were subsequently subjected to centrifugation (Du Pont Instruments^R SS-automatic centrifuge) using 10 000 rev./min for 15 min to remove pore water. The pore water samples were treated as per the water samples above. Pore water samples were collected and analysed to show how the selected OCPs and PCBs settle, concentrate and partition themselves between the pores of the sediment. The sediment and soil samples were transferred to aluminium foil and air-dried before being ground with a mortar and pestle and sieved for homogenisation and increasing surface area. A 60 g sample of sieved sediment or soil underwent soxhlet extraction for 24 hours with 300 mL of HPLC-grade toluene (EPA method 3540, 2007). The obtained extracts were concentrated with a rotary evaporator to nearly 5 mL

and air-dried for subsequent clean-up. The clean-up and concentration procedures were carried out as mentioned above with a florisil column containing 10 g of anhydrous Na_2SO_4 on top and eluting with 4 aliquots of 20 mL of hexane-DCM solvent system. The extracts were air-dried and made up to 2 mL with DCM for analysis using gas chromatography/mass spectrometry (GC/MS).

3.9.1.3 *Msunduzi River water samples*

A 500 mL aliquot of the water sample was transferred into a rinsed separating flask, 40 mL of dichloromethane was added, and the sample was extracted by shaking the separatory flask for 5 minutes with periodic venting. The organic layer was allowed to separate from the aqueous phase by letting the flask stand for 15 minutes. The organic layer (dichloromethane) with the extract was removed, and 10 grams of anhydrous sodium sulphate was added to the dichloromethane extract and the solution filtered. The procedure was repeated two more times and the filtered extract fractions were combined and reduced to 2 mL using rotary evaporation. Samples collected from the wastewater treatment plant were filtered through a vacuum glass funnel to remove the solids and suspended particles due to the high turbidity of the water sample prior to the extraction.

For the analysis of OCPs and PCBs in the Msunduzi River a multi-layer silica gel column was used as recommended by the United States Environmental Protection Agency (EPA method 1668B, 2008) and Kumar et al. (2012) for clean-up of anthropogenic and PCB pollutants. The column (Quickfit D1/11 England 50 mL) was packed from bottom to top with 2.5 g silica gel, 1.5 g basic silica gel, 2.5 g silica gel, 5.0 g acidic silica gel, 2.5 g silica gel, activated copper powder and 10 g anhydrous sodium sulphate (Na_2SO_4). The column was pre-eluted with 20 mL n-hexane before the extract was loaded; 50 mL of n-hexane/DCM/ toluene solvent combinations were used for the elution in the ratio 2.5: 1.5: 1. The eluent was reduced to 2 mL using rotary evaporation and thereafter evaporated to dryness. The samples were reconstituted to 2 mL with n-hexane and filtered through a 4 µm disc filter prior to GC-MS analyses.

Preparation of the basic silica gel for the clean-up column

Pellet samples consisting of 4 g sodium hydroxide (NaOH) were placed into a beaker, distilled water was added and the solution stirred until homogeneous. The solution was then transferred into a 100 mL volumetric flask and made up to the mark with distilled water to make 1 N NaOH. About 33 g of this solution was combined with 67 g of silica gel in a beaker. The aggregates were broken up with a stirring rod until a uniform mixture was obtained. The mixture was transferred into a 500 mL conical flask, covered with aluminium foil and agitated for 2 hours on the mechanical shaker, thereafter activated in the oven overnight at 250°C and cooled in a desiccator prior to use.

Preparation of the acidic silica for the clean-up column

A 44 g sample of concentrated sulfuric acid was thoroughly mixed with 66 g of activated silica gel in a clean 250 mL beaker. The aggregates were broken up with a stirring rod until a uniform mixture was obtained, transferred into a 500 mL conical flask and covered with aluminium foil and agitated for 2 hours on a mechanical shaker. The mixture was activated in an oven overnight at 250°C, and cooled in a desiccator prior to use.

3.9.1.4 *Msunduzi River soil and sediment samples*

The dried and homogenized sample (20 g) was mixed with 10 g of anhydrous sodium sulfate to remove excess moisture from the sample. An ultrasonic bath (UMC 20, 90022112 Kenmare) was used for extraction due to its improved efficiency when extracting organic pollutants, shorter turnaround time, and the need to use less solvent when compared to the conventional soxhlet extraction method (Daso et al. 2011). Soil and sediment samples were extracted with three aliquots of 20 mL dichloromethane (DCM), at 30°C for 30

minutes each. Extracts were combined, filtered and concentrated to 2 mL using rotary evaporation and thereafter subjected to clean-up as described for the water samples.

3.9.2 Methodology for analysis of OCPs and PCBs using GC-MS

Sample analyses were carried out in triplicate using an Agilent 6890 series gas chromatography system attached to a mass spectrometer detector (MSD5973) as well as a Shimadzu QP-2010 Ultra (Japan). Both GC systems were equipped with a ZB-5MS capillary column, 0.25 mm i.d., 0.25 µm film thickness and 30 m length (Hewlett Packard; Houston, TX). The MS was operated using the selective ion monitoring acquisition mode (SIM). The carrier gas was purified helium. For the Agilent GC-MS a splitless mode was used to inject 2 µL of sample into the GC-MS with injector and detector temperatures set at 250 and 280°C respectively. The oven temperature for analysis of OCPs and PCBs was initially 120°C and then increased to 290°C at a ramping rate of 14 ^{o}C/min and held for 2 min. The total run time was 14.14 min. The MS source was operated at 250°C and the quad at 200°C. The electron ionisation energy was 70 eV. For the Shimadzu GC-MS a splitless mode was also used. The injector and detector temperatures were set at 220°C and 320°C respectively. The oven temperature was set at 150°C held for 2 min, raised to 295°C at a ramping rate of 14°C /min and held for a further 2 min. An injection volume of 1 µL was used.

3.9.3 Method validation

The processes used to ensure that all analytical procedures carried out are valid and can be applied to the analysis of the data is presented within this section.

3.9.3.1 *Calculation of limit of detection and limit of quantification*

The definition for limit of detection (LOD) used within this context is the lowest concentration of an analyte in a sample which can be detected and the limit of quantification is the smallest concentration of an analyte in a sample which can be measured and reliable results obtained using a given analytical method (Shrivastava and Gupta 2011, Saadati et al. 2013).

For a linear calibration curve, it is expected that the response “y” recorded by the instrument is linearly related to the standard concentration” x”. This can be expressed by the following equation: (Shrivastava and Gupta 2011)

$$y = mx + b \text{ and therefore: } LOD = 3 * S/m \text{ and } LOQ = 10 * S/m$$

Where: S = standard deviation of the response
m = the slope of the calibration curve
b = y-intercept

The standard deviation can be estimated from y-intercepts of regression lines (Shrivastava and Gupta 2011). Note that in our case the responses were peak areas obtained from the GC-MS.

Example 1: Calculation of LOD and LOQ of PCB 28 in a water sample from the Umgeni River
The calibration standards with six levels of concentrations 0.25, 0.5, 1, 2, 4 and 8 µg/mL were run in triplicate and three calibration curves were obtained. The raw data for PCB 28 is presented in Table 3.19. The data (from Table 3.19) was plotted using Microsoft Excel 2010 and the equation of the line and the R^2 values were obtained. The slope, intercept and R^2 values for the triplicate analysis of PCB 28 is presented In Table 3.20.

The LOQ and LOD were calculated using standard deviation of intercepts (S).

LOD = 3*S/m where m = average of slopes = 75529
= (3*1142.017)/75529
= 0.045 µg/mL

Table 3.19 Peak area for selected standards of PCB 28

Conc. (µg/mL)	Peak area		
0.25	12833	12310	12418
0.5	33331	32762	31820
1	62407	60991	61188
2	139542	130694	134310
4	286689	284660	286701
8	599776	590533	601858

Table 3.20 Parameters extracted from the individual plots of concentration and peak area for PCB 28

Run no	Slope (m)	Intercept (b)	R^2
1	75692	-9593.9	0.9995
2	74734	-10850	0.9994
3	76161	-11874	0.9994
Average	75529	-10772.63333	0.9994
S		1142.017	

LOQ = 10 * S/m
= (10 *1142.017)/75529
= 0.15 µg/mL

One litre (1 L = 1000 mL) of river water was, for a given sample, extracted. After the extraction, clean-up, concentration and drying processes (EPA 1996a, 2007), the obtained extract was reconstituted with exactly 2.00 mL of HPLC-grade dichloromethane (DCM). The dilution factor was:

1000 mL/2 mL = 500; therefore, to obtain LOD and LOQ, for PCB 28 in the water samples, the above-obtained values must be divided by the dilution factor.

Hence: LOD = (0.045 µg/mL)/500
= 9.0×10^{-5} µg/mL
= 0.09 ng/mL
LOQ = (0.15 µg/mL)/500
= 0.0003 µg/mL
= 0.3 ng/mL

Finally, the LOD and LOQ of PCB 28 in Umgeni River water samples were 0.09 ng/mL and 0.3 ng/mL respectively.

Example 2: Calculation of LOD and LOQ of HCB in sediment and bank soil samples of Umgeni River

For sediment and soil samples, a portion of 60 g of sediment or bank soil was extracted, cleaned, dried and reconstituted (EPA 2007, 1996b); therefore, the values obtained using calibration standards, were divided by 60 in order to obtain the LOD and LOQ of analytes (PCB and OCPs) in sediment and soil. For example, for HCB (hexachlorobenzene), the values obtained using the above-mentioned equation, were 0.029820808 µg/mL and 0.099402693 µg/mL for LOD and LOQ respectively. The limit of detection and limit of quantification of HCB in soil and sediment samples were obtained as follows:

LOD = (0.02980808 µg/mL x 1 mL)/60 g
= 4.968013×10^{-4} µg/g
= 0.00049680 µg/g
= 0.50 ng/g

LOQ = (0.099402693 µg/mL x 1 mL)/60 g
= 0.00165671155 µg/g
= 1.66 ng/g

Finally, the LOD and LOQ of HCB in surface sediment and bank soil of Umgeni River were 0.50 ng/g and 1.66 ng/g respectively. The LODs and LOQs for all the selected analytes were determined using the same method described above.

3.9.3.2 *Recoveries*

Recoveries for water samples were obtained using tap water. One litre (1 L) of tap water was measured in a measuring cylinder and transferred in a 2 L separating flask and spiked with 1 mL of 10 µg/mL of a multi-standard solution of PCBs or OCPs. In other words, the spiking level was ten micrograms per litre (10 µg/L) and was kept overnight to allow contact time and homogenisation with the matrix. The tap water sample was then extracted 6 times using 50 mL aliquots of fresh DCM (EPA 1996a). The six fractions were combined and concentrated using a rotary evaporator to about 5 mL. The concentrated extract was taken through the exact same procedure as described for the samples in section 3.9.1. Calculations of recoveries are illustrated in example calculation below. Recoveries for sediment and soil samples were obtained using real samples in order to cater for matrix effects within the sediment/soil itself. A sample was divided into two subsamples. The first subsample of 60 g was not spiked while the second portion of the same mass of sediment or soil was spiked with1 mL of 8 µg/mL multi-standard solution of PCB or OCPs (Harry et al. 2008). This means that the spiking level was 0.13 µg/g. Both samples were kept overnight and then were transferred into the extraction thimble which was placed in a soxhlet extractor/ultrasonicator and extracted using 300 mL of toluene for 24 hours. The extracts were treated exactly in the same way as the samples would be treated as described in section 3.9.1. The calculations of recoveries are illustrated by the example shown below. A similar recovery method was used for validating the method used to analyse the Msunduzi River samples except that an acidic/basic silica gel column was used for clean-up.

Example 1: Calculation of recovery of PCB 28 in water of the Umgeni River
The calibration standards: 0.25, 0.5, 1, 2, 4 and 8 µg/mL were used and were run in triplicate. The three calibration curves were obtained and the above-mentioned equation was used to calculate the concentration (See above).

y = mx + b where y = response (peak area); m = mean of slops and b = mean of intercepts

x = concentration =?

The peak areas of PCB 28 obtained for the three runs of the reconstituted extract were:

y1 = 271490; y2 = 271602; y3 = 269130
x1 = (y1 – b)/m = (271490 +10772.63333)/75529 = 3.737142 µg/mL
x2 = (y2 – b)/m = (271602 + 10772.63333)/75529 = 3.738625 µg/mL
x3 = (y3 – b)/m = (269130 + 10772.63333)/75529 = 3.705896 µg/mL

Note that the spiking level was 10 µL/L, this means that since the clean and dry extract was reconstituted to 2.00 mL, if PCB 28 was extracted at 100%, the recovered concentration was supposed to be 5 µg/mL.

The percentage recovery (%R1) can be calculated using the following formula

% R = (C/D) x 100 where %R = percentage recovery; C = concentration obtained;
D= concentration expected (known value for the spike in the sample).

Therefore %R1 = (x1/5) x 100 = (3.737142422/5) x 100 = 74.74
%R1 = (x 2/5) x 100 = (3.738625/5) x 100 = 74.77
%R1 = (x3/5) x 100 = (3.705896 /5) x 100 = 74.12
%R = (74.74 + 74.77 + 74.12)/3 = 74.54

Finally the percentage recovery of PCB28 in water was 74.54% with RSD = 0.37

Example 2: Calculation of recovery of HCB in sediment from Umgeni River
Calculation of concentration of HCB in unspiked subsample:

The subsample was analysed in triplicate and from the three calibration curves, an overall straight line equation was obtained:
y = mx +b

where y = peak area of HCB obtained from unspiked subsample run
m = average of slops from the three runs
b = average of y-intercepts from the three runs
x = concentration of HCB in the unspiked sample = ?

From the triplicate analysis of the unspiked subsample, three peak areas of HCB were obtained and using the overall equation above, the three following concentrations were obtained:

X1 = 2.101780931 µg/mL, X2 = 1.942859828 µg/mL and X3 = 2.084958414 µg/mL

Calculation of concentration of HCB in spiked sub-sample. The three peak areas were also obtained from the spiked subsample. The three concentrations were obtained from the same equation above-mentioned, generated from the same calibration curves.

X4 = 5.298153282 µg/mL, X5 = 5.235797190 µg/mL and X6 = 5.092006955 µg/mL
The percentage recoveries were obtained using the following equation (Harry et al. 2008):

$$\%R = \frac{Xs - Xu}{K} * 100$$

Where: %R = percentage recovery of spiked analyte
Xs = measured value for spiked sample
Xu = measured value for unspiked samples
K= known value for the spike in the sample

NB. The subsample was spiked with 1 mL of 8 µg/mL solution of HCB (see experimental above). Since the extract was reconstituted to 2.00 mL, the expected concentration was 4 µg/mL. In this case the three recoveries were calculated as follows:

$$\% R1 = \frac{X4 - X1}{4} * 100$$
$$= \frac{5.298153282 - 2.101780931}{4} * 100$$
$$= 79.90930877$$

$$\% \text{ R2} = \frac{\text{X5} - \text{X2}}{4} * 100$$
$$= \frac{5.235797190 - 1.942859828}{4} * 100$$
$$= 82.32343405$$
$$\% \text{ R3} = \frac{\text{X6} - \text{X3}}{4} * 100$$
$$= \frac{5.092006955 - 2.084958414}{4} * 100$$
$$= 75.17621352$$

The overall percentage recovery (% R) was obtained by calculating the average of the recoveries % R1, % R2 and % R3.

$$\%\text{R} = \frac{\% \text{ R1} + \% \text{ R2} + \% \text{ R3}}{3}$$
$$= 79.14$$

Finally, the recovery of HCB in sediment collected from Umgeni River was 79.14%. Similar calculations were carried out for recoveries for the Msunduzi River samples. Table 3.21 and Table 3.22 show the LODs, LOQs and recoveries obtained for the selected PCBs and OCPs in this study.

Table 3.21 Validation results: LODs, LOQs and recoveries for PCBs in water, pore water and sediment

Analyte	PCB28	PCB52	PCB77	PCB101	PCB105	PCB138	PCB153	PCB180
Ions monitored (*m/z*)	150 186 256	150 220 292	220 255 292	254 291 326	184 254 326	145 290 360	145 290 360	162 324 394
LOD (ng/mL) in water	0.045	0.015	0.055	0.02	0.015	0.015	0.02	0.01
LOQ (ng/mL) in water	0.15	0.045	0.19	0.06	0.055	0.045	0.06	0.04
LOD (ng/mL) in pore water	0.455	0.13	0.55	0.18	0.17	0.135	0.185	0.115
LOQ (ng/mL) in pore water	1.51	0.43	1.835	0.595	0.56	0.445	0.625	0.39
%Recovery in water and pore water[1]	74.54 ±0.37	76.99 ±0.67	79.27 ±0.83	71.18 ±0.59	73.88 ±0.45	74.67 ±0.40	77.83 ±0.86	82.36 ±0.41
LOD (ng/g in sediment)[2]	0.76	0.21	0.92	0.30	0.28	0.22	0.31	0.19
LOQ (ng/g in sediment)[3]	2.52	0.71	3.06	0.99	1.12	0.75	1.03	0.66
%Recovery in sediment	69.53 ±1.75	80.74 ±2.94	73.67 ±1.22	79.77 ±2.03	78.46 ±1.27	79.39 ±1.05	82.87 ±2.50	84.39 ±1.15

[1] ± number = Standard deviation; this means that the recovery experiments were carried out in triplicate and the mean recovery calculated as well as the standard deviation.
[2] The limit of detection was calculated as three times the signal-to-noise ratio using three calibration intercepts divided by the slope.
[3] The limit of quantification was calculated as ten times the signal-to-noise ratio using three calibration intercepts divided by the slope.

Table 3.22 Validation results: LODs, LOQs and recoveries of OCPs in water, pore water and sediment

Analyte	HCB	HCH	heptachlor	aldrin	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD/dieldrin[4]	endrin	*p,p'*-DDD/*o,p*-DDT[5]	mirex
Ions monitored (*m/z*)	284 249 142	219 183 147	374 272 237	327 293 263	318 284 246	318 281 246	320/380 235/263 165/147	317 263 207	320/235 235/199 165/165	402 272 237
LOD (ng/mL) in water	0.025	0.06	0.03	0.045	0.06	0.07	0.035	0.06	0.075	0.07
LOQ (ng/mL) in water	0.58	0.10	0.10	0.155	0.19	0.125	0.205	0.205	0.245	0.23
LOD (ng/mL) in pore water	0.24	0.295	0.295	0.465	0.06	0.37	0.06	0.615	0.74	0.69
LOQ (ng/mL) in pore water	0.795	0.35	0.975	1.55	0.19	1.24	0.205	2.05	2.47	0.235
%R in water and pore	51.90±0.47	64.38±0.28	32.66±0.67[6]	69.66±0.36	84.36±1.39	87.42±0.68	103.43±0.97	61.08±0.87	75.27±0.19	65.31±0.33
LOD (ng/g) in sediment	0.50	0.50	0.50	0.78	0.96	0.62	1.04	1.02	1.23	1.15
LOQ (ng/g) in sediment	1.66	1.66	1.62	2.59	3.20	2.07	3.45	3.41	4.11	3.83
%R in sediment	79.14±3.64	98.22±3.81	99.15±10.03	116.97±5.36	95.60±12.15	52.73±1.35	90.02±3.59	94.19±14.81	96.68±2.71	109.28±6.19

[4] *o,p*-DDD and dieldrin could not be resolved on the GC and were reported as a single peak.
[5] *p,p*-DDD and *o,p*-DDT could not be resolved on the GC and were reported as a single peak.
[6] Heptachlor was in contact with tap water for some days to allow its contact with matrix before extraction. It may have degraded into heptachlor epoxide by oxidation, photolysis and can also volatilise in air (Callahan, M. A., 1979) and consequently its recovery was low during extraction.

Table 3.23 Calibration data and method validation parameters for LC-MS analysis of pharmaceuticals

						Wastewater		Surface Water		Sediments		
					ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/g	ng/g	
Analytes	***m/z***	**Therapeutic class**	**Calibration Equation**	**R_t /min**	**Linear Range**	**MDL**	**MQL**	**MDL**	**MQL**	**MDL**	**MQL**	**R^2**
ACE	151	Antipyretic	$y=12.42 \times 10^{6} x-3.48 \times 10^{6}$	3.5	0.1000-100	0.0081	0.2733	0.0027	0.0911	0.0162	0.5466	0.9930
ASP	179	Antipyretic	$y=17.63 \times 10^{3} x+1.41 \times 10^{4}$	2.3	2.0000-100	0.8772	2.9238	0.2924	0.9746	1.7544	5.8476	0.9956
CAF	195	Stimulant	$y=7.00 \times 10^{6} x+9.84 \times 10^{5}$	7.2	0.3473-100	0.3120	1.0419	0.1040	0.3473	0.624	2.0838	0.9973
CBZ	237	Anti-epileptic	$y=5.54 \times 10^{7} x-2.19 \times 10^{6}$	14.3	0.2970-100	0.2676	0.891	0.0892	0.2970	0.5352	1.782	0.9960
CLO	327	Anti-psychotic	$y=22.70 \times 10^{6} x-1.91 \times 10^{7}$	12.6	0.4436-100	0.3993	1.3308	0.1331	0.4436	0.7986	2.6616	0.9931
ERY	734	Antibiotic	$y=6.22 \times 10^{8} x+7.86 \times 10^{6}$	12.2	0.0004-100	0.0003	0.0012	0.0001	0.0004	0.0006	0.0024	0.9913
MET	172	Antibiotic	$y=5.22 \times 10^{5} x+5.18 \times 10^{5}$	4.4	0.9619-100	0.8658	2.8857	0.2886	0.9619	1.7316	5.7714	0.9931
IBU	205	Antipyretic	$y=1.00 \times 10^{6} x+1.13 \times 10^{5}$	8.8	0.2710-100	0.2442	0.813	0.0814	0.2710	0.4884	1.6260	0.9928
SMX	254	Antibiotic	$y=7.70 \times 10^{6} x+8.63 \times 10^{6}$	11.7	0.4135-100	0.3720	1.2405	0.1240	0.4135	0.7440	2.4810	0.9944
SMZ	279	Antibiotic	$y=3.93 \times 10^{7} x+1.66 \times 10^{7}$	9.1	0.2271-100	0.2043	0.6813	0.0681	0.2271	0.4086	1.3626	0.9907
TMP	291	Antibiotic	$y=250.01 \times 10^{6} x+7.88 \times 10^{6}$	7.8	0.5000-100	0.1230	0.4110	0.0410	0.1370	0.2460	0.822	0.9952

CHAPTER 4: PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN THE UMGENI AND MSUNDUZI RIVERS

4.1 INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) can be classified as emerging organic contaminants, and include 1000's of manmade organic chemicals used for human health, cosmetics, or animal care (Cizmas et al. 2015). This section presents the results obtained for the concentration of selected pharmaceuticals and personal care products in sediments, surface water and wastewater of the Umgeni and Msunduzi Rivers. These rivers run through some of the most heavily populated urban areas in KwaZulu Natal, South Africa. Selected pharmaceuticals included; antibiotics (nalidixic acid, sulfamethazine, sulfamethoxazole, erythromycin, metronidazole, and trimethoprim), antipyretics (salicylic acid, naproxen, phenacetin, ketoprofen, diclofenac, meclofenamic acid, aspirin, ibuprofen and acetaminophen), one stimulant (caffeine), one anti-epileptic drug (carbamazepine) and one antipsychotic drug (clozapine). These classes of pharmaceuticals were chosen based on their persistent detection in water bodies world-wide as seen from the literature, high annual consumption in South Africa ImpactRx data, and their reported adverse effects.

There were two methods of analysis and detection developed for the analysis of pharmaceuticals, along the Umgeni and Msunduzi Rivers. The first method derivatized the target analytes and used GC-MS and the second method used LC-MS. Part of the work using LC-MS has been published in Matongo et al. 2015b and part of the GC-MS work has been published in Gumbi et al. 2016. The occurrence of personal care products, particularly, polycyclic musks, namely, galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK) in the Umgeni and Msunduzi Rivers were also investigated. Polycyclic musks and musk ketones are considered emerging pollutants and limited studies show they have the potential to be endocrine disruptors as well as cause various illnesses in humans and aquatic life over prolonged exposure.

4.2 DETERMINATION OF PHARMACEUTICAL CONCENTRATIONS USING GC-MS

To evaluate the suitability of the GC-MS method for the analysis of the environmental samples, all of the samples were spiked with a surrogate (SS) and internal standard (IS) prior to and after extraction. If the method did not provide acceptable recoveries and LODs for the surrogate standards, such samples were repeated until acceptable results were achieved and these results are presented in Table 4.1. The developed method gives good chromatographic resolution and ion intensity for target analytes in the environmental matrices. Changes in the retention times and matrix-induced signal suppression was not observed when this method was applied to the environmental samples. Six out of the nine analysed acidic drugs were detected in the Umgeni River system (Table 4.1). Only naproxen, meclofenamic and diclofenac were not detected in the environment, possibly owing to their almost complete degradation by human metabolic processes prior to excretion (Metcalfe et al. 2003b). In nine of the sites studied, only Midmar dam did not have any of the selected acidic drugs even at LOD levels. Besides lack of method sensitivity, another reason maybe the due to the location of Midmar dam, and the surrounding farms, which are relatively far away from possible sources of contamination (sewage), except during heavy rain falls in the summer season. Ibuprofen was frequently detected at eight of the sites, and only quantified at Darvill WWTP inlet, and its concentration was found to be 3.00 ng/mL. Phenacetin was detected at five of the sites and its concentration was higher than ibuprofen. Phenacetin concentration increased as the river flowed towards the ocean, with values at the Darvill WWTP inlet at 1.95 ng/mL, Inanda dam at 2.35 ng/mL and Umgeni estuary at 8.14 ng/mL. This trend was attributed to the informal settlement surrounding the river near the estuary. The Inanda dam was regarded as a relatively polluted site compared to other analysed sites, because most targeted acidic drugs were found in this area.

Table 4.1 Results on the use of GC-MS to identify and quantify selected pharmaceuticals (ng/mL ±RSD) from various points along the Umgeni River

Sampling sites	%SS	salicylic acid	acetyl salicylic acid	nalidixic acid	ibuprofen	phenacetin	naproxen	meclofenamic	ketoprofen	diclofenac
Midmar dam	106	-	-	-	-	-	-	-	-	-
Albert Falls dam	69	D	-	-	D	-		-	-	-
Henley dam	80	-	-	-	-	D	-	-	-	-
Darvill WWTP inlet	77	-	-	-	3.000 ± 0.065	1.95 ± 0.005	D	-	D	-
Darvill WWTP outlet	83	-	-	-	D	D	-	-	-	-
Nagle dam	120	-	-	-	D	-	-	-	-	-
Inanda dam	71	D	1.13± 0.0658	2.53± 0.420	D	2.34 ± 0.204	D	-	0.620 ± 0.0903	D
Northern WWTP inlet	72	D	D	-	D	-	-	-	D	-
Northern WWTP outlet	86	0.0243 ± 0.0019	-	-	D	-	-	-	-	-
Umgeni Estuary	77	-	-	-	D	8.14 ± 0.474	-	-	-	-

D = detected but not quantifiable
SS = surrogate standard

Note: In contrast to GC-MS, LC-MS remains as one of the most favoured analytical methods for the quantification of pharmaceuticals in the environment (Richardson and Kimura 2016) and as such was used for the initial occurrence studies for both Umgeni and Msunduzi Rivers. The LC-MS analysis of the environmental samples, targeted 3 antipyretics (aspirin, ibuprofen, acetaminophen), 5 antibiotics, (erythromycin, metronidazole, sulfamethoxazole, sulfamethazine, trimethoprim), an anti-psychotic drug (clozapine), an anti-epileptic (carbamazepine), and a stimulant (caffeine).

4.3 PHARMACEUTICAL CONCENTRATIONS IN THE UMGENI AND MSUNDUZI RIVERS

4.3.1 Surface water

The concentrations of the selected pharmaceuticals in surface water samples of the Umgeni River and Msunduzi Rivers are shown in Figure 4.1.

