EMERGING AND PERSISTENT CONTAMINANTS/PATHOGENS: DEVELOPMENT OF EARLY WARNING SYSTEM AND MONITORING TOOLS

Report

to the Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

The importance of water quality worldwide, especially for sustainable socioeconomic development, cannot be overemphasised. For the southern African region, which has experienced drought for consecutive years, this scarcity is becoming a reality and water rationing attests to this enormous challenge. The erratic rainfall patterns, global warming and the global challenge of the continuous discharge of both chemical and pathogenic contaminants into water systems remains a threat that requires urgent intervention. Emerging contaminants are frequently detected in environmental waters worldwide, including drinking water, possibly because of the increased use of chemicals due to high levels of industrialisation. Among these are pharmaceuticals, personal care products, pesticides, industrial and manufacturing chemicals, hormones and pathogens. Pathogens encompass bacteria, viruses and protozoa, all of which are normally detected in water and have a detrimental effect on human health. Unlike chemical emerging pollutants, whose effects on humans at low concentrations are still to be established, most pathogenic contaminants are known to cause a wide range of waterborne diseases. The complexity of dealing with this water challenge is that new strains are being unveiled. In order to fully understand the effects of emerging pollutants, a holistic approach of investigating both pathogens and chemical contaminants from the same water bodies is essential.

Thus, it is of great importance that analytical methods for detecting these chemical compounds, as well as biological methods for detecting pathogens, are developed and validated, and also made easily available and adapted by laboratories nationally, regionally or on the continental in general.

RATIONALE

In South Africa, reports on the occurrence of emerging contaminants have highlighted the presence of steroids, analgesics, antiretrovirals (ARVs), antibiotics, antipyretics, non-steroid anti-inflammatory drugs (NSAIDs) and beta-blockers in different water systems. The development of analytical methods for these emerging contaminants is complex since this class of contaminants consists of a large group of compounds with diverse physicochemical properties. Compounded with this is the fact that they are present in very low concentrations, which require very sensitive detectors or very efficient sample pre-concentration methods that are capable of high enrichment factors. Moreover, what is detected in water systems is not only the parent compounds, but also their metabolites, degradation and transformation products. An approach to selecting analytical methods could be to employ top-range, state-of-the-art mass spectrometry platforms, such as liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), which have excellent sensitivity and could easily handle a multiclass mixture of emerging contaminants, even in low concentrations. Alternatively, affordable, simple high-pressure liquid chromatography (HPLC) methods could be chosen, especially for the majority of laboratories whose mandates are purely to monitor water quality. Both approaches are essential and might point to a need for collaboration between smaller laboratories and those equipped with state-of-the-art equipment that can deal with such diverse and complex compounds. The ideal and most desirable strategy would be to develop analytical methods that allow for several classes of compounds (parent, degradation, transformation products and metabolites) to be determined in a single method. This will provide water regulatory bodies with meaningful data that could be used to draw up monitoring protocols and control the quality of water.

OBJECTIVES AND AIMS

The objectives for this project are as follows:

- Make a case for selected analytical methods to detect and monitor emerging contaminants, persistent contaminants and pathogens
- Validate the selected methods that are not validated for adoption in South Africa
- Undertake a literature review on various detection or monitoring methods
- Undertake laboratory experiments with research progress and annual reports
- Determine the cost-benefit analysis for selected methods
- Organise a stakeholder engagement workshop, including researchers and government departments (Department of Water and Sanitation, Department of Health, Department of Environmental Affairs), on the findings

METHODOLOGY

The approach for this research was to first do an extensive and comprehensive review of the analytical methods (from sample preparation to instrumental analysis) that are available in the literature to guide the selection of methods used for detecting emerging contaminants. Since emerging contaminants exist in the environment as multiclass compounds, the single-class determinations were not the focus of the review; hence, the scope of this research was on methods reported in literature worldwide that could detect various classes of compounds in a mixture. As guided by the literature review and in view of the multiclass approach, the methods that were selected as capable and qualifying for emerging contaminants were liquid chromatography quadropole-quadropole time of flight mass spectrometry (LC-QqToF-MS), Orbitrap liquid chromatography high-resolution time of flight mass spectrometry (LC-HRT-MS) and gas chromatography x gas chromatography high-resolution time of flight mass spectrometry (GCxGC-HRT-MS) for other volatile compounds that are usually found in water systems together with emerging contaminants. For the Orbitrap high-resolution liquid chromatography mass spectrometer (LC-MS), two types of columns were investigated: the Waters X-bridge C18 column and the Restek Biphenyl column. This was viewed as important to give flexibility in the developed methods. Waters Oasis® hydrophilic lipophilic balance (HLB) solid phase extraction (SPE) cartridges were incorporated as a sample clean-up and/or pre-concentration method for all samples. Methods were developed using standards to prepare synthetic mixtures, optimised, validated and applied to wastewater collected from selected wastewater treatment plants (WWTPs). Samples from the surrounding rivers and streams were analysed using validated methods to evaluate the performance merits of the method and the impact of the effluent discharged into these rivers. Both targeted and non-targeted approaches were used in this study to understand the extent of water pollution.

Methods used for pathogen analysis were deoxyribonucleic acid (DNA) extraction and polymerase chain reaction (PCR), next-generation sequencing analysis and biomarker analysis. The methods were applied to similar water bodies where chemical analysis was conducted to understand the nature of the pollution holistically. The cost-benefit analysis focused on selected analytical methods for chemical contaminates. It should be noted that the project also developed methods for the removal of contaminants in wastewater using innovative approaches, although this was not part of the objectives. Therefore, details of the study are not included in this report. It is believed that it is not enough to only know the extent of the water contamination, but also to have strategies in place to remove contaminants where possible.

RESULTS AND DISCUSSION

According to the literature review, the bulk of the work worldwide for the multiclass analysis of emerging contaminants is accomplished using LC-MS/MS instruments with the Orbitrap, time of flight (ToF) and triple quadrupole (QqQ) mass analyser. Sample preparation is mainly by SPE, with Waters Oasis® HLB being the most popular sorbent, although Strata has also been used significantly by other researchers.

Using the Orbitrap high-resolution LC-MS/MS validated methods, 71 and 73 compounds were quantified in influent and effluent samples respectively. In river water, 60 and 63 compounds were found in quantifiable amounts in upstream and downstream river water samples respectively, with 42 of these compounds having more than 50% detection frequency in both types of samples. Most of the quantified compounds could be classified under 14 pharmaceutical groups, which included hormones, antibiotics, anti-inflammatories, anticonvulsants, cardiovascular agents, analgesics, anthelmintics, consumer product additives, bronchodilators, NSAIDS and ARVs, some of which are frequently detected compounds globally. These compounds were in concentration ranges of µg ℓ^{-1} to ng ℓ^{-1}. The antibiotics were the predominant group detected in wastewater samples, accounting for 28% of the quantified compounds. For river water samples, endocrine-disrupting hormones were detected with estradiol, estrone, estriol and diethylstilbestrol frequently detected. Estradiol was detected at the highest concentrations of ± 2.2 µg ℓ^{-1}. Paracetamol, ibuprofen, caffeine and sulphamethoxazole were detected at concentrations ranging from 0.059 to 4.14 µg ℓ^{-1}. Several compounds were frequently detected in all the samples, although at lower concentrations. These included NSAIDs (ketoprofen, naproxen and diclofenac) and ARVs (ritonavir and efavirenz). The fact that several compounds were detected in significant amounts in effluent samples confirms that water treatment plants are not designed to completely eliminate organic compounds, but only to reduce them to a certain extent. The emerging contaminants detected in quantifiable amounts in river water samples demonstrate the impact of wastewater effluent in other water bodies. An interesting observation in comparison to previous studies worldwide was that, in this study, ARVs that are usually not detected in Europe and Asia were detected in influent, effluent and river waters, including those that are not directly receiving wastewater. This could be attributed to the high Human Immunodeficiency Virus (HIV) burden in the nation.

Pearson correlation analysis was done to identify the relationship between the compounds and to determine the potential contaminant marker in the wastewater for further evaluation and monitoring purposes. In this study, carbamazepine from anticonvulsant agents, fluconazole from antimicrobial agents and ritonavir from antiviral agents showed good correlation with other compounds. However, other factors, such as consumption rate, frequent detection rate, degradation, and adsorption ability and spatio-temporal dynamics, have also been considered for determining the potential biomarker. For example, based on the frequent detection rate and the high concentration levels, it could also be assumed that caffeine, paraxanthine, ibuprofen, paracetamol, sulphamethoxazole, fluconazole and trimethoprim could be part of the list of biomarkers. Some of these compounds are, in fact, in agreement with the Pearson correlation analysis.

Overall, the results of the correlation analysis suggest that selective compounds from the identified groups can be proposed as anthropogenic tracers subject to their degradation ability and other intrinsic factors.

Non-targeted analysis allowed an average of 624 and 677 compounds to be identified based on accurate mass in influent and effluent samples, respectively. Using additional qualifications with isotopic patterns (with at least 50% isotopes seen), fragmentation patterns (at least one fragment seen) and the retention time of these numbers were reduced to less than 50% of the compounds that were identified using accurate mass alone.

For the pathogen analysis, the next-generation sequencing technology revealed that diverse bacterial communities were present in both influent and effluent samples, which is not possible in culture-dependent methods. *Proteobacteria* and *Firmicutes* were the two dominant phyla recorded across different wastewater samples. Significantly differential abundant operational taxonomic units (OTUs) showed that unique bacterial communities represent both influent and effluent samples. In this study, the canonical correspondence analysis (CCA) was used to identity the relationship between the antibiotics and the bacterial communities identified in the wastewater samples.

Results revealed that members of *Proteobacteria* had high resistance against sulphonamides such as sulphadimethoxine, sulphamonomethoxine, sulphadimethoxine and sulphabenzamide. Again, some of the fluoroquinolones, such as ciprofloxacin and enrofloxacin, had no effect on any of the bacterial members.

The process flow of a cost-benefit analysis that was applied in this case included identifying and listing alternatives, identifying costs and benefits, quantifying costs and benefits, discounting future streams of benefits and costs to calculate the net present value (NPV), as well as a sensitivity analysis. Five options were considered for analysis in which several assumptions were made. The most viable and favourable approach was the first option (do nothing, use available facilities that are currently available at no cost). This means that infrastructure cost is eliminated, and only human resources, consumables and the charges of the sample analysis are considered.

CONCLUSIONS

In conclusion, high-resolution LC- MS has demonstrated its ability as an invaluable analytical tool in the aquatic environmental analysis for both targeted and non-targeted compounds. Invaluable data was generated in this project showing the magnitude of emerging contaminants in our water systems (wastewater and river water). Most importantly, carbamazepine, fluconazole and ritonavir showed good correlation with other compounds and – together with caffeine, paraxanthine, ibuprofen, paracetamol, sulphamethoxazole, fluconazole and trimethoprim – are proposed as early warning biomarkers for contaminated water. In fact, the presence of ritonavir, efavirenz, sulphamethoxazole, fluconazole and trimethoprim are indicators of water systems contaminated with ARVs.

Proteobacteria had high resistance against some sulphonamides, Moreover, some of the fluoroquinolones had no effect on any of the bacterial members. Finally, emerging and opportunistic pathogens with possible antibiotic resistance were recorded.

Based on the cost-benefit analysis, it can be concluded that the first option (do nothing where the existing facilities and infrastructure are used at no cost) is the most beneficial option with a net benefit in excess of R13 million and a benefit cost ratio of above 1.5. Furthermore, even with the sensitivity analysis scenarios, which assumed more pessimistic costs and benefits, the first option results in a net benefit to the community.

RECOMMENDATIONS FOR FUTURE RESEARCH

- There is a need to expand the scope of the study to include several rivers that feed into drinking water treatment plants.
- The level and impact of emerging contaminants can be well understood by including sediments in the study.
- Available and emerging antibiotic-resistant genes in microbial communities present in wastewater treatment plants should be investigated.
- Other microbial communities such as fungi, viruses and protozoans should be investigated to identify the recurrent biomarkers and their toxigenic compounds.
- A systematic approach that simultaneously determines parent compounds, transformation products and degradation products is long overdue. The non-targeted analysis using high-resolution LC-MS affords such an opportunity. The identification of transformation products would lead to the possible synthesis of transformation products that could be used for toxicological studies. The toxicology of emerging contaminants and/or transformation products should be periodised as regulations and polices are written
- A water reference laboratory should be established in South Africa that would support the monitoring laboratories.
- Research should be promoted on new technologies for the removal of emerging contaminants from wastewater.

ACKNOWLEDGEMENTS

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AAS	Anabolic androgen steroid
AGC	Automatic gain control
Aids	Acquired immune deficiency syndrome
APCI	Atmospheric pressure chemical ionisation
API	Atmospheric pressure ionisation
APPI	Atmospheric pressure photoionisation
ARV	Antiretroviral
BAM	2,6-dichlorobenzamide
BPA	Bisphenol A
CAD	Charge Aerosol Detector
CCA	Canonical correspondence analysis
CBA	Cost-benefit analysis
CCL	Candidate contaminant list
CEC	Contaminants of emerging concern
CI	Chemical ionisation
DAD	Diode array detector
DBP	Dibutyl phthalate
DEHP	di-(2-ethylhexyl) phthalate
DEET	N,N′-diethyltoluamide
DEP	Diethyl phthalate
DMP	Dimethyl phthalate
DNA	Deoxyribonucleic acid
DWTP	Drinking water treatment plant
EC	Emerging contaminant
ECD	Electron capture detection
EDC	Endocrine disrupting compound
EI	Electron ionisation

ELISA	Enzyme-Linked Immunosorbent Assay
ENCI	Electron capture negative chemical ionisation
EPA	Environmental Protection Agency
EP	Emerging pollutants
ESI	Electrospray ionisation
FID	Flame ionisation detection
FLD	Fluorescence detector
FPSE	Fabric phase sorptive extraction
FTICR	Fourier transform ion cyclotron resonance
GC	Gas chromatograph(y)
GC-FID	Gas chromatography flame ionisation detector
GC-MS	Gas chromatography-mass spectrometry
GC-MS/MS	Gas chromatography-mass spectrometry/mass spectrometry
GCxGC	Gas chromatography x gas chromatography (two-dimensional)
GCxGC-HRT-MS	Gas chromatography x gas chromatography high resolution time of flight mass spectrometry
GCxGC-MS	Gas chromatography x gas chromatography-mass spectrometry
GCxGC-ToF-MS	Gas chromatography x gas chromatography time of flight mass spectrometry
HCD	High-energy collision dissociation
HESI	Heated electrospray ionisation
HIV	Human Immunodeficiency Virus
HLB	Hydrophilic lipophilic balance
HPLC	High-pressure liquid chromatography
HRMS	High-resolution mass spectrometry
IT	Injection time
KO	KEGG Orthology
KW	Kruskal-Wallis
LC	Liquid chromatograph(y)
LC-HRT-MS	Liquid chromatography high-resolution time of flight mass spectrometry

LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
LC-QqToF-MS	Liquid chromatography quadropole-quadropole time of flight-mass spectrometry
LC-UV/Vis-MS	Liquid chromatography-ultraviolet/visible-mass spectrometry
LDA	Linear discriminant analysis
LEfSE	Linear discriminant analysis effect size
LLE	Liquid liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LTQ	Quadrupole linear ion trap
LVI	Large volume injection
MAE	Microwave-assisted extraction
Mac	*Mycobacterium avium* complex
MALDI-ToF-MS	Matrix-assisted laser desorption ionisation-time of flight mass spectrometry
MCPA	2-methyl-4-chlorophenoxyacetic acid
MCX	Mixed-mode polymeric sorbent
MDL	Method detection limit
MHD	10,11-dihydro-10-hydroxy carbamazepine
MIP	Molecular imprinted polymers
MS	Mass spectrometry
MS/MS	Tandem mass spectometry
MTB	MALDI-ToF-MS biotyping
NCBI	National Centre for Biotechnology Information
NCE	Normalised collision energy
NPD	Nitrogen-phosphorus detection
NPV	Net present value
NRF	National Research Foundation
NSAID	Non-steroid anti-inflammatory drug
NSTI	Nearest Sequenced Taxon Index

OTU	Operational Taxonomic Unit
PAH	Polycyclic aromatic hydrocarbons
PBDEs	Polybrominated diphenyl ethers
PBS	Phosphate saline buffer
PCB	Polychlorinated biphenyls
PCoA	Principle Coordinate Analysis
PCPs	Personal care products
PCR	Polymerase chain reaction
PDMS	Polydimethylsiloxane
PFAS	Perfluorinated alkylated substance
PFE	Pressurised fluid extraction
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulphonic acid
PHWE	Pressurised hot water extraction
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
PLE	Pressurised liquid extraction
POP	Persistent organic pollutant
POTW	Publicly owned treatment works
PPCP	Pharmaceutical personal care product
PPHCP	Pharmaceutical personal health care product
Q-LIT	Quadrupole tripple linear
qPCR	Real-time polymerase chain reaction
QqQ	Tripple quadrupole
qRT-PCR	Real-time reverse transcriptase polymerase chain reaction
Q-ToF	Quadrupole time of flight
Q-ToF-MS	Quadrupole time of flight mass spectrometry
QTRAP	Hybrid tripple quadrupole mass spectrometer
rRNA	Ribosomal RNA
RSD	Relative standard deviation

SBSE	Stir-bar sorptive extraction
SIC	Single-ion chromatogram
SPE	Solid phase extraction
SPME	Solid-phase micro-extraction
SRA	Sequence Read Archive
TE	Tris-EDTA
ToF	Time of flight
UAE	
UCHIME	
UHP	Ultra-high pressure
UHPLC	Ultra-high-pressure liquid chromatography
UPGMA	
UPLC	Ultra-high-pressure liquid chromatography
UPLC-ESI-QToF	Ultra-high-pressure liquid chromatography electrospray ionisation quadrupole time of flight
UV	Ultra-violet
VWM	Vienna Water Monitoring Solutions
WHO	World Health Organisation
WWE	Wastewater effluent
WWTP	Wastewater treatment plant
WWTW	Wastewater treatment works

CHAPTER 1: INTRODUCTION

1.1   BACKGROUND

For many decades, most of the environmental research worldwide has focused on the presence of industrial or agricultural chemicals such as polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and dioxins. These compounds and others, termed persistent organic pollutants (POPs), are subject to regulation due to their toxicity and bioaccumulation (European Commission, 2008).

The increase in the development of new industrial, agricultural and pharmaceutical substances means that more and more unknown contaminants are potentially discharged into the environment, which could present a threat to human health and/or the environment. These pollutants are included in the group called contaminants of emerging concern (CECs), emerging contaminants (ECs) or emerging pollutants (EPs). The occurrence of organic pollutants in aquatic environmental systems such as wastewater, surface water, underground water and drinking water has become an important research topic over the past few decades. Emerging contaminants can be defined as any synthetic or naturally occurring chemical or chemicals that are persistent in the environment or any microorganism that is not commonly monitored in the environment, but has the potential to enter the environment and cause known or suspected adverse ecological and/or human health effects (Rodil et al., 2009). This group also includes previously unknown or unrecognised contaminants that have recently been identified as being present in the environment, but are not included in existing environmental regulations, as well as contaminants that are not currently routinely monitored, but are seen as posing a possible threat to human and animal health and/or that pose ecological risks. These contaminants might have detrimental effects if they find their way into water systems. It has thus become vital to understand their occurrence, fate and pathways in the environment for the development of meaningful monitoring protocols, especially in water targeted for human consumption.

The emerging contaminants (persistent contaminants and pathogens) include the following:

- Pharmaceuticals
- Pesticides (such as herbicides, insecticides and fungicides)
- Hormones (synthetic and naturally occurring)
- Endocrine disruptors
- Disinfection by-products
- Personal care products
- Industrial and manufacturing chemicals
- Recreational and non-controlled drugs
- Pathogens

The list of contaminants of emerging concern is extensive, and encompasses diverse groups of compounds, including pharmaceuticals and personal care products (PCPs), illicit drugs, steroids and hormones, endocrine-disrupting compounds, surfactants, perfluorinated compounds, phosphoric ester flame retardants, industrial additives and agents, siloxanes, artificial sweeteners and gasoline additives (Rodil et al., 2009). This list is not exhaustive and other classes, such as algal and cyanobacterial toxins and nanomaterials, have most recently been included as emerging pollutants. Pharmaceutical drugs include analgesics, antibiotics, contraceptives, lipid regulators, beta blockers and steroidal hormones. These organic compounds are part of the emerging organic pollutants that are now being detected in water systems, probably as a result of their increased use and/or the improvements that have taken place in analytical techniques. Information on the source and occurrence of these emerging contaminants seems to now be abundant. However, aspects that still require more research efforts are their toxicity, bioaccumulation, transportation, transformation and degradation mechanisms, which are crucial in evaluating their possible human health risks.

Another challenge is the large number of compounds that are involved and that make relevant monitoring very complex. As reported in the literature, of the 3,200 pharmaceutical drugs registered in Europe and North America, less than 10% has been detected in environmental samples (Howard and Muir, 2011). This means that 90% still requires further investigation. Many of the pharmaceutical personal care products (PPCPs) detected in waters around the world are common and are also registered in South Africa under the Medicines and Related Substances Act of 1965 (Republic of South Africa, 1965). Obviously, designing or developing protocols for monitoring a vast number of emerging pollutants requires informed decisions, especially within regions or nations.

An overwhelming number of pharmaceutical products and emerging pollutants, in general, and their diverse forms require well-formulated strategies and protocols for their detection and monitoring. Many countries have approached this challenge by prioritising the drugs and focusing on target analytes. There is no single guideline on how a priority list should be constructed, which means that various nations may present different types of priority lists as guided by their own environments.

Criteria that usually influence the selection of drugs in the priority list may thus vary from nation to nation. However, some of the following fundamental points are usually considered (Osunmakinde et al., 2012):

- Prescription volumes
- The toxicity of parent compounds, as well as their metabolites and transformation products
- Adverse health effects on both humans and animals (such as carcinogenicity, mutagenicity and endocrine disruption)
- The stability or persistence of the drugs in the environment
- The removal efficiencies of the drugs when treated using conventional water treatment systems
- Degradation and photolysis

The 50 most prescribed drugs, as guided by the private and public health prescription volumes in Gauteng, South Africa, have previously been suggested (Osunmakinde et al., 2012). According to this priority list, the most prevalent groups of pharmaceuticals from both the public and private health sector were analgesics, hypertension drugs, antihistamines, vitamins, ARVs, NSAIDs, antidiabetics and antibiotics. It is noted that the pharmaceutical residues that have been detected in South African water systems also fall within this suggested list of target compounds. A more generic protocol for prioritising compounds that could be used in the monitoring of organic pollutants in drinking was suggested by Ncube et al (2011).

In addition to the criteria listed above, the following were also considered:

- The potential of finding the compound in drinking water
- The availability of standards and guidelines for regulation
- The ease of monitoring in the drinking water value chain
- The potential of the contaminant to cause aesthetic water quality problems
- The potential to increase customer perception risk.

Again, in 2012, Ncube et al. prioritised organic contaminants into three classes: short term, medium term and long term (Ncube et al., 2012), with those falling in the short term requiring urgent attention. Their approach was quite comprehensive as it covers several criteria and was informed by consultations with various stakeholders who were critical in guiding the process of constructing such a list.

For the last two decades, many countries in the world have been working in this area. This is reflected by several publications on emerging contaminants or persistent contaminants or pathogens (Kasprzyk-Hordern et al., 2008a; Batt et al., 2008; Esesteban-Lor et al., 2011; Gracia-Lor et al., 2012; Alvarez et al., 2005).

In a previous report, the researchers highlighted several methods of identification and quantification of pharmaceutical personal health care products (PPHCPs) in the aquatic environment (Osunmakinde et al., 2012). Most importantly, common and affordable methods that could be easily utilised within the South African context were identified. The diversity of emerging contaminants, persistent contaminants or pathogens has added to the complexity of monitoring the analyte. Therefore, analytical methods are required that are selective and can detect analytes at very low concentrations. The tremendous progress made with respect to analytical techniques for trace levels enables the researchers to generate the required data.

Although the levels of many of these compounds in the environment are orders of magnitude below known acute toxicity levels, the health impact of long-term exposure at low levels is mostly unknown. The effects of repeated long-term exposure to low doses of emerging pollutants on human and animal health are still to be assessed. There is also the issue of the potential for increased toxicity due to the interaction of various PPCPs through synergistic effects (Jones et al., 2005). Comprehensive reviews on the risks of emerging contaminants have been published (Rizzo et al., 2013; Lei et al., 2015). Although many studies are not conclusive, the emerging contaminants presented suspected mutagenicity, teratogenicity and carcinogenicity to humans and other animals.

According to Hughes et al. (2013), more than 200 different pharmaceuticals alone have been reported in river waters globally, even after water treatment. This is mainly due to the combination of the limitations of existing conventional water treatment plants in the removal of these unidentified contaminants, as well as the ever-increasing list of new chemicals being introduced. Today, water quality is a critical issue, especially for sustainable socioeconomic development. The presence of emerging contaminants in water systems is consequently a cause for concern and calls for quality control action. Thus, monitoring and evaluating concentrations of contaminants is a topic of growing interest from both research and regulatory perspectives.

1.2 AIMS AND OBJECTIVES

The scope of this study was to develop a comprehensive analytical method for the determination of emerging contaminants in water and to identify early warning biomarkers for the contaminated aquatic environment.

The objectives for this project were to do the following:

- Make a case for selected analytical methods to detect and monitor emerging contaminants, persistent contaminants and pathogens
- Validate the selected methods that are not validated for adoption in South Africa
- Undertake a literature review on various detection or monitoring methods
- Undertake laboratory experiments with research progress and annual reports
- Determine the cost-benefit analysis for selected methods
- Organise a stakeholder engagement workshop, including researchers and government departments (Department of Water and Sanitation, Department of Health, Department of Environmental Affairs), on the findings

CHAPTER 2: SOURCES, OCCURRENCE AND FATE OF EMERGING CONTAMINANTS IN THE ENVIRONMENT

Emerging contaminants enter the environment, specifically water, through a variety of pathways that can be categorised as point source (municipal sewage, industrial wastewaters and landfill) and non-point source (agricultural run-off, wash-off from roadways and underground contamination). After use by humans and animals, many drugs are excreted without being metabolised by the patients and consequently enter wastewater through the sewage systems either in their parent or their metabolised form (Fatta-Kassinos et al., 2011). As expressed by some researchers (Petrović, et al., 2003; Bolong et al., 2009), most of current WWTPs are not designed to remove most of the emerging contaminants. Consequently, a high portion of emerging compounds and their metabolites can escape and enter the environment via sewage effluents. Thus, it is obvious that the development of more advanced technologies may be crucial to fulfil the requirements. However, that subject is beyond the scope of this review.

Other sources of pollutants in the environment are biosolids that are generated during water treatment procedures such as anaerobic digestion. Sludge can act as a concentrating medium for some chemicals during wastewater treatment and is often applied to agricultural land as a fertilizer without analysis for emerging contaminants as no legislation currently controls the use of biosolids on agricultural land with respect to the concentration of emerging contaminants (Diaz-Cruz et al., 2009). In one study, it was shown that, despite lengthy digestion (20 to 30 days) and outdoor storage for up to six months following treatment of WWTP sludge, some emerging contaminants were persistent (Isaacson et al., 2009).

2.1 ANALYTICAL APPROACHES FOR DETERMINING EMERGING POLLUTANTS

Current research strategies have been aimed at improving and developing new efficient analytical procedures for complex matrices that focus on advancements in both sample preparation and instrumental analysis. Work done in recent years has resulted in refined methods for various classes of emerging contaminants, simpler and faster sample preparation methods, as well as development of new multi residue analytical methods with lower detection limits. Detection at sub-parts per trillion (ng ℓ^{-1}) concentration levels is becoming routine for many organic analytes and methods that achieve the detection of a few hundred femtograms of some analytes have been reported (Petrović et al., 2010).

The analytical challenges of measuring emerging contaminants in the environment have been a major research focus of scientists for the last 20 years as there have been several complexities to overcome. Firstly, the detection of emerging contaminants in environmental matrices is at trace levels (µg/ℓ or even ng/ℓ), which creates a challenge for most affordable analytical instruments. This is often countered by using a pre-analysis sample clean-up and/or enrichment step, thus achieving higher recoveries, thereby minimising interferences and pre-concentrating analytes to detectable levels (Wu et al., 2010). Secondly, because emerging contaminants exhibit a broad range of activities, they have a diverse range of physiochemical characteristics; hence, the difficulty of identifying and quantifying a large number of compounds in a single analysis. This presents a great challenge as it would be quite expensive, labour intensive and time consuming to analyse groups of similar compounds at the same time in environmental monitoring. Therefore, today, in analytical chemistry, there is a clear trend to expand existing methods to enable the determination of multiple classes of compounds in one analysis (Gros et al., 2006a; Gómez et al., 2007; Gómez et al., 2009).

Publications related to multiclass residue analytical methodologies have increased over recent years as this has become the preferred approach for the environmental monitoring of pollutants (Kantiani et al., 2009; Richardson, 2009; Richardson, 2010; Richardson, 2011a; Diaz-Cruz et al., 2009; Isaacson et al., 2009; Comerton et al., 2009; Buchberger, 2011; Richardon and Ternes, 2011; Richardson, 2011b; Lebedev, 2013).

Among the reported techniques for identifying and quantitating emerging contaminants, gas chromatography-mass spectrometry (GC-MS), LC-MS, or tandem mass spectrometry (gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS), LC-MS/MS and gas chromatography x gas chromatography-mass spectrometry (GCxGC-MS) methods are at the forefront. Liquid chromatography and gas chromatography techniques, coupled with mass spectrometry, provide extremely powerful analytical tools by combining the intrinsic properties of the individual techniques. As such, the major research has been on the improvement of various facets of these analytical techniques for the eventual development of robust environmental monitoring systems.

One of the major challenges in the environmental analysis of emerging contaminants is that, due to the number of parent contaminants, a great number of metabolites, degradation and transformation products of unknown toxicity and persistence is expected to exist. It would be impossible to know and have standards for all the transformation products and metabolites, making their determination impossible using targeted approaches (Helbling et al., 2010). There are already some reviews that focus on the analysis of emerging contaminants, including their transformation products, with particular emphasis on LC-MS-based techniques, which describe the state-of-the-art instrumentation and highlight gaps and future needs (Hübner et al., 2015; Postigo and Barceló, 2015). One of the most notable advancements in analytical techniques with the potential to overcome this challenge has been the introduction of high-resolution mass spectrometry (HRMS). This technique has the potential to be used to determine an unlimited number of emerging contaminant compounds due to its sensitivity and accurate masses capability, without requiring the pre-selection of analytes or the need for reference standards.

2.1.1 Sample preparation techniques

Sample preparation plays a fundamental role in developing analytical methodology for the trace analysis of organic contaminants in complex and diverse environmental sample matrices. Sample preparation steps are labour-intensive and time-consuming components of an analytical process

Solid-phase extraction is still the most utilised technique for the extraction of liquid samples or for the purification and fractionation of raw extracts from solid samples (Vazquez-Roig et al., 2013). Among the different commercially available sorbents, Waters Oasis® HLB is the leader in multiclass clean-up and concentration from various waters (Loos et al., 2009; Ferrer et al., 2010; Ferrer and Thurman, 2012; Gurke et al., 2015). This is because the sorbent exhibits both hydrophilic and lipophilic retention characteristics, thus allowing for the simultaneous extraction and pre-treatment of analytes that have different polarities. However, there are still various classes of pharmaceuticals that cannot be enriched efficiently using multiclass SPE procedures. Much effort has been exerted recently to improve adsorbent materials, of which the most relevant are advanced materials such as molecularly imprinted polymers (MIPs) (Hoshina et al., 2009) and nanomaterials (Moliner-Martinez et al., 2014), which have been used for the selective extraction of compounds from water samples. The major drawbacks of SPE include the need to process relatively high sample volumes (100 to 5,000 mℓ) in order to achieve the sufficient limits of quantification (LOQs) that are required in most cases, as well as the relatively large volumes (typically 5 to 50 mℓ) of organic solvents that are needed to condition and elute the cartridges (Hu et al., 2011). The global mandate to lessen environmental pollution by reducing or avoiding the use of toxic organic solvents has led to substantial efforts to miniaturise existing sample preparation methods, resulting in micro-extraction techniques. Among these techniques are sorbent-based micro-extraction techniques, which have reached commercial status in several formats.

Some methods make use of automated sample preparation units, coupled with separation and detection systems. Online methods for the extraction, purification and/or concentration of compounds from samples have the advantages of reducing solvent consumption, having less exposure to hazardous solvents, and reducing sample manipulation and total analytical time (Valsecchi et al., 2015).

Online methods currently used in environmental analyses are based on SPE (López-Serna et al., 2010; Esteban et al., 2014) and solid phase micro-extraction (SPME) (Araujo et al., 2008). The first and, to date, most popular sorbent-based SPME method is fused silica. The commercial format of SPME consists of a fibre covered with a small amount of sorbent, which comes into contact with the sample containing the target analyte(s) for a specific time. It is then directly desorbed in the gas chromatograph (GC) for analysis. Its main limitations are the cost of commercial fibres and the low amount of sorbent that leads to high LOQ values in some cases. The SPME can be considered to be a fully solventless technique if combined with GC, while a combination with liquid chromatography (LC) has not been as successful as that of SPE using LC methods.

Stir-bar sorptive extraction (SBSE) was introduced to address some of the shortcomings of SPME, such as low extraction recoveries (Baltussen et al., 1999). The main advantage of SBSE is its speed, the minimal use of organic solvents and its applicability for multi-residue analysis as it significantly increases detection limits (Prieto et al., 2007). Like SPME, SBSE is commercially available and consists of a magnetic stirrer covered by the sorbent. A limitation is that only non-polar polydimethylsiloxane (PDMS)-coated bars are commercially available. Therefore, polar compounds are poorly extracted. Despite this, SBSE has shown an increasing demand for the analysis of micro-pollutants in water and has been successfully applied in pre-concentrations of PPCPs, PAHs and pesticides (Giordano et al., 2009; Sánchez-Avila et al., 2010), as well as some polar organic contaminants (Quintana et al., 2007).

Fabric phase sorptive extraction (FPSE), developed by Kabir and Furton (2014) is the newest, yet very promising member of the sorbent-based sorptive micro-extraction techniques. The method has excellent extraction sensitivity and improved speed of extraction. The technique incorporates a high volume of sol-gel hybrid inorganic-organic sorbents into permeable fabric substrates. It has been successfully applied for the extraction of oestrogens (Kumar et al., 2014) and NSAIDs (Racamonde et al., 2015) from water. Compared to other sorbent-based micro-extraction techniques, FPSE has several unique advantages, such as simplicity in device fabrication at low cost, high extraction efficiencies and field deployability.

2.1.2   Instrumental analysis

Although LC and GC have advanced individually as techniques, the most improvements achieved in terms of sensitivity are due to the development of hyphenated chromatography-mass spectrometry techniques and HRMS. Traditionally, less expensive detection techniques such as ultra-violet (UV), diode-array detection (DAD) and fluorescence detection (FLD) for LC (Kasprzyk-Hordern et al., 2008b; Benito-Peña et al., 2006; Garcia et al., 2009; Kim et al., 2013; Seifrtová et al., 2008; Wu and Hu, 2009; Zgoła-Grześkowiak, 2010), flame ionisation detection (FID), electron capture detection (ECD) and nitrogen-phosphorus detection (NPD) for GC (Es'haghi, 2009; García-López et al., 2008; Liu et al., 2009; Farhadi et al., 2009) have been utilised in the analysis of pharmaceuticals in aquatic environments. Generally, low sensitivities are obtained using these detection techniques, limiting their use when it comes to matrices that contain many organic contaminants such as pharmaceuticals (e.g. wastewaters). They are, however, still a low-cost technology that is suited for the environmental analysis of emerging contaminants where highly sophisticated and expensive technologies such as MS are not available. Regardless, the need for higher sensitivities in more complex environmental matrices has resulted in MS techniques being today's methods of choice for the determination of trace organic analytes in environmental samples. As such, advancements in LC and GC techniques, coupled with MS, are the focus of this discussion.

2.1.2.1   LC-MS analysis

The combination of LC and MS offers the possibility of taking advantage of both LC as a powerful and versatile separation technique and MS as a powerful, sensitive detection and identification technique.

The more recent developments of this analytical technique that have greatly benefitted environmental analysis include ultra-high-pressure liquid chromatography (UHPLC), tandem mass spectrometry (MS/MS), as well as updated MS source designs and improved detection. This has resulted in LC-MS/MS methods becoming the main regulatory methods of choice for emerging contaminant identification and quantification (Farhadi et al., 2009).

The UHPLC (or just UPLC) method uses chromatographic columns with a smaller particle size (under 2.0 µm) for the separation of analytes. The UPLC method has led to significantly improved separations of compounds in complex matrices by providing better resolution, increased peak height and a significant reduction in sample analysis time, as well as reduced mobile phase consumption when compared to traditional LC (Primel et al., 2012). Such improved resolution power is essential when dealing with a multitude of compounds in environmental samples and when a multi-residue approach is the preferred analytical method.

In MS, improvements in source design have made this method of analysis much more sophisticated and efficient. Atmospheric pressure ionisation (API) techniques have proved to be very useful in the analysis of a broad range of compounds, including non-volatile, thermally labile and polar species. As such, the interfaces most widely used for the LC-MS analysis today make use of API. Among the API sources, electrospray ionisation (ESI) is more suited for the analysis of polar compounds, while atmospheric pressure chemical ionisation (APCI) is highly effective in the analysis of medium- and low-polarity substances. While both ionisation techniques have been widely used for the analysis of molecules in environmental samples, ESI is by far the most commonly used (Martinez Bueno et al., 2007; Gros et al., 2008; López-Serna et al., 2010).

The ESI and APCI techniques, however, have some limitations in ionising certain classes of compounds, e.g. in the case of some steroids and non-polar compounds like PAHs. Adducts with common cations such as Na^+ and K^+ can also frequently be formed during ESI, leading to an increase in chemical background and/or reduction of analyte signals (Hanold et al., 2004). Another API technique, atmospheric pressure photoionisation (APPI), has the capability to ionise compounds with various polarities, while being remarkably tolerant of matrix additives (Cai et al., 2005; Viglino et al., 2008). It has thus proven to be a valuable alternative for analytes that are poorly or not ionised by ESI and APCI. In recent work, APPI with four different dopants (acetone, anisole, chlorobenzene and toluene), heated electrospray ionisation (HESI) and APCI were evaluated based on method detection limits (MDLs) and recoveries from different aqueous matrixes. Results indicated that APPI, using toluene as dopant, provided exceptional ionisation capabilities for a broad range of compounds for hormones and steroids compared to APCI and HESI (Wang and Gardinali, 2012). The use of APPI for the analysis of environmental samples has the potential to expand the detection and quantification of a wider range of compounds in a single study.

2.1.2.2   GC-MS analysis

The GC methods appear less attractive than the LC methods as they are limited to compounds that are volatile, thermally labile or that can easily be derivatised to become volatile without any by-products. A definite advantage to be considered is that matrix effects may be less serious for the ionisation modes like electron ionisation (EI) or chemical ionisation (CI) that are typically used for MS hyphenated with GC than for ionisation modes like ESI that are used in LC-MS. The GC procedures may therefore be robust routine methods for certain classes of pharmaceuticals and should not necessarily be replaced by HPLC in all cases.

The most notable improvement in GC has been the introduction of two-dimensional gas chromatography (GC×GC). Although the principles and the first system for comprehensive GC×GC were developed in the late 1980s (Phillips et al., 1985), over the years, its use in environmental analysis have expanded greatly (Herrero et al., 2009; Botitsi et al., 2011).

The main advantages offered by GC×GC systems when compared to conventional GC are fast run times, increased peak capacity, improved resolution and enhanced mass sensitivity (Banerjee et al., 2007). This allows for the separation of closely related compounds and/or the resolution of target compounds from impurities and interferences in environmental samples (Wang et al., 2010; Marriott et al., 2012). Such peak capacity and improved resolution make this technique very attractive and versatile for emerging contaminants, their metabolites and transformation compounds. Sample preparation protocols can often be minimised thanks to the high separation power thus afforded.

The GC×GC technique requires coupling to fast detectors and the availability of sophisticated and powerful software to obtain, evaluate and present the data collected. Time of flight MS with its high acquisition rates (up to 500 spectra per second) are often coupled to GC×GC instruments to enable the efficient analysis of extremely complex samples. It is possible to simultaneous determine possibly thousands of pollutants at low levels in a single analysis (Pani and Górecki, 2006; Cortes et al., 2009; Ieda et al., 2011). Recent studies have demonstrated the power of the gas chromatography x gas chromatography time of flight mass spectrometry (GC×GC-ToF-MS) technique for the separation of complex environmental mixtures, including PAHs and PCBs (Hashimoto et al., 2011; Muscalu et al., 2011; Matamoros et al., 2009), as well as pharmaceuticals and other organic contaminants (Matamoros et al., 2009) .

2.1.2.3   *High-resolution mass spectrometry*

The great benefit of MS, even in its early stages, has been its ability to identify and quantify many different analytes in one run. Mass analysers have further evolved over the decades with phenomenal improvements in sensitivity and selectivity for environmental trace analysis. The MS/MS methods have made analysis of many micro-pollutants in the environment samples possible at nanogram and even possibly picogram levels in routine analyses. Currently, multi methods are typically carried out using LC systems coupled to QqQ-MS. The QqQ-MS is exceptional for target analyte determination because of its high sensitivity and selectivity and comparatively low cost. However, QqQ has its limitation because it is intended for targeted acquisitions (i.e. only analytes included in the MS acquisition method will be detected), thus the number of analysed compounds is limited as reference standards are prerequisites for precise determinations. With the growing interest in the screening and quantification of this diverse group of pharmaceuticals, their metabolites, degradation and transformation products, and lack of reference standards for transformation products, in particular, present challenges. Screening and the identification of unknown compounds are quite impossible when using QqQ instruments (Kellmann et al., 2009). This has resulted in the need for instrumentation that is capable of determining known and unknown compounds (non-target methods) in environmental samples.

For non-target analysis, instruments must be able to generate enough information for the elucidation of residues, such as accurate mass, from which empirical formulae can be deduced. The HRMS instruments (e.g. ToF and Orbitrap instruments) provide high-quality information by combining sensitive full-spectrum data with high mass resolution and mass accuracy (Richardson and Ternes, 2011; Bletsou et al., 2015). Full-spectrum HRMS, such as ToF and Orbitrap instruments allow the investigation of both known and unknown compounds, including degradation, transformation and metabolism products (Chitescu et al., 2012; Diaz et al., 2011; Krauss et al., 2010; Müller et al., 2011). There is no prior need for reference standards when using full-spectrum HRMS instruments because the identification of compounds is based on accurate mass acquisition and fragmentation patterns Krauss et al., 2010). Previously, the most common HRMS instruments had resolving power of 10 to 20,000, while the newer technologies can reach values of 40,000 to 60,000 for quadrupole time of flight (Q-ToF), 100,000 to 1,000,000 for Orbitrap and up to 1,000,000 for Fourier transform ion cyclotron resonance (FTICR), with high mass accuracy (up to 2 ppm) and a sensitivity in the femtogram to picogram range (Krauss et al., 2010). This allows for enhanced selectivity when screening for molecular ions and their MS/MS fragments in complex matrices.

Several types of hybrid instruments are commercially available, which offer powerful combinations of mass detectors (e.g. Q-ToF, quadrupole tripple linear (Q-LIT) or quadrupole linear ion trap (LTQ) (Orbitrap), with Q-ToF being the most frequently employed instrument (Masiá et al., 2013).

2.2   APPLICATION OF LC-MS IN ENVIRONMENTAL SAMPLES

This section emphasises how the advancements in analytical techniques that were highlighted in the previous sections have impacted on the occurrence and monitoring studies of contaminants in various environmental matrices. These studies are obviously important as they provide water resource managers and environmental regulators with new and valuable knowledge for developing sound policies regarding the occurrence and distribution of emerging contaminants. Reports that have provided insights into the use of various techniques and/or monitoring strategies are discussed further below. Efforts have been made to avoid tedious sample pre-concentration techniques by performing a direct injection of the sample. Large volume injection (LVI) was combined with UPLC and HRMS in a suspect screening strategy (Vergeynst et al., 2014). The method was successfully used for the target quantification of the 69 multiclass pharmaceuticals in the analysis of river water samples. Results revealed the occurrence of 17 pharmaceuticals in a concentration range of 17 ng ℓ^{-1} to 3.1 µg ℓ^{-1}.

From several reported studies, it can be noted that there is mostly a strong link between surrounding major activities (e.g. industrial, urban or agricultural) in an area and the compounds found. The HPLC-MS/MS was used to study 73 multiclass pharmaceuticals by comparing their levels in the effluents of hospitals with those in the corresponding WWTPs (Verlicchi et al., 2012a). The analysis revealed nine substances that posed a high risk at the concentrations detected in the hospital effluents, with five of them exhibiting high ecotoxicity. Antibiotics were viewed as the compounds of most concern because the treatment plants showed poor removal of these compounds.