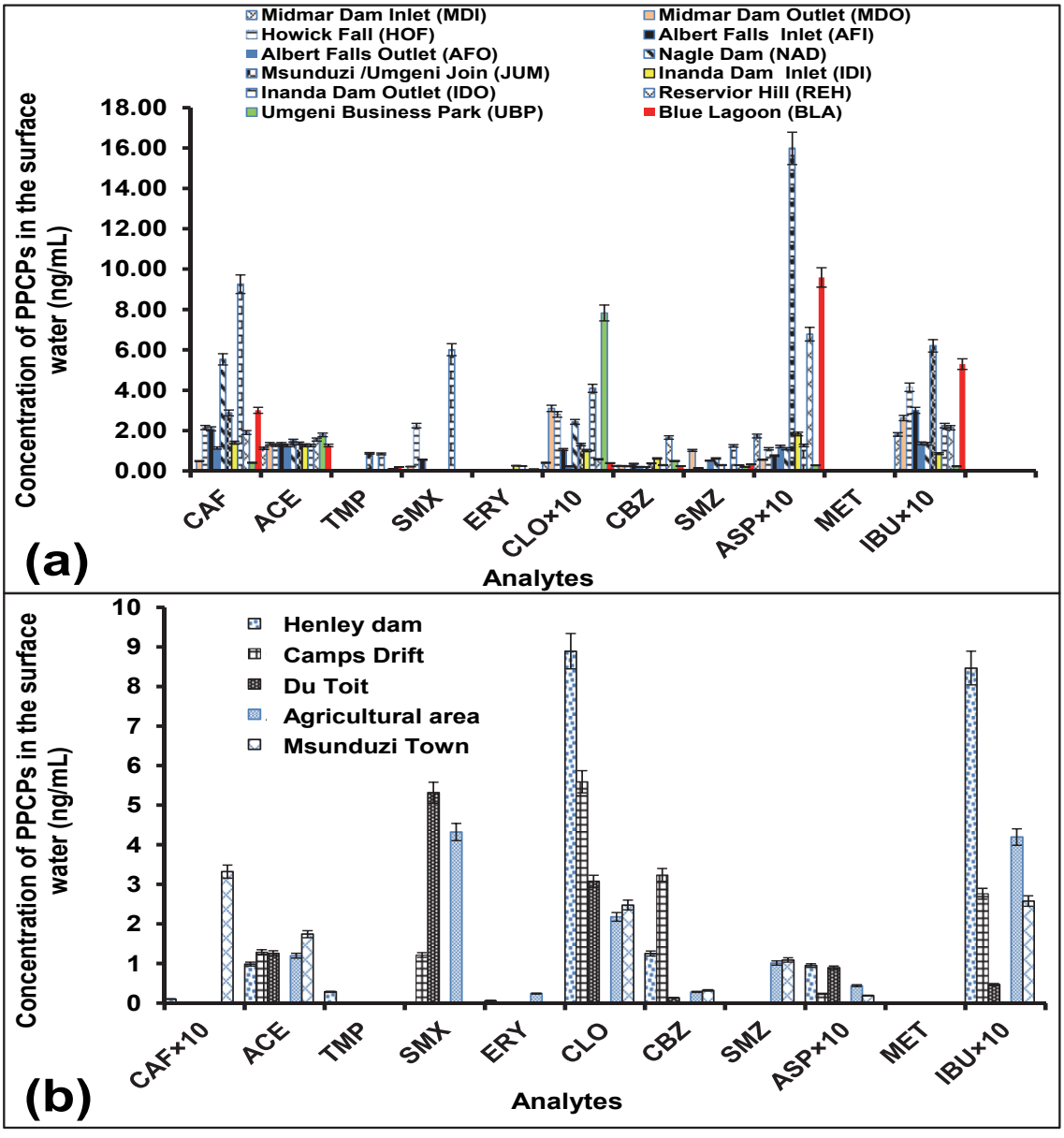


Figure 4.1 PPCPs ((caffeine (CAF), acetaminophen (ACE), trimethoprim (TMP), sulfamethoxazole (SMX), erythromycin (ERY), clozapine (CLO), carbamazepine (CBZ), sulfamethazine (SMZ), aspirin (ASP), metronidazole (MET) and ibuprofen (IBU))) present in Umgeni River (a) and (b) Msunduzi River surface water

Aspirin (ASP) and ibuprofen (IBU) were found in high concentrations in the surface water of both the Umgeni (Table 4.2) and Msunduzi Rivers (Table 4.3). These correspond with the figures obtained from ImpactRX data which show that these two drugs are among the highest consumed drugs in South Africa and also these drugs can be obtained over the counter without a doctor's prescription. The ASP value was the highest of all the detected analytes with a concentration of 150.90 ± 0.18 ng/mL at the joining point of the Msunduzi tributary with the Umgeni River. This was due to the combination of the two rivers thus increasing the concentrations of all the analytes.

It is also important to note that the high concentration of ASP might be also due to its broad usage, especially considering it is easily available and can be purchased at most shops (mainstream big chain grocery stores-small family owned convenience stores). For acetaminophen (ACE) the data sourced from ImpactRX indicated that ACE is being sold in high quantities but it was detected as the lowest concentration of the three antipyretic drugs (aspirin, ibuprofen, acetaminophen) which were investigated. The reason for this could be due to its high removal rate from the WWTP of up to 98% (Martín et al. 2015, Papageorgiou et al. 2016) and degradation in the environment (Marchlewicz et al. 2015, Ortiz de García et al. 2014). Further work should probably be done in terms of identifying the occurrence and levels of the transformation products, e.g. 4-aminophenol (Marchlewicz et al. 2015).

Trimethoprim, sulfamethoxazole, and erythromycin were detected in a few sites in low concentrations. In contrast sulfamethazine was only found in half of the water sampling sites. This result differs to what was obtained in Kenyan surface water (30 ng/mL) (Kenneth et al. 2012). The values of SMX (0.22-6.01 ng/mL) and TMP (0.10-0.82 ng/mL) for all the water sampling sites were close to that reported in Kenya at 22 ng/mL and 6 ng/mL, respectively (Kenneth et al. 2012) but for SMX the values were higher than that reported in the USA (0.400 ng/mL) except at the MDO (0.22 ng/mL) sampling site (Ferguson et al. 2013). This might be due to the high rate of human immune deficiency virus (HIV) infection rate for which SMX is used in the treatment of secondary infections related to HIV in developing countries like South Africa especially in the KwaZulu-Natal Province compared to developed countries such as the USA. SMZ used for veterinary therapy was found at IDO, MDO, AA and MT where there is a high incidence of animal rearing within the vicinity of the sampling point and therefore its prevalence in these areas was expected.

Carbamazepine was detected at low concentrations of 0.21-3.24 ng/mL in both the Umgeni and Msunduzi River sampling sites. The results were consistent with similar investigations in Europe and North America where CBZ was one of the commonly detected pharmaceuticals in river water (Clara et al. 2004). Hence, CBZ can be selected as a drug which can be used as a marker for PPCP contamination in surface water as reported by Clara et al. (2004).

Caffeine was detected in almost all of the sites sampled with concentrations ranging from ND – 33.2 ng/mL. This was expected since it is the main ingredient of hot beverages such as coffee and stimulant drinks such as Red Bull and Monster which is consumed by a large number of the population of South Africa. Caffeine can also be used as an anthropogenic marker for wastewater contamination of surface water (Kurissery et al. 2012).

CLO was detected at a frequency of 87% in all the sampling sites with a maximum concentration of 78.3 ng/mL. Its high frequency detection was attributed to its medicinal use in the treatment of mental disorders. About a third of the population of South Africa have been reported to be suffering from a mental disorder according to the Sunday Times (Beauregard Tromp et al. 2014).

Table 4.2 Concentrations of the selected pharmaceuticals in the Umgeni River surface water (ng/mL) ± RSD (%). Analyte codes are caffeine (CAF), acetaminophen (ACE), trimethoprim (TMP), sulfamethoxazole (SMX), erythromycin (ERY), clozapine (CLO), carbamazepine (CBZ), sulfamethazine (SMZ), aspirin (ASP), metronidazole (MET) and ibuprofen (IBU)

Site code*	CAF	ACE	TMP	SMX	ERY	CLO x 10	CBZ	SMZ	ASP x 10	MET	IBU x 10
MDI	ND	1.13±4.83	ND	ND	ND	0.40±9.43	0.25±5.44	ND	1.73±0.41	ND	1.81±0.78
MDO	0.49± 2.89	1.34±1.05	ND	0.22±3.29	ND	3.10±4.42	0.24±3.01	1.02±1.37	0.56±1.25	ND	2.62±0.81
HF	2.15±0.98	1.28±1.10	ND	2.25±0.63	ND	2.80±1.25	0.22±6.05	0.15±8.84	1.10±6.15	ND	4.15±0.51
AFI	2.09±0.67	1.33±0.53	ND	0.56±2.57	ND	1.06±9.92	0.36±1.94	0.05±32.64	0.75±1.86	ND	3.00±1.19
AFO	1.14±1.23	1.26±0.56	ND	ND	ND	0.22±6.15	0.21±6.43	0.51±2.72	1.21±1.16	ND	1.37±1.04
NAD	5.53±0.38	1.50±4.88	ND	ND	ND	2.43±0.87	0.19±7.07	0.62±2.24	1.10±6.15	ND	1.35±0.52
JUM	2.87±2.00	1.36±3.55	0.87±1.64	ND	0.03±20.20	1.31±3.31	0.38±5.81	0.29±4.71	15.99±0.18	ND	6.20±1.73
IDI	1.40±9.43	1.27±1.12	ND	ND	0.26±14.63	1.01±13.89	0.62±6.53	ND	1.83±0.77	ND	0.85±5.17
IDO	9.25±0.46	1.26±2.75	0.85±0.83	6.01±1.28	0.24±6.15	4.10±1.37	0.29±6.96	1.24±0.57	1.26±1.11	ND	2.24±0.32
REH	1.91±0.37	1.55±3.56	ND	ND	ND	0.58±2.48	1.65±0.85	0.28±7.19	6.78±0.21	ND	2.14±1.67
UBP	0.41±4.99	1.78±1.57	0.10±3.56	ND	0.09±7.44	7.83±0.18	0.49±5.55	0.22±4.77	0.27±5.05	ND	0.23±6.43
BLA	3.01±0.70	1.26±1.11	0.20±1.57	ND	0.03±14.78	0.39±3.54	0.24±20.20	0.32±6.33	9.59±0.22	ND	5.29±1.08

*Midmar dam inlet (MDI), Midmar dam outlet (MDO), Howick Falls (HOF), Albert Falls inlet (AFI), Albert Falls outlet (AFO), Nagle dam (NAD), Joining point Umgeni-Msunduzi Rivers (JUM), Inanda dam inlet (IDI), Inanda dam outlet (IDO), Reservoir Hills (REH), Umgeni business park (UBP), Blue Lagoon (BLA)

Table 4.3 Concentrations of the selected pharmaceuticals ((caffeine (CAF), acetaminophen (ACE), trimethoprim (TMP), sulfamethoxazole (SMX), erythromycin (ERY), clozapine (CLO), carbamazepine (CBZ), sulfamethazine (SMZ), aspirin (ASP), metronidazole (MET) and ibuprofen (IBU))) in the Msunduzi River surface water (ng/mL) ± RSD (%)

Analyte	HND*	CMD*	DUT*	AGR*	MST*
CAF x 10	0.11 ±3.45	ND	ND	ND	3.32 ±0.98
ACE	0.99 ±5.35	1.29 ±0.57	1.26 ±3.47	1.20 ±0.43	1.74 ±4.35
TMP	0.29 ±048	ND	ND	ND	ND
SMX	ND	1.22 ±3.75	5.32 ±0.63	4.32 ±0.56	ND
ERY	0.06 ±13.56	ND	ND	0.24 ±9.93	ND
CLO	8.89 ±4.56	5.59 ±0.33	3.08 ±1.84	2.18 ±0.57	2.48 ±7.65
CBZ	1.26 ±7.65	3.24 ±0.67	0.13 ±0.79	0.29 ±3.95	0.32 ±2.54
SMZ	ND	ND	0	1.02 ±4.05	1.09 ±3.45
MET	ND	ND	ND	ND	ND
IBU x 10	8.46 ±6.65	2.76 ±0.63	0.47 ±1.43	4.20 ±0.42	2.58 ±0.76

*Henley dam (HND), Camps Drift (CMD), Du Toit (DUT), Agricultural area (AGA), Msunduzi Town (MST)

4.3.2 Sediment

Sediment samples were analysed at the various sampling points along both rivers and the concentrations for the target analytes along the Umgeni River and Msunduzi River are presented in Figure 4.2. The findings for Umgeni and Msunduzi Rivers are also shown in Table 4.4 and Table 4.5, respectively.

Metronidazole was not detected in surface water of both the Umgeni and Msunduzi Rivers but was found in appreciable quantities (up to 1253.46 ng/g) in the sediment of some sampling sites along both rivers (Figure 4.2). Ibuprofen were also found in high concentrations in the sediment which is an indicator of its wide spread use. Ibuprofen is an over the counter drug and is used frequently without the need for a prescription. Clozapine, used to treat mental disorders, was also found in the sediment of all sampling sites along the Umgeni River and in the Msunduzi Town sampling site.

Caffeine was found in high concentrations of 187-22435 ng/g. The amount in sediment was much higher than that found in the surface water at the same sites. Caffeine with a log K_{OW} of -0.07 tends to adsorb onto organic matter hence the possible reason for its higher concentration in the sediment compared to surface water. The high values in sediments were in line with what had been previously found for the K_d values of CAF which were in the range 250-1900 L/Kg by Lin et al. 2010.

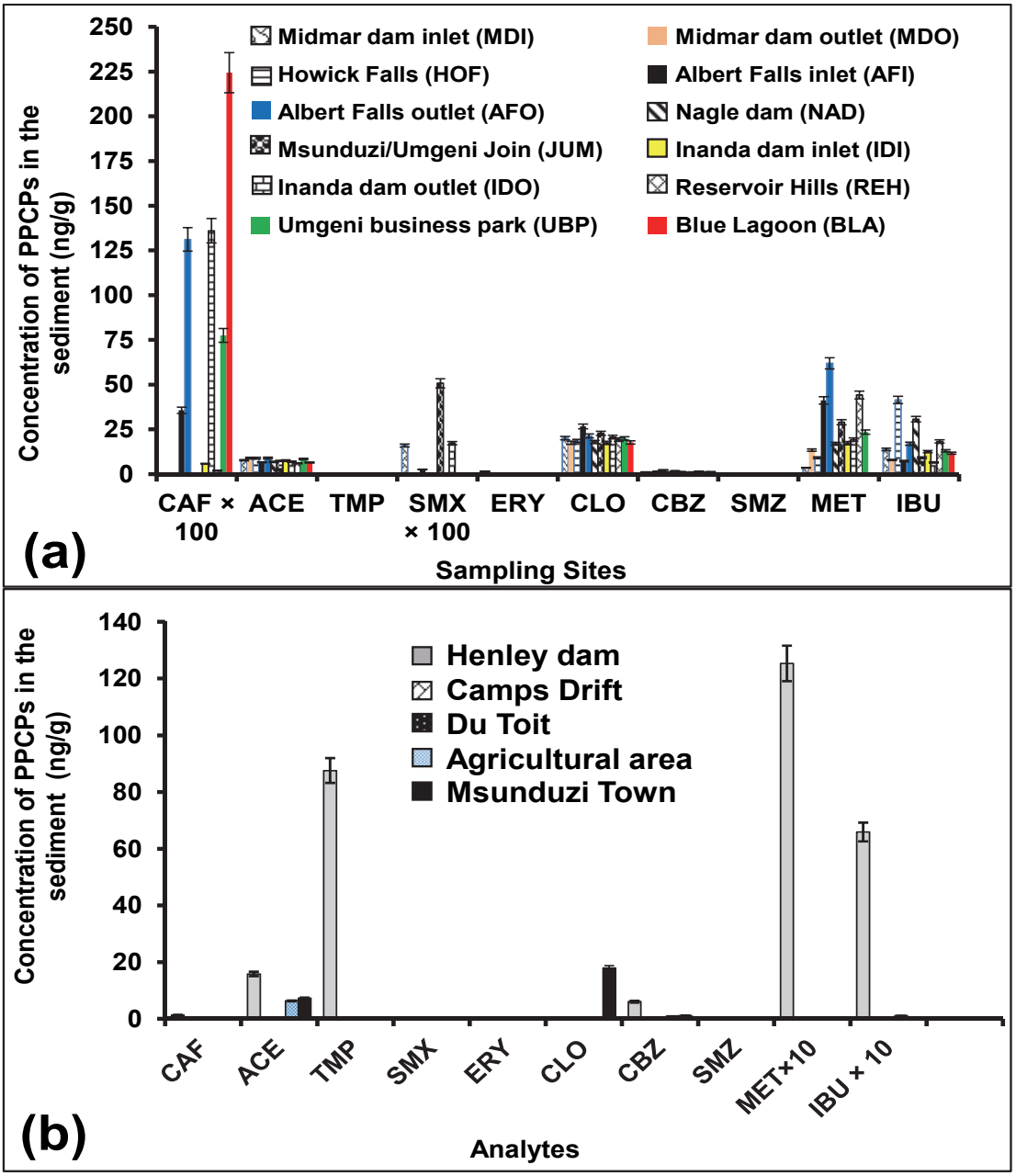


Figure 4.2 Concentrations of caffeine (CAF), acetaminophen (ACE), trimethoprim (TMP), sulfamethoxazole (SMX), erythromycin (ERY), clozapine (CLO), carbamazepine (CBZ), sulfamethazine (SMZ), aspirin (ASP), metronidazole (MET) and ibuprofen (IBU) in (a) Umgeni River sediment, and (b) Msunduzi River sediment.

Table 4.4 Concentrations of the selected pharmaceuticals (caffeine (CAF), acetaminophen (ACE), trimethoprim (TMP), sulfamethoxazole (SMX), erythromycin (ERY), clozapine (CLO), carbamazepine (CBZ), sulfamethazine (SMZ), aspirin (ASP), metronidazole (MET) and ibuprofen (IBU)) in the Umgeni River sediment (ng/g) ± RSD (%)

Site code*	CAF x 100	ACE	TMP	SMX x 100	ERY	CLO	CBZ	SMZ	MET	IBU
MDI	ND	7.75±0.18	ND	15.95±0.44	1.57±0.45	20.1±2.51	1.09±5.39	ND	3.48±0.82	13.80±6.65
MDO	ND	8.92±0.32	ND	ND	ND	17.52±4.67	1.02±3.38	ND	13.40±2.69	7.89±1.17
HOF	ND	8.87±0.32	ND	ND	ND	18.46±7.93	1.43±7.49	ND	9.16±0.31	41.41±0.74
AFI	35.67±0.24	6.57±0.22	ND	2.64±0.54	0.14±10.88	26.65±4.80	2.32±3.28	ND	41.19±1.59	7.25±0.20
AFO	131.19±0.63	8.96±0.32	ND	ND	ND	21.34±1.47	1.16±4.14	ND	61.93±0.47	16.99±1.34
NAD	ND	6.77±0.95	ND	ND	ND	17.96±4.51	1.75±0.89	ND	16.88±0.04	30.66±0.28
JUM	ND	7.36±0.19	ND	50.73±0.03	ND	22.78±0.69	1.26±2.28	ND	29.01±0.15	9.09±1.16
IDI	5.72±0.49	7.54±0.56	ND	ND	ND	17.38±0.61	1.03±2.69	ND	17.22±0.12	12.51±0.90
IDO	136.01±0.17	6.98±0.41	ND	17.26±0.08	ND	20.67±0.38	1.09±0.49	ND	19.31±0.22	6.53±0.75
REH	1.87±0.76	6.03±2.31	ND	ND	0.15±15.71	19.26±0.48	1.44±11.12	ND	44.13±2.74	18.18±1.04
UBP	77.55±0.60	8.31±1.10	ND	ND	0.12±5.66	19.94±0.28	1.11±3.93	ND	23.47±1.80	13.00±2.41
BLA	224.35±0.31	6.43±1.31	ND	ND	ND	17.69±0.60	1.32±2.18	ND	ND	11.70±2.90

*Midmar dam inlet (MDI), Midmar dam outlet (MDO), Howick Falls (HOF), Albert Falls inlet (AFI), Albert Falls outlet (AFO), Nagle dam (NAD), Joining point Umgeni-Msunduzi Rivers (JUM), Inanda dam inlet (IDI), Inanda dam outlet (IDO), Reservoir Hills (REH), Umgeni business park (UBP), Blue Lagoon (BLA)

Table 4.5 Concentrations of caffeine (CAF), acetaminophen (ACE), trimethoprim (TMP), sulfamethoxazole (SMX), erythromycin (ERY), clozapine (CLO), carbamazepine (CBZ), sulfamethazine (SMZ), aspirin (ASP), metronidazole (MET) and ibuprofen (IBU) in the Msunduzi River sediment (ng/g) ± RSD (%)

Analyte	HND	CMD	DUT	AGR	MST
CAF	1.32±0.27	<MDL	<MDL	<MDL	<MDL
ACE	15.8±0.82	<MDL	<MDL	6.33±0.76	7.30±1.04
TMP	87.55±4.88	<MDL	<MDL	<MDL	<MDL
SMX	<MDL	<MDL	<MDL	<MDL	<MDL
ERY	<MDL	<MDL	<MDL	<MDL	<MDL
CLO	<MDL	<MDL	<MDL	<MDL	17.89±3.55
CBZ	6.07±1.44	<MDL	<MDL	1.03±6.92	1.16±1.32
SMZ	ND	ND	ND	ND	ND
MET x 10	125.35±4.33	<MDL	<MDL	<MDL	<MDL
IBU x 10	65.90±1.23	<MDL	<MDL	1.13±4.76	0.53±1.04

***Henley dam (HND), Camps Drift (CMD), Du Toit (DUT), Agricultural area (AGA), Msunduzi Town (MST)**

4.3.3 A comparison of pharmaceuticals in wastewater treatment sites

The Darvill wastewater treatment plant, located in Pietermaritzburg, receives and treats raw municipal wastewater, treated industrial wastewater, and storm water. It was designed to process ±75 ML/day (Manickum and John 2014). The three sampling sites at Darvill were influent (DWin), after treatment (DWaft) and discharge point (DWout). In Durban, the Northern Waste Water Treatment Works is one of the biggest sewage treatment plants in Durban. The plant was designed to process 45 ML/day and receives raw sewage and industrial wastewater (Olaniran et al. 2012). The three sampling sites were influent (NWin), after treatment (NWaft), and discharge point (NWout). The Estcourt wastewater treatment sample points were the influent (Ewin), after treatment (EWaft), and discharge point (EWout). The spatial concentration distribution of selected pharmaceuticals from the three wastewater treatment plants is presented in Figure 4.3.

Acetaminophen was effectively removed by the Darvill WWTP to below the method detection limit and up to 51.9% at Northern WWTP. The value of 3.27 ng/mL acetaminophen in Northern effluent wastewater was lower than that reported in Spain (37.3 ng/mL) (Camacho-Muñoz et al. 2014) and slightly lower to what was reported by Agunbiade and Moodley (2014) (10 ng/mL) at the same sampling point between February and March, 2013 using the HPLC-DAD technique.

The effluent concentrations of aspirin (NWout, 42.21 ng/mL: DWout, 53.80 ngm/L) and ibuprofen (NWout, 12.94 ng/mL; DWout, 58.71 ng/mL) were high. The high values of these pain killers were consistent with the data obtained from ImpactRX data where a large amount of these drugs were consumed in 2013. It should also be noted that ASP and IBU are over-the-counter tablets hence the amount consumed is above the prescribed amount. IBU Darvill effluent maximum concentrations (58.71 ng/mL) was greater than that found in effluent from a WWTP in Gauteng, South Africa (24.60 ng/mL) (Amdany 2013). The range of concentrations of aspirin and ibuprofen (23.26-117.46 ng/mL) found at Darvill and Northern WWTPs were close to that previously reported in Spanish wastewaters (1.5-85 ng/mL) (Camacho-Muñoz et al. 2014). ASP and IBU were found to be below method detection limits in influent and effluent concentrations of Estcourt Wastewater treatment plant.

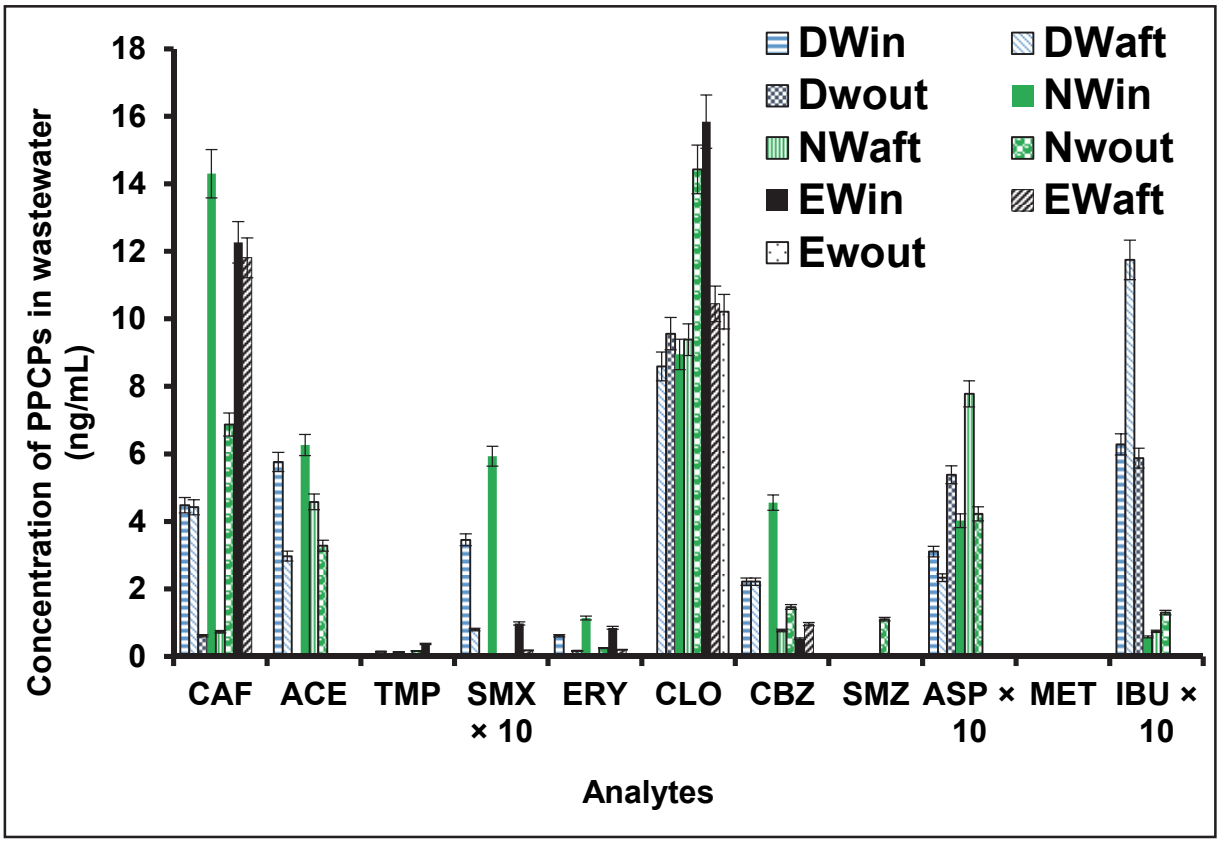


Figure 4.3 Graph of spatial concentration distribution of selected pharmaceuticals in wastewater samples of influent ('in'), after treatment ('aft') and discharge point ('out') of Darvill, Estcourt and Northern WWTP

This anomaly together with high values of ASP and IBU at Darvill and Northern WWTP after treatment in comparison to influent values can be attributed to the variations of pharmaceutical loading wastewater that reaches WWTPs. The pharmaceutical load in wastewater is determined by toilet flushes and wastewater from households and industries. The variation of pharmaceutical load in wastewater can cause a significant difference in influent and effluent concentrations in a range of minutes (Ort et al. 2010, Ort 2014).

Metronidazole was not detected in wastewater of the three treatment plants (Figure 4.3). Similar results were also found in Kenya (Kenneth et al. 2012). Effluent concentrations of TMP and SMX most used in combination as co-trimoxazole were found to be in the range of 0.162-1.76 ng/mL in all three WWTPs studied. Sulfamethazine (SMZ) was not detected in the inlet of all the three WWTPs which was expected since SMZ is not used in humans. Similar results were found in Germany and the United States (Hirsch and Kratz 1999). SMZ effluent concentration of 1.10 ng/mL for Northern WWTP was obtained because this sampling point is where the WWTP effluent discharges its water into the Umgeni River which also combines with the Umkhumbane River which is highly contaminated itself.

SMX seemed to be removed effectively by the active sludge process. It was removed by 98.9% at Darvill WWTP, 81.9% Estcourt WWTP and 93.7% at Northern WWTP. It was detected having the highest value from the selected antibiotics at NWin (59.28 ng/mL). The influent values of SMX were in line with population numbers which the WWTPs serve, being in the order NWWTP > DWWTP > EWWTP. NWWTP is situated in the EThekwini municipality, DWWTP is in the Msunduzi municipality and EWWTP is situated in the Umtshezi municipality with populations of 3 442 361; 618 536 and 83 153 respectively (http://beta2.statssa.gov.za/ retrieved on the 07/08/2014).

CBZ was detected in all three wastewater treatment plants with effluent concentrations (0.96 -1.46 ng/mL) similar to the previous reported values found in urban wastewater in Spanish and Canadian WWTP's (0.12-6.30 ng/mL) (Camacho-Muñoz et al. 2014, Metcalfe et al. 2003a, Metcalfe et al. 2003b). CBZ is one of the most commonly detected analytes in WWTP effluents; hence its presence is now used to confirm whether contamination has taken place in aquatic bodies (Clara et al. 2004).

Figure 4.3 shows that at Northern and Darvill WWTPs CLO appears to increase from the inlet to the outlet point. This can be explained by the hypothesis that during the treatment process bio-solids which contain adsorbed CLO result in them dissolving hence exposing CLO which results in the concentration increasing in the water. This can also be further explained by the variation in pharmaceutical load of the wastewater within a range of minutes, however further investigation is needed to determine the actual cause (Ort et al. 2010).

The effectiveness on the removal of PPCPs on the three WWTPs was investigated using the following equation:

$$\text{Percentage Removal Efficiency (PRE)} = (W_{out}-W_{in})/W_{in} \times 100\%$$

Where Wout is the concentration of the analyte at the discharge point and Win is the concentration of the analyte at the effluent point.

For CAF the WWTP removal efficiency varied as follows: Estcourt (97.5%), Northern (51.9%) and Darvill (86.4%) (Table 4.6). In the case where one of the concentrations was below the detection limit, the value for MDL was used to calculate PRE.

Table 4.6 Percent Removal Efficiency for Darvill, Northern and Estcourt WWTPs

Analytes	Darvill WWTP	Northern WWTP	Estcourt WWTP	Average
CAF	86.40	51.90	97.50	78.60
ACE	98.00	47.70	<MDL	72.90
SMX	98.90	93.70	81.90	91.50
ERY	73.33	78.76	99.86	83.98
CLO	-56.40	-618.8	34.10	-213.70
CBZ	48.80	67.90	<MDL	58.40
MET	N.D	N.D	N.D	-
ASP	-73.53	4.97	0	-22.85
SMZ	N.D	N.D	N.D	-
IBU	6.55	-124.42	<MDL	-39.29
TMP	<MDL	-0.29	99.88	33.19

4.4 CONCENTRATIONS OF POLYCYCLIC MUSKS AND MUSK KETONES

Due to the difficulty in developing a method for the analysis of polycyclic musks, the application of the developed method focused on sediment samples which have higher concentrations of the analyte compared to surface water. Thus, only results obtained for sediment from the Umgeni and River and Msunduzi Rivers will be presented.