Another research team extracted PPCPs and perfluorinated alkylated substances (PFASs) in marine sediments using the United States Environmental Protection Agency (EPA) method 1694 (Long et al., 2013). The concentrations of both PPCPs and PFASs in sediments were mostly very low to non-detectable for most compounds. Fourteen of the 119 PPCPs and only three of the 13 PFASs were quantifiable in sediments. Diphenhydramine (an antihistamine) was most frequently detected with a maximum concentration of 4.81 ng/g dry weight. Triclocarban (an antibacterial) was detected in 35.0% of the samples with a maximum concentration of 16.6 ng g^{-1} dry weight. The PFASs were less frequently detected, with the highest concentrations in this group observed for perfluorooctane sulphonate (1.5 ng g^{-1}). It was noted that the detected concentrations were often highest within the industrial harbour in Bellingham Bay and near the cities of Seattle and Bremerton, USA.

Other researchers have extended studies from wastewater and river waters to less studied coastal and sea water. Jiang et al (2014) utilised SPE LC-MS/MS in detecting 13 emerging contaminants in coastal waters. The median concentrations for the 13 emerging contaminants detected ranged from 1.47 ng ℓ-1 to 156 ng ℓ-1 (Jiang et al., 2014). In another study, effluent from four large publicly owned treatment works (POTWs) and seawater collected near the respective POTW outfall discharges and a reference station were collected quarterly over one year and analysed for 56 CECs. Several CECs were detected in effluents, with naproxen, gemfibrozil, atenolol, and tris (1-chloro-2-propyl) phosphate most frequently detected and with the highest concentrations (more than 1 mg ℓ-1). Gemfibrozil and naproxen also had the highest seawater concentrations (0.0009 and 0.0007 mg ℓ-1) and were among the most frequently detected compounds (Vidal-Dorsch et al., 2012). Table 2.1 highlights numerous current studies that have been done in monitoring emerging contaminants in various parts of the world and the analytical methodologies used.

Table 2.1: **Studies on the determination and/or monitoring of emerging contaminants in various parts of the world**

Country	Matrix	Family of target substances	Sample pre-treatment, pre-concentration method	Instrumental method	Ionisation mode	Mass analyser	Number of compounds detected and concentration	Source
America, Canada and Latin America								
USA	Surface water sediments	54 multiclass (PPCPs, hormones)	SPE, Oasis® HLB	LC-MS/MS	ESI	QqQ	32 in surface water: 0.3-230 ng ℓ⁻¹ 30 in sediment: 3.9-350 ng g⁻¹	Blair et al., 2013
Canada	Stream and combined sewer overflow sediment samples	10 multiclass (NSAIDs, an anti-epileptic, beta blocker, stimulant, bronchodilator, steroid hormones, an artificial sweetener and PCPs)	SPE, Oasis® HLB	UHPLC-MS/MS	APCI		0.13-22 ng/g in stream bed sediment 98-427 ng g⁻¹ in combined sewer overflow sediment	Hajj-Mohamad et al., 2014
USA	Drinking water, ground water, surface water, and wastewater	100 multiclass pharmaceuticals and their degradants	SPE, Oasis® HLB	LC-MS/MS	ESI	Q-ToF	35, 21-455 ng ℓ⁻¹	Ferrer et al., 2010
USA	Hospital effluents, WWTP influents/effluents	Analgesics, cardiac drugs, antibacterials, antidiabetics, diuretics, antiepileptics, calcium channel blockers, antihistamines, anti-inflammatories, anti-thrombotics, lipid-modifying agents, disinfectants, nasal decongestants, steroid hormones, beta agonists, beta blockers, psycholeptics	-	LC/MS/MS-direct injection	ESI	QqQ	102-118 for hospital effluent, 324,000-965,000 ng ℓ⁻¹ 101-112 for WWTP influent, 259,000-573,000 ng/ℓ for WWTP influent 52-102 for WWTP effluent, 19,000-118,000 ng ℓ⁻¹	Oliveira et al., 2015
Spain	WWTP influent and effluent samples	100 multiclass (pharmaceuticals, personal care products, pesticides and metabolites)	Liquid liquid extraction (LLE) SPE	LC-MS/MS GC-MS	ESI EI	Hybrid tripple quadrupole mass spectrometer (QTRAP) Q-LIT Quadrupole	Influent: 90, 7-59,000 ng ℓ⁻¹ Effluent: 88, 5-32,000 ng ℓ⁻¹	Bueno et al., 2012

Spain	Wastewater, seawater, pore water and sediment	30 multiclass (betablockers, lipid regulators, fragrances, UV filters, phthalates)	Pressurised hot water extraction (PHWE) followed by SBSE	GC-MS	EI	Quadrupole	Wastewater influent: 6-10. 630 ng ℓ^{-1} Wastewater effluent: 6-1,600 ng ℓ^{-1} Seawater: 9-1,700 ng ℓ^{-1} Pure water: 50-10,000 ng ℓ^{-1} Sediment: 0.6-148 ng g^{-1}	Pintado-Herrera et al., 2013
USA	Wastewater, soil	Multiclass, pesticides, organohalogens	LLE UAE for soil	GC×GC-MS	EI	ToF	Phenol: 6.400-32.000 ng ℓ^{-1} in soil; 6,100-75,000 ng ℓ^{-1} in water	Prebihalo et al., 2015
USA	Lake water	19 multiclass PPCPs	SPE, Oasis® HLB	LC-MS/MS	ESI	QqQ	0.94-31 ng ℓ^{-1}	Ferguson et al., 2013
USA	Drinking water treatment plant (DWTP) waters	30 multiclass pharmaceuticals, PCPs endocrine-disrupting compounds (EDCs), herbicides	SPE, Oasis® HLB	LC-MS/MS	ESI	QTRAP	120-640 ng ℓ^{-1} in river, 180-700 ng ℓ^{-1} in reservoir, 90-470 ng ℓ^{-1} after flocculation/sedimentation, 60-170 ng ℓ^{-1} after ozonation, 15-180 ng ℓ^{-1} in drinking water	Jones et al., 2005
Spain	Wastewater, surface water, tap water, mineral water, river sediments	21 multiclass (PPCPs and illicit drugs)	SPE, Strata-X 33U	UHPLC-MS/MS	ESI	QqQ	WWTP influents: 20, 2.3-4374 ng ℓ^{-1} Effluents: 11, 11-127 ng ℓ^{-1} River water: 20, 1-830 ng ℓ^{-1} Tap water: 16, 2-39 ng ℓ^{-1} Mineral water: 19, 1-40 ng ℓ^{-1} River sediments: 19, 2-313 ng g^{-1}	Carmona et al., 2014

Europe								
Belgium	Drinking water and surface water	16 multiclass pharmaceuticals	-	LVI-UPLC – HRMS		Q-ToF	17 17-3,100 ng ℓ^{-1}	Vergeynst et al., 2014
England	Crude wastewater, final effluent and river water	90 multiclass including UV filters, parabens, plasticisers, steroid estrogens,antibacterials/antibiotics, hypertension drugs, NSAIDs, lipid regulators, anti-histamines, anaesthetics, anti-depressants, anti-epileptics, calcium channel blockers, hypnotics, anti-psychotics, analgesics, stimulants, opioids and metabolites	SPE, Oasis® HLB microwave-assisted extraction (MAE)-SPE, Oasis® mixed-mode polymeric sorbent (MCX)	UPLC-MS/MS	ESI	QqQ	Crude wastewater: 1.1-146 500 ng ℓ^{-1} Effluent water: 1.2-19 784 ng ℓ^{-1} River water: 7.3-2 318 ng ℓ^{-1}	Petrie et al., 2016
Croatia	WWTP influents and effluents, as well as in river water	29 multiclass (analgesics and NSAIDs, lipid regulators, psychiatric drugs, anti-histamines, anti-ulcer agent, antibiotics and beta blockers)	SPE	LC-MS-MS	ESI	QqQ	Surface waters: 17, <250 ng ℓ^{-1} Effluent wastewaters: 18, < 5,990 ng ℓ^{-1} Influent wastewaters: 18, 26,090 ng ℓ^{-1}	Gros et al., 2006b
Spain	Effluent wastewater and surface water	50 multiclass analgesics, anti-inflammatories, lipid regulators, antidepressants, anti-ulcer agents, psychiatric drugs, ansiolitics, cardiovasculars, antibiotics	SPE	UPLC-MS/MS	ESI	QqQ	Surface waters: 34;12-2,850 ng ℓ^{-1} Effluent wastewaters: 40; 11-201,000 ng ℓ^{-1}	Esesteban-Lor et al., 2011
Germany	STP influent and effluent	56 multiclass pharmaceuticals and metabolites	SPE, Abimed ASPEC XL with Oasis® HLB	LC-MS/MS	ESI	QqQ	41 (influent) <29,700 ng ℓ^{-1} 42 (effluent) < 22,700 ng ℓ^{-1}	Gurke et al., 2015
Spain	River water	10 natural and synthetic estrogens Two antimicrobials/disinfectants Four preservatives Bisphenol A (BPA) Eight alkylphenolic compounds and their metabolites Two anticorrosives Three organophosphorus-based flame retardants	Online-EQuan column Hypersil GOLD™	LC-MS/MS	ESI	QqQ	19, 2-5,928 ng ℓ^{-1}	Esteban et al., 2014
Switzerland	Influent, effluent, primary sludge and secondary sludge matrices	59 multiclass (toxaphenes, polychlorinated naphthalenes, organochlorine pesticides, PCBs, polybrominated diphenyl ethers (PBDEs))	SPE, Supelco C18 pressurised fluid extraction (PFE)	GC×GC GC×GC-MS	Electron capture negative chemical ionisation (ENCI)	µECD ToF	Influent water and particles: 0.5-40 ng ℓ^{-1} Effluent water and particles: 0.2-10 ng ℓ^{-1} Primary sludge: 0.9-400 ng g^{-1} Secondary sludge: 0.6-600 ng g^{-1}	Dimitriou-Christidis et al. 2015

Switzerland	Groundwater, surface water, and wastewater	88 multiclass (pesticides, biocides, pharmaceuticals, corrosion inhibitors and some transformation products)	Online-SPE-mixed bed multilayer-Oasis® HLB, Strata-X-AW Strata-X-CW, Isolute ENV+	LC-MS	ESI	QqQ	36; 0.1-600 ng ℓ^{-1}	Huntscha et al., 2012
European Union: Austria, Belgium, Czech Republic, Cyprus, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, The Netherlands, Portugal, Slovenia, Spain, Sweden, Switzerland	WWTP effluents	152 multiclass (PPCPs, veterinary antibiotics, PFASs, organophosphate ester flame retardants, pesticides (and some metabolites), benzotriazoles, iodinated X-ray contrast agents, and gadolinium magnetic resonance imaging agents)	LLE, SPE	LC-MS-MS GC-HRMS	ESI	QqQ	125, 0.1 ng ℓ^{-1} -76,000 ng ℓ^{-1}	Loos et al., 2013
Switzerland	Wastewater and surface water	Multiclass (biocides, pesticides, and pharmaceuticals)	Online SPE	LC-MS/MS	ESI	QqQ	Biocides and pesticides: 10-1,010 ng ℓ^{-1} Pharmaceuticals: 50-1,450 ng ℓ^{-1}	Singer et al., 2010
Sweden	Infiltration ponds, raw water and drinking water	22 (anatoxins, cylindrospermopsins and microcystins)		UPLC-MS-MS	ESI	QqQ	Surface water: 520-660,000 ng ℓ^{-1} Infiltration ponds: 690-1,780 ng ℓ^{-1}	Pekar et al., 2016
Greece	WWTPs influents and effluents	18 multiclass PPCPs	Oasis® HLB	Liquid chromatography-ultraviolet/ visible mass spectrometry (LC-UV/Vis-MS) LC-HRMS	ESI	Orbitrap	WWTP influents: 20, 2.3-4,374 ng ℓ^{-1} Effluents: 11, 11-127 ng ℓ^{-1}	Kosma et al., 2014

Romania	River water	67 multiclass (sulphonamides, quinolones, fluoroquinolines, antihelminthics, benzimidazole, macrolides, NSAIDs, azole antifungal, conazole fungicides, triazole antifungals)	SPE-Strata-X	LC-HRMS	HESI	Orbitrap	23; 2-166 ng ℓ^{-1}	Chitescu et al., 2015
Switzerland	Sediments	180 multiclass (PPCPs, pesticides, biocides, additives, corrosion inhibitors, musk fragrances, UV light stabilisers, and industrial chemicals)	Pressurised liquid extraction (PLE) LLE	LC-HRMS	ESI APPI	Orbitrap		Chiaia-Hernandez et al., 2012
Finland	Wastewater effluent samples	84 pesticides and pharmaceuticals	SPE-Oasis® MCX, Strata-X	UPLC-MS	ESI	TOF	11, 39-2 200 ng ℓ^{-1}	Nurmi and Pellinen, 2011
Spain	River waters and wastewater effluent (WWE)	47 multiclass pharmaceuticals (analgesic and anti-inflammatories, cholesterol-lowering statin drugs and lipid regulators, antidepressants, antiulcer agents, psychiatric drugs, ansiolitics, cardiovasculars, antibiotics)	SPE-Oasis® HLB	UPLC–MS/MS	ESI	QqQ	River water: 31, 12 ng ℓ^{-1} – 2,580 ng ℓ^{-1} WWE: 37, 9-201,000 ng ℓ^{-1}	Gracia-Lor et al., 2012
Asia and Australia								
Australia	Estuary waters	Multiclass (PPCPs, a food additive and pesticides)	SPE, Strata-X 33	LC-MS/MS	ESI	Qtrap	16; 1.1-55 ng ℓ^{-1}	Birch et al., 2015
Australia	STP influents and effluents	11 PPCPs	SPE, Oasis® HLB	LC-MS/MS, GC-MS/MS	ESI	QqQ	Influents: 2.1-3,544 ng ℓ^{-1} Effluents: 4-1-760 ng ℓ^{-1}	Roberts et al., 2016
China	WWTPs influents, effluents and sludge	48 PPCPs	SPE	LC-MS/MS	ESI	QqQ	Influents: 0.7-5,850 ng ℓ^{-1} Effluents: 0.8-702 ng ℓ^{-1} Sludge: 8.2-4,020 ng ℓ^{-1}	Sun et al., 2016

There have also been many reports targeting metabolites, degradation and the transformation products of emerging contaminants. Reemtsma et al. (2013) developed a method to determine 150 pesticide metabolites in surface water and groundwater using direct injection LC-MS. This method provided important information of previously unknown or undetectable metabolites of multiclass pesticides in the environment; 142 of the metabolites were quantifiable at 0.1 µg ℓ^{-1} or below. Michael et al. (2012) developed a UPLC-ESI-QToF for the identification of the transformation products of the solar photocatalytic oxidation of trimethoprim. More than 20 compounds were detected, and their structures were tentatively assigned through accurate mass measurements and fragmentation patterns via MS/MS.

The more regular and improved study of degradation and/or transformation products has provided some valuable insights into environmental pollutants. In a study to determine 56 pharmaceuticals and metabolites in different sewage samples using LC-MS/MS, it was noted that the metabolite-like 10, 11-dihydro-10-hydroxy carbamazepine (MHD) was found in higher mass loads than its corresponding parent compound in the sewage samples (Gurke et al., 2015). Some metabolites and their parent compounds also behaved differently in the sewage treatment process. While MHD was detected with a lower mass load in the effluent than in the influent, oxcarbazepine showed the contrary pattern.

Gómez et al. (2010) identified transformation products of acetaminophen (p-aminophenol) and azithromycin (unnamed compound) by non-targeted screening using Q-ToF-MS. The key result in the study was that transformation products can be more toxic than the parent compound as is the case with p-aminophenol (Gómez et al., 2010). This highlights the fact that the removal of the parent emerging contaminant does not necessarily translate into the removal of toxicity environmental concerns, particularly if they are biologically active. In a more recent study, the fate of steroidal compounds in WWTP processes was evaluated by a non-targeted approach based on GCxGC-ToF-MS (Kopperi et al., 2016). In addition to the wide variety of steroidal compounds, many transformation products were tentatively identified, and it would be beneficial to consider them in studies where the fate of steroids is evaluated.

Other significant research contributions are on expanded monitoring processes in an attempt to have a better understanding of spatial and temporal trends of emerging contaminant occurrence. Ferguson et al. (2013) conducted a study to quantify the spatial and temporal variation of PPCP concentrations in near-shore habitats of Lake Michigan, as well as to identify factors related to and influencing concentrations. Sample extraction was performed using Waters Oasis® HLB cartridges and detection and quantification using LC-MS/MS. Their findings indicated that sampling date and location, and not sample depth, influenced concentrations of the compounds. Sulphamethoxazole was found to have significant seasonal variation (Ferguson et al., 2013).

Guerra et al. (2014) investigated 62 antibiotics, analgesic/anti-inflammatory and antifungal compounds using an LC-MS/MS method equipped with a QqQ-MS. The study compared five different WWTP processes: facultative and aerated lagoons, chemically enhanced primary treatment, secondary activated sludge and advanced biological nutrient removal. The PPCPs were found in all final effluents at median levels ranging from 3.6 to 4,200 ng ℓ^{-1} with higher values detected during winter. Removal efficiencies ranged between 45 and 120%, depending on the compound, WWTP type and season. It was also shown that the fate of analgesic/anti-inflammatory compounds was predominantly biodegradation during biological treatment, while antibiotics and antifungal compounds were more likely to sorb to sludge. However, some PPCPs remained soluble and were detected in effluent samples. Overall, this study highlighted the occurrence and behaviour of a large set of PPCPs and determined how their removal was affected by environmental or operational factors in different WWTPs (Guerra et al., 2014). Blair et al. (2013) studied the trends of 54 PPCPs and hormones over a two-year period from surface water and sediment samples. Some 32 PPCPs were detected in Lake Michigan and 30 were detected in the sediment, with numerous PPCPs being detected up to 3.2 km away from the shoreline. The most frequently detected PPCPs in Lake Michigan were metformin, caffeine, sulphamethoxazole, and triclosan.

In a European Union-wide monitoring survey on emerging polar organic contaminants in WWTP effluents, 156 chemicals were measured, and four different toxicity assays were conducted on selected samples (Loos et al., 2013). The obtained results showed the presence of 125 substances (80% of the target compounds) in European wastewater effluents in concentrations ranging from low nanograms to milligrams per litre. The most relevant compounds in the effluent waters with the highest median concentration levels were the artificial sweeteners acesulphame and sucralose, benzotriazoles, several organophosphate ester flame retardants and plasticisers, pharmaceutical compounds such as carbamazepine, tramadol, telmisartan, venlafaxine, irbesartan, fluconazole, oxazepam, fexofenadine, diclofenac, citalopram, codeine, bisoprolol, eprosartan, antibiotics (trimethoprim, ciprofloxacine, sulphamethoxazole, and clindamycine), the insect repellent, N,N′-diethyltoluamide (DEET), the pesticides 2-methyl-4-chlorophenoxyacetic acid (MCPA) and mecoprop, perfluoroalkyl substances (such as perfluorooctanesulphonic acid (PFOS) and perfluorooctanoic acid (PFOA), caffeine and gadolinium.

Robles-Molina et al. (2014) carried out an extensive survey to monitor 373 compounds belonging to priority organic substances (regulated by the EU Directive 2008/105/EC) and pollutants of emerging concern (not yet regulated). The most frequently detected priority substances were chlorpyrifos ethyl, diuron and hexachlorobenzene. Within the other groups, the most frequently detected compounds were terbuthylazine, oxyfluorfen, desethyl terbuthylazine, diphenylamine (pesticide family), fluorene, phenanthrene, pyrene (PAHs group), codeine, paracetamol (pharmaceuticals compounds), caffeine and nicotine (lifestyle compounds).

The examples above are just some of the highlights of how the selected analytical methodologies in this review have made inroads into the environmental monitoring and assessment of emerging contaminants. In summary, most of the work makes use of both LC-MS, LC-MS/MS and GC-MS techniques, and HRMS methods are finding more application. This trend is expected to continue. Generally, for both river and wastewaters spanning across studies all over the world, the most frequently detected compounds belong to the PPCPs, more specifically anti-inflammatories, analgesics and antibiotics. These include acetaminophen, carbamazepine, trimethoprim, ibuprofen, triclosan, caffeine and sulphamethoxazole, which have been detected in numerous studies (Gros et al., 2006b; Gracia-Lor et al., 2012; Petrie et al., 2016; Chitescu et al., 2015; Stuart et al., 2011).

2.3   THE CURRENT STATE IN AFRICA AND SOUTH AFRICA

As seen from the previous section, environmental monitoring studies have mostly been conducted in developed countries around the world. The occurrence of emerging contaminants in developing countries, particularly in Africa, is largely unknown, although problems regarding water quantity and quality are often even more severe than in more developed regions. Of the few studies reported in literature, even fewer involved monitoring studies.

In one of the occurrence studies, researchers in Kenya developed a multi-residue analytical method that provided the first data on the environmental occurrence of human pharmaceuticals in Africa (K'oreje et al., 2012). Based on pharmaceutical consumption data available for the Nairobi region, the study focused on 43 "priority" active pharmaceutical compounds. The analytical methodology included SPE using Waters Oasis® HLB and HPLC, coupled with a double-focusing magnetic sector HRMS. The detected compounds belong to different classes, i.e. antibiotics, analgesic/anti-inflammatories and anti-epileptic drugs, antimalarials and ARVs. Ibuprofen, paracetamol, sulphamethoxazole and zidovudine had the highest concentrations (10-30 µg ℓ^{-1}). Among the antibiotics and antimalarials, trimethoprim and sulphamethoxazole were the most abundant with indicative concentrations up to 5 and 20 µg ℓ^{-1}, respectively. According to the authors, the detected levels for the ARVs lamivudine, zidovudine and nevirapine were significantly higher than those reported in the literature from other parts of the world.

They attributed this to the high prevalence of specific diseases like the HIV/Acquired immune deficiency syndrome (Aids) infection in developing countries, which may possibly present different trends in the occurrence of emerging contaminants in the environment to the more developed regions.