4.4.1 Umgeni River

The highest concentrations of galaxolide (HHCB) were found at the Inanda inlet (518.0 ng/g) and at the WWTP after chlorination (329.0 ng/g) (Table 4.7). Musk ketone was found in the second highest overall concentration with its highest concentration at Reservoir Hills (373.8 ng/g), WWTP (241.8 ng/g) and at Blue Lagoon (213.6 ng/g). Tonalide (AHTN) was found in relatively lower concentrations as compared to HHCB and MK at all sampling sites with its highest concentration at the Inanda inlet (163.7 ng/g) (Figure 4.4). The Inanda inlet site has the highest average concentration of the three selected musks. This site was also found to have higher concentrations of other emerging contaminants and the geography of the surrounding environment as well as the soil type are thought to influence the build-up of organic pollutants found at this site. More research will need to be conducted to further investigate this. It is worth noting that the Inanda Dam is the main source of water that is treated before discharge to the eThekwini municipality for distribution to the rest of Durban and surrounding areas for consumption so there is a need to further investigate this area.

Table 4.7 Concentrations of galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK) in sediment samples of the Umgeni River

Sampling Sites	HHCB (ng/g)	AHTN (ng/g)	MK (ng/g)	Σ (ng/g)	Average (ng/g)
Midmar inlet	6.7	5.7	12.6	25	8.33
Midmar outlet	11.6	5.5	6.9	24	8.00
Albert inlet	11.8	4.5	37.8	54.1	18.03
Albert outlet	8.5	3.0	4.0	15.5	5.17
Nagle dam	4.3	3.2	4.1	11.6	3.87
Joining point Msunduzi/Umgeni	10.7	6.9	109.8	127.4	42.47
Inanda inlet	518.0	163.7	95.0	776.7	258.90
Inanda outlet	15.9	13.0	186.7	215.6	71.87
Reservoir hills	121.5	36.0	373.8	531.3	177.10
Business park	13.6	9.0	18.0	40.6	13.53
EThekwini after chlorination	329.0	49.0	241.8	619.8	206.60
Blue lagoon	22.0	3.6	213.6	239.2	79.73

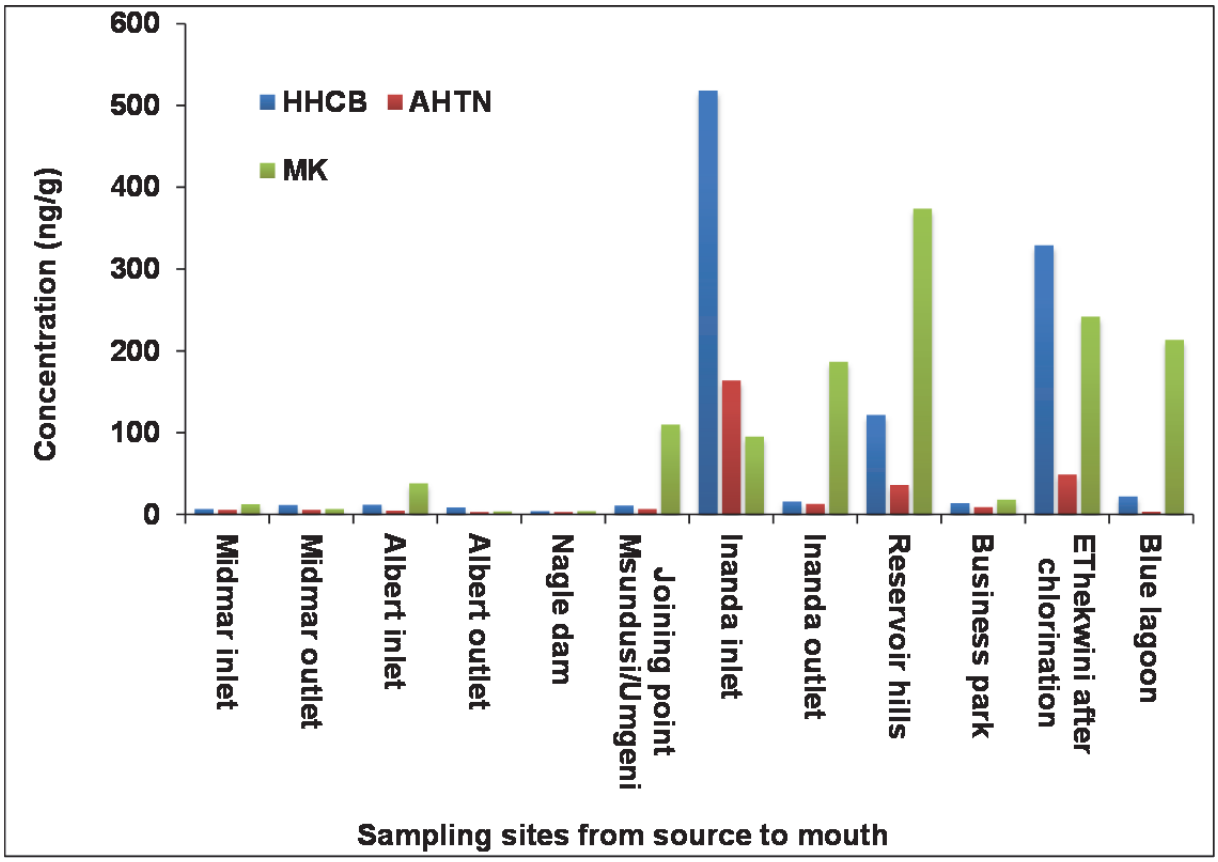


Figure 4.4 Concentrations of galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK) in ng/g across sampling sites of the Umgeni River

4.4.2 Msunduzi River

Table 4.8 and Figure 4.5 show the results for the concentrations of galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK) in selected sediment samples of the Msunduzi River. The preliminary results for the Msunduzi River show that the Msunduzi Town sampling site was the most contaminated site of the three sites sampled with the highest concentration for all the selected musks investigated. Musk ketone was found to be in the highest concentration at this site (250.9 ng/g) followed by HHCB (219.2 ng/g) and AHTN (114.9 ng/g). The average concentration of all musks at the Msunduzi Town was in the same region as the most contaminated site of the Umgeni River (Inanda inlet and WWTP). These high concentrations could possibly indicate leakage from water pipes in the area or poor sanitation infrastructure. These results are the first reported results and this work is still ongoing.

Table 4.8 Concentrations of galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK) in sediment samples of the Msunduzi River

Sampling Sites	HHCB (ng/g)	AHTN (ng/g)	MK (ng/g)	Sum (ng/g)	Average (ng/g)
Camps Drift	BDL	BDL	BDL	BDL	BDL
Du Toit	8.8	9.1	11.9	29.8	9.93
Agricultural area	10.1	4.7	11.4	26.2	8.73
Msunduzi town	219.2	114.9	250.9	585	195.00

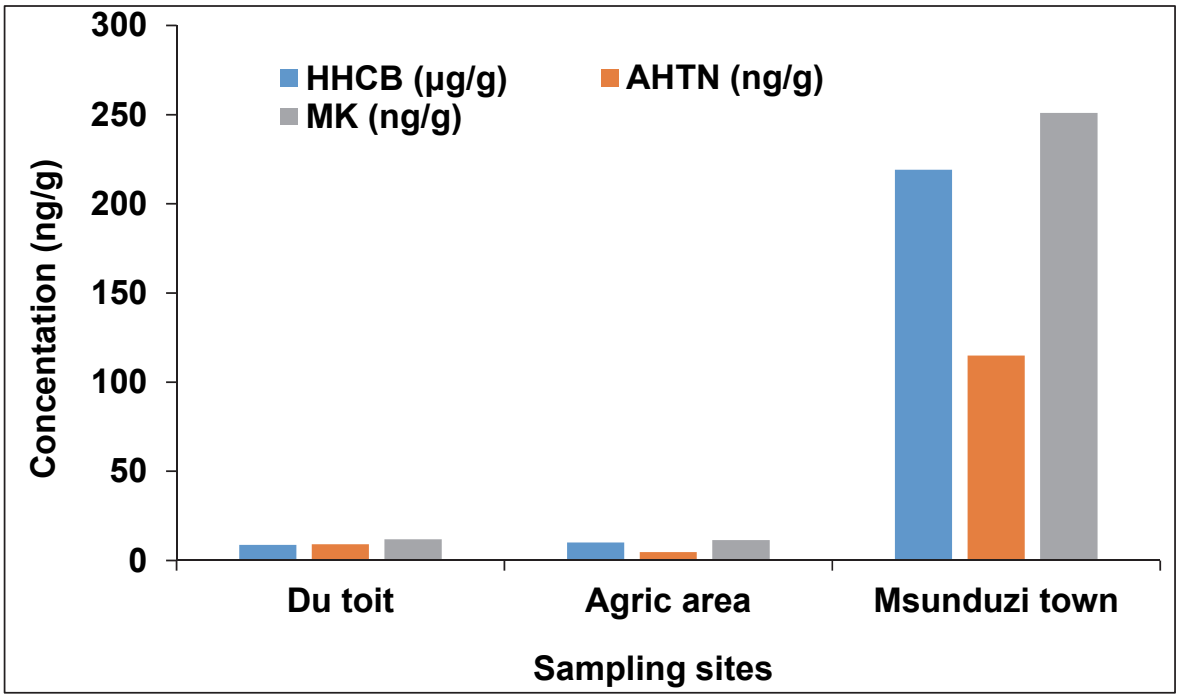


Figure 4.5 Concentrations of galaxolide (HHCB), tonalide (AHTN) and musk ketone (MK) in ng/g across three sampling sites of the Msunduzi River

4.5 SUMMARY OF FINDINGS

Two methods, using GC-MS and LC-MS, were successfully developed and validated for the analysis of PPCPs. The GC-MS method developed used derivatisation and was applied to the analysis of surface water samples from Umgeni River only. The highest concentrations of some analytes were detected at the Darvill WWTP and the Inanda dam. The LC-MS method was applied for the analysis of surface water, wastewater and sediment from both the Umgeni and Msunduzi Rivers. The results from LC-MS showed that ASP, IBU and CAF were found in high concentrations with other analytes such as CLO and SMX also present in significant amounts. SMX was the antibiotic found in highest concentrations and is used to treat HIV related infections. The wastewater treatment plants showed some removal of selected pharmaceuticals though in some cases the outlet had significant amounts of the pollutant being returned to the river. A further study of the wastewater treatment plants must be conducted. A GC-MS method has been developed for the analysis of selected musks and musk ketones in sediment. The highest concentrations were found in the lower reaches of the Umgeni River; the Inanda dam inlet, Reservoir Hills, WWTP after chlorination and the Blue lagoon as well as the Msunduzi town along the Msunduzi River.

CHAPTER 5: **POLYCHLORINATED BIPHENYLS (PCBS) IN THE UMGENI AND MSUNDUZI RIVERS**

5.1 INTRODUCTION

This section focuses on the analysis and monitoring of polychlorinated biphenyls (PCBs), in various samples collected from the Umgeni and Msunduzi Rivers. Different environmental matrices such as surface water, pore water, sediment and soil were investigated in order to fully understand the occurrence and significance of the above-mentioned contaminants in these rivers. Part of the results for the concentrations of PCBs in the different matrices of the Umgeni River has already been published (Gakuba et al. 2015).

5.2 PCB CONCENTRATIONS IN THE UMGENI RIVER

5.2.1 Surface water

Table 5.1 shows the summary of findings on the occurrence of PCBs in Umgeni River water samples. All investigated PCBs were detected in all sites. The concentration of PCB 180 (log K_{ow} = 6.82) was highest in all sites while that of PCB 28 (log K_{ow} = 5.71) was lowest. The relatively high log K_{ow} value for PCB 180 corresponds to its low solubility in water and it was therefore expected to be present in lower concentrations in water. However, the higher concentrations of PCB 180 in water may be explained by the strong affinity of this high molecular weight PCB with total suspended solids (TSS) and dissolved organic carbon (DOC). The water samples were unfiltered in order to determine the concentrations that animals and humans are exposed to when they directly consume it. Hence the water samples contained high TSS and DOC to which PCB 180 could partition to resulting in high PCB 180 concentrations in water (Aparna et al. 2014, Matyas et al. 2015, Zhang et al. 2011).

Furthermore, PCB 180 has a higher number of chlorine atoms (7) compared to other investigated congeners, and consequently was more difficult to degrade, lasting longer in the aquatic environment (de Voogt et al. 1990, Nhan et al. 2001). This suggests that its presence in the environment was due to accumulation over time rather than point source entry. This is in contrast to PCB 28 which has 3 chlorine atoms and was found to be present in the lowest concentration. The maximum value for the individual PCB congeners was 7.34 ng/mL, for PCB 180, from the Northern Wastewater Works treatment plant influent, NWTI (Figure 5.1). The lowest value observed was 0.42 ng/mL for PCB 28 at Howick Falls (HOF). A clear spike in the total amount of PCB congeners is seen at the entrance point for the Northern Wastewater treatment works (sample site labelled NWTI on Figure 5.2), and can be attributed to the fact that the treatment plant receives wastewater from residential, industrial and commercial sources. At the point (pipe) where treated water is released back into the river the sum is 9.22 ng/mL, which means on average the plant is unable to degrade or transform all of the PCB congeners analysed in this study. Note, simply using 21.43 – 9.22 = 12.21 ng/mL, is not a true reflection of the amount of PCB congeners removed by the plant, because the sample at the outlet pipe was not taken at a time that takes into account the residence time associated with the treatment process. Also, this study has only looked at 8 PCB congeners as opposed to 209 possible PCB congeners.

Table 5.1 Concentrations of PCB congeners (ng/mL±SD) in Umgeni River water samples

Site	PCB 28	PCB 52	PCB 77	PCB 101	PCB 105	PCB 138	PCB 153	PCB 180	∑PCBs
MDI	0.73±0.16	1.33±0.12	0.87±0.10	1.15±0.11	1.13±0.15	1.15±0.14	1.26±0.12	2.19±0.08	9.81±0.98
MDO	0.74±0.13	1.35±0.09	0.90±0.11	1.15±0.09	1.15±0.09	1.17±0.10	1.26±0.09	2.21±0.06	9.93±0.76
HOF	0.42±0.23	0.97±0.09	0.70±0.15	0.80±0.10	0.80±0.07	0.81±0.09	0.90±0.10	2.26±0.00	7.66±0.83
AFI	0.85±0.11	1.44±0.04	0.67±1.33	1.25±0.03	1.26±0.04	1.27±0.04	1.38±0.02	2.37±0.03	10.49±1.64
AFO	0.71±0.10	1.29±0.04	0.82±0.08	1.11±0.06	1.13±0.07	1.13±0.06	1.23±0.06	2.53±0.05	9.95±0.52
NAD	0.83±0.05	1.46±0.03	0.96±0.06	1.26±0.04	1.26±0.03	1.28±0.04	1.37±0.03	2.53±0.02	10.95±0.30
JUM	1.02±0.05	1.68±0.00	1.24±0.02	1.50±0.01	1.49±0.01	1.50±0.03	1.62±0.02	2.08±0.01	12.13±0.15
IDI	0.84±0.10	1.41±0.07	1.01±0.11	1.24±0.07	1.25±0.05	1.25±0.06	1.36±0.06	1.54±0.03	9.9±0.55
IDO	0.98±0.07	1.57±0.05	1.09±0.09	1.40±0.06	1.40±0.06	1.41±0.05	1.53±0.06	1.77±0.05	11.15±0.49
REH	0.94±0.12	1.50±0.06	1.12±0.05	1.37±0.08	1.25±0.06	1.26±0.05	1.40±0.06	2.74±0.05	11.58±0.53
UBP	0.81±0.03	1.38±0.03	0.96±0.03	1.21±0.02	1.16±0.01	1.16±0.01	1.31±0.02	2.48±0.01	10.47±0.16
NWTI	1.09±0.05	1.61±0.02	2.94±0.28	4.01±0.04	1.51±0.04	1.39±0.03	1.54±0.03	7.34±0.02	21.43±0.51
NWTT	0.66±0.11	1.27±0.08	1.31±0.07	1.11±0.08	1.06±0.05	1.05±0.02	1.19±0.04	1.74±0.02	9.39±0.47
NWTE	0.72±0.12	1.39±0.06	0.92±0.09	1.15±0.05	1.14±0.06	1.13±0.04	1.26±0.03	1.51±0.02	9.22±0.47
BLA	0.60±0.04	1.20±0.02	1.11±0.03	1.03±0.04	1.01±0.02	1.02±0.04	0.90±0.87	2.30±0.01	9.17±1.07
∑PCBs	11.94±1.47	20.85±0.80	16.62±2.60	20.74±0.88	18±0.81	17.98±0.80	19.51±1.61	37.59±0.46	163.23±9.43
Min	0.42±0.23	0.97±0.09	0.67±1.33	0.80±0.10	0.80±0.07	0.81±0.09	0.90±0.10	1.51±0.02	7.66±0.83
Mean	0.80±0.10	1.39±0.05	1.11±0.17	1.38±0.06	1.2±0.05	1.20±0.05	1.30±0.11	2.51±0.03	10.88±0.63
Max	1.09±0.05	1.68±0.00	2.94±0.28	4.01±0.04	1.51±0.04	1.50±0.03	1.62±0.02	7.34±0.02	21.43±0.51

(MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

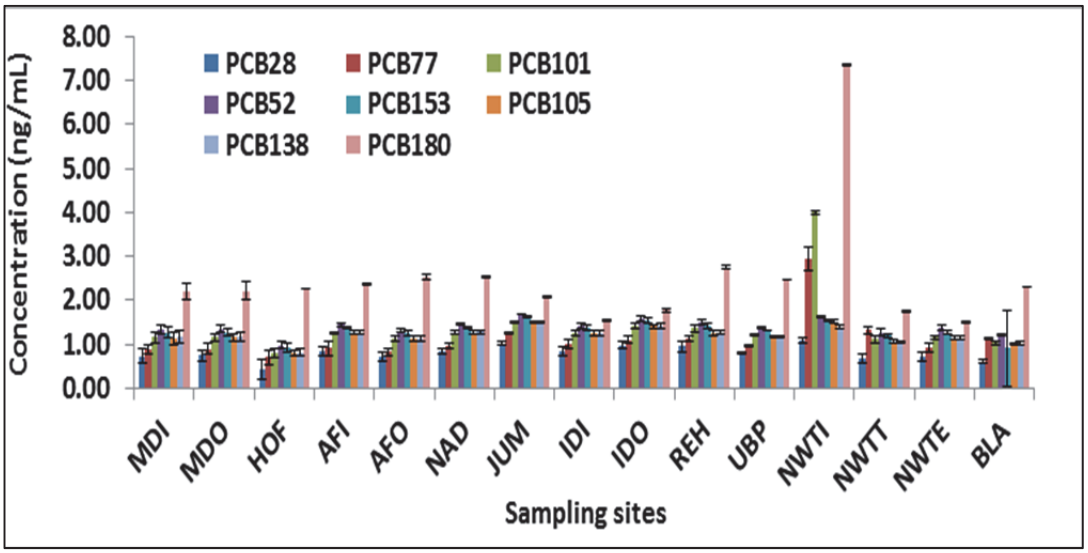


Figure 5.1 Levels of PCB congeners, in ng/mL, in water samples from various points along the Umgeni River (MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

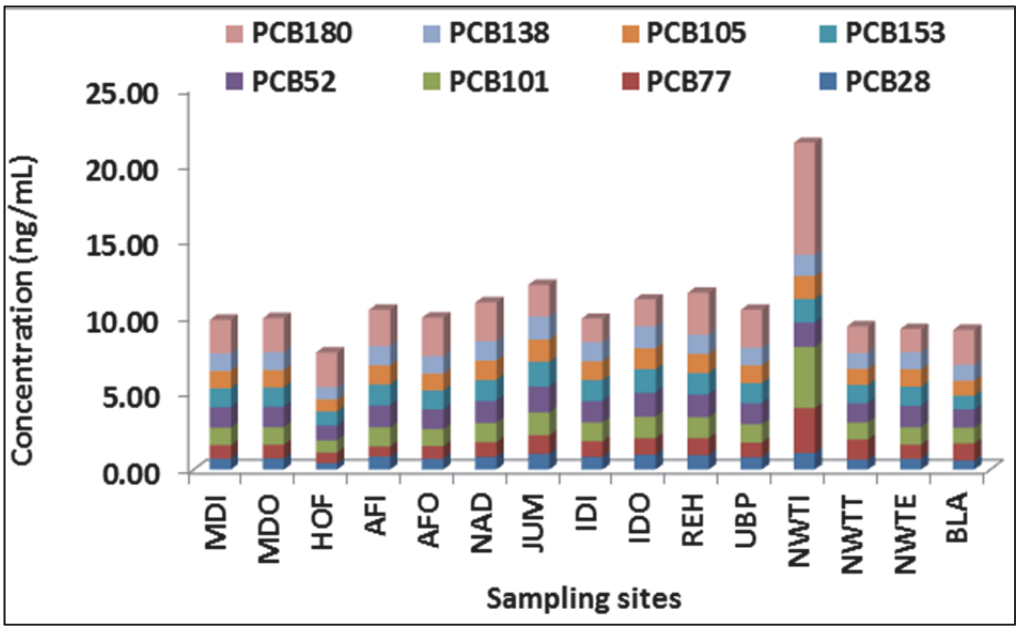


Figure 5.2 Total concentrations of PCB congeners in water samples at each site sampled along the Umgeni River (MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

5.2.2 Pore water

The concentrations of PCBs in the Umgeni River's pore water ranged from not detectable level for PCB 28 at MDO and AFO to 52.30 ng/mL for PCB 77 at UBP with the site NWTE having the highest sum of concentrations of studied PCBs (Figure 5.3). The concentrations were generally lower towards the source of the river and increased towards the mouth (Figure 5.3). This may be due to the increase in industrial activities as the river flows down towards the city of Durban or also an accumulation effect of the PCBs as the river flows downstream towards the mouth carrying with it TSS and DOC with PCBs partitioned to it. PCB 180 was again the most abundant PCB in pore water in almost all sampling sites confirming its strong affinity with TSS and DOC whose concentrations were greater in unfiltered pore water than unfiltered surface water (Aparna, et al. 2014, Matyas, et al. 2015, Zhang, et al. 2011). Another possible reason is its highly chlorinated structure which makes it less volatile and lipophilic allowing it to be preferentially retained in the sediment pore water (de Voogt, et al. 1990). In addition, early work carried out by Bunce et al. (1978) suggested that PCB 180 could be in high concentrations in the environment due to photodegradation of higher chlorinated PCBs. Early studies have shown that highly chlorinated PCBs were more photolabile leading to PCB 180 as a degradation product which could account for the high concentrations of PCB 180 observed in this study. The total PCB levels in pore water varied from 35.65 to 203.77 ng/mL with a mean of 116.14 ng/mL, which is higher than in water (Table 5.2). The higher concentrations in pore water is expected because PCBs are hydrophobic and tend to partition onto organic materials found in sediments rather than dissolve in water (Julia et al. 2012). In addition, the hydrophobic PCBs in sediment may also re-suspend from the sedimentary phase to the pore water (Zhang et al. 2003). Studies on sediment-pore water distribution models of POPs have also confirmed higher POP concentrations in pore water than in water (Persson et al. 2005).

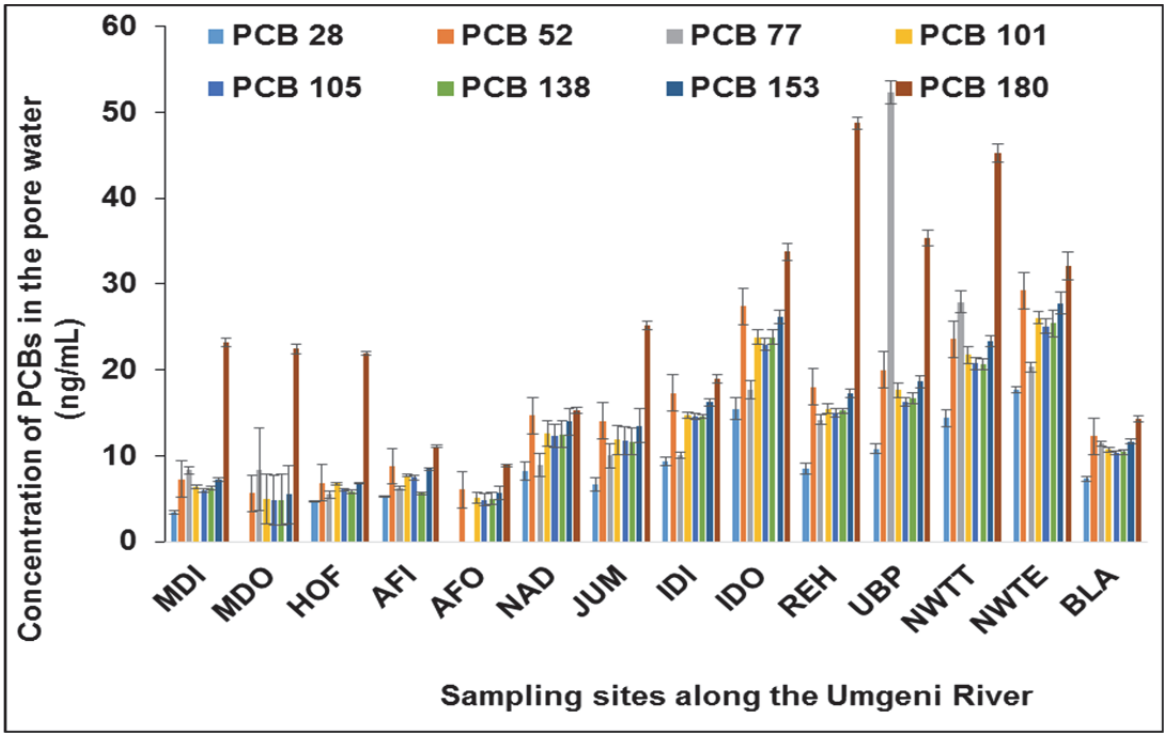


Figure 5.3 Trends of PCBs in pore water along the Umgeni River ((MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon))

Table 5.2 Concentrations of PCBs (ng/mL±SD) in Umgeni River pore water

Site	PCB 28	PCB 52	PCB 77	PCB 101	PCB 105	PCB 138	PCB 153	PCB 180	∑PCBs
MDI	3.42 ±0.26	7.29 ±0.24	8.30 ±0.41	6.39 ±0.18	5.98 ±0.26	6.31 ±0.21	7.17 ±0.22	23.17 ±0.50	68.03 ±2.28
MDO	nd	5.63 ±3.28	8.41 ±4.84	5.00 ±2.92	4.83 ±2.89	4.90 ±2.92	5.49 ±3.35	22.47 ±0.58	56.73 ±20.78
HOF	4.71 ±0.06	6.84 ±0.15	5.48 ±0.46	6.78 ±0.12	6.06 ±0.16	5.84 ±0.24	6.81 ±0.10	21.91 ±0.20	64.43 ±1.49
AFI	5.20 ±0.07	8.78 ±0.13	6.24 ±0.19	7.72 ±0.19	7.47 ±0.28	5.62 ±0.11	8.47 ±0.13	11.10 ±0.15	60.60 ±1.25
AFO	nd	6.05 ±0.74	nd	5.13 ±0.60	4.88 ±0.70	5.04 ±0.66	5.69 ±0.76	8.86 ±0.09	35.65 ±3.55
NAD	8.21 ±1.11	14.69 ±1.73	8.92 ±1.35	12.60 ±1.46	12.35 ±1.32	12.48 ±1.54	13.95 ±1.52	15.33 ±0.35	98.53 ±10.38
JUM	6.71 ±0.75	14.07 ±2.15	10.05 ±1.42	11.89 ±1.69	11.76 ±1.64	11.67 ±1.60	13.50 ±1.98	25.22 ±0.50	104.87 ±11.73
IDI	9.30 ±0.50	17.31 ±0.48	10.09 ±0.33	14.67 ±0.34	14.56 ±0.34	14.57 ±0.18	16.27 ±0.43	18.98 ±0.45	115.75 ±3.05
IDO	15.44 ±1.27	27.39 ±0.54	17.72 ±1.09	23.84 ±0.89	22.99 ±0.70	23.82 ±0.87	26.17 ±0.82	33.79 ±1.00	191.16 ±7.18
REH	8.48 ±0.67	18.00 ±0.53	14.22 ±0.52	15.47 ±0.57	15.03 ±0.48	15.23 ±0.28	17.30 ±0.52	48.72 ±0.73	152.45 ±4.30
UBP	10.82 ±0.54	19.99 ±0.58	52.30 ±1.32	17.64 ±0.84	16.26 ±0.51	16.70 ±0.57	18.64 ±0.71	35.37 ±0.86	187.72 ±5.93
NWTT	14.38 ±0.99	23.61 ±0.66	27.92 ±1.32	21.73 ±0.94	20.77 ±0.71	20.70 ±0.64	23.32 ±0.68	45.26 ±1.05	197.69 ±6.99
NWTE	17.73 ±0.34	29.25 ±1.16	20.33 ±0.54	26.09 ±0.66	25.09 ±.85	25.42 ±□1.55	27.76 ±1.29	32.10 ±1.63	203.77 ±8.02
BLA	7.36 ±0.28	12.28 ±0.30	11.41 ±0.28	10.71 ±0.24	10.35 ±0.23	10.46 ±0.26	11.66 ±0.29	14.35 ±0.34	88.58 ±2.22
∑PCBs	111.76±6.84	211.18±12.67	201.39±14.07	185.66±11.64	178.38±11.07	178.76±11.63	202.20±12.80	356.63±8.43	1625.96±89.15
Min	3.42±0.26	5.63±3.28	5.48±0.46	5.00±2.92	4.83±2.89	4.90±2.92	5.49±3.35	8.86±0.09	35.65±3.55
Mean	9.31±0.57	15.08±0.91	15.49±1.08	13.26±0.83	12.74±0.79	12.77±0.83	14.44±0.91	25.47±0.60	116.14±6.37
Max	17.73±0.34	29.25±1.16	52.30±1.32	26.09±0.66	25.09±0.85	25.42±1.55	27.76±1.29	48.72±0.73	203.77±8.02

(MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

The total concentrations of congeners at each site showed that the levels of PCBs were highest at NWTT and NWTE due to the accumulation of contaminants from different sources that make their way to the treatment plant. The higher levels of total PCBs at sites IDO and UBP were attributed to the low water flowrate at these sites, allowing time for the TSS onto which the pollutants were partitioned, to settle in sediment and therefore be extracted in its pore water. PCB 77 was in high concentrations at UBP (Figure 5.3) which suggested a possible input of this congener at this site from sources such as transformer liquids, incineration of waste or from construction material (this site was under construction and was being used by heavy machinery at the time of sampling).