The same sentiments were expressed in the work of Sorensen et al. (2015) in their study to determine the occurrence and seasonal variations of a broad range of emerging contaminants (n > 1,000) in urban and periurban settings in Kabwe, Zambia, using a multi-residue GC-MS method. Their results showed that there was a general absence of personal care products, lifestyle compounds and pharmaceuticals, which are commonly detected in the aquatic environment in the developed world. The absence of these compounds could possibly be due to unaffordability and unavailability. The highest detection frequencies were within the classes of antibiotics and ARVs with >1 mg/ℓ of the ARV nevirapine detected in shallow wells used as drinking water. A total of 27 organic compounds were identified in groundwater samples, with the most prevalent compound being the insecticide DEET in both seasons. Results showed that the insecticide DEET was prevalent in groundwater at concentrations up to 1.8 mg ℓ^{-1}. Other compounds with notable concentrations included triclosan (up to 0.03 mg ℓ^{-1}), trihalomethanes (up to 50 mg ℓ^{-1}) and the surfactant 2,4,7,9-tetramethyl-5-decyne-4,7-diol (up to 0.6 mg ℓ^{-1}). Emerging contaminants were most prevalent in shallow wells situated in low-cost housing areas. It was suggested that this was a result of poor sanitation and household waste disposal, as well as a lack of structures to seal off wells properly. The onset of seasonal rains resulted in a five-fold increase in median DEET concentration. Five other insecticides that were absent in the dry season were detected during the wet season at concentrations up to 0.31 mg ℓ^{-1}. Three herbicides were detected, in addition to the dichlobenil metabolite 2,6-dichlorobenzamide (BAM), with atrazine found at the highest concentration.

K'oreje et al. (2016) also conducted a survey on concentrations and loads of 24 pharmaceuticals including antibiotics, ARVs, analgesics, anti-inflammatories and psychiatric drugs in three WWTPs, three rivers and three groundwater wells in Nairobi and Kisumu, Kenya. The samples were pre-treated using SPE, followed by analysis using an HPLC coupled to a magnetic sector HRMS. Overall, the most frequently detected compounds were ARVs (nevirapine and zidovudine) and antibiotics (metronidazole, sulphamethoxazole and trimethoprim). High concentrations, with values up to 160 mg ℓ^{-1} for compounds like paracetamol (wastewater) and lamivudine (river water) were detected. It was determined that, at some locations, the total measured river water concentrations (up to 320 mg ℓ^{-1}) were similar to or even higher than in untreated wastewater.

Many studies in South Africa have mostly focused on investigating the occurrence of a single class of compounds in the environment using less sophisticated analytical methodologies (Table 2.2). Some studies have successfully determined multiclass contaminants using lower resolution detectors. Agunbiade and Moodley (2014) investigated the determination of the occurrence of nine antibiotics, five antipyretics, atenolol, bezafibrate and caffeine in wastewater and surface water samples from the mNgeni River. Quantification was done using HPLC-DAD after the compounds were extracted from water samples using Waters Oasis® HLB and C-18 cartridges for the acidic and neutral drugs, respectively. With the increased accessibility of hyphenated instruments, more multi-class studies have been done in the last few years.

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Table 2.2: **Studies on the determination of contaminants on the environmental matrices done in South Africa**

Matrix	Family of target substances	Sample pre-treatment, pre-concentration method	Instrumental method	Number. of compounds detected and concentration	Source
Wastewater	Steroid hormones and EDCs, 17-β-estradiol, estrone, estriol, 17-α-ethinylestradiol, testosterone and progesterone	C18 SPE	Enzyme-linked immunosorbent assay (ELISA)	Progesterone: 408 ng ℓ^{-1} Testosterone: 343 ng ℓ^{-1} 17-β-estradiol: 119 ng ℓ^{-1} Estrone: 84 ng ℓ^{-1} 17-α-ethinylestradiol: 30 ng ℓ^{-1} Estriol: 5 ng ℓ^{-1}	Manickum and John, 2014
Treated sewage effluent	Steroid hormones, 17-β-estradiol, estrone, estriol	C18 SPE	ELISA	Estradiol: 0.8-4.7 ng ℓ^{-1}, Estrone: 7.2 -10.6 ng ℓ^{-1}, Estriol: <1.1 ng ℓ^{-1}	Swart and Pool, 2007
Soil	Phthalate esters (dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and diethyl hexyl phthalate (DEHP), metals (lead, cadmium, manganese, zinc, iron and calcium) and flame retardants)	Soxhlet extraction, silica gel clean-up	Gas chromatography flame ionisation detector (GC-FID)	Phthalates: 0.030-0.310 ng g^{-1} Metals: 0.070-11,620 ng g^{-1}	Adeniyi et al., 2008
River water	DMP, DEP, DBP and DEHP.	LLE	GC-FID	DEP: 160,000-4,040 000 ng ℓ^{-1} DBP: 3,080,000-10,170,000 ng ℓ^{-1} DEHP: 330,000-2,780,000 ng ℓ^{-1}	Fatoki et al., 2010
River and marine water	Phthalate esters (DMP, DEP, DBP and DEHP)	SPE-C18	GC-FID	30-2,306,000 ng ℓ^{-1}.	Fatoki and Noma, 2002
Sewage influent and effluent	17-β-estradiol, estrone		ELISA	Estrone: ± 28 ng ℓ^{-1} Effluent: -±10 ng ℓ^{-1} River upstream: ± 4 ng ℓ^{-1} River downstream: ±3 ng ℓ^{-1} Estradiol: ±37 ng ℓ^{-1} Effluent:11 ng ℓ^{-1} River upstream: 5 ng ℓ^{-1} River downstream: ± 4 ng ℓ^{-1}	Manickum et al., 2012
Raw water, secondary effluent, final effluent sewage sludge	PBDE congeners (BDE congeners 28, 47, 99 100 153 154 183, and 209) and BB-153	LLE, silica gel column clean-up	GC-ECD	PBDEs; 369-4,370 ng ℓ^{-1} for raw water, 19.2-2,640 ng ℓ^{-1} for secondary effluent, and 90.4 -15 100 ng ℓ^{-1} for final effluent, 13.1-652 ng g^{-1} for sewage sludge BB:153; ND to 18.4 ng ℓ^{-1} for effluents ND to 9.97 ng g^{-1} for sewage sludge	Daso et al., 2012

Matrix	Family of target substances	Sample pre-treatment, pre-concentration method	Instrumental method	Number. of compounds detected and concentration	Source
Landfill leachates	PBDEs (BDE -28, -47, -66, -71, -75, -77, -85, -99, -100, -119, -138, -153, -154, and -183)	LLE, silica gel column clean-up	GC-ECD	ND: 9.793 ng ℓ^{-1}	Odusanya et al., 2009
Wastewater and sludge	Antiretrovirals, nevirapine and efavirenz		GC-HRT-MS	5,500-14,000 ng ℓ^{-1} efavirenz in influents; 17 and 43 mg kg^{-1} in sludge	Schoeman et al., 2017

Matongo et al. (2015a) studied selected pharmaceuticals, including antibiotics, antipyretics, a stimulant, an antiepileptic and an antipsychotic drug in wastewater, surface water and sediments. Separation and quantification of the compounds was achieved using HPLC-MS/MS after clean-up and pre-concentration using Waters Oasis® HLB SPE cartridges. Ultrasonic assisted liquid extraction was utilised to extract the compounds from sediments. Results showed that ibuprofen (an antipyretic) and clozapine (an antipsychotic) were the most abundant, with concentrations as high as 62 and 78.33 µg ℓ^{-1} respectively. Lower average concentrations (<10 µg ℓ^{-1} or 10 ng g^{-1}) were observed for the other compounds (sulphamethazine, sulphamethoxazole, erythromycin, metronidazole, trimethoprim, acetaminophen, caffeine and carbamazepine). Significant concentrations of caffeine (2243.52 ng g^{-1}) and sulphamethoxazole (507.34 ng g^{-1}) were detected in sediment samples. The antibiotic metronidazole was only detected in the sediment samples. The authors also carried out a similar study on a different river system and wastewater treatment plant (Matongo et al., 2015b). Similar to their earlier results, ibuprofen had significant concentrations and had the highest concentrations in wastewater (117 µg ℓ^{-1}), surface water (84.60 µg ℓ^{-1}) and sediment (659 ng g^{-1}). Metronidazole was again only detected in the sediments with concentrations of up to 1253.50 ng g^{-1}. Previously, in our research using an HPLC-Charge Aerosol Detector (CAD), we reported the occurrence on ribavirin (19.60 µg ℓ^{-1} influent and 0.042 µg ℓ^{-1} effluent) and famciclovir (19.00 µg ℓ^{-1} influent and 0.055 µg ℓ^{-1} effluent) in wastewater (Osunmakinde et al., 2012).

Madikizela and Chimuka (2016), Madikizela and Chimuka (2017) and Madikizela et al. (2018) have studied the occurrence of NSAIDs in river waters using different sample preparation techniques. Wood et al. (2015) have developed a UPLC-MS/MS method for the countrywide monitoring of 12 ARVs (zalcitabine, tenofovir, abacavir, efavirenz, lamivudine, didanosine, stavudine, zidovudine, nevirapine, indinavir, ritonavir and lopinavir) in surface water. Sample clean-up and pre-concentration was achieved by SPE using Waters Oasis® HLB. They reported that matrix effects were substantial in the samples, achieving a method detection limit of 90.4 ng ℓ^{-1}. The average concentrations for the detected compounds ranged between 26.5 and 430 ng ℓ^{-1}.

In an extensive monitoring study, Odendaal et al. (2015) presented data on a survey of potential CECs in the drinking water of major South African cities. The study was conducted over four seasons and included approximately 700 multiclass compounds. The HPLC system used in the work was coupled to a QTRAP hybrid QqQ-MS. A qualitative analysis identified 29 potential CECs. Quantification was done for atrazine, terbuthylzine and carbamazepine, which were detected in more than 60% of the drinking water samples. However, the concentration levels of these CECs were lower than the maximum levels proposed by the World Health Organization (WHO) and the United States EPA. The study also revealed seasonal variation for some compounds, e.g. atrazine was higher in summer when it is used as a herbicide for summer crops. However, other herbicides, such as terbuthiuron and terbuthylazine, were consistently present in drinking water throughout the year.

Archer et al. (2017) conducted a study on PPCPs, illicit drugs and EDCs in WWTP influent and effluent, together with river water. Their results showed 55 emerging contaminants in the influent surface water of wastewater treatment works (WWTW), 41 emerging contaminants in effluent, and 40 emerging contaminants in environmental waters located upstream and downstream of the plant. A recent review (Madikizela et al., 2017) still highlights that significantly less work has been done in Africa and South Africa on assessing multiclass emerging contaminants in the environment compared to other countries in the world. It is, however, important to highlight that some regulatory organisations have recognised the importance of evaluating and monitoring emerging contaminants in the environment and have engaged researchers in the development of analysis and monitoring strategies (Osunmakinde et al., 2012).

The review of instrumentation used in studies conducted over the past five years reveals that there is an increase in the use of MS detectors with some researchers, even using HRMS instruments.

2.4   CONCLUSIONS

The review of work done in other parts of the world reveals that there is a need to expand the studies on emerging contaminants in Africa, including South Africa. While several examples of extensive work on multiclass emerging contaminant analysis has been done in other continents, Africa still lags behind in this research space. Only a handful of papers demonstrate the use of high-resolution equipment (LC-MS/MS) for the determination of a mixture of different classes of emerging contaminants. Most of the work reported focuses on single-class methods, which use low-end instruments (e.g. HPLC-DAD, GC-MS and low-resolution LC-MS). Therefore, there is a need to develop LC-MS/MS methods that can be validated and adopted by several monitoring laboratories.

The data obtained in South Africa reveals the presence of ARVs in addition to the commonly detected pharmaceutical compounds such as carbamazepine, fluconazole, oxazepam, fexofenadine, diclofenac, citalopram, codeine, bisoprolol, eprosartan, antibiotics (trimethoprim, ciprofloxacin, sulphamethoxazole and clindamycin), the insect repellent DEET, the pesticides MCPA and mecoprop, perfluoroalkyl substances (such as PFOS and PFOA), caffeine and gadolinium.

CHAPTER 3: ANALYTICAL METHODOLOGIES FOR THE DETERMINATION OF CHEMICALS

3.1 INTRODUCTION

The United States EPA has developed selective and sensitive methods as a guide for chemists to detect and quantify pollutants in various environmental samples. For example, EPA method 1694 refers to the determination of PPCPs in water, soil, sediment, and biosolids by HPLC-MS/MS (EPA, 2007). A major limitation with this EPA method is that four distinct LC-MS methods are used to determine different classes of pharmaceutical compounds. This can be tedious and time consuming. Like other EPA methods, the sample preparation and analysis procedures are very specific and limited to the use of particular SPE sorbents, extraction methods, instrument type and ionisation modes. Although the methods are used as a guide, this can present limitations in instances where the required materials and equipment are not available.

In this work, a "one-pot" LC-MS/MS method was developed and validated using a high-resolution accurate-mass LC-MS instrument as a tool for the detection of contaminants in water. This method incorporated Waters Oasis® HLB SPE cartridges as sample preparation and/or clean-up. This method was used to detect emerging contaminants in the Daspoort WWTP and three rivers (two of which were recipients of effluent from WWTPs and the third going through a non-formal settlement).

3.1.1 Chemicals, reagents and materials

A comprehensive list of all compounds used for this study is listed in Appendix A (Table A1). All standards used were at least 95% pure and purchased from Sigma-Aldrich (Steinheim, Germany) or Merck KGaA (Darmstadt, Germany). The LC-MS grade methanol, acetonitrile, hexane, ethyl acetate and formic acid were purchased from Romil or Merck KGaA (Darmstadt, Germany). Ultra-purity water of 18.2 MΩ cm^{-1} was prepared using a Milli-Q Q-POD purification system from Millipore (Bedford, Massachusetts, USA). Waters Oasis® HLB SPE cartridges (12 cc, 500 mg) from Waters (Milford, Massachusetts, USA) was used for all extraction. High-resolution MS was calibrated with a Pierce LTQ ESI positive and negative ion calibration solution purchased from Thermo Fisher Scientific (Rockford, Illinois, USA).

3.1.2 Standard preparation

Stock solutions of 1 mg mℓ$^{-1}$ were prepared by accurately weighing standards using a Mettler Toledo XP6U Comparator microbalance (Greifensee, Switzerland) and dissolving them in an appropriate solvent. The stock solutions were stored in amber vials at -20 °C. The stock solutions of the individual standards were used to prepare working mixture solutions and calibration solutions. Calibration standards were prepared over a concentration range from 0.1 to 500 ng ℓ$^{-1}$ by dilution of the 1 mg mℓ$^{-1}$ stock solutions in either 50:50 methanol/water, or 50:50 acetonitrile/water with 0.1% (v/v) formic acid or hexane, depending on the analysis method.

3.1.3 Sampling sites, samples collection and pre-treatment

Sampling was conducted for a period of approximately three years at the Daspoort WWTW using the grab sampling approach. The WWTW is located on the corner of E'skia Mphahlele Drive (M1) and Bazaar Street in Pretoria (GPS: 25° 43' 16.72" S, 28° 03' 14.07" E), where the Apies River and the Skinnerspruit meet. The Daspoort WWTW draws raw wastewater from the main wastepipe sewer that collects wastewater from the Central Pretoria area at two points. The first inlet of wastewater goes through its Eastern Works and the second inlet through its Western Works.

The Eastern Works is a trickling filter plant, while the Western Works is a conventional biological nutrient 58 removal activated sludge system. The main sewage drainage runs alongside the Apies River past the Daspoort WWTW to the Rooiwal WWTW. Samples were collected from upstream and downstream points on the Apies River, into which effluent from the Daspoort WWTW is discharged (Table 3.1).

Samples were collected upstream and downstream of the rivers into which the Daspoort WWTW discharged. Two other locations were identified for sampling: Muldersdrift se Loop, which has no contribution from WWTP effluents, and the Juskei River, downstream of the Northern WWTP (Table 3.1). Google maps images of these sites are given in Appendix C.

Table 3.1:   Sampling locations and types of samples collected in the study

Location	Type of sample	Coordinates
Daspoort WWTW	Wastewater influent and effluent	-25° 44' 3.933" 28° 10' 38.1318" (influent)
		-25° 43' 58.4358" 28° 10' 20.406" (effluent)
Apies River up and down	Water	25° 44' 00.6" S 28° 10' 42.9" E (Apies River up)
		25° 43' 36.7" S 28° 10' 17.6" E (Apies River down)
Juskei River	Water	25° 57' 10.0" S 27° 57'41.4" E
Muldersdrift se Loop	Water	26° 03' 48.8" S 27° 50' 28.8" E

All glassware, including sampling bottles, were soaked in detergent for 24 hours, rinsed thoroughly with ultra-high-pressure (UHP) water, soaked further in 10% nitric acid (HNO_3) or aqua regia solution for another 24 hours and finally rinsed again with UHP water (18.2 M Ω. cm^{-1}) to minimise the possible contaminants on the glass walls. All glassware, except for volumetric flasks, were then heated to 150 ºC for at least eight hours. After collection, samples were stored in a cold room (±4 ºC) prior to SPE.

3.1.4   Sample clean-up and/or concentration

All water samples were processed through Waters Oasis® HLB SPE cartridges to clean up and/or pre-concentrate. A Thermo Scientific™ Dionex™ AutoTrace™ 280 SPE instrument was used to process water samples (Thermo Fischer, Sunnyvale, California, USA). Before extraction, each cartridge was pre-conditioned with 3 mℓ of methanol, followed by 3 mℓ of UHP water at a flow rate of 10 mℓ min^{-1} with two minutes of equilibration between each solvent. After the conditioning step, 1,000 mℓ of water sample was loaded onto the conditioned cartridge at a flow rate of 10 mℓ min^{-1}. After extraction, the cartridges were washed with 1 mℓ of 5% methanol in water, and air-dried under vacuum for at least 20 minutes. The analyte residues were then eluted from the cartridge with two portions of 5 mℓ methanol. All the extracts were completely evaporated to dryness by a gentle stream of nitrogen using the Biotage Turbovap LV automated evaporation system (Uppsala, Sweden). The dried samples were then reconstituted in the appropriate solvent prior to analysis.

3.1.5   Instrumental parameters

3.1.5.1   LC-HRMS analysis

A Thermo Fischer Q Exactive Plus Orbitrap HRMS (Rs 280,000) was used for analyte detection (Sunnyvale, California, USA). The MS was coupled to a Dionex UltiMate 3000 UHPLC+ focused HPLC (Sunnyvale, California, USA). Two methods were developed and used for this study.

The first method employed a Waters X-Bridge C_{18} column, 2.1 x 100 mm, 3.5 µm. The mobile phase consisted of 0.1% (v/v) formic acid in water (A) and 0.1% (v/v) formic acid in ACN (B). The analysis started with 98% of Eluent A and the composition was linearly decreased up to 5% in 15 minutes. This composition was held for two minutes and increased again up to 98% in one and a half minutes, followed by a re-equilibration time of three minutes (total running time = 21.5 minutes).

The flow rate was 0.3 mℓ min^{-1} and the column temperature was set at 30 °C. The second method utilised a Restek biphenyl C18 column. Solvent A was composed of water with 0.1% formic and 2 mM ammonium formate, while Solvent B was methanol with 0.1% formic and 2 mM ammonium formate. A linear gradient was used starting with 5% B at 0 minutes up to 100% at 14 minutes. This composition was held for one minute before returning to initial conditions in 0.1 minutes. The column was then equilibrated for three minutes before injecting the next sample.

The Q-Exactive Plus was operated in either full MS SIM or full MS/data-dependent (dd-MS^2) in positive and negative mode switching over a scan range from *m/z* 66.7 to 1,000 with a mass accuracy of less than 5 ppm. The mass resolution was set to 7.0×10^4, the automatic gain control (AGC) target was set at 1.0×10^6 with a maximum injection time (IT) of 200 ms. In full MS-SIM, the Orbitrap performed a full MS scan without high-energy collision dissociation (HCD) fragmentation. The precursor ion was fragmented with stepped normalised collision energy (NCE) to generate the resulting dd-MS^2 product ion spectrum using a mass resolution of 35,000, automatic gain control (AGC) target of 2×10^5, maximum injection time (IT) at 100 ms. The MS was calibrated weekly for mass accuracy using Pierce LTQ ESI positive and negative ion mass accuracy standards. A quality control standard (1.00 mg ℓ$^{-1}$ standard) was run every five injections, followed by a blank sample (pure acetonitrile).

3.1.5.2 *GC×GC-HRT-MS analysis*

A LECO PEGASUS 4D GCxGC-HRT-MS coupled to an Agilent 7890A GC was used for the analysis of volatile analytes. The GC used a 30 m x 0.25 mm x 0.25 µm HP-5MS UI column (primary oven) and a 2 m x 0.25 mm x 0.25 µm Rxi-17 Sil MS (secondary oven) column. Helium was used as a carrier gas at a constant flow of 1.35 mℓ min^{-1}. The GC oven temperature program was 1 minute at 50 °C, ramped at 11 °C per minute to 180 °C held for 3 minutes, then 15 °C per minute to 290 °C held for 7 minutes. The secondary oven was maintained +15 °C relative to the primary oven. The GCxGC modulator was operated at a modulation frequency of five seconds with temperature maintained +15 °C relative to the secondary oven. Samples of one microlitre were injected in split mode (Inlet Split Ratio: 5) and EI mass spectra (70 eV) was collected in the high-resolution mode (>25 000 resolution) over the mass range 50-600 Dalton. The transfer line and ion source temperature were set at 300 °C and 250 °C respectively.

3.1.6 Data analysis

Processing of the data from the LC-MS was done using TraceFinder EFS Software Version 3.2 and QualBrowser software (Thermo Scientific). For quantification, detection was based on the presence of the analyte (de)protonated molecule at accurate mass (<5 ppm), retention time agreement with standard (±2.5%) and isotopic pattern (60% fit threshold, 5 ppm mass deviation, 10% intensity deviation). For screening, information from the compound database was used. An analyte was tentatively detected (potential positive) when the (de)protonated molecule at accurate mass (<5 ppm, intensity >5,000) was observed. Tentative identification parameters included the presence of the (de)protonated molecule at accurate mass (<5 ppm, intensity >5,000, 60% fit threshold, 5 ppm mass deviation, 10% intensity deviation) and at least one fragment ion (intensity > 1,000, <10 ppm mass tolerance). For both GC-MS methods, data acquisition was achieved using Chroma ToF software. The peak deconvolution was used to confirm the identity of each compound and the internal standards.

3.1.7 Compound database development for LC-HRMS

The compounds used for the study were primarily selected based on their prescription volumes in South Africa (Osunmakinde et al., 2012). Some of the compounds are also part of those routinely monitored in studies done around the world, and some are most frequently detected pharmaceuticals in WWTP effluent, surface water, and/or sediments. Other compounds were added to include compounds from different groups of the EPA method; hence, their performance could be tested under the generic conditions used in the study.