5.2.3 Sediment

The concentrations along the Umgeni River ranged from 10.16 ng/g (PCB 105 at IDO) to 93.74 ng/g, (PCB 28 at NWTT) with an average of 24.31 ±1.10 ng/g of dry weight (dw) (Figure 5.4 and Table 5.3). All the PCB congeners investigated were detected in all sediment samples. This may be attributed to the strong affinity that exists between the hydrophobic pollutants and sediment organic carbon (Kookana 2011). As in water and pore water, the level of PCB 180 was highest in all sediment samples due to its hydrophobicity in the aquatic environment which is related to its K_{ow} value and therefore its sorption to the organic matter in sediment (Zhou et al. 2005). The distribution of PCB congeners in the aquatic systems may also be assigned to losses of less chlorinated congeners through volatilisation, sedimentation and degradation by microbial activity as well as thermal and UV light degradation (Brown et al. 1987, De et al. 2006, MacDonald et al. 1992, Quensen et al. 1988). PCBs 28 and 77 were in unusually high concentrations at NWTT. This suggested that apart from the waste received by the plant there may be another input of these two congeners at that site such as industrial effluent received by the plant itself (Gioia et al. 2014). The lowest total concentrations of PCBs were observed at IDO while the highest was recorded at NWTT. The relatively low concentrations at the IDO sampling site could be because particular sediment grain sizes at that site do not promote partitioning to the sediment. Zhao et al. (2010) showed that high PCBs levels occur in the fraction of sediment with grain size of 31 to 63 µm. In addition, studies on sorption of hydrophobic pollutants on natural sediment demonstrated that the sand fraction (>50 µm) is considerably less effective in adsorption of hydrophobic pollutants (Carro et al. 2002, Karickhoff et al. 1979, Ke-xin et al. 2003).

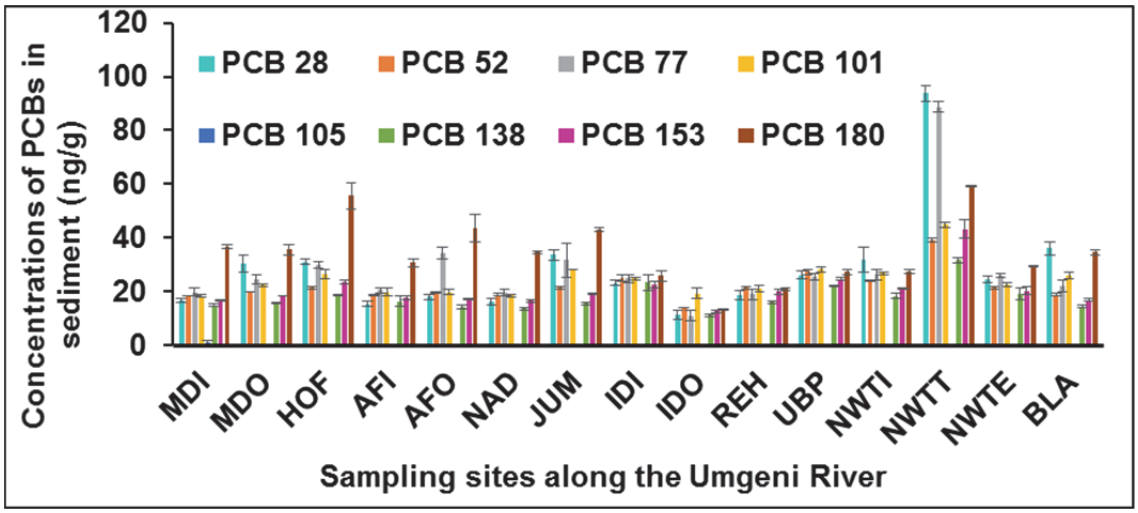


Figure 5.4 Concentrations of PCBs in the sediment of the Umgeni River (MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

Table 5.3 Concentrations of PCBs (ng/g ±SD) in sediment of the Umgeni River

Site	PCB 28	PCB 52	PCB 77	PCB 101	PCB 105	PCB 138	PCB 153	PCB 180	∑PCBs
MDI	16.70 ±0.60	18.41±0.03	19.85 ±1.43	18.43±0.52	15.04±0.08	15.07±0.68	16.64±0.33	36.69±0.83	156.83±4.50
MDO	30.57 ±3.10	19.93±0.15	24.58 ±1.91	22.50±0.59	17.32±0.07	15.73±0.36	18.38±0.03	35.64±2.12	184.65±8.33
HOF	31.18 ±0.83	21.44±0.51	29.91 ±1.27	26.40±1.78	24.11±0.17	18.75±0.26	23.51±0.65	55.78±4.83	231.08±10.30
AFI	15.49 ±0.85	18.83±0.32	20.47 ±1.00	19.91±1.34	13.46±0.56	16.41±1.92	17.73±0.68	30.75±1.66	153.05±8.33
AFO	18.09 ±0.95	19.58±0.20	34.45 ±2.35	19.82±1.01	15.28±0.13	14.38±0.59	17.30±0.16	43.73±5.14	182.63±10.53
NAD	16.40 ±1.22	18.97±0.55	19.61 ±1.14	18.51±0.72	15.76±0.62	13.70±0.41	16.59±0.32	34.66±0.43	154.20±5.41
JUM	33.81 ±1.97	21.49±0.55	31.73 ±6.47	28.34±0.08	18.11±0.84	15.63±0.55	19.16±0.30	43.15±0.79	211.42±11.55
IDI	23.33 ±0.82	24.99±1.30	24.89 ±1.60	24.86±0.56	22.12±1.43	23.49±2.89	22.43±1.21	25.97±1.90	192.08±11.71
IDO	11.41 ±1.66	14.17±0.10	11.09 ±1.87	19.46±1.86	10.16±0.23	11.24±0.33	12.70±0.47	13.42±0.26	103.65±6.78
REH	18.74 ±1.62	21.48±0.66	19.01 ±1.76	21.15±1.23	17.55±0.23	16.11±0.67	19.97±0.82	21.16±0.48	155.17±7.47
UBP	26.23 ±1.43	27.31±0.93	25.47 ±1.35	28.48±0.97	22.51±1.06	22.21±0.36	24.75±0.63	27.32±0.82	204.28±7.55
NWTI	31.86 ±4.74	24.16±0.30	26.37 ±1.78	26.84±0.35	18.82±0.48	18.48±0.91	21.12±0.20	27.70±0.78	195.35±9.54
NWTT	93.74 ±2.79	39.42±0.69	88.73 ±2.11	44.89±1.05	26.67±0.41	31.69±1.04	43.36±3.49	59.34±0.33	427.84±11.91
NWTE	24.41 ±1.24	21.53±0.48	26.01 ±0.66	22.66±0.57	18.05±0.64	19.31±2.08	20.29±1.48	29.35±0.21	181.61±7.36
BLA	36.17 ±2.56	18.92±0.60	22.01 ±2.21	26.19±1.31	14.25±0.48	14.40±0.46	17.02±0.61	34.61±0.83	183.57±9.06
∑PCBs	428.13±26.38	330.63±7.37	424.18±28.91	368.44±13.94	269.21±7.43	266.60±13.51	310.95±11.38	519.27±21.41	2917.41±130.33
Min	11.41±1.66	14.17±0.10	11.09±1.87	18.43±0.52	10.16±0.23	11.24±0.33	12.70±0.47	13.42±0.26	103.65±6.78
Mean	28.54±1.76	22.04±0.49	28.28±1.93	24.56±0.93	17.95±0.50	17.77±0.90	20.73±0.76	34.62±1.43	194.49±8.69
Max	93.74±2.79	39.42±0.69	88.73±2.11	44.89±1.05	26.67±0.41	31.69±1.04	43.36±3.49	59.34±0.33	427.84±11.91

(MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

Therefore, since more than 52% of IDO sediment size was higher than 300 µm (particle sizes determined during grinding and sieving steps of sample preparation), it could not retain much pollutant and hence PCB concentrations in pore water was higher than the sediment itself at this site. For NWTT, the high concentration of PCBs in its bio-solid was expected since this site continually receives treated water before being discharged. Table 5.1 shows that even the treated water still has considerable amounts of PCBs which eventually partition itself in the bio-solid sampled at the NWTT site resulting in the high concentrations detected. The levels of PCBs in the bio-solids of NWTI were generally lower than those in the bio-solids of NWTT. This was because the bio-solid at NWTI was fresh and occasional while the bio-solid at NWTT accumulated over time at that sampling point. Note that in all matrices the levels of PCB concentrations at sites close to and exiting the Northern Wastewater Treatment Works (NWWTW) were high. Other studies have also found that wastewater treatment plants are important possible point sources of POP contamination (Samara et al. 2006).

5.2.4 Umgeni River bank soil

The concentrations of PCBs in soil obtained from the banks of the Umgeni River varied from 10.46 to 89.46 ng/g (Figure 5.5). A high total PCB concentration was observed in the bio-solids collected from the NWTT (542.95 ng/g) due to industrial and residential waste and was the most contaminated site. The high levels of contamination at this site were attributed to the accumulation of pollutants from wastewater since this site stores wastewater before being discharged back into the river. Being a store of wastewater and having an excess of plant life as a result of eutrophication, this site (NWTT) may contain more organic carbon than other sites. This may in turn allow for partitioning of more PCBs into the soil than in the water. According to Table 5.4, high levels of total concentration of PCBs (275.09 ng/g) were observed at sampling site HOF (Howick Falls). This could be attributed to this site being situated in the town of Howick and pollution by industrial wastes is likely which may contain substantial amounts of contaminants, including PCBs.

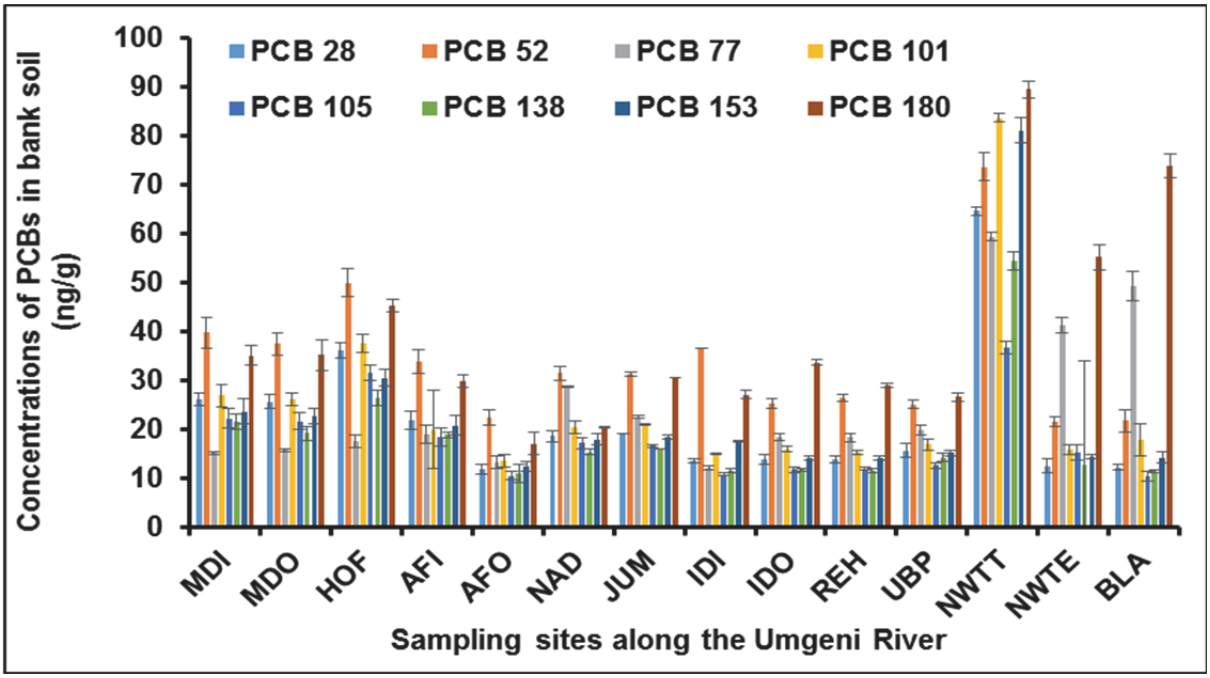


Figure 5.5 Distribution of PCB congeners in the bank soil of the Umgeni River (MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

Table 5.4 Concentrations of PCBs (ng/g±SD) in Umgeni River bank soil

Site	PCB28	PCB52	PCB77	PCB101	PCB105	PCB138	PCB153	PCB180	∑PCB
MDI	26.13±1.28	39.83±3.14	15.27±0.25	26.96±2.31	22.24±1.96	21.65±1.60	23.68±2.59	35.03±2.01	210.78±15.12
MDO	25.70±1.49	37.55±2.30	15.76±0.20	26.17±1.19	21.69±1.73	19.26±1.38	22.76±1.65	35.25±3.17	204.15±13.83
HOF	36.09±1.61	49.95±2.90	17.52±1.25	37.60±1.87	31.69±1.58	26.49±1.63	30.49±1.71	45.27±1.23	275.09±14.77
AFI	21.98±1.81	33.98±2.40	19.09±1.88	20.00±8.00	18.53±1.79	18.90±0.61	20.87±2.11	29.79±1.33	183.13±19.93
AFO	12.02±1.00	22.50±1.57	13.43±1.28	13.64±1.34	10.60±0.84	11.05±1.84	12.48±1.01	17.08±2.24	112.79±11.12
NAD	18.66±1.20	31.49±1.46	28.61±0.14	20.54±1.28	17.25±1.10	15.35±0.60	18.02±1.12	20.43±0.23	170.35±8.14
JUM	19.09±0.05	31.31±0.32	22.58±0.26	21.05±0.22	16.67±0.24	16.02±0.12	18.45±0.40	30.62±0.07	175.79±1.67
IDI	13.59±0.31	36.59±0.13	12.20±0.42	15.09±0.21	10.79±0.29	11.62±0.34	17.70±0.15	27.15±0.90	144.73±2.76
IDO	13.99±0.97	25.35±1.04	18.56±0.69	15.93±0.58	11.85±0.53	11.85±0.25	14.08±0.41	33.68±0.55	145.29±5.03
REH	13.90±0.72	26.54±0.70	18.38±0.91	15.35±0.51	11.96±0.32	11.68±0.38	14.11±0.39	29.01±0.38	140.93±4.62
UBP	15.70±1.41	25.10±0.89	19.87±0.91	16.95±1.15	12.64±0.60	14.19±0.87	15.22±0.59	26.67±0.85	146.34±6.58
NWTT	64.56±0.72	73.66±2.96	59.36±0.86	83.59±0.82	36.73±1.18	54.49±1.81	81.11±2.52	89.46±1.78	542.95±13.36
NWTE	12.54±1.41	21.64±1.02	41.25±1.63	15.87±1.01	15.23±1.57	12.8±21.27	14.38±0.48	55.19±2.57	188.91±10.03
BLA	12.32±0.46	21.75±2.25	49.31±3.11	17.79±3.28	10.46±1.11	11.41±0.31	14.23±1.16	73.77±2.46	211.03±14.09
∑PCB	306.26±0.42	477.23±23.09	351.19±14.80	346.51±24.50	248.34±14.85	256.76±13.01	317.58±16.27	548.40±20.77	2852.28±141.04
Min	12.02±1.00	21.64±1.02	12.20±0.42	13.64±1.34	10.46±1.11	11.05±1.84	12.48±1.01	17.08±2.24	112.79±11.12
Mean	21.88±0.98	34.09±1.65	25.08±0.06	24.75±1.75	17.74±1.06	18.34±0.93	22.68±1.16	39.17±1.48	203.73±10.07
Max	64.56±0.72	73.66±2.96	59.36±0.86	83.59±0.82	36.73±1.18	54.49±1.81	81.11±2.52	89.46±1.78	542.95±13.36

The most abundant PCB congener in the river bank soil was again found to be PCB 180 (17.08-89.46 ng/g) with a mean concentration of 39.17 ng/g (Table 5.4 and Figure 5.5). This is probably due to its strong affinity with organic matter (Log K_{ow} = 6.70-7.21) in soil to which it strongly adsorbs (Preda et al. 2010). PCB 180 has 7 chlorine atoms in its structure which makes it relatively stable and resistant to degradation and volatilisation from soil to air, compared to other investigated congeners (de Voogt, et al. 1990, Vesna et al. 2006). The second most abundant congener was PCB 52 (21.64-73.66 ng/g). This PCB is of lower complexity having only four chlorine atoms and together with its relatively lower Log K_{ow} (5.79-6.09) value, it tends to adsorb less strongly to soil and would therefore mean relatively lower concentrations in the soil. Therefore, the higher than expected concentrations suggest a possible input of this congener into the surrounding area or possible degradation of higher chlorinated PCBs into this PCB. The other congeners were also present in significant amounts: PCB 28 (12.02-64.56 ng/g), PCB 77 (12.20-59.36 ng/g), PCB 101 (13.64-83.59 ng/g), PCB 105 (10.46-36.73 ng/g), PCB 153 (12.48-81.11 ng/g) and PCB 138 (11.05-54.49 ng/g) (Table 5.4).

5.2.5 Seasonal trends of PCBs in the Umgeni River

Sampling for PCBs in the Umgeni River was done during winter, spring, summer and autumn months. These seasonal sampling campaigns also took into account the various rainfall levels typically seen with the KwaZulu-Natal province. The highest total concentrations of PCBs were found in winter and spring for all sites (Figure 5.6). This was attributed to a number of factors. The ambient and water temperatures (12.3-32.8°C and 11.6-25.0°C) were generally low during winter and spring and the level of vaporisation of pollutants from water to air is also expected to be low due to the cooler temperatures. Hence the concentrations of PCBs in the environmental matrices were higher during the cooler months than during the warmer months.

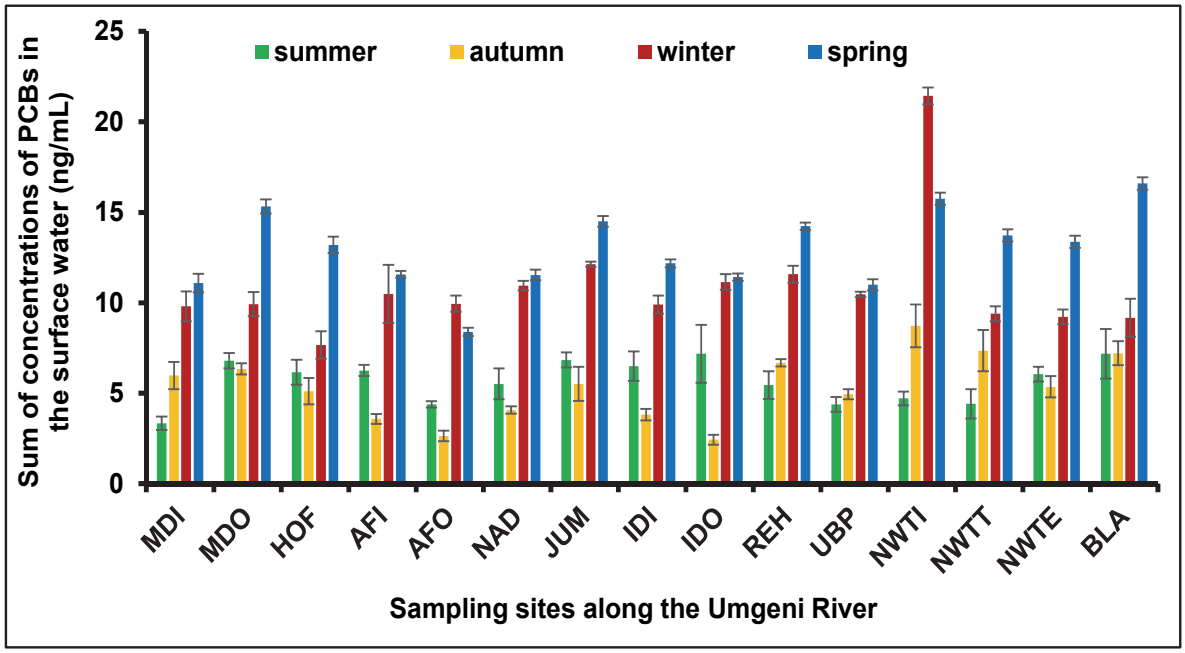


Figure 5.6 Seasonal trends of PCBs in surface water across the Umgeni River sampling sites

A similar trend as for surface water was observed for pore water (Figure 5.7(a)) and sediment (Figure 5.7(b)) during the different seasons. Winter was found to have the higher concentrations of PCBs in both pore water and sediment and summer had the lower concentration for both matrices. This was due to the lower temperatures during winter that encouraged deposition rather than evaporation of PCBs into the atmosphere as well as the South African winter is a dry season with minimal to no rainfall which led to substantial build-up of pollutants and very little dilution which is rather seen in the summer season due to the heavy rainfall leading to reduced concentrations in summer.

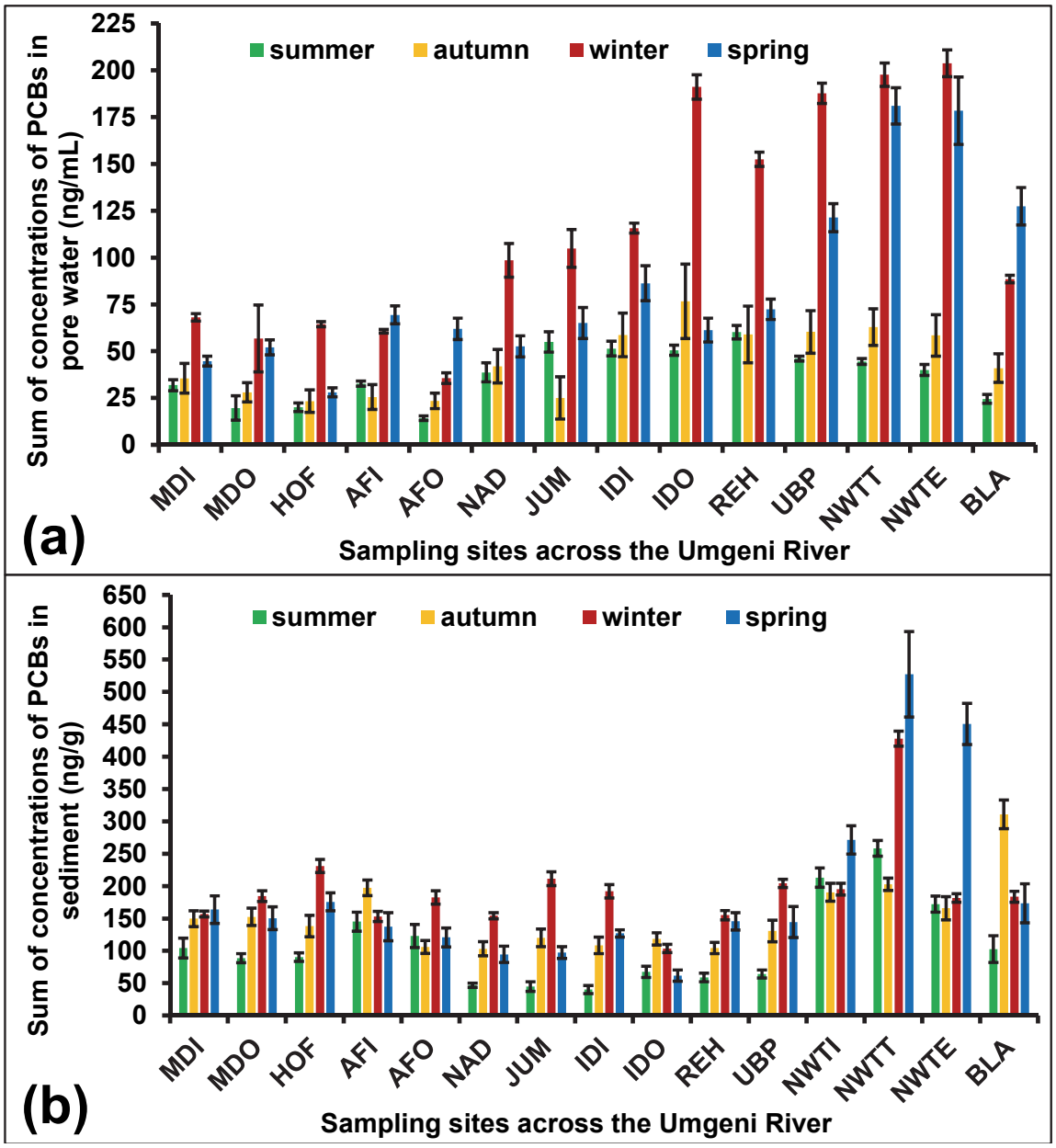


Figure 5.7 Seasonal trends of PCBs in (a) pore water (b) sediment across the Umgeni River sampling sites

PCBs may also be deposited from the atmosphere through diffusive air-water exchange (Meijer et al. 2006). Unlike in winter and spring, the summer ambient and water temperatures were high (28.8-38.6°C and 21.8-30.6°C) at all sites favouring the evaporation and dissolution processes. Volatilisation was found to be a major phenomenon through which PCBs are lost by the water column in some regions during summer while deposition increased the PCB concentration during cool seasons (Hornbuckle et al. 1994). Furthermore, the volatilisation from contaminated waters during warmer seasons was confirmed to be an important source of organic pollutants into ambient air (Rowe et al. 2007, Totten et al. 2001). Another factor which would have influenced the concentrations of PCBs in water was precipitation. The South African winter is a dry season with very little precipitation and therefore there was no dilution of the concentration of various pollutants in water; instead the concentration tends to increase as the volume of water decreased. This resulted in the river water being concentrated in contaminants during winter since there was no rain to dilute the river water.

5.3 PCB CONCENTRATIONS IN THE MSUNDUZI RIVER

The Msunduzi River is the main river in the Pietermaritzburg area and eventually feeds into the Umgeni River. The Msunduzi flows past industrialised areas, informal settlements, semi-urban areas, rural and agricultural areas as well. Investigating the levels of OCPs and PCBs within this river system may provide some insights into the trends, levels and patterns observed with the Umgeni River.

5.3.1 Surface water

The concentration of PCB congeners along the Msunduzi River during the winter sampling campaign is presented in Table 5.5 and Figure 5.8. The PCB concentrations in the surface water of the Msunduzi River ranged from 0.09 ng/mL at the agricultural sampling site to 22.37 ng/mL at the Darvill wastewater treatment plant inlet (WWT 1). Table 5.5 shows that the Darvill wastewater treatment plant had the highest sum of concentrations (78.71 ±2.70 ng/mL) of the selected PCBs followed by Nagle dam (64.94 ±2.56 ng/mL) and Henley dam (51.30 ±2.87 ng/mL). The high concentrations of PCBs found at WWT 1 was expected because this site receives both domestic and industrial effluent from the surrounding areas. The lowest sum of concentrations of PCBs was found at the agricultural sampling site which was expected since there is no industrial activity at that site that could contribute to the levels of PCBs and any concentrations found would be due to transportation of PCBs from areas upstream that could have made its way to this site. PCB 101 was the PCB found in highest concentration in the Msunduzi surface water with an average value of 14.55 ±0.15 ng/mL.

Table 5.5 Concentrations of PCBs (ng/mL±SD) in the surface water of the Msunduzi River

Site Code	PCB 28	PCB 52	PCB 77	PCB 101	PCB 105	PCB 138	PCB 153	PCB 180	∑PCBs
HND	12.46±0.04	7.03±0.13	4.07±0.61	15.28±0.21	0.17±1.45	2.06±0.01	8.37±0,41	1.86±0.01	51.3 ±2.87
CMD	9.69±0.74	5.6±0.07	4.58±1.29	21.35±0.29	0.19±1.25	1.86±0.11	3.95±0.08	1.66±0.31	48.88 ±4.14
DUT	12.97±0.32	7.8±1.00	2.81±0.49	14.45±0.01	0.31±0.4	0.91±2.58	3.96±1.91	2.13±0.22	45.34 ±6.93
WWT 1	6.82±0.18	22.37±0.65	3.18±0.18	15.06±0.26	5.73±0.19	0.68±1.15	19.33±0.09	5.54±0.001	78.71 ±2.70
WWT 2	7.06±0.03	13.12±0.12	1.91±1.52	9.58±0.17	0.66±1.69	0.56±2.42	6.61±0.04	4.32±0.20	43.82 ±6.19
AGA	7.27±0.42	2.85±0.18	1.45±2.51	11.66±0.09	0.09±0.16	1.78±0.38	1.21±0.43	3.95±0.41	30.26 ±4.58
MST	5.99±0.09	8.2±0.58	2.02±0.99	6.86±0.13	4.33±0.82	5.13±0.72	1.7±1.85	5.52±0.53	39.75 ±5.71
NGD	3.9±0.22	12.91±0.17	5.8±0.27	21.77±0.14	5.05±0.5	9.00±0.19	1.67±1.01	4.84±0.06	64.94 ±2.56
UMJ	8.03±0.16	2.84±0.5	4.82±0.31	14.95±0.03	3.34±0.57	5.37±0.00	0.2±2.02	4.84±0.00	44.39 ±3.59
∑PCBs	74.19 ±2.20	82.72 ±3.40	30.64 ±8.17	130.96 ±1.33	19.87 ±7.03	27.35 ±7.56	47.00 ±7.84	34.66 ±1.74	447.39 ±39.27
Min	3.90±0.22	2.84±0.50	1.45±2.51	6.86±0.13	0.09±0.16	0.56±2.42	0.2±2.02	1.66±0.31	30.26±4.58
Mean	8.24±0.24	9.19±0.38	3.40±0.91	14.55±0.15	2.21±0.78	3.04±0.84	5.22±0.87	3.85±0.19	49.71±4.36
Max	12.97±0.32	22.37±0.65	5.8±0.27	21.77±0.14	5.73±0.19	9.00±0.19	19.33±0.09	5.54±0.001	78.71±2.70

(HND, Henley dam; CMD, Camps Drift; DUT, Du Toit; WWT 1, Darvill Wastewater Treatment influent; WWT 2, Darvill Wastewater Treatment effluent; AGA, Agricultural area; MST, Msunduzi Town; NGD, Nagle dam; UMJ, Umgeni/Msunduzi joining point)

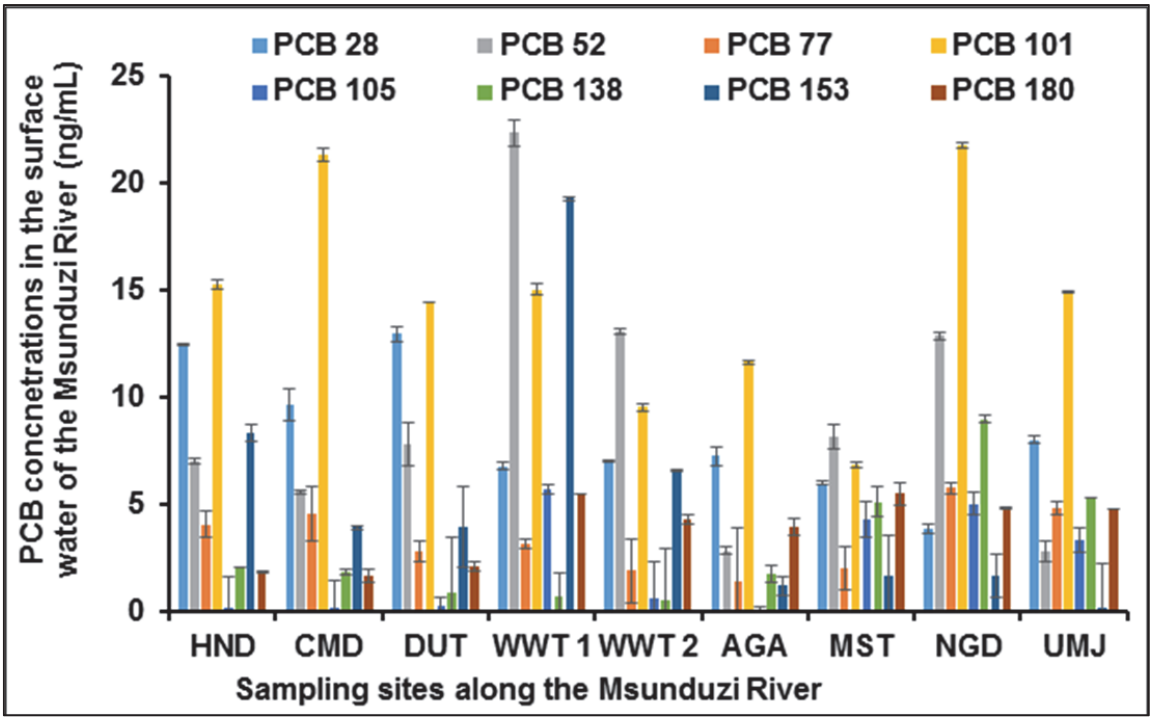


Figure 5.8 PCB concentrations in the surface water of the Msunduzi River

5.3.2 Sediment

The total sum of concentrations of PCBs across the various sampling sites of the Msunduzi River ranged from 644.63 ng/g at the agricultural area to 3530.62 ng/g at the Du Toit sampling site (Table 5.6). The second highest concentration of PCBs (3424.07 ng/g) was found at the WWT 1 site (Darvill wastewater treatment plant inlet) which was the most contaminated site for PCBs in surface water as well. The high concentrations of PCBs at the Du Toit sampling site indicate the high level of pollution that is close to this site in the form of industries as well as this site is close to the city centre and the N2 freeway. As a result, there is a lot of vehicular activity around this site which has possibly resulted in the high PCB concentrations observed. Previous studies have shown that these activities lead to PCB contamination of the environment (Zhang et al. 2003; Rissato et al. 2006; Nasir et al. 2014).

The lowest sediment concentration observed at the agricultural area was also seen in the surface water which again confirms that not much PCBs are used in the agricultural areas (Table 5.6 and Figure 5.9). The sediment concentrations are much higher than in the corresponding sites surface water which is due to the preferential partitioning of these PCBs to the organic matter in sediments. Most PCB partition coefficients are greater than 5 which means that they prefer to partition to the organics in the sediment rather than dissolve in the water column. Hence sediments serve as “sinks” for PCB accumulation. Generally, the higher chlorinated PCBs were found in higher concentrations with PCB 138 having the highest average concentration of 447.59 ng/g. The total average PCB levels obtained were much lower than the interim fresh water sediment quality guidelines (ISQG) of 21 500 ng/g (dw) and the probable effect level (PEL) of 189 000 ng/g (dw) as provided by the Canadian quality sediment guidelines (CCME, 2002).

Table 5.6 Concentrations of PCBs (ng/g±SD) in the sediment of the Msunduzi River

Site Code	PCB 28	PCB 77	PCB 101	PCB 52	PCB 153	PCB 105	PCB 138	PCB 180	∑PCBs
HND	37.43±1.45	282.44±0.15	272.8±0.09	376.42±0.06	244.09±0.51	347.80±1.5	665.93±0.21	745.88±0.40	2972.79±4.37
CMD	287.80±0.04	329.57±0.83	324.94±0.38	311.89±0.15	80.45±1.73	242.53±0.14	418.20±1.20	574.36±0.09	2569.74±4.56
DUT	549.19±0.03	438.18±0.18	610.45±0.38	422.55±0.15	354.49±0.32	552.58±0.46	388.97±0.53	214.21±2.52	3530.62±4.57
WWT 1	88.85±0.99	226.01±0.14	67.54±0.95	49.45±1.46	656.86±0.19	683.77±0.53	933.34±0.12	718.25±0.16	3424.07±4.54
WWT 2	21.93±0.35	130.8±0.24	14.67±0.24	38.86±0.03	15.04±2.58	22.40±2.15	282.67±1.14	142.81±0.80	669.18±7.53
AGA	28.24±1.16	172.32±0.69	8.87±0.55	145.57±0.51	106.94±0.25	29.46±2.09	146.71±0.29	6.52±1.87	644.63±7.41
MST	120.08±0.30	390.1±0.04	10.58±0.78	112.8±2.12	42.78±0.96	11.92±1.30	380.94±1.04	111.99±0.43	1181.19±6.97
NGD	215.33±0.17	420.46±0.02	27.65±0.56	333.57±0.01	228.75±0.14	41.72±0.22	127.75±0.57	208.6±1.42	1603.83±3.11
UMJ	535.37±0.28	265.83±0.63	611.92±0.16	136.06±0.39	36.45±2.23	449.05±0.08	683.77±0.14	156.39±1.05	2874.84±4.96
∑PCBs	1884.22±4.77	2655.71±2.92	1949.42±4.09	1927.17±4.88	1765.85±8.91	2381.23±8.47	4028.28±5.24	2879.01±8.74	19470.89±48.02
Min	21.93±0.35	130.80±0.24	8.87±0.55	38.86±0.03	15.04±2.58	11.92±1.30	127.75±0.57	6.52±1.87	644.63±7.41
Mean	209.36±0.53	295.08±0.32	216.60±0.45	214.13±0.54	196.21±0.99	264.58±0.94	447.59±0.58	319.89±0.97	2163.43±5.34
Max	549.19±0.03	438.18±0.18	611.92±0.16	422.55±0.15	656.86±0.19	683.77±0.53	933.34±0.12	745.88±0.40	3530.62±4.57

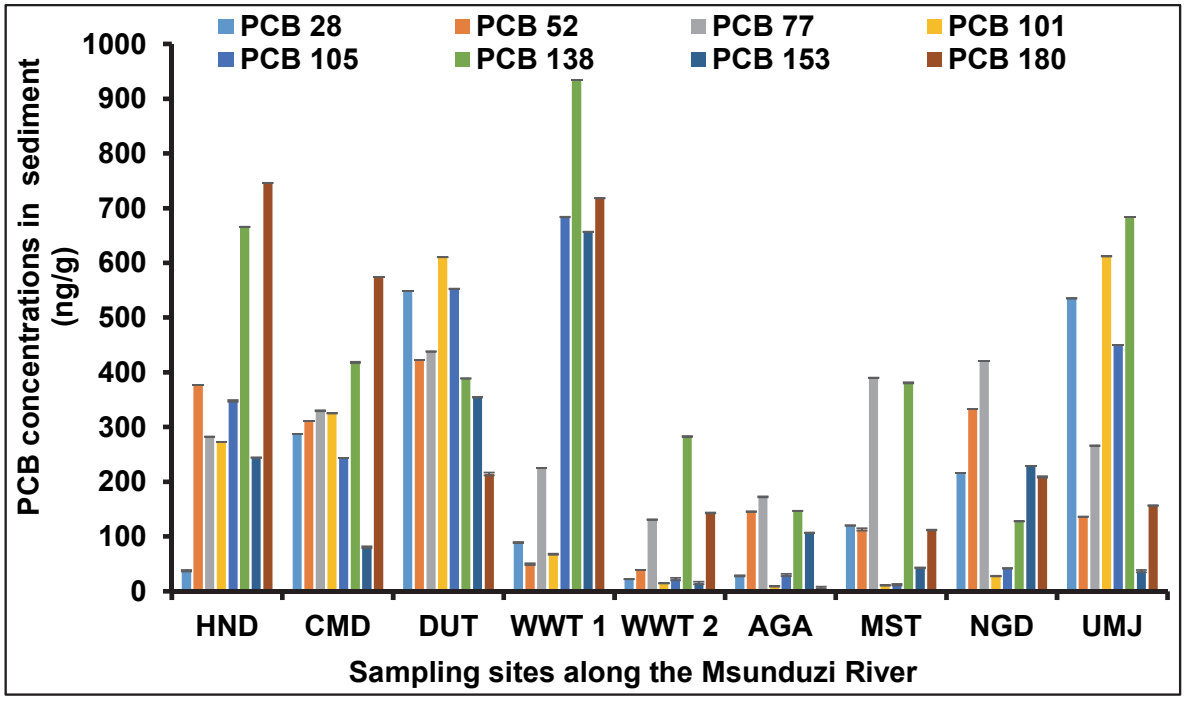


Figure 5.9 PCB concentrations in the sediment of the Msunduzi River

5.3.3 Soil

The concentration of PCBs in the soil of the Msunduzi River ranged from 817.13 ng/g at the Henley dam sampling site to 1427.22 ng/g at the Msunduzi town site (Table 5.7). The high concentrations at the Msunduzi town site could be due to the high vehicular traffic at that site which was close to a taxi rank. In addition the high concentrations of PCBs in this area could be due to burning of fuels during winter to keep homes warm. The PCB in highest concentration was PCB 52 with an average concentration of 275.20 ng/g. Its highest concentration of 343.44±0.01 ng/g was found at the Msunduzi town sampling site (Figure 5.10). The trend of the PCB concentrations in the environmental media in this study also agreed with previous research carried out by Aydin and Yurdun (1999), which showed that organic pollutants are more lipophilic and hydrophobic in nature and therefore bio-accumulate more in the organic matter than in surface water which is a possible reason for the high concentrations of pollutants observed in the sediment and soil than in the water.

Table 5.7 Concentrations of PCBs (ng/g±SD) in the soil of the Msunduzi River

Site Code	PCB 28	PCB 77	PCB 101	PCB 52	PCB 153	PCB 105	PCB 138	PCB 180	∑PCBs
HND	124.56±0.05	200.66±1.23	200.47±0.02	46.82±0.61	49.18±0.89	99.68±0.10	53.03±0.15	42.73±0.25	817.13±3.30
CMD	146.54±0.03	117.05±1.03	188.44±0.14	324.35±0.18	172.89±0.03	96.48±0.18	49.11±0.17	96.92±0.41	1191.78±2.17
DUT	164.55±0.07	158.03±0.01	139.11±0.03	302.82±0.01	175.74±0.16	96.06±0.00	59.66±0.00	120.75±o.14	1216.72±0.42
AGA	146.97±0.41	36.78±0.89	232.27±0.09	282.67±0.69	174.52±0.21	110.14±0.18	55.25±0.18	97.45±0.32	1136.05±2.97
MST	165.54±0.03	83.49±0.80	397.75±0.01	343.44±0.05	177.42±0.06	76.53±0.21	80.4±0.41	102.65±0.10	1427.22±1.67
NGD	147.29±0.23	168.4±0.14	217.75±0.33	341.07±0.02	159.66±0.02	85.44±0.79	111.42±0.35	134.51±0.22	1365.54±2.10
UMJ	86.33±0.55	164.66±0.02	90.88±0.72	285.2±0.07	190.8±0.10	54.67±1.76	25.64±0.36	118.1±0.16	1016.28±3.74
∑PCBs	981.78±1.37	929.07±4.12	1466.67±1.34	1926.37±1.63	1100.21±1.47	619.00±3.22	434.51±1.62	713.11±1.60	8170.72±16.37
Min	86.33±0.55	36.78±0.89	90.88±0.72	46.82±0.61	49.18±0.89	54.67±1.76	25.64±0.36	42.73±0.25	817.13±3.30
Mean	140.25±0.20	132.72±0.59	209.52±0.19	275.20±0.23	157.17±0.21	88.43±0.46	62.07±0.23	101.87±0.23	1167.25±2.34
Max	165.54±0.03	200.66±1.23	397.75±0.01	343.44±0.01	190.8±0.10	110.14±0.18	111.42±0.35	134.51±0.22	1427.22±0.42

Note: Soil samples were not available from the Darvill Wastewater Treatment Plant for this sampling campaign.

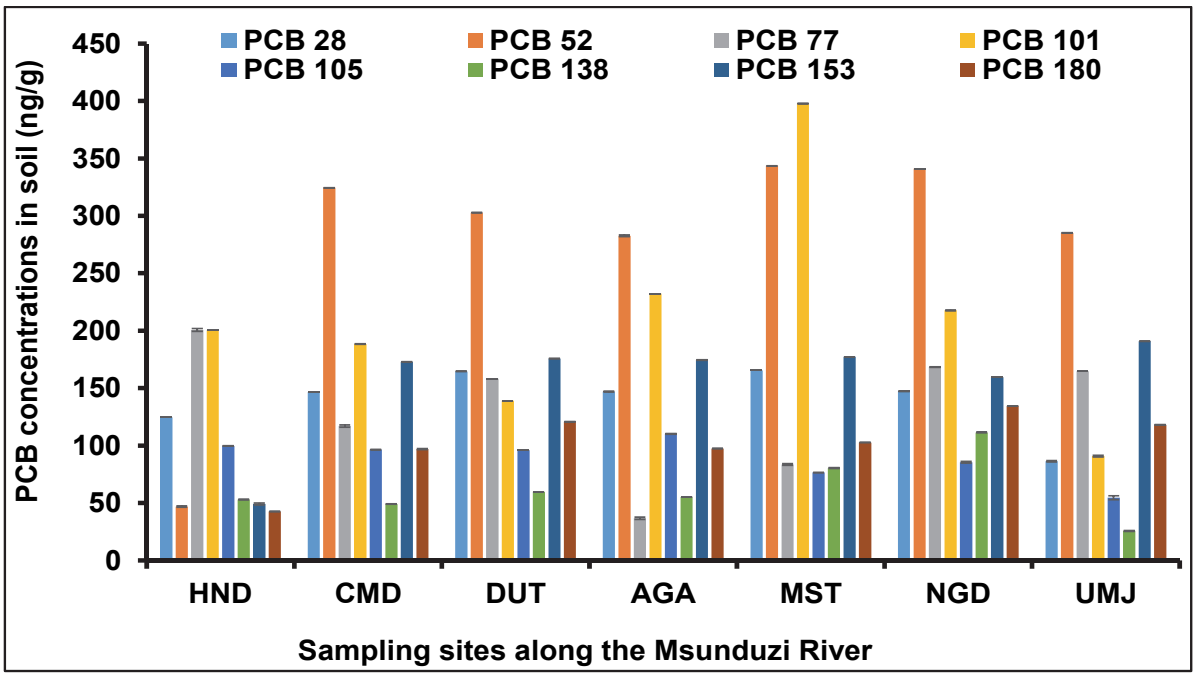


Figure 5.10 PCB concentrations in the soil of the Msunduzi River

5.3.4 Seasonal trends of PCBs in Msunduzi River

The seasonal trends of PCBs in the Msunduzi River show that the highest concentrations of PCBs were found generally during the winter season and the lowest concentration found during the summer season (Figure 5.11). The general high concentration during winter is due to the low rainfall experienced during winter. KwaZulu-Natal experiences a fairly dry winter season with little to no rainfall which results in lower water levels along the river resulting in a concentration of the PCBs. Compared to summer when KwaZulu-Natal has most of its rainfall, the river levels increase thus diluting the concentrations of PCBs.

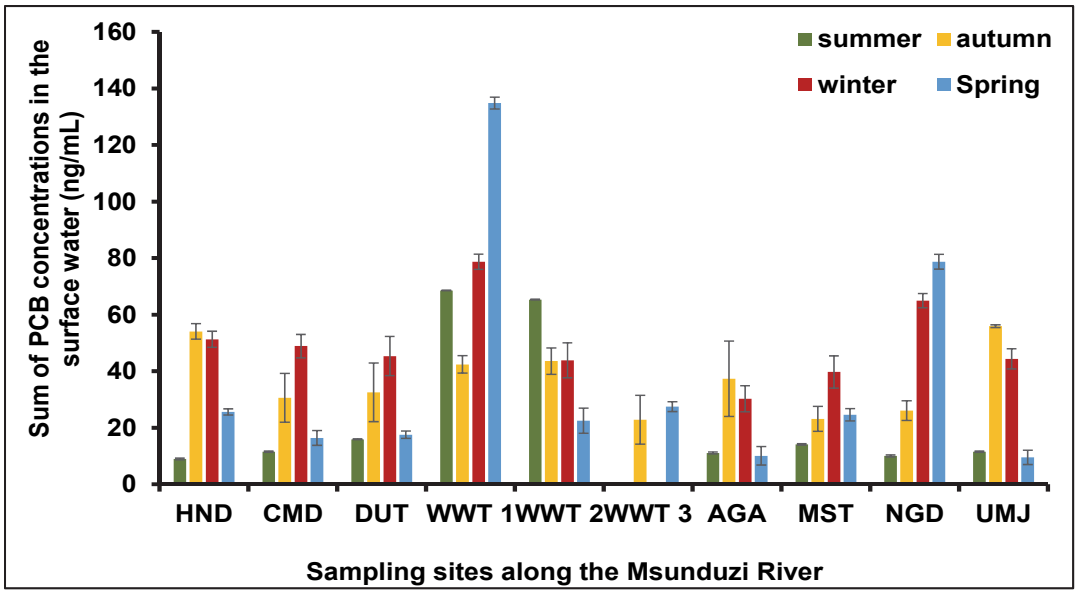


Figure 5.11 Seasonal trends of PCBs in the surface water of the Msunduzi River

In addition, the summer season has fairly high temperatures which encourage vaporisation into the atmosphere further reducing the concentrations in the river water. In winter, the cooler temperatures tend to encourage deposition of pollutants from the atmosphere into the river water leading to higher concentrations of PCBs in the river water. The sediment samples had much higher concentrations of PCBs than the surface water samples across all seasons. Again, the winter season showed the highest concentrations of PCBs followed by spring, autumn and summer (Figure 5.12). The reasons for the high concentrations in winter are the same as described above for the surface water. The spring showed the second highest concentration because spring follows winter but has a little more rainfall with slightly warmer temperatures which results in a decrease in the concentrations compared to winter. The autumn concentrations are also similar or slightly higher than the summer concentrations as autumn follows summer but has less rainfall with milder temperatures leading to slightly higher concentrations than seen in summer.

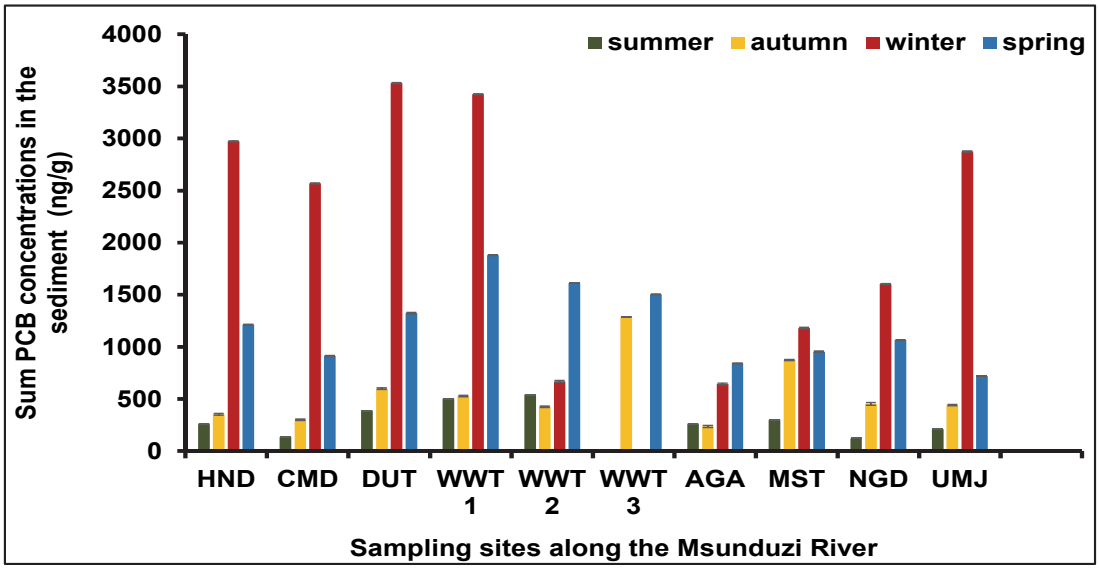


Figure 5.12 Seasonal trends of PCBs in the sediment of the Msunduzi River

5.4 SUMMARY

PCBs can be considered emerging organic pollutants within a South African framework, since the legislation that covers any monitoring and/or reporting is still being developed. Further information on PCB use and current and upcoming legislation can be found at sheqAfrica (http://sheqafrica.com/), Chemical and Allied Industries' Association (http://www.caia.co.za/), and The Department of Environmental Affairs (https://www.environment.gov.za/content/home). One thing to note from the previously mentioned resources is that PCBs are most likely still in use in South Africa; however, it is not clear if they are being manufactured intentionally or inadvertently produced as by-products of certain processes. From our results, we found that the sum of PCBs ranged from 7.66-21.43 ng/mL, which was much lower than the values from the Msunduzi sampling points (6.49-141.68 ng/mL). In general, the total average PCB level in both the Umgeni and Msunduzi River sediments were lower than the interim fresh water sediment quality guidelines (ISQG) of 21 500 ng/g (dw) and probable effect level (PEL) of 189 000 ng/g (dw) permitted by the Canadian quality sediment guidelines (CCME, 2002). However, according to Ontario sediment quality guidelines, the total average of PCBs in the sediment of Umgeni River was higher than the lowest effect level (LEL) (70 ng/g, dw) but far less than the severe effect level (SEL) (530 000 ng/g, dw) (Persaud et al. 1993). Hence the PCB levels are high enough to affect aquatic life and may increase over time but at present are lower than the current guidelines available.

CHAPTER 6: **ORGANOCHLORINE PESTICIDES (OCPs) IN THE UMGENI AND MSUNDUZI RIVERS**

6.1 INTRODUCTION

This section focuses on the analysis and monitoring of organochlorine pesticides (OCPs) in various samples collected from the Umgeni and Msunduzi Rivers. Different environmental matrices such as surface water, pore water, sediment and soil were investigated in order to fully understand the occurrence and significance of the above-mentioned contaminants in these rivers.

6.2 OCP CONCENTRATIONS IN THE UMGENI RIVER

6.2.1 Surface water

The results for the concentrations of OCPs in the Umgeni River surface water are shown in Table 6.1. The concentrations of individual pesticides ranged from a non-detectable level for aldrin at Reservoir Hills (REH) to 3.48 ng/mL for endrin at Northern Wastewater Treatment works influent (NWTI). The levels of pesticides were higher at the sites surrounding the wastewater treatment works and the point of discharge back into the Umgeni River. This was expected because the wastewater treatment plant receives residential waste which may contain residues of pesticides from food sold in supermarkets, such as fish, fruits and vegetables onto which they are known to accumulate (Barnhoorn et al. 2015, Gómez-Pérez et al. 2015, González-Curbelo et al. 2012, Vuković et al. 2012). Researchers have found that wastewater treatment plants can be considered as a source point of persistent organic pollutants (Katsoyiannis and Samara 2005).

The results showed that *o,p*-DDE and *p,p*-DDE were among the main OCPs in the Umgeni River water with average concentrations of 1.50 and 1.62 ng/mL, respectively. The presence of these DDT degradation products suggest that DDT was the common pesticide in use before it was banned in 1983 in South Africa. Today DDT is allowed to be used in a controlled manner but only by government, for the purpose of malaria control (Rother and Jacobs 2008) and therefore may be present in food stuff such as meat, fish, and vegetables transported from DDT-affected areas (McHugh et al. 2011) such as Limpopo and Mpumalanga (Dalvie et al. 2004a; Dalvie et al. 2004b; Naudé and Rohwer 2012a; Van Dyk et al. 2010) to the area investigated in this study.

The present study found no significant difference in total concentrations of pesticides at each site (Figure 6.1), from the source of the river at MDI (10.99 ng/mL) downstream to NAD (9.73 ng/mL), however JUM, the joining point of the Msunduzi River (tributary) with the Umgeni River, showed a slightly higher concentration (12.69 ng/mL) compared to upstream sites. This could be due to an added pollution load where the pollutants from the Msunduzi River now mix with the Umgeni River thus increasing the total concentration. The NWTI site also had high total concentrations of OCPs due to the same reasons discussed earlier.