The LC-MS does not have standardised spectral libraries like the GC-MS. The spectral libraries in GC-MS are a result of the fact that, under electron ionisation at 70eV, the spectra of compounds are reproducible, which is not the case for LC-MS spectra. This is mainly due to differences in the ionisation interfaces and, in addition, variability depending on mobile phase composition, additives or the voltages applied. It is therefore necessary to build compound databases relevant to specific operating and analysis conditions to facilitate the proper identification and screening of compounds. The databases often contain information on the molecular formula, the exact mass of the neutral and (de)protonated molecule, the theoretical isotopic pattern expected for each compound, characteristic fragment ions, possible adducts with CH_3CN^+, Na^+ or H_3O^+ and retention time matching reference standards. All this information combined enables the positive identification and quantification of contaminants in environmental samples. This has been done and successfully demonstrated by some researchers over the last five years in the determination of mixed class compounds (Gómez-Pérez et al., 2012).

A compound database with approximately 1,400 compounds was available on the instrument. To build information for the compound database for our study, full-scan spectra were acquired for each compound to identify the precursor ion before fragmentation using individual standards. The MS parameters used in the study were as generic as possible to allow for "optimal" ionisation conditions of most compounds entering the source and to include as many analytes as possible for a multi-residue method. Table A1 (see Appendix A) shows data available for each compound in the database, such as ionisation mode, exact mass for (de)protonated compounds and the mass(es) of the main fragment(s) ions observed. Additional data such as the retention time for the different columns was added to the compounds for further identification and quantification purposes. Among the compounds used in this study, 57 compounds (highlighted in red in Table A1) were not found in the database and complete information, as described above, was added to the database.

3.2 METHOD DEVELOPMENT AND VALIDATION

Three methods were developed for the determination of contaminants based on high-resolution LC-MS using Waters X-Bridge and Restek Bipheyl columns, and high-resolution GCxGC-HRT-MS. In this section, the focus is on the high-resolution LC-MS/MS methods.

3.2.1 High-resolution LC-MS methods using X-Bridge and Biphenyl columns

Two methods were developed and validated using Waters X-Bridge and Restek Bipheyl columns for several compounds (Table A2 and Table A3 in Appendix A). The validation data for the Waters X-Bridge column method is shown in Appendix A, where linear regression analysis showed good linearity for most compounds with the range of 0.9901-0.9990. However, there were a few compounds (about 3%) that had a linearity of less than -0.99 (shown in Table A2 in Appendix A). The limit of detection (LOD) and LOQ values ranged from 0.003 to 8.41 ng ℓ^{-1} and 0.01 to 28.0 ng ℓ^{-1}, respectively. A second method was developed and validated that used a Restek Biphenyl column. Table A3 (in Appendix A) shows the validation data using the Restek Biphenyl column method. Linearity ranges were from 0.9528-0.9997, while the LOQ values ranged from 0.014 to 4.51 ng ℓ^{-1} and the LOD values ranged from 0.002 to 1.86 ng ℓ^{-1}. Azithromycin showed the poorest linearity range of 0.9528.

3.2.2 GCxGC-HRT-MS method

The GC×GC has become one of the most important tools in the detection and qualification of some groups of environmental compounds. In this work, we have applied this approach for several important groups of environmental compounds that are of concern. The incorporation of high resolution in the GCxGC-HRT-MS is an important aspect of environmental science due to its suitability for both targeted and untargeted analysis. The HRT-MS has excellent sensitivity in full-scan acquisition mode and high mass accuracy. Therefore, the use of the exact masses in combination with the mass spectral library increases the confidence in identifying non-target compounds. The developed method was validated and the data presented in Table A4 (see Appendix A).

The LOD and LOQ values of the analytes ranged from 0.016 to 0.284 µg ℓ^{-1} and from 0.053 to 0.95 µg ℓ^{-1}, respectively. The coefficient of determination (R^2) ranged from 0.9905 to 0.9997 and the calibration curves were linear in the concentration range presented in Table A4.

The precision of the method was evaluated based on repeatability and reproducibility. For repeatability studies, the percentage relative standard deviation (RSD) was determined through repeated injections (duplicates) of five replicates of a spiked extract at three concentration levels for each analyte, three times during the same day. On the other hand, reproducibility was determined as described for repeatability, but over a period of five consecutive days. The repeatability, expressed as a percentage RSD, was between 3.41 and 11.72%, while reproducibility ranged from 2.88 to 9.91% over the three concentrations evaluated over five days. These results indicated that the proposed method has acceptable precision and can be applied to real wastewater samples.

3.2.3   Conclusions

The two methods based on Orbitrap high-resolution LC-MS/MS using Water X-Bridge and Restek Bipheyl columns were developed and validated for emerging contaminant compounds. The performance of the two columns was very similar, hence providing for flexibility. Using both methods good linearity, LOD values and LOQ values were achieved. The methods were successfully applied to rivers and WWTP influent and effluent. The sensitivity in full-scan acquisition mode and high mass accuracy was well demonstrated when the method was applied to real wastewater and river water.

Using the GCxGC-HRT-MS method, the LOD and LOQ values of the analytes ranged from 0.016 to 0.284 ng ℓ^{-1} and from 0.053 to 0.95 ng ℓ^{-1}, respectively. The coefficient of determination ranged from 0.9905 to 0.9997. The validated method was successfully applied to emerging contaminant compounds. In addition, the incorporation of GCxGC high-resolution chromatography to HRMS was an added advantage.

CHAPTER 4: APPLICATION OF VALIDATED METHODS TO REAL WATER SAMPLES

4.1 QUANTIFICATION OF COMPOUNDS IN THE WATER SAMPLES

4.1.1 High-resolution LC-MS Orbitrap analysis of WWTP influents and effluents

We have reviewed the methods that have previously been used to generate the large amount of data worldwide that used a clean-up and/or sample preparation incorporated into the chromatographic method with several detectors. Mass spectrometry proved itself to be the most suitable of all detectors, coupled with the chromatographic system due to its inherent sensitivity and selectivity. Over the last two decades, there has been huge leap in the development of MS. This has resulted in instruments that are not only sensitive, but also have high resolution and tremendous data-processing power. These new developments have increased the capacity to determine as many compounds as possible in "one pot".

The previous chapter demonstrated that both Waters X-Bridge and Restek Biphenyl columns could be used to determine emerging contaminants. However, based on preference, the former was selected for the rest of the application work. In this section the analysis of samples was carried out with the developed and validated high-resolution LC-MS method using the Waters X-Bridge column. Several emerging contaminant compounds, including most compounds of interest in the study, could be quantified. Linear regression analysis showed good linearity for most of the compounds with correlation of determination in the range of 0.9901-0.9990. As previously mentioned, about 3% of the compounds had linearity of less than 0.99. The LOD and LOQ values ranged between 0.003 to 8.41 ng ℓ^{-1} and 0.01 to 28.0 ng ℓ^{-1}, respectively (Appendix A). The reported results were slightly higher than previously reported for multi-residue methods for the analysis of pharmaceuticals (Diaz et al., 2011; Gómez et al., 2011).

Figure 4.1 shows a typical ion chromatogram, which clearly demonstrates the power of the combination of an HRMS and the extracted single-ion chromatogram using some emerging contaminant compounds detected in an effluent wastewater sample. The use of exact masses was used to identify the six diverse compounds, i.e. ritonavir (*m/z* 721.32068), sulphamethoxazole *m/z* 254.05957), efavirenz (*m/z* 316.03482), carbamazepine (*m/z* 237.10237), estradiol (*m/z* 273.18503) and diclofenac (*m/z* 296.02405) present in the effluent wastewater sample. The inherent sensitivity in full-scan acquisition mode and high mass accuracy clearly makes this approach an attractive tool for the detection and monitoring of emerging contaminants in an aquatic environment. In this work, during the validation of the developed method, high-resolution LC-MS clearly demonstrated its ability to monitor a large and diverse number of emerging contaminant compounds in the so-called "one-pot" analysis.
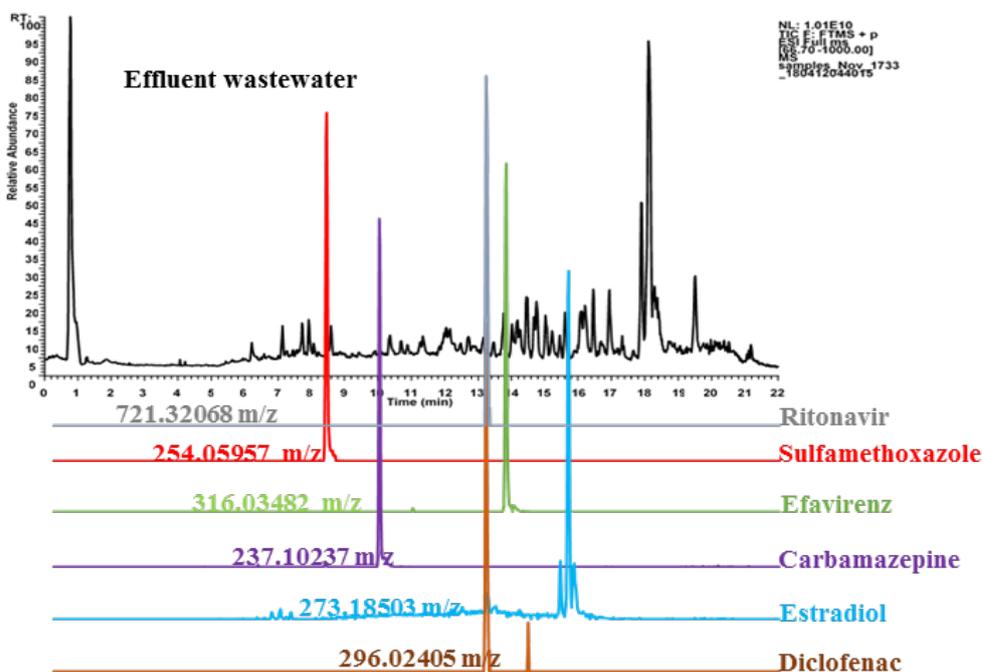
Figure 4.1: Extracted single-ion chromatograms (SICs) of some compounds detected in an effluent wastewater sample

The compounds detected in WWTP influent and effluent samples covered 14 classes: antibiotics, ARVs, steroid hormones, NSAIDs, anti-inflammatories, antivirals, antifungals, antidepressants, anticonvulsants, cardiovascular agents, analgesics, anthelmintics, consumer product additives and bronchodilators (Figure 4.2). In this study, 71 and 73 PPCP compounds were quantified in influent and effluent samples, respectively. Table 4.1 shows a summary of the results obtained and the data for each compound detected in the influent and effluent samples.

The compounds found with the highest mean concentrations in the influent samples (above 1 µg ℓ^{-1}) included caffeine (28.17 µg ℓ^{-1}), paraxanthine (16.79 µg ℓ^{-1}), ibuprofen (15.83 µg ℓ^{-1}), paracetamol (6.21 µg ℓ^{-1}), estradiol (1.20 µg ℓ^{-1}) and efavirenz (1.17 µg ℓ^{-1}). These compounds were found in all influent samples. The other compounds had varied concentrations ranging from medium ng ℓ^{-1} concentrations (i.e. sulphamethoxazole of 512.4 ng ℓ^{-1} and valsartan of 649.1 ng ℓ^{-1}) to low ng ℓ^{-1} (metoprolol of 0.003 ng ℓ^{-1} and verapamil of 0.023 ng ℓ^{-1}). Similarly the emerging contaminant compounds detected with the highest mean concentrations in the effluent samples with concentrations above 1 µg ℓ^{-1} included caffeine (1.53 µg ℓ^{-1}), paraxanthine (1.26 µg ℓ^{-1}), ibuprofen (2.50 µg ℓ^{-1}), paracetamol (< 0.03 µg ℓ^{-1}), estradiol (2.42 µg ℓ^{-1}) and efavirenz (1.04 µg ℓ^{-1}). Overall, 32% of the compounds were found in all samples and included compounds such as sulphamethoxazole, carbamazepine, diclofenac, fluconazole, methylparaben, mefenamic acid, ritonavir, valsartan, triclorban and tonalid. Some 44 compounds were above 50% detection frequency, while some compounds, such as clarithromycin, amitryptiline, sarafloxacin and verapamil, were detected in 15% or less of the analysed samples. Antibiotics were the predominant class detected in the WWTP influent samples, accounting for about 28% of the compounds quantified. This is in agreement with the findings of a previous report where antibiotics were found to be the highest consumed drugs in the South African community.

Table 4.1: **Summary of occurrence and concentrations of contaminants in WWTP influent and effluent samples**

Compound	Concentration (ng ℓ⁻¹)				Frequency of detection	
	WWTP influent		WWTP effluent		WWTP influent (n = 28)	WWTP effluent (n = 16)
	Range	Mean	Range	Mean		
Albendazole	nd-17.58	0.695	nd-0.157	0.001	9	1
Amitriptyline	nd-5.614	0.234	nd-19.55	1.775	3	9
Atazanavir	nd	-	nd-308.2	75.12	-	5
Bufexamac	nd-3.196	0.316	nd-10.69	2.073	7	14
Caffeine	1,170-60,136	28171	85.76-4,878	1533	28	16
Carbamazepine	1.775-115.7	30.89	16.39-416.3	193.6	28	16
Ciprofloxacin	nd-77.04	35.32	nd-5.590	1.03	27	3
Clarithromycin	nd-10.06	0.451	nd-75.44	9.837	3	10
Dexamethasone	nd	-	nd-0.924	0.079	-	2
Diclofenac	12.16-246.3	147.5	5.561-243.6	74.44	28	16
Diethylbestrol	nd-91.11	13.56	nd-547.7	143.1	9	15
Digoxigenin	nd-3.532	0.380	nd	-	5	-
Efavirenz	50.98-2,169	1171	210.1-2,042	1,036	28	16
Enalapril	nd-32.53	5.936	nd-3.100	0.824	20	13
Enrofloxacin	nd	nd	nd-0.737	0.125	-	4
Erythromycin	nd	nd	nd-11.89	4.006	-	8
Estradiol	66.45-2,206	1,204	154.1-7,133	2,415	28	16
Estriol	53.23-1313	250.3	56.53-779.1	275.1	28	16
Estrone	nd-35.96	7.951	nd-60.83	20.04	20	11
Famciclovir	nd-17.67	0.863	nd-7.165	1.211	9	6
Fenoprofen	nd	nd	nd-207.6	46.80	-	10
Fluconazole	13.54-396.4	165.9	14.78-307.6	159.6	28	16
Flumequine	nd-3.341	0.562	nd-0.175	0.011	6	1
Gabapentin	nd-146.4	18.43	2.910-41.79	18.70	23	16
Gemfibrozil	nd-598.6	258.4	3.776-479.4	189.3	27	16
Ibuprofen	568.7-76,377	15,831	nd-7,652	2,504	28	15
Ifosfamide	nd-2.122	nd	nd-5.426	1.035	4	11
Indometacin	nd-42.52	11.84	0.273-18.70	8.248	25	16
Isoniazide	nd-31.55	6.957	nd-27.77	12.62	18	15
Ketoprofen	nd-23.10	8.052	nd-49.48	13.38	23	15
Lamivudine	nd-1001	267.5	nd-323.4	28.07	22	13
Lidocaine	nd-93.29	9.395	nd-424.6	37.86	19	15
Lincomycin	nd-2.801	0.100	nd-20.65	1.526	1	2
Mebendazole	nd-61.83	9.942	nd-29.36	6.676	11	11
Medroxyprogesterone	nd-16.85	4.043	nd-4.788	1.628	22	9
Mefenamic acid	11.30-91.15	31.95	4.789-55.05	19.99	28	16
Mestranol	nd-123.4	14.30	nd-110.0	14.13	4	3
Methylparaben	1.649-600.4	300.6	nd-110.0	36.07	28	14
Metoprolol	nd-0.091	0.003	nd-2.215	0.603	1	8
Naproxen	16.85-546.1	64.07	13.09-349.6	122.3	28	16
Nevirapine	0.310-26.34	9.383	0.352-80.53	10.20	28	16
Norfloxacin	nd-31.70	3.116	nd-9.833	0.614	6	1
Ofloxacin	24.66-67.50	36.39	11.54-86.51	42.05	28	16
Oxolinic acid	nd-0.187	0.018	nd-0.205	0.034	6	6
Oxytetracycline	nd-21.01	2.943	nd-1.365	0.304	12	4
Paracetamol	155.3-22,889	6,209	nd-106.8	31.32	28	13
Paraxanthine	4,963-35,286	16,790	9.704-8,452	1258	28	16
Penciclovir	nd-22.94	4.241	16.31-104.8	56.05	12	16
Phenacetin	0.315-68.58	16.08	0.466-25.81	3.256	28	16
Pindolol	nd-2.757	0.389	nd-18.41	2.153	18	11
Prednisolone	nd-7.383	1.951	nd-36.17	8.510	18	12
Procaine	nd-15.47	6.466	nd-1.825	0.403	26	9
Progesterone	nd-14.52	3.711	0.244-4.025	1.408	22	16
Ractopamine	nd-2.294	0.326	nd-0.938	0.182	15	9
Ritonavir	4.084-393.9	72.77	14.43-675.9	128.5	28	16
Salbutamol	nd-5.171	0.345	nd-8.599	1.872	16	9

Compound	Concentration (ng ℓ^{-1})				Frequency of detection	
	WWTP influent		WWTP effluent		WWTP influent (n = 28)	WWTP effluent (n = 16)
	Range	Mean	Range	Mean		
Salicylamide	5.472-563.5	198.5	4.864-112.9	28.51	28	16
Sarafloxacin	nd-8.33	-	-	-	2	-
Sulphadiazine	nd-0.416	0.050	-	-	4	-
Sulphadimethoxine	nd-0.643	0.020	nd-0.409	0.056	6	3
Sulphadoxin	nd-6.750	0.741	nd-1.256	0.442	22	11
Sulphaguanadin	nd-11.47	1.433	-	-	5	-
Sulphamethazine	nd-26.72	1.399	nd-41.88	3.837	9	9
Sulphamethoxazole	52.92-2,405	512.4	34.93-504.4	204.3	28	16
Sulphanilamide	nd-4.003	0.414	nd-10.00	1.665	7	8
Sulphapyridine	nd-110.2	22.12	nd-23.22	5.387	11	5
Terbutaline	nd-1.444	0.148	nd-0.448	0.145	13	10
Testosterone	nd-44.09	18.19	nd-5.826	0.591	23	4
Thiabendazole	nd-1.684	0.312	nd-10.01	1.780	9	6
Tonalid	0.211-80.16	71.30	nd-28.57	7.247	28	15
Tramadol	nd-77.16	10.88	0.718-289.8	95.68	18	16
Triclocarban	8.973-276.1	159.9	4.566-44.89	21.56	28	16
Triclosan	nd-97.78	7.944	1.828-26.96	8.446	26	16
Trimethoprim	16.61-577.6	108.7	nd-136.6	50.40	28	15
Valsartan	99.37-1289	649.1	106.2-762.4	319.7	28	16
Venlafaxine	nd-7.585	1.881	nd-39.60	15.14	16	15
Verapamil	nd-0.472	0.023	nd-1.209	0.149	3	3
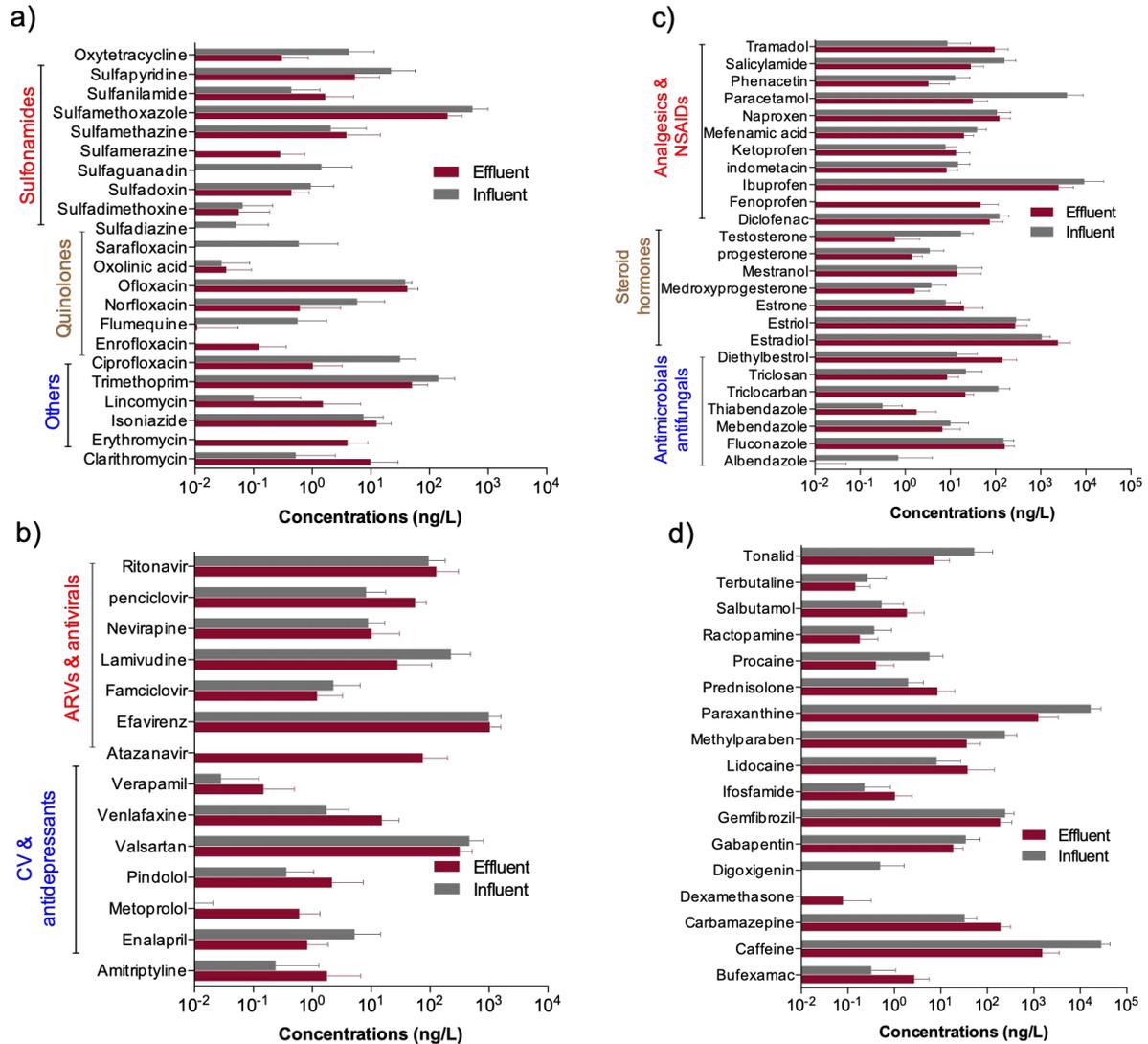
Figure 4.2: Compounds and their classes quantified in the Daspoort WWTP influent and effluent samples: a) sulphonamides, quinolones and others; b) ARVs, antivirals, antidepressants and cardiovascular agents; c) antibiotics, antifungals, steroids hormones, analgesics and NSAIDs; d) miscellaneous

After going through the water treatment process, there was generally a reduction rather than an elimination of most of the compounds detected in the influent water samples, highlighting the inadequacy of water treatment techniques to remove emerging contaminants. It was also noted that some compounds increased in concentration from the influents to the effluents.

The removal efficiency for the different chemicals was calculated based on their WWTP influent and effluent concentrations calculated as:

$$\text{Removal efficiency} = \frac{Cinf - Ceff}{Cinf} \, x \, 100 \, \%$$

where: $Cinf$ and $Ceff$ are the concentrations of the compound in the influent and effluent of the WWTP in ($\mu g \, \ell^{-1}$). The removal efficiencies for the quantified compounds showed great variability. Compounds were grouped into three categories: negative removal (where compounds showed an increase in concentration from influent to effluent or were only detected in the effluent), low-to-moderate removal (0-70%) and significant removal efficiency (>70%), as shown in Table 4.2.

Some compounds, such as efavirenz, terbutaline and fluconazole, were among those with the lowest removal efficiency rates (≤15%) and acetaminophen, ibuprofen and caffeine were some that showed the highest (>85%) removal efficiency rates. Steroidal hormones, including estradiol and estriol, showed negative removal efficiency rates. Some compounds showed an increase in concentration levels in effluent. These included atazanavir (0.08 µg ℓ^{-1}), which was not detected in the influent, carbamazepine (0.19 µg ℓ^{-1}), diethylbestrol (0.14 µg ℓ^{-1}), estradiol (2.42 µg ℓ^{-1}), which doubled its concentration, estrone (0.020 µg ℓ^{-1}) and tramadol (0.096 µg ℓ^{-1}) (Figure 4.2). This negative removal phenomenon has previously been attributed to one or a combination of possible transformation, recombination and/or accumulation of compounds during the treatment processes (Verlicchi et al., 2012b). Antiretrovirals were represented in this category by atanazavir, nevirapine and ritonavir. Carbamazepine also showed a negative removal efficiency rate. This has previously been highlighted due to its accumulation and in efficient removal in WWTPs (Tarpani and Azapagic, 2018). Negative removal and variable removal efficiencies of emerging contaminants in full-scale WWTPs could simply be due to sampling schemes such as grab sampling, which takes the concentration of emerging contaminants in the instant the water sample is taken (Baalbaki et al., 2017).

Table 4.2: Removal efficiencies of the various contaminants in the WWTP

Negative removal	0-70% removal	>70% removal
Amitriptyline, atazanavir, bufexamac, carbamazepine, clarithromycin, diethylbestrol, enalapril, estradiol, estriol, famciclovir, gabapentin, ifosfamide, isoniazide, ketoprofen, lidocaine, lincomycin, metoprolol, naproxen, nevirapine, ofloxacin oxolinic acid, phenacetin, prednisolone, ritonavir, salbutamol, sulphadimethoxine, sulphamethazine, sulphanilamide, thiabendazole, tramadol, triclosan, venlafaxine, verapamil	Diclofenac, efavirenz, fluconazole, gemfibrozil, indomethacin, mebendazole, medroxyprogesterone, mefenamic acid, mestranol, penciclovir, progesterone, ractopamine, sulphadoxin, sulphamethoxazole, terbutaline, trimethoprim, valsartan	Albendazole, caffeine, ciprofloxacin, digoxigenin, enrofloxacin, erythromycin, flumequine, ibuprofen, lamivudine, methylparaben, norfloxacin, oxytetracycline, paracetamol, paraxanthine, pindolol, procaine, salicylamide, sarafloxacin, sulphadiazine, sulphaguanadin, sulphapyridine, testosterone, tonalid, triclocarban,

4.1.2 River water samples

Overall, the data from the river water samples showed the presence of endocrine-disrupting hormones, which are already classified as pollutants. The most detected groups of compounds were estradiol, estrone, estriol and diethylstilbestrol. Estradiol was detected with the highest concentrations of 2.21 µg ℓ^{-1}. Paracetamol, ibuprofen, caffeine and sulphamethoxazole were detected at concentration levels ranging from 0.059 to 4.14 µg ℓ^{-1}. Several compounds at lower concentrations were frequently detected in all the samples. These included NSAIDs (ketoprofen, naproxen and diclofenac) and ARVs (ritonavir and efavirenz). Indicators/markers for ARVs, mainly fluconazole, trimethoprim and sulphamethoxazole, are always detected in the presence of ARVs.

4.1.2.1 *Apies River upstream and downstream*

The Apies River is the recipient of effluent from the Daspoort WWTP. Samples were collected upstream, i.e. at the discharge point of the effluent, and downstream to assess the impact of the WWTP effluent on the river. Table 4.3 shows the data of the occurrences of contaminants upstream and downstream of the Apies River. Higher levels of contaminants were detected downstream of the river with concentration means of 4,139 ng ℓ^{-1}, 2,979 ng ℓ^{-1}, 506 ng ℓ^{-1}, 433 ng ℓ^{-1}, 329 ng ℓ^{-1}, 108 ng ℓ^{-1} and 35 ng ℓ^{-1} for ibuprofen, caffeine, estradiol, paracetamol, efavirenz, carbamazepine and ritonavir, respectively.

Some 60 and 63 compounds were found in the upstream and downstream river samples, respectively. Some 42 chemicals had over 50% detection frequency in both the upstream and downstream samples. The highest mean concentrations observed in both samples were for caffeine (2.98 µg ℓ^{-1} downstream; 1.42 µg ℓ^{-1} upstream) and paraxanthine (1.22 µg ℓ^{-1} downstream; 0.798 µg ℓ^{-1} upstream).

Compared to the downstream samples, upstream samples had significant contamination, considering that no effluent is discharged into it. This suggests that there are other significant sources of contamination in the river besides the WWTP effluent.

Table 4.3: **Occurrence and concentrations of contaminants in the Apies River upstream and downstream**

Compound	Concentration (ng ℓ⁻¹)				Frequency of detection	
	Apies River upstream		Apies River downstream		Apies River upstream (n = 7)	Apies River downstream (n = 7)
	Range	Mean	Range	Mean		
Amitriptyline	nd-1.158	0.355	nd-2.272	0.815	5	6
Bufexamac	0.155-3.188	1.098	0.487-2.389	1.323	7	7
Caffeine	4.098-2,785	1,424	823.8-7,718	2,979	7	7
Carbamazepine	8.774-176.0	58.508	23.42-240.7	107.7	7	7
Ciprofloxacin	nd	nd	nd-5.590	2.262	-	2
Clarithromycin	nd-5.480	3.194	0.982-10.44	4.390	5	6
Desipramine	nd-0.620	0.194	nd	-	2	-
Dexamethasone	nd-0.365	0.052	nd-0.707	0.101	-	2
Diclofenac	5.642-81.98	20.38	9.488-24.20	17.81	7	7
Diethylbestrol	nd-249.1	73.79	nd-368.4	144.3	6	7
Efavirenz	116.7-345.3	208.9	170.9-514.6	328.9	7	7
Enalapril	0.517-2.891	2.421	0.277-1.533	0.680	7	7
Enrofloxacin	nd	nd	nd-1.835	0.262	-	1
Erythromycin	nd-6.589	1.570	nd-9.713	2.286	2	3
Estradiol	134.7-644.0	362.2	134.7-931.1	505.9	7	7
Estriol	81.30-244.5	134.7	83.30-546.0	296.7	7	7
Estrone	7.124-63.04	30.39	nd-46.95	22.46	7	5
Famciclovir	nd-8.693	2.507	nd-3.107	1.000	3	3
Fenoprofen	nd-67.98	15.86	nd-418.1	104.3	3	4
Fluconazole	10.67-81.87	48.98	26.52-200.8	78.26	7	7
Flumequine	nd	-	nd-0.932	0.266	-	2
Gabapentin	2.061-18.62	9.138	4.703-17.86	11.56	7	7
Gemfibrozil	8.505-173.9	63.15	41.98-545.2	138.6	7	7
Ibuprofen	nd-8,651	3,192	1548-12,812	4,139	6	7
Ifosfamide	nd-0.106	0.015	0.109-1.149	0.458	1	7
Indometacin	nd-4.403	1.959	nd-8.555	2.679	6	5
Isoniazide	nd-3.638	1.644	nd-5.873	2.206	5	6
Ketoprofen	nd-8.853	4.289	0.561-39.49	9.388	6	7
Lamivudine	nd-8.912	2.528	nd-10.38	3.831	4	4
Lidocaine	1.292-49.59	13.57	3.125-112.4	23.77	7	7
Medroxyprogesterone	nd-6.711	2.019	nd-9.822	3.750	4	6
Mefenamic acid	2.239-91.15	12.87	5.861-19.60	10.62	7	7
Mestranol	nd-19.55	2.793	nd-81.59	11.66	1	1
Methylparaben	4.376-16.04	10.02	nd-110.0	19.46	7	7
Metoprolol	nd-0.217	0.048	nd-0.114	0.031	2	2
Naproxen	30.33-137.9	99.59	64.61-486.9	231.6	7	7
Nevirapine	0.389-7.332	3.087	0.274-10.99	3.304	7	7
Norfloxacin	nd	-	nd-9.675	1.382	-	1
Ofloxacin	nd--4.654	0.665	nd-30.70	13.34	1	6
Paracetamol	nd-323.0	54.17	nd-1683	432.8	7	7
Paraxanthine	262.9-1245	797.9	204.7-2907	1218	7	7
Penciclovir	nd-18.66	3.338	nd-33.94	22.34	2	6
Phenacetin	0.337-2.174	1.439	0.322-2.746	0.825	7	7
Pindolol	nd-0.421	0.194	0.062-0.701	0.301	6	7
Prednisolone	nd-25.27	9.548	nd-36.12	12.32	6	6
Procaine	nd-15.47	0.102	nd-0.261	0.078	4	4
Progesterone	0.161-2.204	0.807	0.206-3.588	1.207	7	7
Ractopamine	nd-0.211	0.114	nd-0.654	0.159	4	3
Ritonavir	nd-58.84	25.54	5.0-52.57	35.04	6	7
Salbutamol	nd-0.939	0.196	nd-1.326	0.389	4	6
Salicylamide	nd-26.37	13.62	nd-40.81	16.59	6	6
Sulphadimethoxine	nd-0.859	0.272	nd-1.830	0.498	3	4

Compound	Concentration (ng ℓ⁻¹)				Frequency of detection	
	Apies River upstream		Apies River downstream		Apies River upstream (n = 7)	Apies River downstream (n = 7)
	Range	Mean	Range	Mean		
Sulphadoxin	nd-0.351	0.050	nd-0.721	0.367	1	6
Sulphamethazine	nd-1.768	0.253	nd-4.891	2.949	1	6
Sulphamethoxazole	nd-237.4	103.5	52.97-297.4	178.5	6	7
Sulphanilamide	nd-0.300	0.043	nd-0.418	0.146	1	2
Sulphapyridine	nd	-	nd-1.151	0.164	-	1
Terbutaline	nd-0.090	0.023	nd-0.283	0.063	2	3
Testosterone	nd	-	nd-2.381	0.340	-	1
Thiabendazole	nd	-	nd-<loq	-	-	3
Tonalid	0.133-3.535	1.705	0.158-7.445	2.244	7	7
Tramadol	6.056-25.26	14.32	8.361-40.38	29.95	7	7
Triclocarban	3.494-28.99	14.60	nd-11.35	5.198	7	5
Triclosan	nd-11.52	4.037	0.587-8.975	3.970	6	7
Trimethoprim	6.9011-114.8	49.76	17.76-171.3	85.32	7	7
Valsartan	81.61-143.0	105.6	54.01-322.1	143.0	7	7
Venlafaxine	0.167-2.035	1.200	0.972-5.142	2.924	7	7

Data for the upstream and downstream river samples was also compared to that from the effluent discharged into the river. This could give an indication of the impact the effluent discharge has on contamination in the river. It should be noted that as grab samples were used for this analysis, the data presented only provides some insight rather than a complete analysis as potential daily variations in effluent discharge may present different results.

The WWTP effluent showed the highest sum concentration of contaminants compared to the river samples. The upstream samples had the lowest sum of chemical concentration, although the same number of contaminants were detected in the downstream samples (Figure 4.3). The sum concentration of the chemical groups profile was quite similar in the upstream and downstream samples with the most prevalent chemical classes in the effluent being analgesics and NSAIDs (>45% in both samples), although paraxanthine and caffeine present in the group of "other compounds" were the highest individual contributors to the contamination among the detected chemicals.
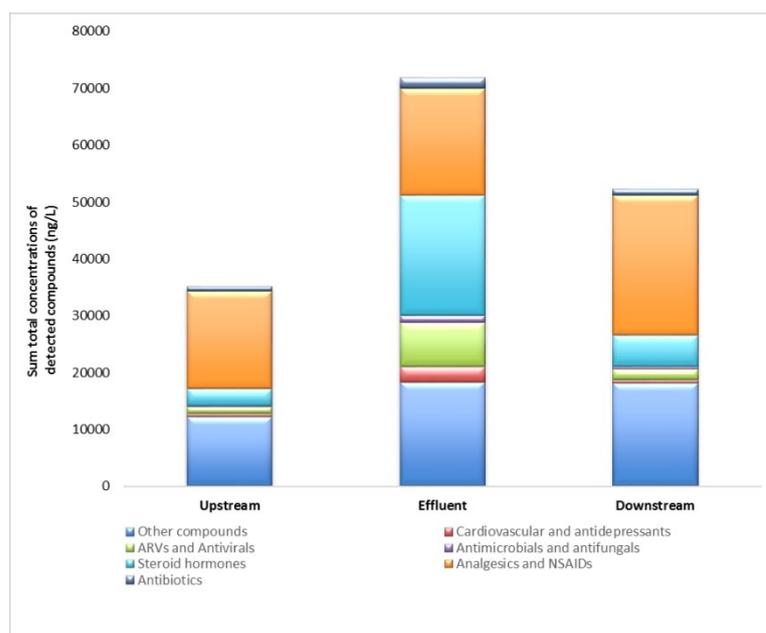
Figure 4.3: **The sum concentration of compound classes detected from the Daspoort WWTP effluent together with that collected upstream and downstream of the Apies River**

The profile was quite different for effluent samples with three classes (steroid hormones, analgesics and NSAIDs) and "other compounds" accounting for more than 25% each of the sum concentration of contaminants. About 10% of the contaminants was due to the ARVs and antivirals class, while the rest of the groups contributed less than 4% each to the total concentration of the compounds.

4.1.2.2 Muldersdrift se Loop and Juskei River

Data from samples collected from other rivers, mainly the Juskei River and Muldesdrift se Loop, was compared with data from samples from the Apies River. The Juskei River is a recipient of the Northern WWTP, the biggest wastewater treatment plant in South Africa, while Muldesdrift se Loop has no direct WWTP effluent discharge. Some 23 and 32 chemicals were found in each river sample for Muldersdrift se Loop and the Juskei River, respectively (Table 4.4). This included caffeine, carbamazepine, efavirenz, fluconazole, ibuprofen, methylparaben, gabapentin, tonalid and paraxanthine. The highest mean concentrations were observed for ibuprofen (4.486 ng ℓ^{-1}), caffeine (3,722 ng ℓ^{-1}), paraxanthine (2,777 ng ℓ^{-1}), efavirenz (1,094 ng ℓ^{-1}) and estradiol (900.0 ng ℓ^{-1}) in the Juskei River. For Muldersdrift se Loop, ibuprofen (2260 ng ℓ^{-1}), estradiol (361.0 ng ℓ^{-1}), caffeine (246.6 ng ℓ^{-1}), paracetamol (181.9 ng ℓ^{-1}) and estriol (115.9 ng ℓ^{-1}) were the compounds found with mean concentrations above 100 ng ℓ^{-1}.

Table 4.4 summarises data of the compounds detected from the Juskei River and Muldersdrift se Loop.

Table 4.4: Occurrence and concentration of contaminants in the Juskei River and Muldersdrift se Loop

Compound	Concentration (ng ℓ^{-1})				Frequency of detection	
	Muldersdrift se Loop		Juskei River		Muldersdrift se Loop (n = 8)	Juskei River (n = 14)
	Range	Mean	Range	Mean		
Albendazole	nd	-	nd	-	-	-
Amitriptyline	nd	-	nd-9.727	1.775	-	12
Atazanavir	nd	-	nd-308.2	75.12	-	5
Bufexamac	nd	-	0.170-7.622	4.567	-	14
Caffeine	128.2-403.4	246.6	2,788-5,040	3,722	8	14
Carbamazepine	13.08-166.3	57.62	20.44-266.4	119.4	8	14
Clarithromycin	nd	-	nd-15.84	5.527	-	7
Desipramine	nd	-	nd-8.143	1.095	-	4
Dexamethasone	nd-0.535	0.156	nd	-	4	-
Diclofenac	nd-10.44	6.505	35.66-149.9	92.80	7	14
Diethylbestrol	nd-68.13	13.56	20.82-291.1	144.1	7	14
Efavirenz	29.27-88.77	53.44	140.9-1968	1094	8	14
Enalapril	nd		0.299-8.363	3.759	-	13
Erythromycin	nd	nd	nd-7.075	1.167	-	5
Estradiol	71.55-632.4	361.0	150.8-2,096	900	8	14
Estriol	45.94-269.2	115.9	86.26-563.6	192.8	8	14
Estrone	1.413-15.27	8.418	nd-55.87	13.03	8	10
Famciclovir	nd-3.354	1.708	nd-6.118	1.109	7	3
Fenoprofen	nd-6.500	0.812	nd-387.7	50.88	1	7
Fluconazole	7.066-28.47	11.80	36.21-175.6	109.2	8	14
Gabapentin	2.195-10.49	6.717	48.98-151.8	78.51	8	14
Gemfibrozil	14.84-177.7	72.27	nd-660.1	237.2	8	13
Ibuprofen	156.5-4912	2260	1,352-10,978	4,486	8	14
Ifosfamide	nd	-	nd-0.759	0.292	-	8
Indometacin	nd	-	4.189-22.50	11.23	-	14
Isoniazide	nd	6.957	nd-9.253	12.62	-	9
Ketoprofen	nd-21.04	9.264	nd-35.57	8.138	7	13
Lamivudine	nd	-	3.112-110.2	44.61	-	14
Lidocaine	nd	-	nd-79.28	8.677	-	10

Compound	Concentration (ng ℓ⁻¹)				Frequency of detection	
	Muldersdrift se Loop		Juskei River		Muldersdrift se Loop (n = 8)	Juskei River (n = 14)
	Range	Mean	Range	Mean		
Mebendazole	nd	-	nd-0.215	0.054	-	6
Medroxyprogesterone	nd-4.238	1.417	nd-4.936	3.204	3	15
Mefenamic acid	4.726-11.52	6.451	14.93-37.25	28.59	8	14
Mestranol	nd	-	nd-51.48	10.64	-	3
Methylparaben	6.834-44.63	18.00	4.015-73.90	33.50	8	14
Metoprolol	nd	-	nd-0.789	0.191	-	9
Naproxen	26.31-96.6 8	54.21	52.19-328.8	185.9	8	14
Nevirapine	0.147-0.997	0.448	0.518-31.92	5.23	8	14
Norfloxacin	nd	-	nd-9.744	0.696	-	1
Ofloxacin	nd	-	nd-25.60	15.39	-	14
Paracetamol	41.39-414.5	181.9	nd-2441	472.1	8	13
Paraxanthine	97.08-508.6	305.3	890.5-8,452	2,777	8	14
Penciclovir	nd-16.11	9.649	28.44-47.68	35.25	5	14
Phenacetin	0.315-68.58	0.299	0.435-3.877	1.621	5	14
Pindolol	nd	-	0.071-1.391	0.612	-	14
Prednisolone	0.267-5.273	3.054	nd-16.92	8.877	8	12
Procaine	nd	-	0.084-14.51	2.550	-	14
Progesterone	nd-0.665	0.355	0.115-14.51	5.827	6	14
Ractopamine	nd		nd-0.780	0.283	-	11
Rifampicin	nd	-	nd-24.46	6.667	-	7
Ritonavir	nd-19.14	6.085	14.3-473.4	178.8	5	14
Salbutamol	nd-0.334	0.078	nd-1.546	0.118	3	2
Salicylamide	3.568-12.92	9.021	nd-39.39	16.81	8	13
Sulphadoxin	nd	-	0.143-14.22	4.901	-	14
Sulphamethazine	nd	-	Nd-2.330	0.860	-	9
Sulphamethoxazole	12.48-36.58	21.96	267.3-1082	530.1	8	16
Sulphanilamide	nd	-	nd-3.602	0.712	-	8
Terbutaline	nd	-	nd-0.976	0.228	-	9
Testosterone	nd-1.651	0.382	nd-2.471	0.359	5	3
Tonalid	0.237-1.696	0.713	0.125-24.98	10.36	8	14
Tramadol	7.496-17.27	12.76	21.71-196.8	112.1	8	14
Triclocarban	nd-21.33	6.813	nd-28.71	16.78	5	13
Triclosan	nd-3.969	1.358	1.227-38.81	16.62	4	14
Trimethoprim	1.116-4.201	2.805	1.936-157.4	81.75	8	14
Valsartan	32.10-54.33	39.58	236.1-966.6	559.4	8	14
Venlafaxine	0.464-3.891	1.894	nd-88.15	37.02	8	10
Verapamil	nd	-	nd-0.510	0.059	-	2

A closer look at the contamination of the river samples showed that WWTP effluents have significantly contributed to the contaminant load into the Juskei and Apies rivers in comparison with Muldersdrift se Loop, which is not a direct recipient of WWTP effluents (Figure 4.4). Muldersdrift se Loop was initially selected as control as it was not expected to be contaminated. However, to the researchers' surprise, they detected several environmental compounds. There are some informal settlements along the river, which may be a contributing factor to the contaminants observed, considering the type of chemicals detected. Contamination was also observed upstream of the Apies River, also suggesting possible contamination due to the non-formal settlements through which the river passes. In urban or mixed-use areas, such as those used in this study, the contaminants in surface waters can possibly be attributed to stormwater drains, septic systems and damaged sewer pipes (Schenck et al., 2015).