Table 6.1 Concentration of OCPs (ng/mL ±SD) in Umgeni River surface water

Site Code	HCB	HCH	heptachlor	aldrin	*o,p*-DDE	*p,p'*-DDE	*o,p*-DDD/dieldrin	endrin	*p,p'*-DDD/*o,p*-DDT	mirex	∑OCPs
MDI	0.41±0.12	0.65±0.21	0.48±0.11	1.21±0.21	1.37±0.11	1.52±0.11	1.64±0.04	1.52±0.28	1.41±0.14	0.79±0.06	10.99±1.39
MDO	0.48±0.17	0.69±0.29	0.47±0.17	1.16±0.12	1.37±0.13	1.48±0.07	1.61±0.12	1.28±0.26	1.36±0.09	0.90±0.15	10.80±1.56
HOF	0.70±0.09	0.53±0.23	0.36±0.10	1.02±0.19	1.21±0.13	1.34±0.08	1.41±0.11	1.42±0.25	1.25±0.10	0.74±0.13	9.97±1.41
AFI	0.53±017	0.73±0.24	0.43±0.10	1.17±0.11	1.40±0.15	1.53±0.11	1.64±0.12	1.14±0.18	1.36±0.09	0.94±0.13	10.87±1.40
AFO	0.74±0.06	0.50±0.13	0.34±0.06	1.02±0.16	1.21±0.13	1.35±0.13	1.43±0.08	0.88±0.08	1.23±0.13	0.78±0.19	9.47±1.13
NAD	0.43±0.16	0.59±0.20	0.35±0.11	1.05±0.18	1.31±0.15	1.41±0.15	1.47±0.10	1.06±0.11	1.25±0.10	0.79±0.07	9.73±1.31
JUM	0.69±0.16	0.81±0.21	0.53±0.11	1.43±0.21	1.67±0.19	1.84±0.05	1.84±0.11	1.30±0.08	1.52±0.04	1.06±0.07	12.69±1.34
IDI	0.50±0.05	0.60±0.05	0.40±0.03	1.20±0.16	1.37±0.05	1.55±0.05	1.54±0.21	0.92±0.04	1.31±0.04	0.90±0.03	10.31±0.62
IDO	0.56±0.04	0.69±0.05	0.41±0.1	1.20±0.03	1.48±0.03	1.64±0.07	1.65±0.17	1.06±0.02	1.46±0.01	0.98±0.02	11.12±0.45
REH	0.53±0.03	1.57±0.40	0.81±0.21	nd	1.61±0.01	1.80±0.06	1.87±0.10	1.00±0.02	1.44±0.16	1.06±0.02	11.69±1.03
UBP	0.730.08	1.97±0.43	0.71±0.20	0.67±0.02	1.82±0.06	1.97±0.04	1.99±0.05	1.11±0.02	1.56±0.16	1.24±0.10	13.76±1.18
NWTI	1.04±0.21	1.26±0.29	1.91±0.42	2.73±0.08	2.32±0.09	2.01±0.04	1.92±0.07	3.48±0.07	1.72±0.09	1.02±0.06	19.41±1.43
NWTT	0.49±0.11	0.64±0.10	2.07±0.13	1.37±0.11	1.58±0.03	1.81±0.02	1.82±0.08	1.53±0.10	1.32±0.06	1.74±0.07	14.36±0.80
NWTE	0.77±0.06	0.53±0.13	0.32±0.08	1.35±0.02	1.37±0.08	1.49±0.04	1.79±0.11	1.90±017	1.35±0.07	1.38±0.05	12.26±0.81
BLA	0.80±0.03	0.53±0.05	0.35±0.08	1.40±0.10	1.41±0.10	1.54±0.03	1.78±0.09	1.24±0.18	1.36±0.14	0.96±0.06	11.38±0.85
∑OCPs	9.40±0.55	12.27±3.00	9.95±1.92	17.98±1.60	22.51±1.43	24.29±1.11	25.41±1.57	20.83±1.86	20.88±1.45	15.29±1.21	178.80±16.71
min	0.41±0.12	0.50±0.13	0.32±0.08	nd	1.21±0.13	1.34±0.08	1.41±0.11	0.88±0.08	1.23±0.13	0.74±0.13	9.47±1.13
mean	0.63±0.10	0.82±0.20	0.66±0.13	1.20±0.11	1.50±0.10	1.62±0.07	1.69±0.04	1.39±0.12	1.39±0.10	1.02±0.08	11.92±1.11
max	1.04±0.21	1.97±0.43	2.07±0.13	2.73±0.08	2.32±0.09	2.01±0.04	1.99±0.05	3.48±0.07	1.72±0.09	1.74±0.07	19.41±1.43

(MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

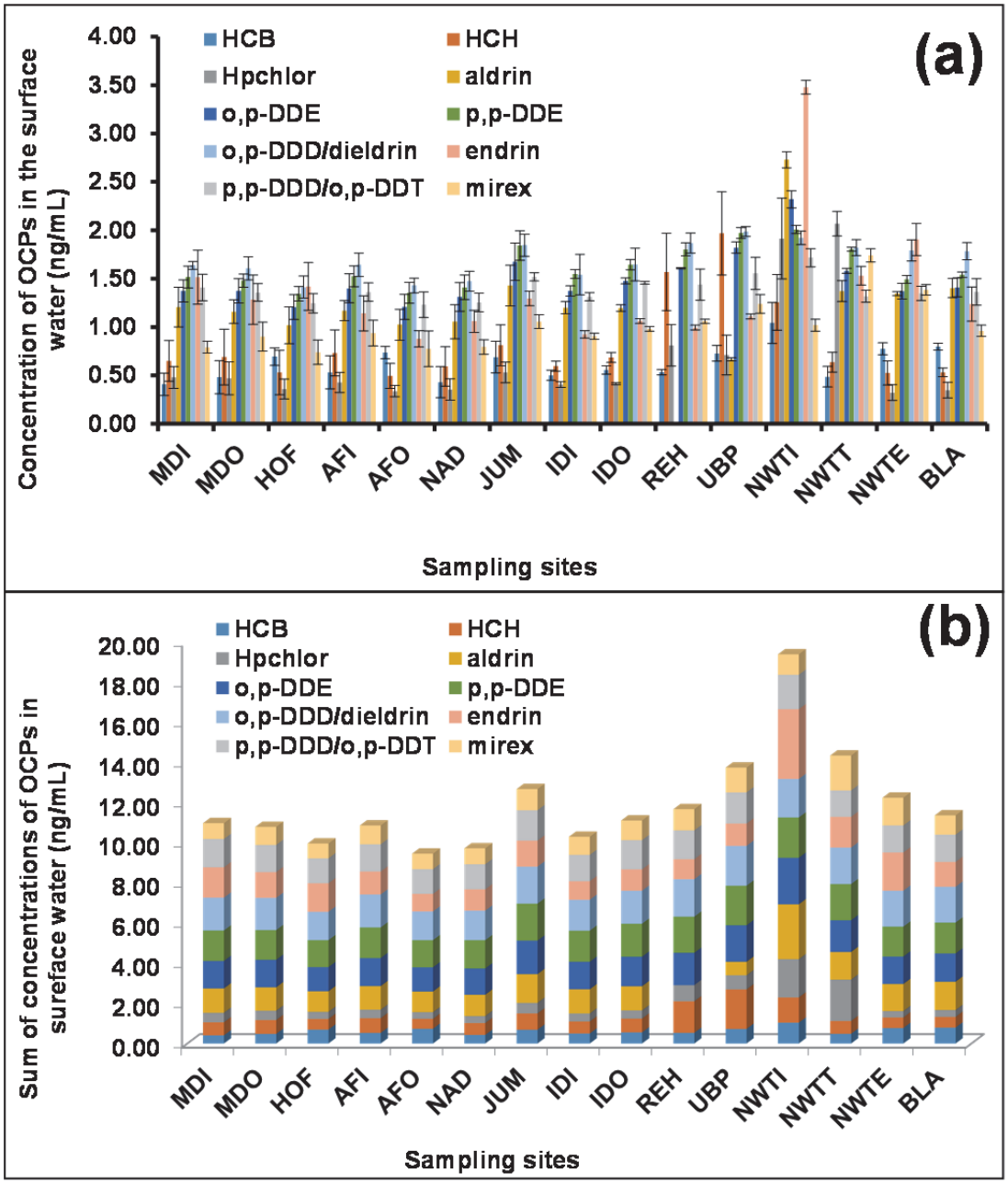


Figure 6.1 (a) Individual OCP concentrations and (b) Total concentration of OCPs in surface water samples at each site of the Umgeni River (MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

The increase in total concentration of OCPs may also be explained by the physical parameters that were recorded during the sampling trip. The higher levels of total suspended solids and total dissolved solids is an indication of pollution (Mahananda et al. 2010) which is seen in the higher conductivity and higher TDS values. There was very little increase in total dissolved solids and conductivity from the source at MDI (TDS = 49 mg/L, conductivity = 83.7 µs/cm) to NAD (66 mg/L, 114.0 µs/cm), with the corresponding total OCP concentrations ranging from 9.47±1.13 to 10.99±1.39 ng/mL. However, at JUM, the values of TDS and conductivity increased considerably to 214.0 mg/L and 367.0 µs/cm, respectively. The significant increase in TDS and conductivity corresponded to the large increase in OCP concentration at that site (12.69 ng/L)

which could be explained by the high preference of the OCPs to adsorb onto the dissolved organic matter (high TDS) at this site. Furthermore, the total OCP concentration at this site was increased by the joining of the tributary (Msunduzi River) and may be contributing to the pollution load at that site. The middle to lower reaches of the river from IDI to NWTI had the highest values in TDS (568.0 mg/L) and conductivity (970.0 µs/cm) and corresponded to the highest total OCP concentrations (19.41 ng/mL) at NWTI (Figure 6.1b) A and B showed a decrease in total concentration of OCPs from NWTI to BLA which is attributed to dilution effects because the treated water is discharged into the Umgeni River at NWTE.

Research has shown that there is a strong affinity between organic carbon and hydrophobic compounds that have high Log K_{ow} values such as organochlorine pesticides (Guan et al. 2009, Luo et al. 2009). Since the present study analysed water samples that were unfiltered in order to determine the concentrations of OCPs that humans and animals were exposed to by direct consumption, it was expected to contain high levels of organic carbon and hence the reason for the presence of high concentrations of total OCPs observed at the downstream sampling sites.

6.2.2 Pore water

The levels of individual OCPs varied from 0.76 ±0.01 for HCH at site MDO to 34.92 ±4.01 ng/mL for o,p-DDD+dieldrin at site IDO (Table 6.2). The highest mean concentration of individual contaminants across all sampling sites include *o,p*-DDE (14.79 ±0.88 ng/mL), *p,p*-DDE (17.09 ±0.92 ng/mL), endrin (10.38 ±1.09 ng/mL) and aldrin (12.61 ±0.77 ng/mL) in pore water samples. The total concentration of OCPs in pore water was ten times higher than in surface water. The reason for this is the high organic matter content in sediment pore water to which the OCPs preferentially adhere. The high Log K_{ow} values for these selected OCPs range from 5.46-6.89 (Shen and Wania 2005) which also indicates its preference to partition itself to organic matter rather than dissolve in water and hence is the reason for the higher concentrations of OCPs in pore water than in surface water. As a result, these OCPs tend to have long-term deposition and accumulation in sediment (Josefsson 2011)

There was a general increase in concentration of OCPs in pore water from the source to the mouth of the river (Figure 6.2 (a) and (b)) which corresponds with the increase in TDS observed in the same direction. Increase in the TDS values downstream could imply an increase of TOC, as well as other ionic, mineral and colloidal species. The organic carbon content can affect the partitioning of OCPs (Montes et al. 2012, Tan et al. 2009); however, further analysis in terms of ascertaining TOC values in the water and pore water samples, sediments collected, as well as analysing the size distribution of particulates are needed to understand the observed trends.

It was observed that the concentration of OCPs decreased at Blue Lagoon (mouth of the river) which may be attributed to dilution effects because of the close proximity of the Indian Ocean at that site. The highest total concentration of OCPs was observed at the middle to lower reaches of the river such as NWTE (166.23 ng/mL, 12%), NWTT (162.88 ng/mL, 11%), IDO (162.88 ng/mL, 11%), UBP (141.22 ng/mL, 10%), REH (140.18 ng/mL, 10%) and IDI (128.30 ng/mL, 9%) (Figure 6.2). The high OCP concentrations at the REH site is of concern because this sampling site is a few kilometres upstream of an informal settlement whose residences may be exposed to these OCPs if they collect their drinking water too close to the sediment, as the OCPs can re-suspend from the sediment into the water column (Chau 2006, Elena et al. 2011).

Table 6.2 Concentrations of OCPs (ng/mL ±SD) in sediment pore water of the Umgeni River

Site Code	HCB	HCH	heptachlor	aldrin	*o,p*-DDE	*p,p*-DDE	*o,p*-DDD + dieldrin	endrin	*p,p*-DDD + o,p-DDT	mirex	∑OCPs
MDI	1.90±0.41	1.92±0.07	3.81±0.33	6.99±0.16	8.52±0.16	9.88±0.21	9.02±0.64	6.10±0.90	7.50±0.34	3.99±0.12	55.63±3.34
MDO	1.78±0.08	0.76±0.11	2.19±1.13	4.11±2.32	5.18±3.22	5.73±3.28	7.15±4.01	4.17±2.71	5.37±4.16	2.47±1.33	34.32±22.36
HOF	2.93±0.19	1.62±0.12	5.04±1.26	11.87±048	8.47±0.19	9.30±0.43	8.91±0.28	5.41±0.70	7.73±0.19	6.71±2.66	61.28±6.53
AFI	1.69±0.10	2.61±0.43	4.07±0.35	7.91±0.78	8.45±0.60	9.41±0.05	11.42±0.48	8.22±2.10	7.57±0.19	3.54±0.20	61.35±5.29
AFO	1.60±0.25	1.24±0.01	3.13±0.29	5.79±0.71	6.67±1.27	7.44±0.83	9.30±1.09	4.61±0.72	6.06±0.58	2.74±0.35	45.29±6.24
NAD	2.94±0.49	3.56±0.62	7.08±0.84	11.56±1.17	13.89±1.18	16.07±1.29	19.64±1.95	6.86±0.88	12.79±1.53	6.28±0.82	94.40±10.78
JUM	3.08±0.89	2.97±0.51	7.69±0.32	11.31±1.37	13.87±1.41	17.20±2.01	20.69±3.01	13.68±0.63	13.11±1.97	6.97±1.12	103.61±13.24
IDI	1.59±0.19	5.93±0.61	8.92±0.17	15.69±0.58	18.61±0.41	21.53±0.67	26.53±1.28	13.01±1.10	16.82±0.12	9.22±0.55	128.30±5.64
IDO	4.61±0.20	5.55±0.27	11.43±0.93	18.89±0.85	24.39±0.51	27.76±0.76	34.92±1.05	13.00±1.93	22.34±0.71	10.59±0.24	162.88±7.47
REH	4.24±1.13	5.88±0.14	10.41±1.79	16.33±0.87	20.32±0.70	23.50±0.55	29.27±0.83	11.98±0.72	18.26±1.16	10.03±0.37	140.18±8.27
UBP	3.84±0.30	4.25±0.41	9.72±0.86	16.65±0.81	19.43±0.82	22.87±0.23	27.96±1.66	19.21±0.60	17.28±0.62	10.27±1.19	141.22±7.50
NWTT	6.08±0.94	6.80±1.11	11.35±1.97	21.96±0.37	23.27±0.49	26.75±1.31	32.54±0.30	13.10±0.29	21.02±0.96	12.18±0.90	162.88±8.65
NWTE	5.28±0.37	5.20±0.69	12.63±1.37	17.90±0.10	24.16±0.87	27.93±0.57	33.38±0.71	19.08±1.30	20.66±0.86	11.11±0.34	166.23±7.16
BLA	2.91±0.39	3.43±0.55	5.71±0.21	9.53±0.16	11.89±0.49	13.93±0.69	16.70±0.26	6.95±0.61	10.64±0.48	5.84±0.58	81.69±4.41
∑OCPs	43.61±6.04	51.72±5.64	103.18±11.84	176.49±10.74	207.12±12.33	239.29±12.89	287.44±17.54	145.27±15.21	185.14±13.88	101.95±10.76	1541.22±116.89
min	1.59±0.19	0.76±0.11	2.19±1.13	4.11±2.32	5.18±3.22	5.73±3.28	7.15±4.01	4.17±2.71	5.37±4.16	2.47±1.33	34.32±22.36
mean	3.11±0.43	3.69±0.40	7.37±0.85	12.61±0.77	14.79±0.88	17.09±0.92	20.53±1.25	10.38±1.09	13.22±0.99	7.28±0.77	102.80±8.35
max	6.08±0.94	6.80±1.11	12.63±1.37	21.96±0.37	24.39±0.51	27.93±0.57	34.92±1.05	19.21±0.60	22.34±0.71	12.18±0.90	166.23±7.16

(MDI, Midmar dam inlet; MDO, Midmar dam outlet; HOF, Howick Falls; AFI, Albert Falls inlet; AFO, Albert Falls outlet; NAD, Nagle dam; JUM, Joining Point of Msunduzi and Umgeni Rivers; IDI, Inanda dam inlet; IDO, Inanda dam outlet; REH, Reservoir Hills; UBP, Umgeni Business park; NWTI, Northern Wastewater Treatment inlet; NWTT, Northern Wastewater Treatment after chlorination; NWTE, Northern Wastewater Treatment effluent; BLA, Blue Lagoon)

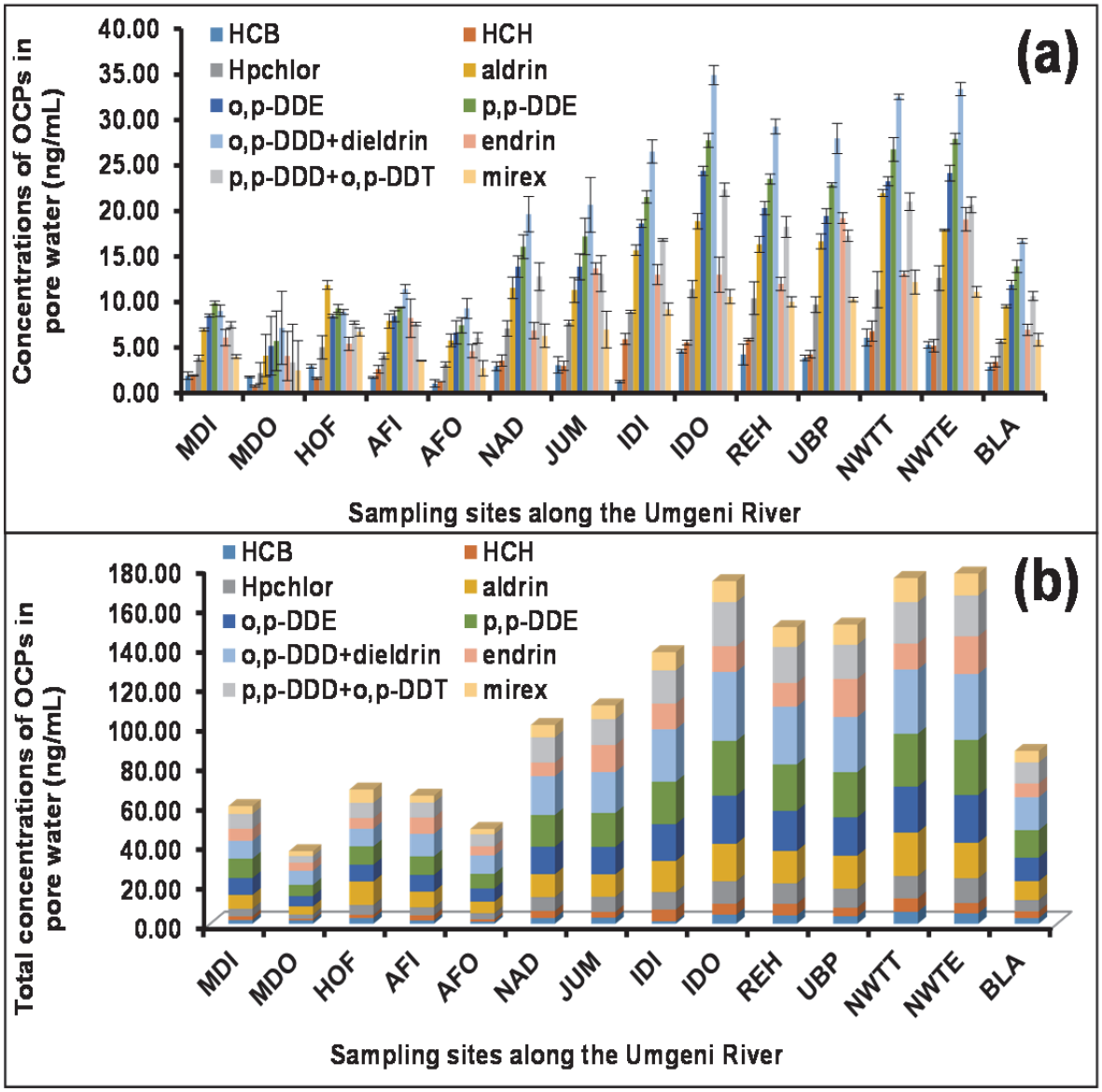


Figure 6.2 (a) Concentrations of individual OCPs in pore water, and (b) Total concentrations of OCPs at each site of the Umgeni River

6.2.3 Sediment

The OCPs investigated were detected in the sediment samples from all sites. The total concentrations of pesticides at each site are shown in Table 6.3. The total concentrations varied from 183.63-495.21 ng/g, with an average concentration of 308.70 ng/g, dw. The highest concentrations of pesticides were obtained at NWTT (495.21 ±32.38 ng/g, 11%), BLA (417.49 ±23.58 ng/g, 9%) and HOF (353.39 ±41.71 ng/g, 8%).The highest individual mean concentration in sediment was obtained for endrin (55.57±7.11 ng/g) which has a high octanol-water coefficient (Log K_{ow} = 5.6) and high persistence allowing it to remain in the environment for up to 14 years or more (USEPA, 2008). The analysis of bio-solids collected from NWTT (post-chlorination) (11%) showed a higher concentration than that collected from NWTI (before process treatment) (7%).

Table 6.3 Concentrations of OCPs (ng/g, dw ±SD) in sediment of the Umgeni River

Site code	HCB	HCH	heptachlor	aldrin	*o,p*-DDE	*p,p'*-DDE	*o,p*-DDD/dieldrin	endrin	*p,p'*-DDD/*o,p*-DDT	mirex	∑OCPs
MDI	6.23±0.93	4.34±0.91	26.67±2.85	21.52±0.04	17.78±0.11	20.61±0.13	23.60±1.14	36.86±2.23	19.17±0.01	6.83±0.52	183.63±8.87
MDO	4.51±1.24	30.36±4.02	26.70±2.93	22.70±2.29	26.38±2.11	26.45±2.50	23.96±0.46	44.43±6.61	27.58±0.64	48.86±2.28	281.93±25.07
HOF	10.75±1.69	37.49±5.06	30.06±2.22	19.59±7.55	25.56±4.12	34.40±4.50	29.94±1.16	73.84±8.69	33.44±3.77	58.31±2.94	353.39±41.71
AFI	6.81±1.32	23.49±3.17	26.38±8.10	17.45±7.90	22.34±2.08	27.23±2.69	29.93±2.54	63.70±7.76	21.98±0.77	61.22±1.38	300.53±34.93
AFO	7.41±1019	34.81±6.84	30.06±4.54	8.27±4.94	24.33±1.60	27.60±2.37	29.80±1.22	60.50±9.06	22.78±0.98	22.00±6.58	267.58±39.25
NAD	9.06±1.23	8.68±2.16	30.30±7.71	23.48±0.29	27.07±1.03	29.25±1.19	32.57±1.65	38.77±8.33	24.78±0.90	70.50±7.35	294.45±31.45
JUM	14.12±3.99	4.40±1.23	34.60±8.26	30.96±5.71	27.13±2.50	32.45±0.76	30.49±1.05	59.25±6.47	27.56±0.52	31.07±0.79	292.04±32.55
IDI	7.30±2.38	39.52±4.05	32.35±6.50	13.81±2.61	23.20±2.92	29.12±3.36	24.06±3.08	78.51±2.99	24.05±1.77	45.13±1.39	317.06±30.13
IDO	2.29±0.57	25.04±1.82	18.50±3.08	19.18±7.01	25.80±6.31	23.43±5.52	23.46±0.48	67.20±10.67	20.67±0.85	44.34±1.03	269.91±39.64
REH	5.49±1.58	16.83±3.83	18.46±3.67	23.9±77.68	18.52±1.19	20.95±1.56	23.79±0.94	32.74±9.38	19.18±3.14	56.35±4.83	236.28±35.37
UBP	11.35±0.48	41.94±4.31	43.31±1.68	13.80±2.34	23.78±1.43	21.20±0.20	22.97±0.97	28.10±1.05	18.51±0.71	50.32±2.82	275.30±15.55
NWTI	7.07±1.15	87.87±4.25	37.15±9.15	14.32±6.63	27.33±2.07	28.68±0.77	25.64±1.68	33.90±3.72	20.47±0.27	22.29±7.45	304.74±37.04
NWTT	49.44±2.45	82.96±3.13	26.83±3.78	37.80±6.91	50.70±1.40	38.72±2.44	26.40±1.81	75.84±9.60	27.84±0.18	78.66±0.20	495.21±32.38
NWTE	10.57±0.49	93.02±3.50	24.25±3.66	36.42±1.29	19.10±0.87	25.80±1.22	34.46±1.07	52.11±7.02	18.91±0.67	26.38±3.04	341.01±23.58
BLA	45.26±2.24	83.47±2.73	50.77±3.32	31.12±2.84	35.10±1.09	27.59±1.36	24.98±0.75	87.72±13.14	20.22±0.92	11.27±1.44	417.49±30.07
∑OCPs	197.68 ±23.30	614.24 ±51.00	456.38 ±71.40	334.40 ±64.00	394.12 ±30.80	413.49 ±29.60	406.06 ±20.00	833.51 ±106.70	347.14 ±16.50	633.52 ±44.00	4630.55 ±457.60
min	2.29±0.57	4.34±0.91	18.46±3.67	8.27±4.94	17.78±0.11	20.61±0.13	22.97±0.97	28.10±1.05	18.51±0.71	6.83±0.52	183.63±8.87
mean	13.18±1.56	40.95±3.40	30.43±4.76	22.29±4.27	26.27±2.05	27.57±1.97	27.07±1.33	55.57±7.11	23.14±1.10	42.23±2.94	308.70±30.50
max	49.44±2.45	93.02±3.50	50.77±3.32	37.80±6.91	50.70±1.40	38.72±2.44	34.46±1.07	87.72±13.14	33.44±3.77	78.66±0.20	495.21±32.38

A possible explanation could be that at NWTT, the pollutants from the WWTW accumulated over years in the bio-solid at the bottom of the pit where treated water is held before being discharged, while at NWTI, the bio-solid collected was not allowed to accumulate over long periods but were occasional since it is periodically removed from influent water. The levels of OCPs in sediments were higher than in water and in pore water. This was expected since POPs are known to prefer partitioning to organic material in sediment rather than dissolving in water (Zhou and Rowland 1997).

All the OCPs investigated were detected in all sampling sites and their individual concentrations ranged from 2.29 to 93.02 ng/g (Figure 6.3 and Table 6.3). Endrin (28.10-87.72 ng/g) was the most abundant OCP in almost all sites investigated. This was attributed to endrin's low mobility in soil and its long half-life. Its leaching into ground water and evaporation to air is very limited due to its very strong adsorption to soil particles (Log K_{ow} = 5.6) and low vapour pressure respectively (EPA, 2009).

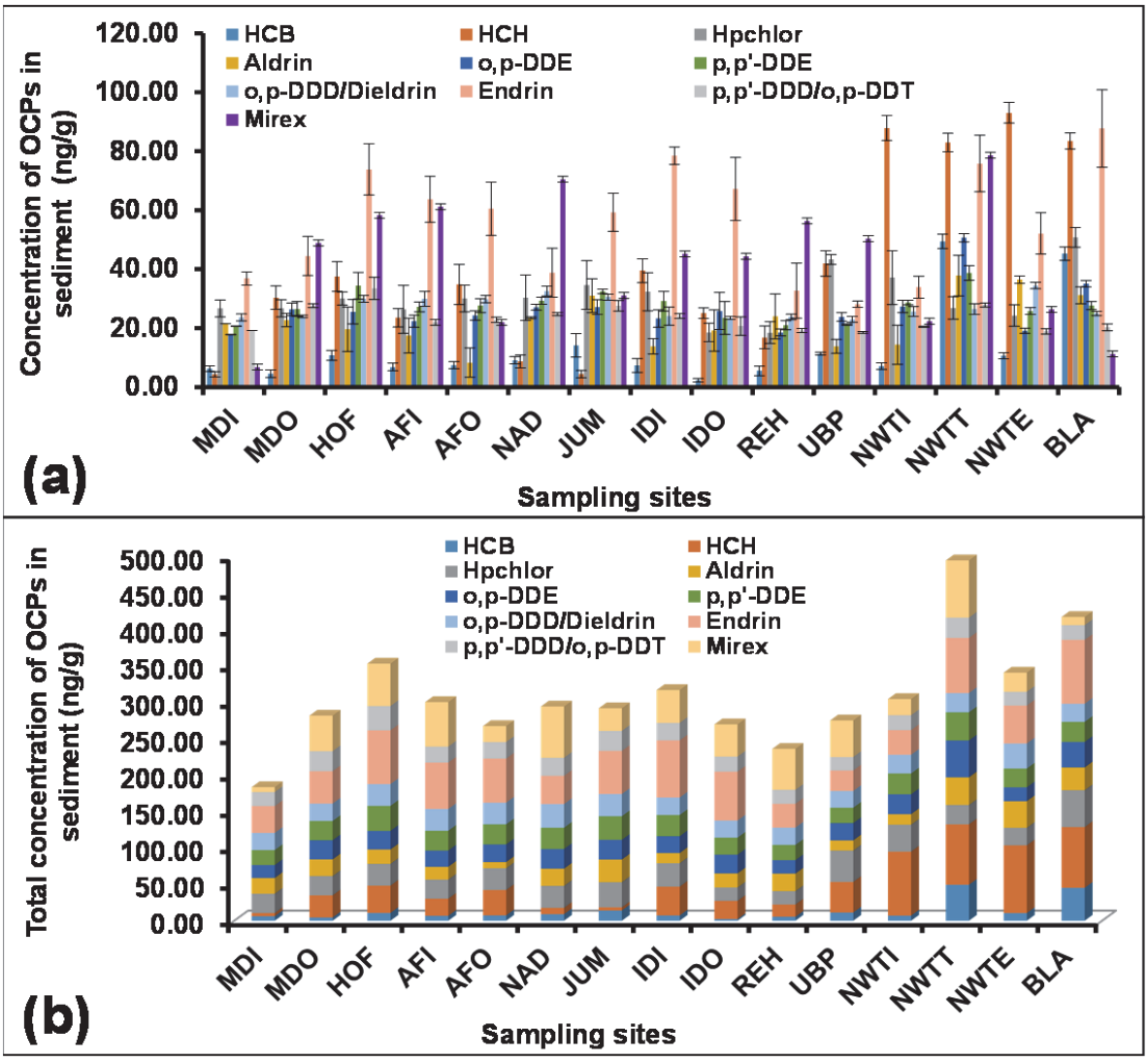


Figure 6.3 (a) Individual OCP concentrations in sediment at different sites along the Umgeni River, and (b) total concentrations (ng/g, dw) of OCPs in sediments

Other OCPs such as DDDs and DDEs were also detected in substantial amounts. The high concentrations of residues of these break-down products of DDT may be an indication of its extensive use in past years. The concentration of HCH in the sediment of the Umgeni River (4.34-93.02 ng/g, dw) was higher than levels of HCH observed in the sediments of the Qinhe River (nd-13.72 ng/g) in China (Fei et al. 2013) and in the sediment samples collected from the Old Yellow River estuary in China (0.001-14.85 ng/g,dw) (Da et al. 2014). However, the individual levels of OCPs in Umgeni River sediment (2.29-93.02 ng/g), were comparable to individual levels in four rivers running through an intensive agricultural area in Kilimanjaro in Tanzania (nd – 132 ng/g) (Hellar-Kihampa 2011). The levels in this study were below the results obtained from water (0.1-48.6 ng/mL) and sediment (0.10-163.00 ng/g) collected from the Densu River basin in Ghana (Kuranchie-Mensah et al. 2012).