The contaminant load of the Juskei River, which is impacted on by the Northern WWTP, was higher than that observed for the Daspoort WWTP with more compounds detected, as well as higher concentrations recorded for most compounds. Analgesics and NSAIDs were the biggest contributor to contaminants in the Apies River and Muldersdrift se Loop.

Other compounds, mostly due to the high concentration of caffeine and paraxanthine, had the highest contribution in the Juskei River. The highest concentrations detected (>500 ng ℓ^{-1}) were for ibuprofen, paracetamol and caffeine. Estradiol had the highest concentration of 2,096 ng ℓ^{-1}. In addition to this, there may be issues of the illegal dumping of expired drugs or waste chemicals. At one of the sampling sites on the Juskei River, various bottles of ARV drugs were seen in the area close to the river (Figure 4.5).
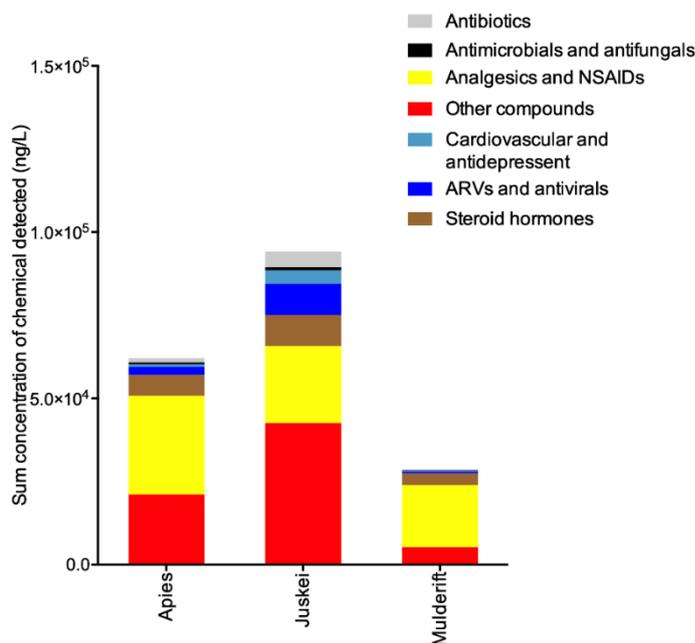
Figure 4.4:   The sum concentration of compound classes detected downstream of the Apies, Juskei and Muldersdrift se Loop rivers

Figure 4.5:   Photographs of drug bottles found next to the Juskei River sampling site

4.1.3   Comparison of study results with other studies

According to a recent review, significantly more studies are needed to better assess the presence of contaminants as the current data for developing countries is far less compared to that of developed countries. The detection of pharmaceuticals in the environment does not only vary between countries, but also between different regions of the same country. Detectable pharmaceuticals in one country or region may not appear in other countries or regions where they are not highly prescribed (Ebele et al., 2017). Differences in climate, population demographics, pharmaceutical usage statistics and sewage treatment methods highlight the need to collect data locally.

Generally, for both rivers and wastewaters spanning studies all over the world, the most frequently detected water contaminants are pharmaceutical compounds, more specifically anti-inflammatories, analgesics and antibiotics. These include paracetamol, carbamazepine, trimethoprim, ibuprofen, diclofenac, triclosan, caffeine and sulphamethoxazole, and have been detected in numerous studies (Osunmakinde et al., 2012; Bolong et al., 2009; Diaz-Cruz et al., 2009). In this study, similar results were observed. There was, however, substantial variation in the concentration levels of the detected compounds in comparison to other studies performed in Asia, Europe and the Americas. One example is that of carbamazepine, which is frequently detected in significant concentrations in Europe and other Western countries. However, its concentrations in this study were not as high. Notably, ARVs such as ritonavir, efivarenz and nevirapine were frequently detected, which is not common in other studies around the world. This can be attributed to the high HIV burden experienced in several African countries, including South Africa (Esesteban-Lor et al., 2011) compared to other regions in the world.

Removal efficiencies for the studied emerging contaminants varied widely from negative removal to almost 100%. This is in line with other studies, where removal rates are varied depending on the compound and the WWTP.

Although far fewer studies on environmental assessment and the monitoring of emerging contaminants have been conducted in South Africa compared to the rest of the world, the studies have provided great insight into the current situation in the country. The results obtained within the present work complements already published data relating to the presence of emerging contaminants in South African waters. Wood et al. (2015) conducted a study focused on the Roodeplaat Dam system, which captures the effluent water from two treatment plants (Zeekoegat and Baviaanspoort). Their study provided significant insight into the water quality in South Africa, while demonstrating the utility of low-resolution LC-MS/MS in environmental water analysis. They found that prednisolone and ritonavir had the highest concentrations of 623 and 429 µg ℓ^{-1}, respectively. They also reported that caffeine, lamotrigine and nevirapine were the most frequently detected contaminants in the water. Like our study of WWTP effluent-impacted rivers, caffeine was mostly detected and – in addition – had fairly high concentrations in surface water.

4.1.4   Identifying correlations and potential contamination markers

The WWTP effluents are significant sources of pharmaceutical residues in surface waters, where high concentrations of diverse compounds are detected. Using the quantified compounds, a Pearson correlation analysis was done to identify the relationship between the compounds and to determine the potential contaminant marker in the wastewater for further evaluation and monitoring purposes. Results of the correlation analysis revealed that a number of identified compounds had positive correlations with other compounds (Figure 4.6). For instance, carbamazepine (an anticonvulsant) showed strong relationship with fluconazole, ritonavir, fenoprofen, clarithromycin, tramadol and trimethoprim. Similarly, the compound fluconazole (an antifungal agent) exhibited positive correlations with carbamazepine, paraxanthine, ritonavir, sulphadoxin, tramadol and trimethoprim. With reference to ARVs, the compound ritonavir had the highest association with other compounds, including carbamazepine, fluconazole, paraxanthine, procaine, sulphadoxin, tramadol, trimethoprim, valsartan and venlafaxine. Studies reported that carbamazepine was one of the most persistent pharmaceuticals in the environment and generally accepted as a stable indicator of water contamination in some regions of the world (Isaacson et al., 2009). In this study, carbamazepine from anticonvulsant agents, fluconazole from antimicrobial agents and ritonavir from antiviral agents showed good correlation with other compounds. However, other factors, such as consumption rate, frequent detection rate, degradation and adsorption ability, and spatio-temporal dynamics, have also been considered in determining the potential biomarker. Overall, the results of the correlation analysis suggest that selective compounds from the identified groups can be proposed as anthropogenic tracers subject to their degradation ability and other intrinsic factors.
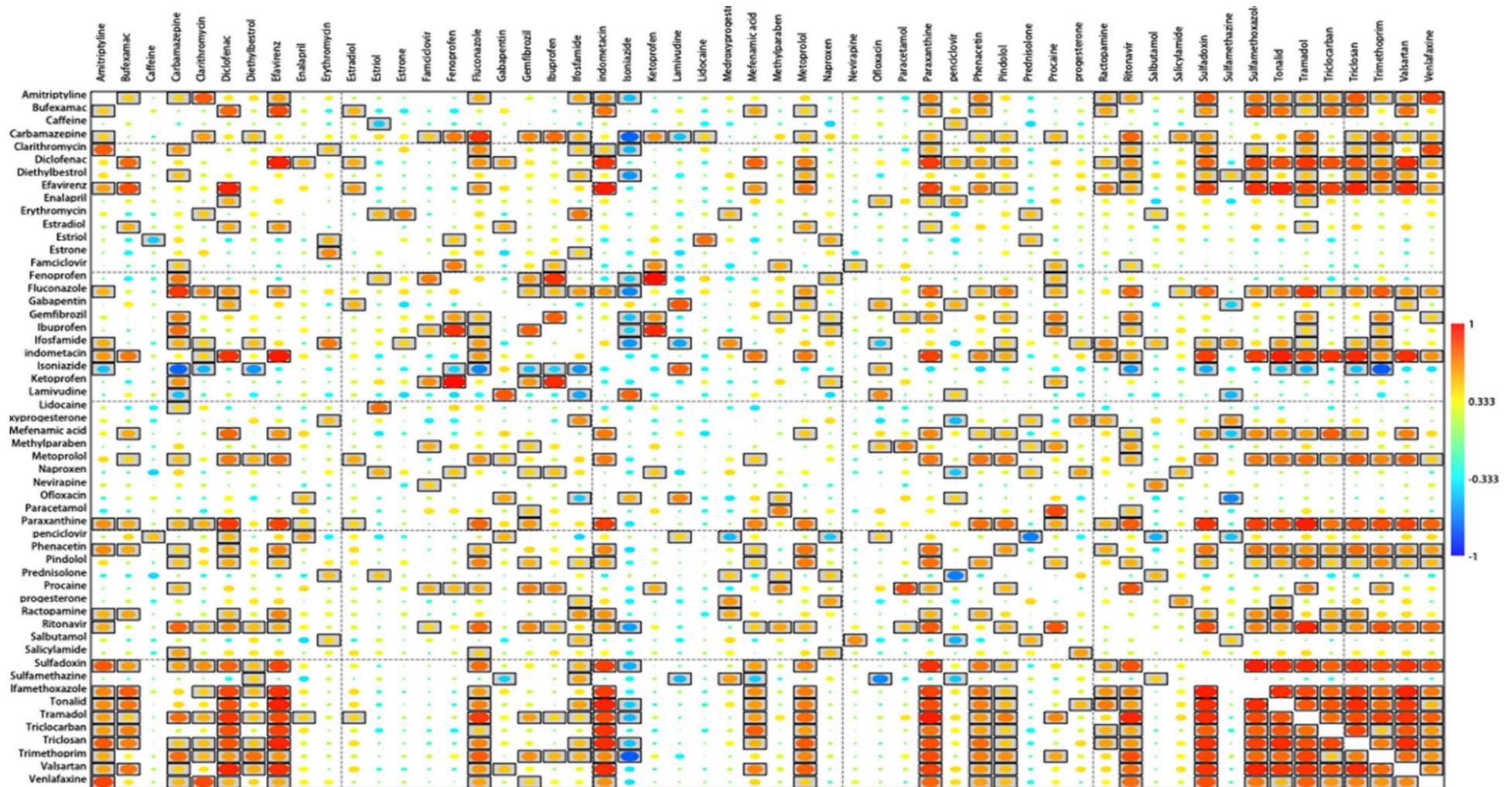
Figure 4.6: Correlations between quantified compounds and potential markers for contamination

4.1.5   Screening of contaminants in the water samples

The development of high-resolution LC-MS methods are a prerequisite for non-targeted screening. Non-targeted screening was also carried out to appreciate other contaminants that may be present in the water samples, thus providing a more realistic and broader perspective of water pollution. The developed and validated methods were extended to acquire information on the non-target compounds present in our water system.

4.1.5.1   High-resolution LC-MS analysis

The screening was conducted using the available compound database, which contained more than 1,500 multiclass compounds of interest. Contaminants were tentatively identified based on three levels: using their accurate mass, using accurate mass and isotopic patterns, and using accurate mass, isotopic patterns and – where available – fragmentation patterns. This was done to narrow down the potentially relevant contaminants that are most likely to be present in the waters and that would need to be prioritised for quantification in future studies. It is therefore important to note that this is not to say that compounds identified using fewer criteria are not present in the samples.

An average of 624 and 677 compounds were identified based on accurate mass in influent and effluent samples, respectively. Using additional qualification with isotopic patterns (with at least 50% isotopes observed) and fragmentation patterns (with at least one fragment observed), these numbers were reduced to less than 50% identified using accurate mass alone. The data for compounds confirmed was further assessed for the effluent and river water samples.
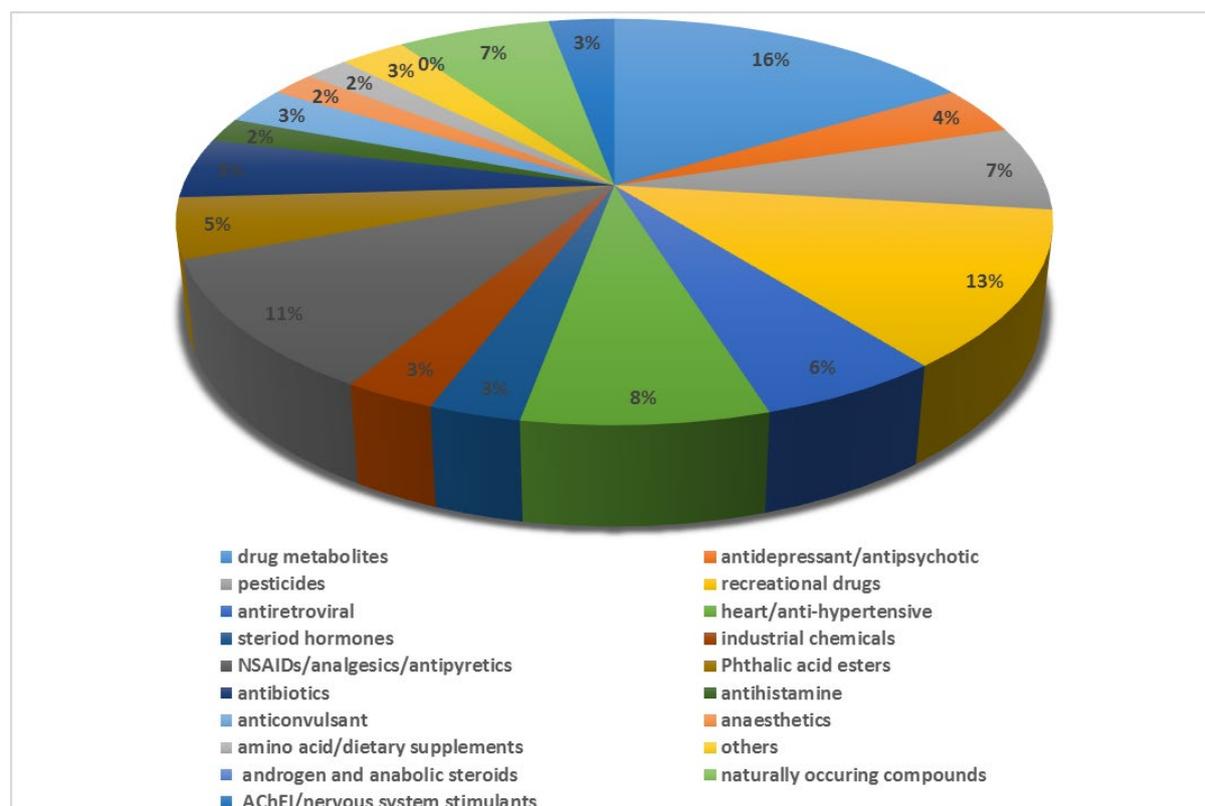
Figure 4.7:   Representation of compounds tentatively identified using suspect screening in effluent water samples

Figure 4.7 shows a representation of the compounds observed in the study that belonged to many different classes of chemicals. Drug metabolites were the major group identified in both the influent and effluent wastewater. Some examples of active metabolites detected include paraxanthine, O-desmethyltramadol and O-desmethylvenlafaxine that are metabolites of caffeine (a central nervous system stimulant), tramadol (an opiod analgesic) and venflaxine (an antidepressant), respectively. All the parent compounds were detected in the effluent water. Other metabolites identified were those of cocaine (egconine methyl ester and anhydroegconine) and anabolic androgen steroids (AASs) (e.g. 3 hydroxystanozolol and 19-noretiocholanolone) compounds. There is an overall significant concern with respect to secondary products (metabolites, degradation and transformation products) as some have been found to be present at higher concentrations (Isaacson et al., 2009) and at times exhibit higher toxicity than their respective parent compounds (Comerton et al., 2009). The other major groups of compounds were recreational and illicit drugs, which are mostly addictive psychedelics and stimulants. The only study to have quantified illicit drugs in South African waters (cocaine, mephedrone and methamphetamine) was done by Archer et al. (2017). These compounds were some of those identified in this screening, together with tetrahydrocanabinnol and dimethylcathinone, to mention a few.

Several AASs, such as stanozolol and methandionone, were identified in the influent wastewater. These compounds, together with some of the stimulants, were also identified in this study and could be found in sport and fitness supplements (Buchberger, 2011; Richardson and Ternes, 2011). Although the AASs were not identified in effluent wastewater, phendimetrazine (a stimulant and appetite suppressant), which is often used in combination with AASs, was detected in the effluent water. Other main groups identified included pesticides, ARVs, NSAIDs and drugs for heart-related conditions. As previously mentioned, ARVs are not frequently detected in more developed countries but more so in African countries due to the high HIV burden. In addition to the quantified ARVs, atanazavir, emtricibatine, nevirapine and zidovudine were also identified in the screening. Among the compounds identified, NSAIDs of interest include codeine, which has been rated as one of the most abused over-the-counter drug in South Africa (Richardson, 2011b). Other identified compounds of interest include dextrorphan. Although used primarily as an antitussive or cough suppressant, it can also be abused as it has psychoactive and dissociative hallucinogenic effects. Compounds from anaesthetics and dietary supplements were not identified in influents, but are seen in effluents. It is possible that they have been masked by other high-concentration compounds in the influent or also accumulate in the system and are hence detectable in effluents and not in influents.

Table 4.5: **Compounds identified in wastewater effluents using accurate mass, isotopic ratios and fragmentation patterns**

Classes	Compounds
Illicit or recreational drugs and their metabolites	3,4-dimethylmethcathinone, 4-carboxydihydromephedrone, 4-carboxymephedrone, 4'-methyl-alpha-pyrrolidinobutiophenone, 4-methylethcathinone, butylone, ecgonine, ecgonine methyl ester, ethylone, MDMA, MDPBP, mephedrone, methaqualone, methedrone, methoxetamine, N-ethylbuphedrone, pentedrone, cannabidiol, delta-9-tetrahydrocannabinol
AASs and their metabolites, hormonal steroids	19-norandrosterone, 19-noretiocholanolone, estrendione, stanozolol, 3- hydroxystanozolol, methandionone
Other metabolites	Paraxanthine, metanephrine, carbamazepine epoxide, 4-aminophenol, 4-butoxyphenylacetic acid, 4-acetamidoantipyrine, 10,11-dihydro-10-hydroxy carbamazepine, N-desmethyl mephenytoin N-desmethylvenlafaxine, O-desmethyltramadol, O-desmethylvenlafaxine,
NSAIDs, antipyretics, analgesics	Codeine, alminoprofen, tramadol, 4-acetamidophenol, antipyrine, levorphanol, meperidine, beta-hydroxyfentanyl
ARVs, antidepressants, antipyschotics	Atazanavir, emtricibatine, nevirapine, zidovudine, citalopram, escitalopram, amisulpride, venlafaxine
Antibiotics, antifungals, corticosteroids	Metronidazole, fluconazole, fluocinolone acetonide
Beta blockers, anaesthetics	Alprenolol, atenolol, bisoprolol, oxprenolol, lidocaine, etomidate

Classes	Compounds
Industrial chemicals	Phthalic acid, bis(2-ethylhexyl) ester, phthalic acid, bis-butyl ester, phthalic acid, bis-ethyl ester, phthalic acid, bis-iso-butyl ester, phthalic acid, bis-propyl ester, 2-acetamidophenol, 3,3-dimethoxybenzidine, benzophenone, triclosan, tricloban
Pesticides	Atrazine-2-hydroxy, benomyl, butopyronoxyl, carbofuran, isoprocarb, mexacarbate, tebuthiuron
Antihistamines, cough suppressants	Dextrorphan, fexofenadine, doxylamine, cimetidine
Anticonvulsants, heart-related treatments	Oxcarbazepine, primidone, telmisartan, theobromine, valsartan, trimetazidine, irebsartan
Others	Neostigmine bromide, phendimetrazine, verrucarol, tryptophan, L-tyrosine tranexamic acid, pseudocapsaicin, melatonin, mescaline, ethyl pentadecanoate, eugenol

Overall, the screening process provided significant information on the potential compounds of interest in wastewaters. Confirmation of the presence of these tentatively identified compounds and their quantification can be done by obtaining reference standards. This data, together with that obtained from quantitative studies, also sheds light on the health and lifestyle of the urban community whose waste feeds into this treatment plant. Most importantly, it provides information on compounds that may be of interest in future studies. In Table 4.5, the compounds identified through screening in the effluent waters are shown. This data is of great interest for future research as the effluent from the Daspoort WWTP is discharged into a river that flows through various informal settlements and is therefore a source of water for several communities.

4.1.5.2   GCxGC-HRT-MS analysis

The application of the GCxGC-HRT-MS method developed in this study on wastewater effluent samples revealed numerous compounds, in addition to those PPCPs under study. Some of these compounds may have environmental relevance. The identification of compounds used in the peak finding and library search was carried out using the ChromaToF software, and the NIST MS library enabled the positive identification of non-targeted compounds. The retention time and fragmentation patterns of hits with similarity matches equal to or greater than 900 were used to identify the compounds. The exact mass capability played an essential role in the identification (Table 4.6). The developed and validated GCxGC-HRT-MS was used for the non-targeted analysis of effluent wastewater samples. The peak identification was based on the NIST MS library and exact masses.