6.2.4 Umgeni River bank soil

The total concentrations of OCPs were higher at Howick Falls (HOF) (284.09 ng/g), Inanda Dam inlet (IDI) (284.82 ng/g) and at NWTT (323.92 ng/g) and NWTE (320.60 ng/g) (Table 6.4). The high concentrations at HOF were probably due to leaching or long range transportation of agricultural pesticides from surrounding farms mainly sugar cane and wood plantations. Similarly, the high concentrations at Inanda Dam inlet, may be due to agricultural runoff and regular spraying of a mixture of herbicides. This spraying was observed during one of the sampling campaigns. The high levels of contaminants at NWTT and NWTE were expected since the wastewater treatment works receives residential and industrial waste which may contain many of these pollutants. The samples collected from these sites were mainly made of bio-solids which may have accumulated more pollutant than soils obtained from the banks of the river. Endrin was found in highest concentration in almost all sampling sites (Figure 6.4) which may be attributed to its persistence and long half-life. High concentrations of HCH were also observed at UBP, NWTE and BLA.

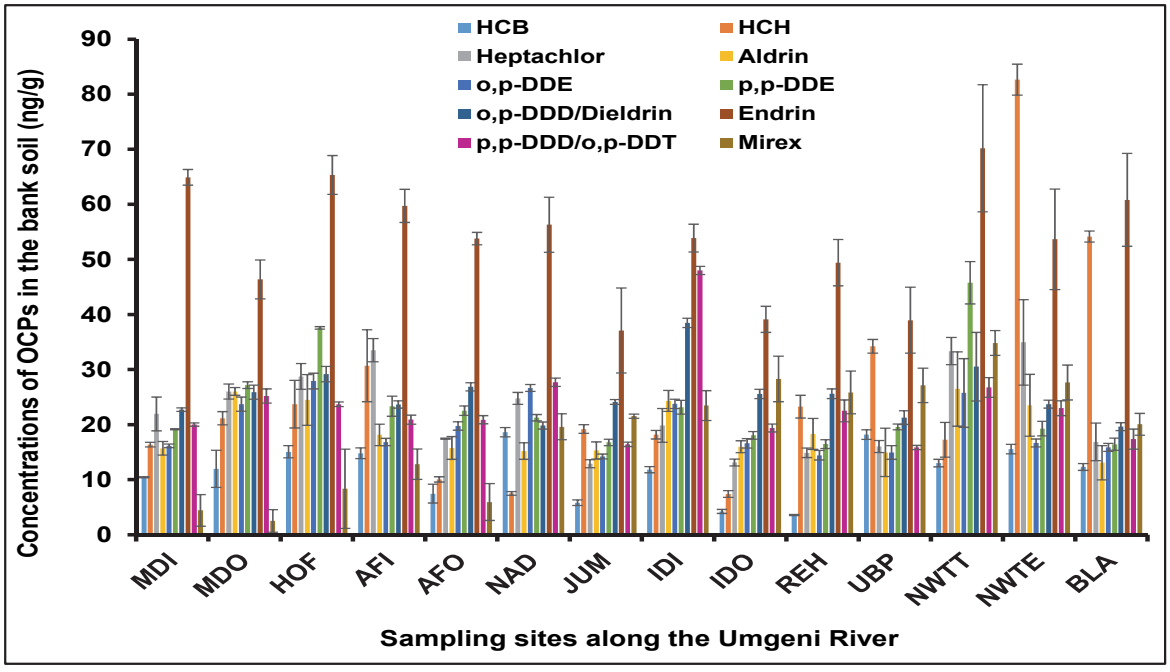


Figure 6.4 Concentrations of OCPs in the bank soil of the Umgeni

Table 6.4 Concentration of OCPs (ng/g ±SD) in the bank soil of the Umgeni River

Site Code	HCB	HCH	heptachlor	aldrin	*o,p'*-DDE	*p,p'*-DDE	*o,p'*-DDD/Dieldrin	endrin	*p,p'*-DDD/ *o,p'*-DDT	mirex	∑OCPs
MDI	10.42±0.10	16.35±0.42	21.95±3.06	15.72±1.24	16.15±0.33	19.17±0.10	22.71±0.33	64.90±1.42	20.02±0.30	4.43±2.86	211.82±10.15
MDO	11.97±3.38	21.17±1.20	26.00±1.37	26.02±0.70	23.71±1.28	27.17±0.60	25.90±1.62	46.36±3.52	25.20±1.27	2.52±2.05	236.01±16.97
HOF	15.08±1.10	23.73±4.32	28.75±2.35	24.48±4.62	27.91±1.42	37.57±0.23	29.17±4.95	65.33±3.53	23.70±0.44	8.36±7.20	284.09±30.18
AFI	14.79±0.98	30.69±6.53	33.51±2.11	18.14±1.95	16.84±067	23.33±1.82	23.66±0.97	59.71±3.00	20.91±0.82	12.81±2.77	254.40±21.62
AFO	7.45±1.70	10.07±0.48	17.43±0.10	15.75±2.07	19.75±0.75	22.53±0.86	26.87±0.57	53.77±1.12	20.89±0.75	5.93±3.35	200.44±11.76
NAD	18.63±0.86	7.51±0.33	24.78±1.08	15.20±1.49	26.66±0.63	21.26±0.59	19.83±0.89	56.30±4.99	27.70±0.76	19.61±2.35	237.48±13.97
JUM	5.81±0.50	19.21±0.77	12.90±0.73	15.31±1.56	14.22±0.45	16.77±0.57	24.13±0.72	37.08±7.72	16.41±0.40	21.54±0.37	183.38±13.79
IDI	11.82±0.56	18.18±0.79	19.84±3.07	24.29±1.91	23.75±0.84	23.15±1.24	38.46±0.70	53.87±2.52	48.00±0.75	23.46±2.69	284.82±15.09
IDO	4.24±0.38	7.41±0.61	13.12±0.63	15.99±1.07	16.61±0.85	18.05±0.70	25.56±0.81	39.10±2.37	19.40±0.72	28.29±4.13	187.76±12.27
REH	3.58±0.09	23.27±2.07	14.88±0.85	18.30±2.81	14.43±0.87	16.45±0.80	25.60±1.10	49.41±4.20	22.50±2.00	25.85±3.90	214.27±18.68
UBP	18.19±0.87	34.24±1.24	16.02±1.09	14.96±4.39	14.94±1.24	19.61±0.52	21.29±0.14	38.97±5.98	15.86±0.38	27.12±3.12	221.21±18.97
NWTT	13.00±0.68	17.26±3.14	33.35±2.47	26.48±6.76	25.76±6.23	45.78±3.85	30.53±0.97	70.18±11.54	26.76±1.78	34.82±2.24	323.92±39.66
NWTE	15.55±0.86	82.65±2.82	34.93±7.77	23.52±5.63	16.67±0.72	19.28±1.33	23.72±0.71	53.65±9.12	22.98±1.34	27.65±3.15	320.60±33.45
BLA	12.32±0.63	54.15±0.99	16.87±3.41	13.09±3.11	15.86±0.72	16.44±1.11	19.62±1.45	60.81±8.43	17.37±1.81	20.06±1.98	246.58±23.65
∑OCPs	162.84±12.69	365.91±25.71	314.33±30.08	267.27±39.32	273.25±17.01	326.55±14.32	357.04±15.94	749.42±69.46	327.70±13.50	262.47±3.01	3406.78±28.20
Min	3.58±0.09	7.41±0.61	12.90±0.73	13.09±3.11	14.22±0.45	16.44±1.11	19.62±1.45	37.08±7.72	15.86±0.38	2.52±2.05	183.38±13.79
Mean	11.63±0.91	26.14±1.84	22.45±2.15	19.09±2.81	19.52±1.21	23.33±1.02	25.50±1.14	53.53±4.96	23.41±0.96	18.75±3.01	243.34±20.01
Max	18.63±0.86	82.65±2.82	34.93±7.77	26.48±6.76	27.91±1.42	45.78±3.85	38.46±0.70	70.18±11.54	48.00±0.75	34.82±2.24	323.92±39.66

6.2.5 Seasonal trends of OCPs in the Umgeni River

Sampling was done during winter, spring, summer and autumn months. These seasonal sampling campaigns also took into account the various rainfall levels typically seen with the KwaZulu-Natal province. The trends observed for OCP concentrations in water, pore water and sediment across all sites are presented in Figures 6.5-6.7. During the winter sampling campaign, the sum of the OCP concentrations in the surface water samples was higher for most sites (Figure 6.5). The exceptions were JUM (Joining Point of Msunduzi and Umgeni Rivers) and BLA (Blue Lagoon). Variations in OCP levels have been correlated with water volume, TOC's, suspended particulates (organic and inorganic) and the K_{ow} values of the pollutant (Luo, et al. 2009). However, some studies have shown very low correlations (Montes, et al. 2012). Despite these differing reports in the literature, the current work does highlight the need for more in-depth studies taking into account all these parameters, and possibly including passive samplers.

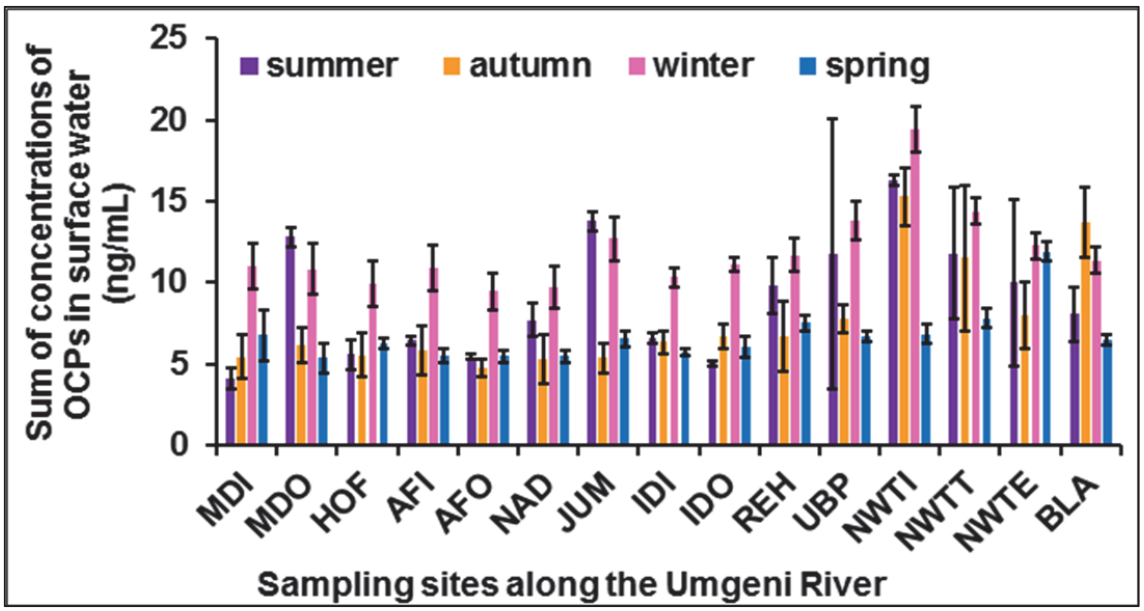


Figure 6.5 Seasonal trend of OCPs in water across the Umgeni River sampling sites

The OCP sediment averages for all seasons were higher than in water and pore water (
Figure 6.7). This was not unexpected because sediment is known to be both a pollutant sink and a carrier and source of contaminants in the aquatic environment (Chau 2006, Chee et al. 1996, Hongwen and Wen 2011, Usha 2013). The highest mean concentration was generally found in winter for all matrices. The winter, autumn and spring mean concentrations did not differ significantly for sediment because, being less mobile, its concentration in OCPs will take a relatively longer time to change significantly, except in the presence of extreme conditions like those in summer. The seasonal climatic mild conditions like those in autumn and spring may not change the sediment concentrations immediately. A monitoring study which covers more than one year is needed to know their temporal change in sediment. The lowest mean concentration was obtained in spring for water samples and in summer for pore water and

sediment samples (Figure 6.7). The mean concentrations of water in summer were a bit high due to an increase in concentration at wastewater treatment sites (NWTI, NWTO and NWTE) during that period probably due to continued release of household wastewater which was not much influenced by climatic conditions but is rather influenced by the type and quantity of influent coming into the wastewater treatment plant at any given time on any given day.

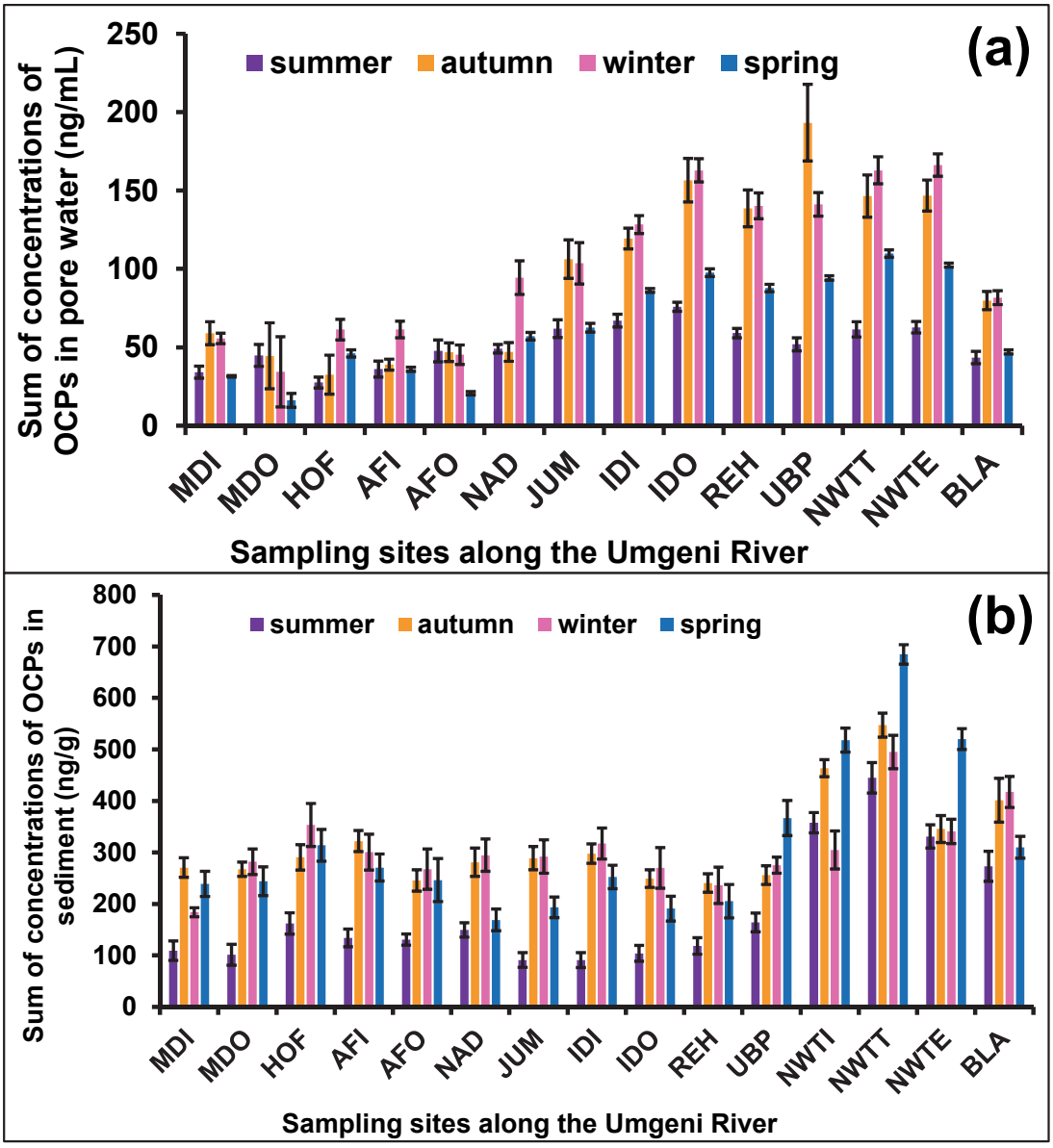


Figure 6.6 Seasonal trends of OCPs in (a) pore water and (b) sediment across the Umgeni River sampling sites

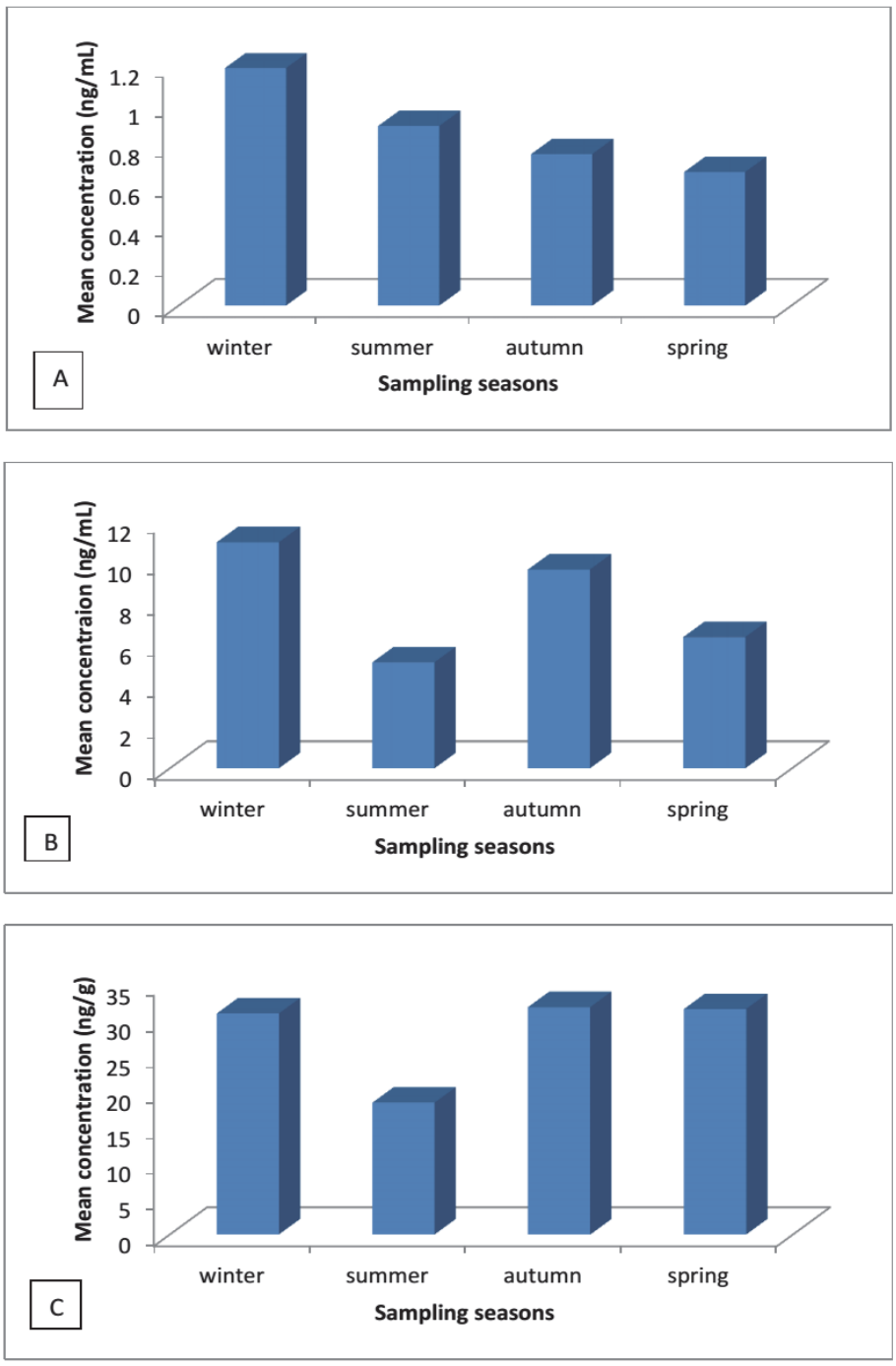


Figure 6.7 Mean seasonal OCP concentrations: A- in water, B- in pore water, C- in sediment of the Umgeni River

6.3 OCP CONCENTRATIONS IN THE MSUNDUZI RIVER

Surface water

Figure 6.8 shows observed concentration of OCPs in the surface water samples collected from Msunduzi River. The pesticide found with the highest average concentration was HCH (66.18 ng/mL) followed by mirex and the metabolites of DDT. The highest individual concentration of HCH was found at WWT 2, followed by Du Toit and Nagle dam. The high concentration of WWT 2 is probably due to the effluent received from domestic and commercial activities. The difference in concentrations between WWT 1 and WWT 2 sites is an indication of the high variability in pollutant concentration at any given time in the wastewater treatment plant. Research has shown that wastewater treatment plants often tend to have high concentrations of OCPs due to receiving household waste (including washings from supermarkets and restaurants) which includes washings of fish, fruit and vegetables onto which OCPs adhere (Barnhoorn 2015; Gómez-Pérez 2015; Vuković 2012). Other sites with high sums of concentrations of OCPs were Du Toit and Nagle dam. HCH is known to be used for increasing the yield of crops and is soluble in water and also volatile hence its high concentrations in the wastewater treatment plant are expected. However its significantly high concentrations compared to the other OCPs investigated is a cause for concern and further studies are required. The total sum of OCPs in the surface water of the Msunduzi River ranged from 81.38 ng/mL at the Msunduzi town sampling site to 471.48 ng/mL at the WWT 2 (Darvill wastewater treatment plant exit) (Table 6.5).

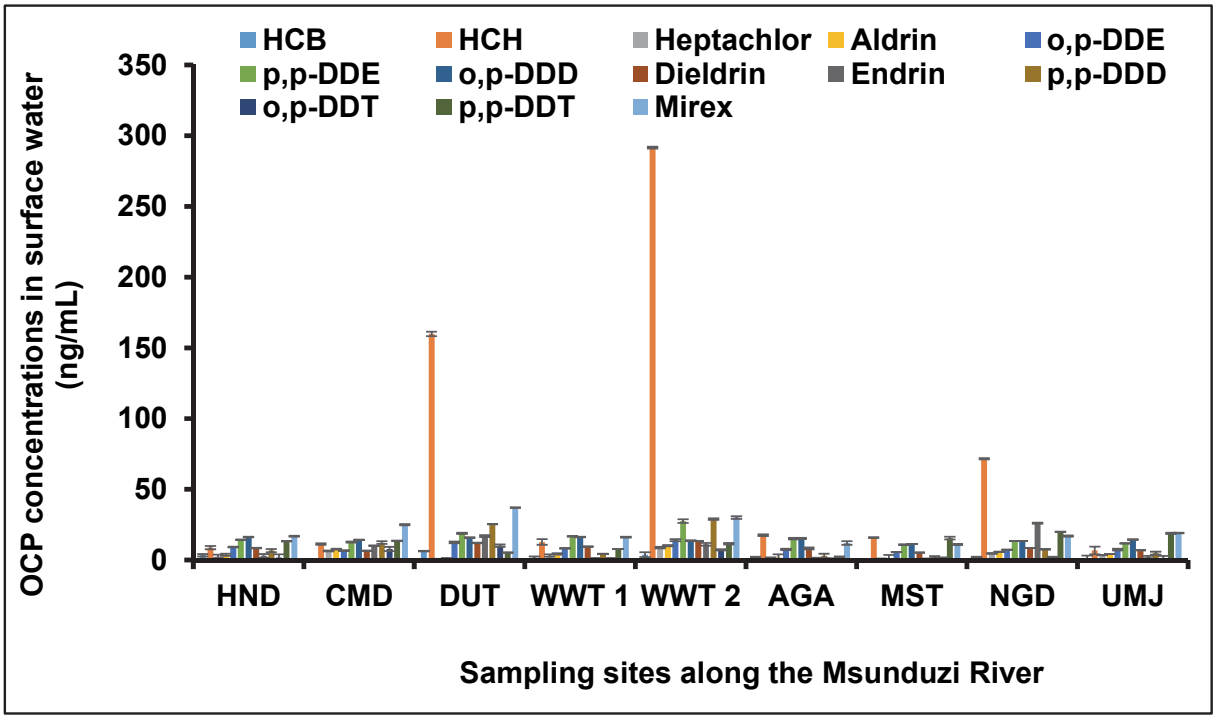


Figure 6.8 OCP concentrations in the surface water of the Msunduzi River

Table 6.5 Concentrations of OCPs (ng/mL±SD) in the surface water of the Msunduzi River

Site Code	HCB	HCH	heptachlor	aldrin	o,p-DDE	p,p-DDE	o,p-DDD	dieldrin	endrin	p,p-DDD	o,p-DDT	p,p-DDT	mirex	∑OCPs
HND	3.31±0.86	8.77±1.15	2.55±1.14	3.72±0.80	9.17±0.3	14.36±0.29	16.36±0.23	8.39±0.19	2.95±1.32	6.71±1.21	1.94±2.16	13.24±0.23	16.79±0.27	108.26±10.15
CMD	0.76±0.02	11.30±0.50	6.42±0.31	7.71±0.27	6.73±0.32	12.60±0.44	14.24±0.05	6.54±0.28	10.15±0.26	12.35±0.96	7.76±1.50	13.53±0.31	24.98±0.41	135.07±5.63
DUT	6.34±0.16	159.81±1.57	0.73±0.14	0.55±0.84	12.40±0.66	18.85±0.37	15.77±0.32	12.00±0.20	16.96±0.65	25.19±0.11	10.11±0.9	5.23±0.25	37.00±0.16	320.94±6.33
WWT 1	1.54±0.90	12.71±1.99	3.10±0.80	4.29±0.60	8.24±0.17	16.76±0.18	16.23±0.21	9.51±0.15	0.56±0.86	4.37±0.13	0.85±0.68	7.53±0.00	16.17±0.29	101.86±6.96
WWT 2	3.82±1.67	291.39±0.63	8.80±0.46	10.16±0.41	14.04±0.68	27.50±1.19	13.59±0.37	13.50±0.11	11.17±0.93	28.87±0.48	7.21±0.40	11.46±0.50	29.97±0.89	471.48±8.72
AGA	1.73±0.49	17.63±0.66	0.06±1.86	1.89±2.16	7.48±0.47	15.06±0.51	15.14±0.43	8.14±0.64	0.37±1.31	2.93±1.55	0.98±0.24	2.09±0.52	12.08±1.33	85.58±12.17
MST	0.30±0.15	15.84±0.17	0.90±0.21	1.03±2.50	5.56±0.05	10.89±0.11	11.46±0.04	5.30±0.20	0.73±0.33	2.01±0.82	0.89±1.08	15.50±0.91	10.97±0.50	81.38±7.07
NGD	2.09±0.14	71.53±0.38	4.58±0.21	5.82±0.17	7.27±0.03	13.24±0.02	13.8±0.04	8.65±0.02	26.05±0.37	7.47±0.07	1.97±0.37	19.95±0.12	16.86±0.44	199.28±2.38
UMJ	1.13±1.99	6.67±2.75	3.44±0.12	4.64±0.09	7.48±0.62	11.81±0.10	14.55±0.12	6.84±0.18	2.04±0.83	4.82±1.13	1.52±1.50	18.84±0.50	19.06±0.13	102.84±10.06
∑OCPs	21.02±6.38	595.65±9.80	30.58±5.25	39.81±7.84	78.37±3.30	141.07±3.21	131.14±1.81	78.87±1.97	70.98±6.86	94.72±6.46	33.23±8.83	107.37±3.34	183.88±4.42	1606.69±69.47
Min	0.30±0.15	6.67±2.75	0.06±1.86	0.55±0.84	5.56±0.05	10.89±0.11	11.46±0.04	5.30±0.20	0.37±1.31	2.01±0.82	0.85±0.68	2.09±0.52	10.97±0.50	81.38±7.07
Mean	2.34±0.71	66.18±1.09	3.40±0.58	4.42±0.87	8.71±0.37	15.67±0.36	14.57±0.20	8.76±0.22	7.89±0.76	10.52±0.72	3.69±0.98	11.93±0.37	20.43±0.49	178.52±7.72
Max	6.34±0.16	291.39±0.63	8.80.46	10.16±0.41	14.04±0.68	27.50±1.19	16.36±0.23	13.50±0.11	26.05±0.37	28.87±0.48	10.11±0.90	19.95±0.12	37.00±0.16	471.48±8.72

6.3.1 Sediment

The sediment OCP concentrations were much higher than the OCP concentrations for surface water throughout the Msunduzi River (Figure 6.9). The sum of the concentrations across all sites ranged from 1819.29 ng/g at Nagle dam to 7008.51 ng/g at WWT 1 (Table 6.6). The second highest sum of concentrations of OCPs was found at WWT 2 (5771.14 ng/g) followed by DUT (3135.84 ng/g) and CMD (3110.98 ng/g). The high concentrations at WWT 1 and WWT 2 are due to the high effluent loads received from industrial, commercial and residential activities. In addition, the sediment at these sites are expected to have the highest amounts of OCPs due to them partitioning themselves to the organic matter in the sediment. DDT and its metabolites along with dieldrin, endrin and mirex were found to be the OCPs with the highest concentrations. P,p-DDE had an average concentration of 419.48 ng/g followed by p,p-DDT with an average concentration of 419.30 ng/g. DDT has been banned from use today and is only used in a controlled manner by specific authorities in the control of the malaria vector in certain areas of South Africa such as northern KwaZulu-Natal, Limpopo and Mpumalanga (Dalvie et al. 2004b, Naudé and Rohwer 2012a; Van Dyk et al. 2010). The high concentrations of DDT found in the sediment confirms its persistent nature in the environment as well as could be due to trans-boundary effects from the areas currently sprayed with DDT to the current area of study.

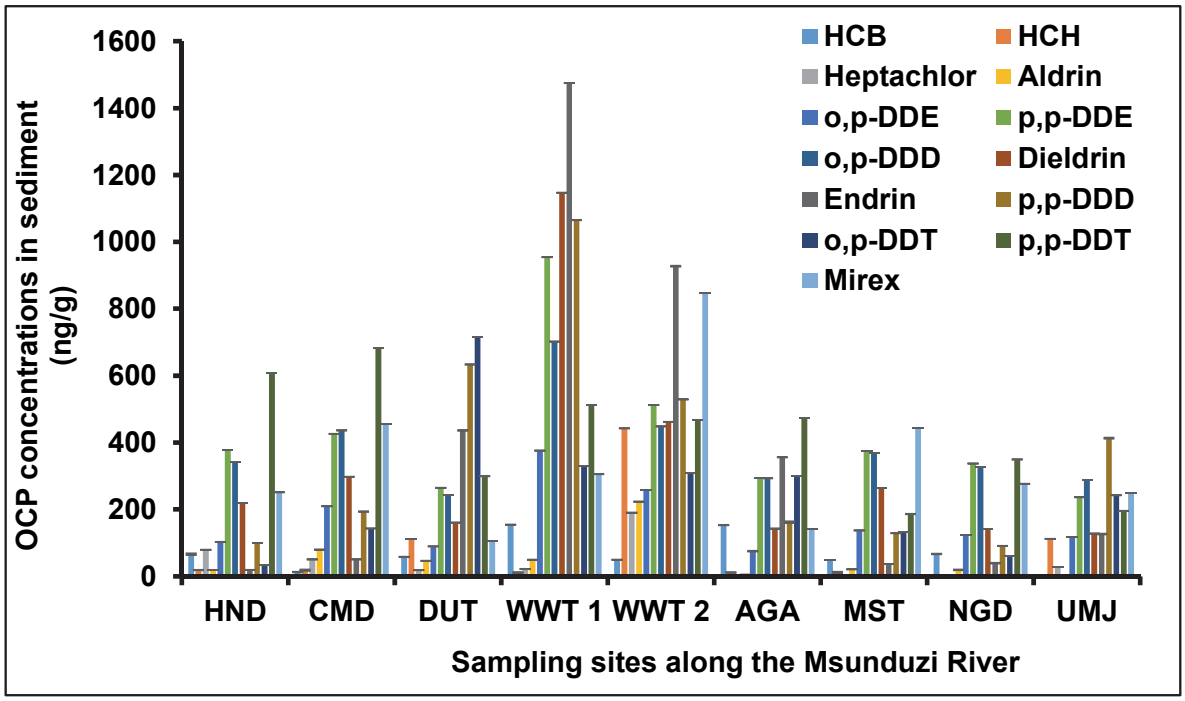


Figure 6.9 OCP concentrations in the sediment of the Msunduzi River

Table 6.6 Concentrations of OCPs (ng/g±SD) in the sediment of the Msunduzi River

Site Code	HCB	HCH	heptachlor	aldrin	o,p-DDE	p,p-DDE	o,p-DDD	dieldrin	endrin	p,p-DDD	o,p-DDT	p,p-DDT	mirex	∑OCPs
HND	1.39±2.63	18.73±1.16	79.53±0.45	17.73±0.04	103.34±0.47	378.08±0.10	342.82±0.02	220.09±0.11	19.20±0.71	98.27±0.13	34.72±0.25	606.55±0.14	251.50±0.30	2171.95±6.51
CMD	65.61±0.70	18.54±1.90	50.92±1.39	79.82±0.92	209.40±0.07	425.75±0.34	436.24±0.13	297.18±0.36	50.48±0.62	193.54±0.38	142.90±0.43	683.50±0.07	457.10±0.03	3110.98±7.34
DUT	12.64±0.60	110.96±0.04	18.5±0.35	46.40±0.14	89.38±0.859	264.04±0.39	243.66±0.09	160.39±0.22	436.50±1.30	633.44±0.48	714.61±0.10	299.50±0.88	105.82±0.16	3135.84±5.60
WWT 1	58.04±0.66	10.89±1.65	21.85±0.66	49.86±0.30	376.23±0.57	954.21±0.48	701.87±0.67	1146.79±0.47	1474.96±0.21	1065.03±0.58	329.90±0.24	513.56±0.07	305.32±0.06	7008.51±6.62
WWT 2	154.49±0.93	442.36±0.16	190.23±0.58	223.45±0.51	258.91±0.14	512.05±0.38	448.43±0.43	461.24±0.56	927.40±1.46	529.19±0.64	310.20±0.14	466.23±0.06	846.96±0.04	5771.14±6.08
AGA	49.06±0.76	11.55±1.01	0.45±2.77	3.87±1.75	75.05±1.27	292.77±0.25	293.28±0.35	142.86±0.88	355.70±0.47	161.94±2.63	300.33±0.06	473.30±0.26	139.88±0.03	2300.04±12.49
MST	152.89±0.06	10.94±2.65	2.09±1.03	21.01±0.42	136.82±0.57	374.73±0.02	367.43±0.02	263.96±0.08	36.98±0.04	129.27±0.42	131.40±0.32	186.19±0.85	444.58±0.08	2258.29±6.56
NGD	48.71±0.79	1.85±1.06	1.01±0.214	19.65±1.24	124.45±0.34	337.09±0.58	327.33±0.08	142.42±0.04	39.37±1.44	90.49±0.34	61.09±0.013	349.56±0.71	276.27±0.41	1819.29±7.27
UMJ	66.64±0.22	111.70±0.26	27.00±0.04	2.86±0.407	117.73±0.01	236.58±0.21	288.68±0.18	128.20±0.78	126.27±1.24	412.99±1.81	242.46±0.29	195.27±0.22	249.41±0.14	2205.79±5.79
∑OCPs	609.47±7.35	737.52±9.89	391.58±7.48	464.65±5.72	1491.31±4.35	3775.30±2.75	3449.74±1.90	2963.13±3.50	3466.86±7.49	3314.16±7.41	2267.61±1.89	3773.66±3.28	3076.84±1.25	29781.83±64.26
Min	1.39±2.63	1.85±1.06	0.45±2.77	2.86±0.40	75.05±1.27	236.58±0.21	243.66±0.09	128.20±0.78	19.20±0.71	90.49±0.34	34.72±0.25	186.19±0.85	105.82±0.16	1819.29±7.27
Mean	67.72±0.82	81.95±1.10	43.51±0.83	51.63±0.64	165.70±0.48	419.48±0.31	383.30±0.29	329.24±0.39	385.21±0.83	368.24±0.82	251.96±0.26	419.30±0.36	341.87±0.14	3309.09±7.14
Max	154.49±0.93	442.36±0.16	190.23±0.58	223.45±0.51	376.23±0.57	954.21±0.48	701.87±0.67	1146.79±0.47	1474.96±0.21	1065.03±0.58	714.61±0.10	683.50±0.07	846.96±0.04	7008.51±6.62

6.3.2 Soil

Results obtained showed that mirex was the most abundant OCP (Figure 6.10) found with an average concentration of 510.99 ng/g. Mirex showed a significant difference in total percentage concentrations in water compared to sediment and soil. This could be attributed to the fact that mirex is very insoluble in water and binds strongly to the aquatic soil and sediment. Mirex has no natural origin but historically it has been used in South Africa as a termite control and produced industrially through catalytic processes of dimerization of hexachlorocyclopentadiene, and majorly used as an insecticide, flame retardant in plastics, paper and electrical appliances (ATSDR 1995, Ritter et al. 2005). Its production has since been banned, but the residues of this compound may still remain in the environment due to its long half-life, historical use, open dumping, burning and leaching from old materials. Both endrin and dieldrin (half-life of approximately 5 years in temperate soil for dieldrin) were also found in appreciable concentrations in the environmental media and their source could possibly be traced to extensive usage in agriculture for the control of soil insect vectors of diseases before they were banned. Aldrin and heptachlor as well as HCH concentrations were lower compared to others, however the lower concentration of aldrin could possibly be due to its instability in the environment as aldrin is readily and rapidly converted to dieldrin leading to high concentrations of dieldrin (UNEP 2001; Ritter 2005). In this study, the indicative ratios of aldrin/dieldrin were 0.16, 0.14 and 0.50 in sediment, soil and water, respectively which confirms aldrin's degradation to dieldrin.

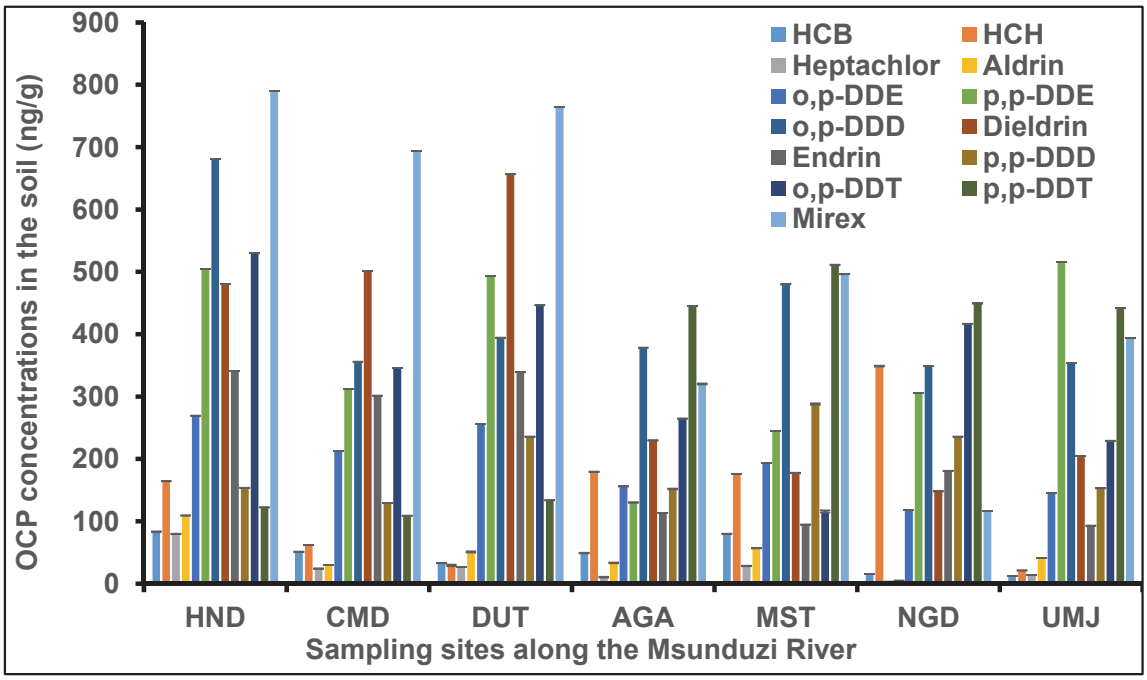


Figure 6.10 OCP concentrations in the soil of the Msunduzi River

The metabolites of DDT were again found in high concentrations though not as high as mirex. The total percentage of DDT metabolites were 60.68 in sediment, 57.96 in soil, and 49.37% in water. Technical

DDT (DDT mixtures) in general contain 75% *p,p*-DDT, 15% *o,p*-DDT, 5% *p,p*-DDE, and < 5% others (Bopp et al. 1982). Possible pollution sources of DDT can be assessed using relative total concentrations of DDTs together with its biological metabolites such as DDD and DDE which is an indicative index of pollution assessment. DDT is susceptible to degradation under aerobic conditions to DDE and under anaerobic condition to DDD (Bossi et al. 1992). A ratio of DDE+DDD/DDTs > 0.5 indicates long term-weathering while a ratio of DDD/DDE > unity (1), indicates that the sediments were dominated by DDD which results from anaerobic degradation of DDT (Hitch and Day 1992; Doong et al. 2002a; Doong et al. 2002b).

The ratio of DDD/DDE was determined to be 1.28 and 1.12 in sediment and soil, respectively. They were greater than unity, which indicated that both the sediment and soil of Msunduzi River are both dominated by *p,p*-DDD, a product of anaerobic degradation of *p,p*-DDT (Zhou et al. 2006). The ratios of (DDE+DDD)/DDTs in both surface sediment and soil were 1.99 and 1.80, which were also greater than 0.5. This result revealed that DDT metabolite contamination in soils and sediments of the Msunduzi River may be as a result of long term weathering of DDTs along the river and retained by anaerobic conditions within the sediment and soil in the river. To assess the trend of DDT sources in water, the ratio of DDE/∑DDTs and DDD/∑DDTs were evaluated and values of 1.56 and 1.61 were obtained. The values were greater than unity in water which is an indication that DDT metabolite contamination may be as a result of aged long weathering of DDT in the Msunduzi River.

The total concentrations of OCPs in the soil of the Msunduzi River across all sites ranged from 2461.35 ng/g at the agricultural area to 4307.97 ng/g at Henley dam (Table 6.7). Henley dam was the most contaminated site of all the sampling sites of the Msunduzi River with highest concentrations for HCB (83.43±0.08 ng/g), heptachlor (79.53±0.45 ng/g), aldrin (109.32±0.33 ng/g), o,p-DDE (269.26±0.02 ng/g), o,p-DDD (681.09±0.09 ng/g), endrin (340.35±0.03 ng/g), o,p-DDT (529.75±0.09 ng/g) and mirex (790.30±0.02 ng/g). The Henley dam sampling site is a semi-rural area with large open spaces that are used for farming, hence the runoff from the agricultural activities may have led to the high concentrations observed in the soil samples.

Table 6.7 Concentrations of OCPs (ng/g±SD) in the soil of the Msunduzi River

Analyte	HND	CMD	DUT	AGA	MST	NGD	UMJ	∑OCPs	Min	Mean	Max
HCB	83.43±0.08	50.86±0.49	32.89±0.58	49.06±0.76	79.44±0.48	15.39±0.22	11.60±0.21	322.67±2.82	11.60±0.21	46.10±0.40	83.43±0.08
HCH	163.97±0.15	61.87±0.05	29.12±1.33	179.26±0.34	175.81±0.33	348.72±0.81	21.08±0.28	979.83±3.29	21.08±0.28	139.98±0.47	348.72±0.81
heptachlor	79.53±0.45	24.11±0.12	26.99±0.11	10.42±0.68	28.40±0.33	2.48±1.10	13.92±0.15	185.85±2.94	2.48±1.10	26.55±0.42	79.53±0.45
aldrin	109.32±0.33	29.08±0.01	50.86±1.20	33.09±0.64	56.60±0.17	3.97±1.40	41.00±0.10	323.92±3.85	3.97±1.40	46.27±0.55	109.32±0.33
o,p-DDE	269.26±0.02	213.41±0.00	256.00±0.00	155.97±0.34	193.96±0.01	117.57±0.01	145.72±0.07	1351.89±0.45	117.57±0.01	193.13±0.06	269.26±0.02
p,p-DDE	504.55±0.05	311.80±0.01	493.26±0.10	130.13±0.01	245.68±0.01	305.12±0.01	515.52±0.03	2506.06±0.22	130.13±0.01	358.01±0.03	515.52±0.03
o,p-DDD	681.09±0.09	355.88±0.05	394.23±0.32	378.39±0.43	479.89±0.16	348.79±0.03	353.77±0.07	2992.04±1.15	348.79±0.03	427.43±0.16	681.09±0.09
dieldrin	480.74±0.17	502.12±0.02	656.76±0.02	229.47±0.37	177.45±0.38	147.96±0.39	204.70±0.20	2399.20±1.55	147.96±0.39	342.74±0.22	656.76±0.02
endrin	340.35±0.03	301.44±0.03	339.16±0.06	113.44±0.17	94.68±0.41	180.76±0.29	92.80±0.22	1462.63±1.21	92.80±0.22	208.95±0.17	340.35±0.03
p,p-DDD	153.67±0.11	128.34±0.02	235.63±0.12	151.92±0.64	288.07±1.20	235.40±0.54	153.05±0.29	1346.08±2.92	128.34±0.02	192.30±0.42	288.07±1.20
o,p-DDT	529.75±0.09	345.26±0.01	446.59±0.28	264.76±0.42	115.85±1.85	416.38±0.24	228.71±0.23	2347.30±3.12	115.85±1.85	335.33±0.45	529.75±0.09
p,p-DDT	122.01±0.48	108.85±0.07	133.85±0.12	445.18±0.17	511.20±0.40	449.58±0.54	441.57±0.08	2212.24±1.86	108.85±0.07	316.03±0.27	511.20±0.40
mirex	790.30±0.02	694.71±0.07	764.53±0.04	320.26±0.72	496.31±0.04	117.29±0.07	393.51±0.02	3576.91±0.98	117.29±0.07	510.99±0.14	790.30±0.02
∑OCPs	4307.97±2.07	3127.73±0.95	3859.87±4.28	2461.35±5.69	2943.34±5.77	2689.41±5.65	2616.95±1.95	22006.62±26.36	2461.35±5.69	3143.80±3.77	4307.97±2.07

Note: Soil samples were not available from the Darvill Wastewater Treatment Plant during this sampling campaign.

6.3.3 Seasonal trends of OCPs in the Msunduzi River

Sampling campaigns for OCPs in the Msunduzi River was done over the winter, spring, summer and autumn months. The fate and occurrence of most organic pollutants does depend on temperature, river/water volume, and various other physical and chemical parameters. The seasonal trends observed for the OCPs in the Msunduzi River water are presented in Figure 6.11 for the surface water samples and Figure 6.12 for the sediment samples. The winter season showed the highest concentrations of sum of OCPs at almost all sampling sites with much higher concentrations found in the sediment than in the surface water. The lower concentrations were observed during summer and autumn. The WWT sampling sites may not always show seasonal trends due to its varied input of residential and commercial waste. This is noted in the unusually high concentrations of OCPs in the surface water observed during the spring season

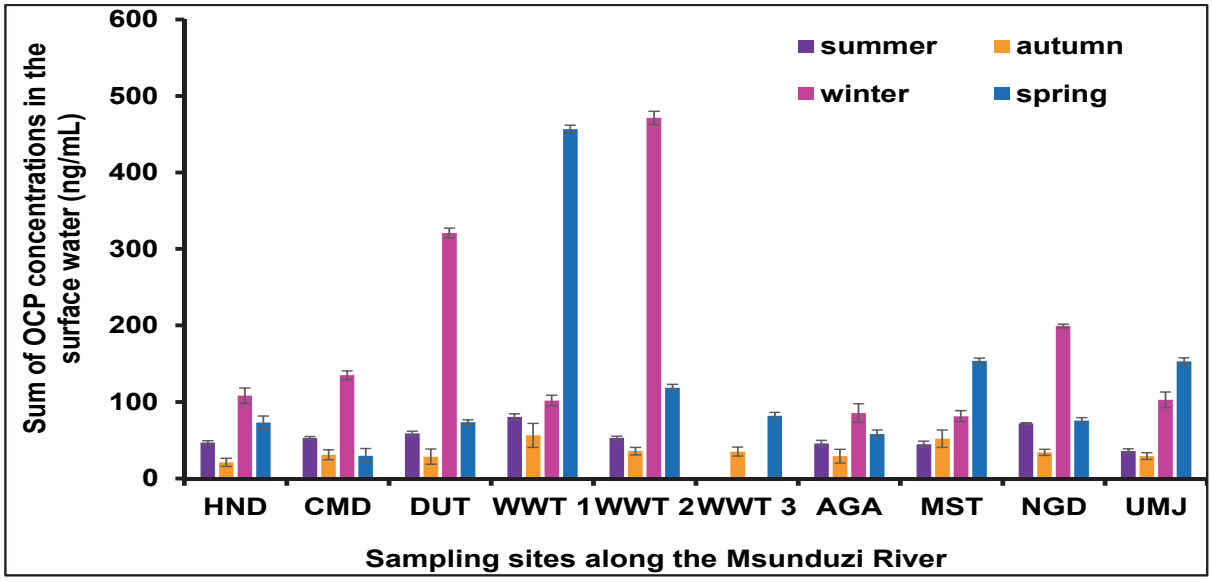


Figure 6.11 Seasonal trends of OCPs in the surface water of the Msunduzi River

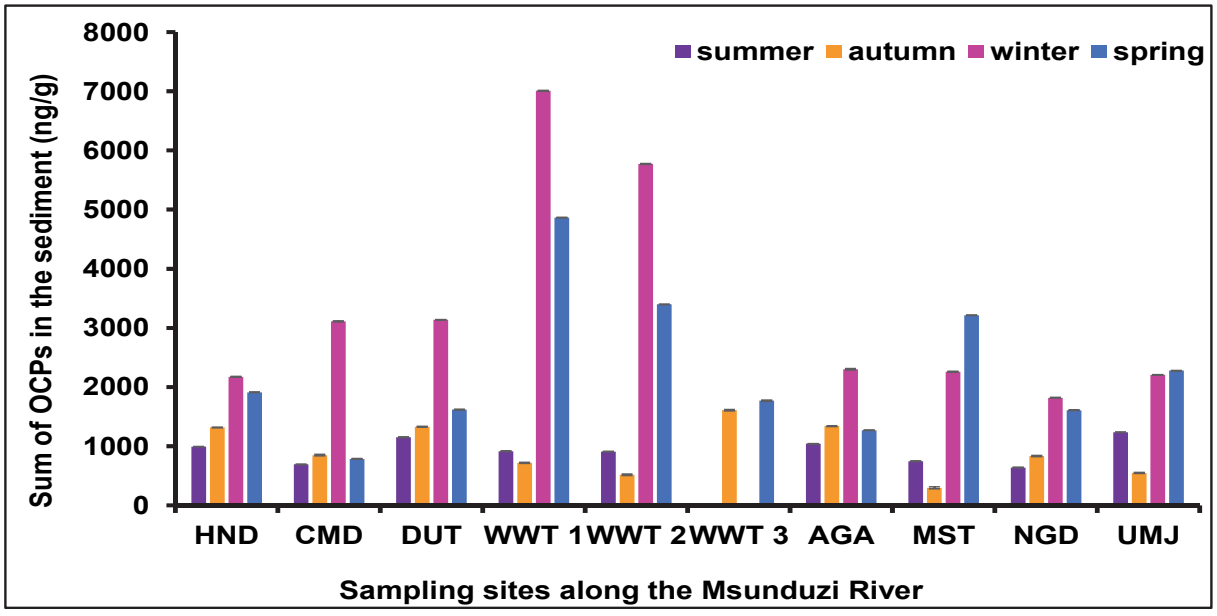


Figure 6.12 Seasonal trends of OCPs in the sediment of the Msunduzi River

6.4 SUMMARY OF FINDINGS

For OCPs, the results obtained for both the Umgeni and Msunduzi Rivers showed that selected OCPs were higher in concentration than the Canadian quality sediment guidelines of (HCB: 0.940 ng/g), (HCH: 0.600 ng/g), (*o,p*-DDE and *p,p´*-DDE: 1.420 ng/g) and Probable Effect Level (PEL) (HCB:1.380 ng/g), (HCH: 2.740 ng/g), (*o,p*-DDE and *p,p´*-DDE: 6.750 ng/g) (CCME, 2002). Almost all OCPs were higher than their LEL value of 20 ng/g. The values for all studied OCPs were significantly lower than the SEL values (HCB: 24 000 ng/g), HCH :12 000 ng/g), (*o,p*-DDE and *p,p´*-DDE: 19 000 ng/g) (Persaud et al. 1993). Thus, the levels of OCPs in the Umgeni and Msunduzi Rivers are of concern as they are above the guidelines and could lead to significant health effects even though they are currently below the severe effect levels.

Generally, the wastewater treatment plants had the highest concentrations for both PCBs and OCPs and could be a possible source of these pollutants back into the Umgeni and Msunduzi Rivers. The soil and sediment matrices had higher concentrations of the selected PCBs and OCPs compared to the surface water which can be attributed to the high partition coefficients for these pollutants which result in them preferring to partition to the organic matter in the sediment and soil rather than dissolve in water. A comparison of the Umgeni River and Msunduzi River results shows that the Msunduzi River is far more contaminated than the Umgeni River in all matrices analysed. The seasonal trends show that the winter season had highest concentrations of PCBs and OCPs due to the low rainfall and cooler temperatures which promote deposition rather than vaporisation.

CHAPTER 7: **CONCLUSIONS AND RECOMMENDATIONS**

7.1 INTRODUCTION

The overall aim of this project was to investigate the presence of certain selected persistent organic pollutants, namely; pharmaceutical and personal care products, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) in the key river systems within KwaZulu-Natal, in particular the Msunduzi and Umgeni Rivers. The specific objectives of the project were as follows:

- Understand and determine the state of the art concerning emerging organic pollutants in Durban and KwaZulu-Natal's water systems
- To determine levels of emerging organic pollutants in selected water bodies and changes in their concentrations from source to domestic outflow
- Development and validation of protocols for the quantification of emerging organic pollutants in surface water and waste samples

7.2 CONCLUSIONS

A GC-MS method using derivatisation was developed, optimized, and used to identify and quantify selected pharmaceutics, namely; salicylic, acetyl salicylic acid, nalidixic acid, ibuprofen, phenacetin, naproxen, meclofenamic, ketoprofen, and diclofenac in surface water samples along various points of the Umgeni River, and selected wastewater treatment plants. Sample preparation and derivatization methods developed were relatively fast and simple, while the GC-MS analyses of the derivatives was achieved in less than 13 minutes. Experimental optimization allowed for the identification of specific analyte fragments, optimum derivatization conditions and suitable adsorbent for accurate determination of analytes in the environment. As demonstrated in method validation for linearity, LOD, LOQ, precision and confirmation of sorbent, the developed method was sensitive and repeatable over established calibration ranges. The detection of selected acidic drugs in Inanda dam, a major source of potable water for the Durban municipality, provides a much needed initial measurement of such EOCs in the environment. The developed method will enable further studies for determination of pharmaceutical fate and long-term exposure in aqueous environment.

A simple multi-residue LC-MS method for the determination of eleven pharmaceutical drug residues in sediments, surface water and wastewater was developed. The method was developed for five antibiotics (sulfamethazine, sulfamethoxazole, erythromycin, metronidazole, and trimethoprim), three antipyretics (aspirin, ibuprofen and acetaminophen), one stimulant (caffeine), one anti-epileptic drug (carbamazepine) and one antipsychotic drug (clozapine). The pharmaceutical products varied widely from site to site, with no general trends in the distribution. In general, along the Msunduzi River system the sediment samples had a higher value than water samples except for sulfamethoxazole, erythromycin, clozapine, and sulfamethazine. While along the Umgeni River system the exceptions were aspirin, trimethoprim, erythromycin, sulfamethazine, and clozapine. The lack of comparable data for aspirin along the Msunduzi means no conclusive statement can be made about whether the selected pharmaceuticals follow a similar trend in both river systems.

The work with the polycyclic musks is on-going, with the key significant milestone being the development of an extraction method and analytical technique for the analysis of three selected musks from simulated spiked sediment samples and preliminary results from real sediment samples of the Umgeni and Msunduzi Rivers show significant contamination of these rivers with the selected musks.

This research study has developed a suitable method that can analyse 8 (out of a possible list of 209) PCB congeners and 13 OCPs in water and sediment samples from the Msunduzi and Umgeni Rivers. All 8 PCBs and 13 OCPs were detected in water and sediment samples and the levels were higher in the Msunduzi River than in the Umgeni River. The levels found for just these 8 PCBs and 13 OCPs in the Msunduzi River is much higher than areas in India, China, Norway and the USA. The sediment samples contained higher concentrations of PCBs and OCPs than the sediment pore water and surface water samples because most of these pollutants have high log K_{ow} values and prefer to partition to organic matter and thus less is dissolved in water. The PCB in highest concentration was identified as PCB180 which has a high log K_{ow} value which again confirms its partitioning to organic matter. It also has a large number of chlorine atoms which makes it more resistant to degradation.

DDT and its metabolites together with mirex and endrin were found in significant concentrations in the surface water, sediment and soil of the Msunduzi River. The DDT metabolites are thought to be as a result of their persistence from previous use, however, currently there is controlled spraying of DDT in some areas of South Africa to reduce and control the Malaria vector. Thus, trans-boundary effects may have contributed to the high levels of DDT metabolites observed. In general, the Msunduzi River was found to be more contaminated by the analytes investigated than the Umgeni River. The WWTPs are also possible sources of organic pollutants in the Msunduzi and Umgeni Rivers. The PCBs were found to be within the Canadian sediment guidelines whereas the OCPs were found in higher concentrations than the guidelines.

Thus, the results of this work have provided a good understanding of the presence of the selected emerging organic contaminants in two major river systems in KwaZulu-Natal which is a major source of water to a large part of the population. Appropriate analytical methods have been developed and validated for the quantification of selected emerging contaminants and the results show the concentrations of these emerging contaminants from the source to the mouth of the two rivers including dams and wastewater treatment plants along the river.

7.3 RECOMMENDATIONS AND FUTURE STUDIES

- Continue monitoring studies with the recommendations that eThekwini includes organic pollutants in its monitoring studies of water bodies.
- The tributaries of the Umgeni River should also be monitored to identify possible sources of pollution load.
- The metabolites of pesticides, PCBs, pharmaceuticals and personal care products, and musk ketones should also be analysed as most of these pollutants maybe broken down into other compounds in the environment or as it passes through the human body.
- The results from this study show that wastewater treatment plants are possible sources of these organic pollutants and it is therefore recommended that the wastewater treatment plants upgrade their processes to include the removal of organic pollutants.
- Future studies should also look at degrading or completely removing organic pollutants from the environment.

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