Table 4.6: **Non-targeted screening using the GCxGC-HRT-MS method**

Compound	Class
1-Dodecanol[#], 1-Hexadecanol[#], 1-Tetradecanol[#], 1-Undecanol[#], 2(3H)-Furanone, 5-ethyldihydro-[#], 2(3H)-Furanone, 5-heptyldihydro-[#], 2(3H)-Furanone, dihydro-5-pentyl-[#], 2-n-Butyl furan[#], Benzeneacetic acid, 4-(1,1-dimethylethyl)-, methyl ester[#], Benzene, 1-ethyl-4-methoxy-[#]Benzoic acid, 4-methoxy-[#]Cyclopentaneacetic acid, 3-oxo-2-pentyl-, methyl ester[#]Ethanone, 1-(2-furanyl)-[#], Geranic acid[#], Heptanoic acid[#], Hexadecanoic acid, methyl ester[#], Hexanoic acid[#], Hexanoic acid, 2-ethyl-[#], Hydrocinnamic acid[#], Methyl stearate[#], n-Hexadecanoic acid[#], Octanoic acid[#], Pentanoic acid, 2-methyl-[#], Phenol, 3,4-dimethyl-[#], Terpineol[α], Tetradecanoic acid[#], Triethyl citrate[#], Undecanoic acid[α,#]	Flavour ingredient
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester[#]	Plasticiser
2,2,3-trimethyl-Pentane[η], 3-methyl-Pentane[#],Methyl isocyanate[η,μ,α,β], Azetidine[α,η], Butyl isocyanatoacetate[α], (R)-(-)-4-Methylhexanoic acid[#], 1,1-Diphenylpropanol[μ], 1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl ester[α,#], 1,2-Benzenediol, o-(1-adamantancarbonyl)-o'(cyclobutanecarbonyl)-[#,α], 1,2-Benzenediol, O-(4-methoxybenzoyl)-O'-(2-furoyl)-[#], 1,2-Benzenediol, o-(4-methoxybenzoyl)-o'-(2,2,3,3,4,4-heptafluorobutyryl)-[#,α,μ,η,β], 1,2-Benzenediol, o-(4-methoxybezoyl)-o'-(5-chlorovaleryl)-[α],1,2-Dimethyl-3-formylindole[#], 1,3-Benzenediol, o-(2-methoxybenzoyl)-o'-ethoxycarbonyl-[α], 1,3-Benzenediol, o-(2-methoxybenzoyl)-o' propargyloxycarbonyl-[α,β,η], 1,3-Benzenediol, O,O'-di(2-methoxybenzoyl)-[α,β,μ],1,3-Dioxolane, 2-(4-methoxyphenyl)-2-methyl-[#], cis-1,4-Cyclohexanediamine[μ], 1,5-Diphenyl-2H-1,2,4-triazoline-3-thione[#,α], 1,6,11-Dodecatriene, (Z)-[#], 1,6-Dioxacyclododecane-7,12-dione[#,α,μ,η], 1,8,11-Heptadecatriene, (Z,Z)-[#], 1-Adamantyl bromomethyl ketone[μ,α], 1-Dodecanol, 3,7,11-trimethyl-[#], 1-Dodecanone, 2-(imidazol-1-yl)-1-(4-methoxyphenyl)-[α,β,η], 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-[#,α], 1-n-Hexyladamantane[#,μ,η,β], 1-Pyrrolidinecarboxaldehyde[#], 2(3H)-Furanone, 5-acetyldihydro-[#], 2-Methoxybenzoic acid, 4-isopropylphenyl ester[α,β,#,μ], 2-Oxo-4-phenyl-6-(4-chlorophenyl)-1,2-dihydropyrimidine[#,α,η], 2-Pentenoic acid, 4-hydroxy-[#], Acetaldoxime[α], Benzene, 1-(chloromethyl)-3-methoxy-[#,α], Benzeneacetic acid[#], Benzenemethanol, à-phenyl-[μ], Cyclopentene, 5-hexyl-3,3-dimethyl-[#], Cyclopropane, 2-(1,1-dimethyl-2-pentenyl)-1,1-dimethyl-[#], Cyclotetradecane[#], Ethanol, 2-[4-(1,1-dimethylethyl)phenoxy]-[#], Ethanone, 1-(4-methoxyphenyl)-2-(4-methyl-1,2,4-triazol-3-ylthio)-[α,β,μ,η], Guanidine[α], Hexadecanoic acid, 15-methyl-, methyl ester[#], Hexadecenoic acid, Z-11-[#], Indolizine, 3-methyl-[#], l-Leucine, N-ethoxycarbonyl-N-methyl-, pentyl ester[#], l-Norvaline, n-butoxycarbonyl-, dodecyl ester[α,μ], N-Cbz-glycylglycine p-nitrophenyl ester[#], Nonanamide[α], o-Anisic acid, 4-benzyloxyphenyl ester[α,η], o-Anisic acid, tridec-2-ynyl ester [α,β,η,#], Oxalic acid, allyl octadecyl ester[#], p-Anisic acid, morpholide [α,β,μ,η,#], Pentafluoropropionic acid, 2-(1-adamantyl)ethyl ester [α,η,#], Pentane, 2,2,3-trimethyl- [α,η], Phosphorus pentafluoride[#], Phthalic acid, 2-isopropylphenyl methyl ester[#], Phthalic acid, 4-bromophenyl hexyl ester[#], Propane, 1,2-dimethoxy-[α], Pyrimidine, 5-methyl- [α], Thieno[2,3-c]pyridine[α], trans-2-Decen-1-ol, trifluoroacetate[#], Tricyclo[5.2.1.0(2,6)]dec-3-en-10-ol[#], Tridecanoic acid, methyl ester[#], Trimethylene oxide[μ]	Others
2-Propanol, 1-(2-methoxy-1-methylethoxy)-[#,α], Pentane, 3-methyl-[α,#,μ]	Intermediate
2-Propanol, 1-(2-methoxypropoxy)- [#,α]	Indirect additives
Benzoic acid[#]	Preservative
Caffeine[#,μ,α]	Central nervous system stimulant
Cholestanol[#]	Cholesterol derivative
cis-Vaccenic acid[#], dodecanoic acid[#]	Fatty acid
Dodecanamide[α], pentanamide[α]	Fatty amide
Ethosuximide[#], valproic acid[#]	Anticonvulsant

Compound	Class
Diethyltoluamide[#,α]	Insect repellent
N,N,N',N'-tetraacetylethylenediamine[#]	Oxidant stabilisers
Phenol[#], phenylethyl alcohol[#]	Antiseptic
n-decanoic acid[#], nonanoic acid[#]	Herbicide
Ethanol, 2-(dodecyloxy)-[#] ethanol, 2-(2-butoxyethoxy)-[#], ethanol, 2-(2-ethoxyethoxy)-[#], ethanol, 2-ethoxy-[#,α]	Cleansing solvents
Tridecanoic acid[#]	Surfactants
Ethanol, 2-butoxy-, phosphate (3:1)	Flame retardants
Ibuprofen	Analgesic
Compounds detected using non-targeted screening in: [#] effluent (Daspoort WWTP), [α]Jukskei River (Heronbridge College), [μ] Apies Rivier (upstream of Daspoort WWTP), [ŋ]Apies River (downstream of Daspoort WWTP), [β]Muldersdrif se Loop	

4.1.6   Conclusions

Using the developed and validated Orbitrap high-resolution LC-MS method, in which a Waters X-Bridge column was used, 71 and 73 PPCP compounds were quantified in influent and effluent samples, respectively. Both influent and effluent samples were heavily contaminated with emerging contaminants such as caffeine, paraxanthine, ibuprofen, paracetamol, estradiol and efavirenz, which were detected at higher concentrations of greater than 1,000 ng ℓ^{-1}. In general, the compounds detected in WWTP influent and effluent samples were antibiotics, ARVs, steroid hormones, NSAIDs, anti-inflammatories, antivirals, antifungals, antidepressants, anticonvulsants, cardiovascular agents, analgesics, anthelmintics, consumer product additives and bronchodilators. Antibiotics were the predominant class detected in the WWTP influent samples, accounting for about 28% of the compounds quantified.

All three rivers under study were contaminated with emerging contaminants, including Muldersdrift se Loop, which is not linked to the WWTP. For this particular river, it can be concluded that the source was the informal settlement where waste could have been discharged directly into the river. The two rivers linked to the WWTPs were clearly highly contaminated, indicating the plant's limitations to completely remove the emerging contaminants. The contaminant load of the Juskei River (Northern WWTP), however, was much higher than for the Apies River (Daspoort WWTP), which had more compounds and at higher concentrations. Notably our water systems seem to be contaminated with ARVs such as ritonavir, efivarenz and nevirapine, in addition to the usual frequently detected emerging contaminants, which seems to be unique to the African context. This can be attributed to the high HIV burden experienced in several African countries, including South Africa, compared to other regions in the world.

Based on the Pearson correlation analysis, carbamazepine, fluconazole and ritonavir showed good correlation with other compounds, These may, therefore, constitute potential biomarkers. In addition, based on the frequent detection rate and the high concentration levels, caffeine, paraxanthine, ibuprofen, paracetamol, sulphamethoxazole, fluconazole and trimethoprim can also be considered compounds that contribute to the early warning system as possible biomarkers for contaminated water.

The non-targeted approach provides invaluable information about the status of the level of contamination. An average of 624 and 677 compounds were identified based on accurate mass in influent and effluent samples, respectively. Using additional qualification with isotopic patterns (with at least 50% isotopes observed) and fragmentation patterns (with at least one fragment observed), these numbers were reduced to less than 50% identified using accurate mass alone. Interestingly, the non-targeted GCxGC-HRT-MS approach revealed additional environmentally related compounds such as plasticisers, flavouring agents, fire retardants, herbicides, surfactants and other compounds that were present together with the emerging contaminants.

CHAPTER 5: ANALYTICAL METHODOLOGIES FOR THE DETERMINATION OF PATHOGENS

5.1 INTRODUCTION

The monitoring of the microbial quality of raw water, drinking water and recreational waters has long been deemed essential. Despite monitoring technology advances day by day, waterborne pathogens still pose a threat to public health. Most of the disease-causing organisms originate from contaminated drinking water. Microbial pathogens are harmful microorganisms and are also classified as emerging contaminants because of their potential hazard when they contaminate water. According to the WHO (2008), the mortality of water-associated diseases exceeds five million people per year. Of these, more than 50% are from microbial intestinal infections, with cholera presenting the highest number of infections (Cabral, 2010). Although a significant proportion of infections and diseases are attributed to "classic" water-related pathogens, such as those causing typhoid and cholera, newly recognised pathogens and new strains of established pathogens are being discovered (Sherchand, 2012). Over the last few decades, emerging infectious diseases caused by unidentified or known microorganisms have increased worldwide (Kot et al., 2015). Taking the above considerations into account, it is of great importance that environmental water systems be regularly monitored to ensure that they are free of harmful microorganisms.

5.2 PATHOGENS AND THEIR OCCURRENCE IN WATER

Four main types of microorganisms can be found in drinking water: bacteria, viruses, fungi and protozoa. These microbes can exist naturally or can occur as a result of contamination from human or animal waste (Health Canada, 2006). There are over 500 multiclass waterborne pathogens of potential concern in drinking waters, identified by the United States EPA through its Candidate Contaminant List (CCL 3 Universe list) (EPA, 2009). Various studies have shown that wastewater effluents into different surface fresh water sources are the major source of faecal microorganisms, including emerging pathogens. Surface waters can also be contaminated through faeces from infected domestic or wild animals, humans, agricultural waste and zoo technical areas, domestic sewage, industrial discharge and wastewaters (Semenza, 2014; Funari et al., 2007). The abundance and importance of pathogens in water depend on factors such as contamination level, the pathogens' persistence in water bodies, biological reservoirs (including aquatic plants and sediments) and the ability of pathogens to be transported through water systems (Dechesne et al., 2006).

5.2.1 Bacteria

The most commonly known bacterial waterborne bacteria include *Vibrio*, *Salmonella* and *Shigella* species, as well as *Escherichia coli* (Cabral, 2010). These have been detected in various environmental waters, including drinking water systems. There have even been reports of bacteria such as *Vibrio cholera*, *Salmonella typhimurium* and *E. coli* in bottled water (Bahrami et al., 2013; Momtaz et al., 2013; Ranjbar et al., 2016). Emerging waterborne bacterial pathogens that have raised concern over the years include *Mycobacterium*, *Helicobacter* and *Legionella* species.

Mycobacterium avium complex (*Mac*) are considered opportunistic human pathogens, particularly in people living with immune-compromised HIV and Aids conditions. *Mac* organisms have been identified in a broad range of environmental sources, including marine waters, rivers, lakes, streams, ponds, springs and piped water supplies. *Mac* organisms have been isolated from natural water and drinking water distribution systems in the USA (Von Reyn et al., 1994).

They are of concern as they can proliferate in water at higher temperatures up to 51 °C and can grow in natural waters over a wide pH range (Health Canada, 2006). Due to their high sporulating ability, they are highly resistant to chlorine and other chemical disinfectants used for the treatment of drinking water and can therefore be reduced rather than eliminated during standard drinking water treatment processes (Cabral, 2010). Unlike gastrointestinal pathogens, where *E. coli* can be used as an indicator to assume their presence, no suitable indicators have been identified to alert increasing concentrations of *Mac* organisms in water systems (Health Canada, 2006).

5.2.2   Viruses

Viruses are the intracellular and smallest of all microorganisms, and their size facilitates transport into many environmental compartments. Among the different microorganisms, viruses are best fit to become emerging pathogens since they can adapt by mutation and/or recombination and are able to infect new hosts and adjust to new environments (La Rosa et al., 2012). Enteric viruses are among the most common and most hazardous waterborne pathogens, causing both sporadic and outbreak-related illnesses. Their presence is therefore a complex problem for environmental engineers because of their prevalence, infectivity and the resistance of viruses to disinfection. Commonly observed waterborne viruses include adenoviruses, enteroviruses, noroviruses and rotaviruses. Emerging waterborne enteric viruses belong to the families *Caliciviridae* (norovirus), *Picornaviridae* (enterovirus and hepatitis A virus) and *Adenoviridae* (adenovirus). Other virus groups are potentially emerging waterborne pathogens and include hepatitis E virus, the viral agent of avian influenza, coronavirus, polyomavirus, picobirnavirus, and papillomavirus (Gall et al., 2015).

5.2.3   Fungi

Compared to bacteria and viruses, less attention has been given to fungal occurrence in aquatic environments. Recently, more attention has been drawn to the presence and identification of fungi in various drinking water sources due to its mycotoxigenic properties. Pereira et al. (2009) reported as many as 49 fungal species being detected in drinking water samples. More recently, Babič et al. (2016) conducted a study that revealed the high occurrence of several human opportunistic fungi, in particular black-pigmented yeasts *Exophiala* spp., *A. melanogenum* and white yeast *C. parapsilosis*.

5.2.4   Protozoans

In general, emphasis has only been placed on bacteria compared to other pathogens. However, the hazard posed by certain protozoan parasites is being increasingly recognised. Researchers have analysed drinking water and detected oocysts of *Cryptosporidium* and cysts of *Giardia sp.* These two protozoans are the main cause of outbreaks of diarrhoea in humans. Although the levels detected are very low and do not represent a health risk, it is still essential and important to analyse the protozoa in surface water systems.

5.3   RISKS ASSOCIATED WITH PATHOGENS IN WATER

Water that is contaminated by pathogens can be the source of large and serious disease outbreaks (Brunkard et al., 2011). Several studies have confirmed that water-related diseases not only remain a leading cause of morbidity and mortality worldwide, but the spectrum of disease is expanding and the incidence of many water-related microbial diseases is increasing (WHO, 2003).

Many health effects on humans are caused by waterborne diseases that vary in severity from mild to severe and even fatal (Marcheggiani et al., 2015). The most common illnesses associated with waterborne pathogens are gastrointestinal upsets (nausea, vomiting and diarrhoea). The course of symptoms is usually of short duration. However, in susceptible individuals such as infants, the elderly and immunocompromised individuals, the effects may be more severe, chronic (e.g. kidney damage) or even fatal.

Bacteria (such as *Shigella* and *Campylobacter*), viruses (such as norovirus and hepatitis A virus) and protozoa (such as *Giardia* and *Cryptosporidium*) are some examples of pathogens that are responsible for severe gastrointestinal illnesses. Other illnesses that can manifest include respiratory symptoms, conjunctivitis, hepatitis, central nervous system infections and chronic diseases (Health Canada, 2006).

5.4 METHODS OF ANALYSIS

To uphold the quality of water supplies, efficient and comprehensive pathogen monitoring systems are of the utmost importance. This includes the development of robust methods to accurately identify the diverse microorganisms that are present in water. Ideal methods should be sensitive, rapid and reliable to avoid delays in identifying contaminating microorganisms, as well as their source, to reduce public health risks and/or curb outbreaks.

5.4.1 Traditional microbiological testing techniques

The traditional strategies for routine microbiological testing include gram staining, colony morphology, microscopic examination, differential growth on selective media and various biochemical tests (catalase and oxidase tests), with either manual or automated methods or – in some cases – commercial kits. Furthermore, secondary phenotypic characterisations complete the microbial identification process (Carroll and Weinstein, 2007). There are several drawbacks to conventional culture assays as routine and robust detection tools for pathogens. The major drawback is that the analyses are slow (they can take between two and seven days) and labour intensive (Sartory and Watkins, 1998) as pathogens need to be cultured and enriched in selective media to isolate specific pathogens from other microorganisms. Moreover, in many instances, pathogenic concentrations may be too low for cultural detection, but may still be high enough to cause infection (Girones et al., 2010). Some studies have even highlighted the fact that the microbial load in water can be significantly underestimated using the traditional plate count method due to the presence of physiologically active bacteria that are unable to form colonies on culture media (Giao et al., 2008). Using culture-based methods, *H. pylori* has not been isolated from environmental sources, including water (Giao et al., 2008). Some pathogenic viruses, such as human noroviruses, also have no available cell line for propagation (Hamza et al., 2011a).

5.4.2 Polymerase chain reaction

Molecular diagnostics are better alternative approaches to culturing techniques for identifying pathogens. Polymerase chain reaction enables rapid bacterial identification by targeting conserved genes such as those coding for the ribosomal RNA (rRNA) of pathogens. The PCR techniques have several advantages over culturing methods. Firstly, PCR allows for the identification of slow-growing organisms and has been used to establish pathogenesis for uncultivable organisms. The PCR has been used successfully to detect the presence of *H. pylori* DNA in drinking water (Giao et al., 2008). Secondly, results are generally obtained within a short time, especially if real-time PCR (qPCR) is used. Real-time reverse transcriptase PCR (qRT-PCR) uses specific probes that generate significant information on the presence, quantity and distribution of classic and new emergent pathogens in water with a high level of sensitivity and specificity. The qPCR displays better specificity, sensitivity and reduced time requirements compared with available culture-dependent methods and has been widely and routinely used to directly detect pathogens in research and clinical diagnosis (Ahmed et al., 2014; Aw and Rose, 2012).

One disadvantage of these molecular biology-based identification techniques is the targeted approach for specific microbial genera or species. Therefore, multiple pathogens can be monitored at a time (Plummer and Long, 2007). Even though PCR is a very sensitive detection technique, it faces challenges with visual identification. The reason behind the challenge of viral identification is the low concentration of viral particles in environmental water and their extraction procedures.

It is a prerequisite that viral pathogens are subjected to a concentration step before the PCR can be done (Girones et al., 2010). Another disadvantage of direct PCR is the ability to detect naked nucleic acids, infectious and non-infectious pathogens. Consequently, direct PCR does not allow for discrimination between infectious and non-infectious viral particles (Hamza et al., 2011b).

5.4.3 Pyrosequencing techniques

Pyrosequencing technology is a revolutionary technique based on DNA sequencing, utilising enzyme-coupled reactions and bioluminescence to monitor the pyrophosphate release that accompanies nucleotide incorporation (Niedringhaus et al., 2011). Unlike PCR, where scientists are limited by known sequence information and must select the pathogens to be considered in each assay, a high-throughput sequencing approach is unbiased and makes it possible to detect novel pathogens. Sequencing technology also has the potential to provide an unbiased detection approach for waterborne pathogens with a single common protocol (Niedringhaus et al., 2011). There are several recent articles reporting the application of pyrosequencing to investigate the diversity of bacterial and viral pathogens in environmental samples (Ye and Zhang, 2011; Kristiansson et al., 2011; Djikeng et al., 2009).

There are commercially available, high-throughput sequencing platforms for the study of microbial diversity in environmental waters such as the Roche 454 pyrosequencing Solexa/Illumina Genome Analyzer, Applied Biosystem SOLiD Sequencing and the Ion Torrent system (Niedringhaus et al., 2011). Although studies are promising, high-throughput sequencing platforms are exploratory and in their infancy for the direct detection of pathogens in water with many technological and methodological challenges that need to be overcome.

5.4.4 Mass spectrometry techniques

Mass spectrometry has emerged as a powerful tool for analysis and proteomics research and the first attempts at utilising it for the characterisation of organisms were made in 1975 (Anhalt and Fenselau, 1975). The soft ionisation Matrix-assisted Laser Desorption Ionisation time of flight mass spectrometry (MALDI-ToF-MS) was particularly useful for large biomarkers (Tanaka et al., 1988). As such, MALDI-ToF-MS can be used to characterise a wide variety of microorganisms, including bacteria, fungi and viruses from water (Siegrist et al., 2007; Lartigue, 2013; Chui et al., 2015; Clark et al., 2013; Cabrolier et al., 2015; Mansson et al., 2015; Welker, 2011).

MALDI-ToF-MS identifies microorganisms by analysing the total protein and generating mass spectra from whole cells and their comparison to reference spectra (Bizzini and Greub, 2010; Fenselau and Demirev, 2001). Two general approaches are used when characterising microorganisms using MALDI-ToF-MS. The first approach is fingerprinting intact microorganisms where intact cells are used to generate unique spectral fingerprints that can be compared with previously collected fingerprints. This is because spectral fingerprints vary between microorganisms and the spectra obtained are reproducible if the bacteria are grown under the same conditions (Carbonnelle et al., 2011). This approach is relatively simple, as it is possible to use minimally processed intact cells.

The second approach is the bioinformatics-enabled approach often referred to as MALDI-ToF-MS biotyping (MTB). Here, masses associated with an unknown microorganism can be identified by comparing them with masses of proteins in protein databases (Demirev et al., 1999). Software algorithms are used to compare the spectra and to generate numerical similarity measures (inter-spectral distances and scores) between experimental and database spectra. These score values are arranged in a ranking list and the best matching database entry is used to determine the identity of the microorganism (Sauer and Kliem, 2010).

Some studies have evaluated MALDI-ToF-MS in microbiology laboratories for routine use. In one study, MALDI-ToF-MS systems (Microflex-Bruker Daltonics/BioTyper™ and Axima-Assurance-Shimadzu/ SARAMISAnagnosTec) were assessed for bacterial identification. Focusing on bacteria that are normally difficult to identify routinely, 296 strains were identified by molecular biology techniques as the gold standard. The MALDI-ToF-MS identification provided the correct results at genus and species level for 94.9% and 83.4%, and for 83.8% and 65.9% of strains with Biotyper and Saramis, respectively (Carbonnelle et al., 2012). Microbial identification protocols using MALDI-ToF-MS are commercially available for various users. These include experimental procedures for microbial cultivation, sample preparation and MS data acquisition, as well as customised mass spectrometers, dedicated software solutions and validated databases that contain mass spectra from several thousands of microbial reference strains (Lasch et al., 2016). In conclusion, identification by MALDI-ToF-MS is well suited and effective in identifying pathogenic microorganisms in routine laboratories, replacing the traditional biochemical or molecular techniques (Nomura, 2015).

5.4.5 Automated online microbial analysers

There is also the challenge that faecal pollution events can hit randomly, so not all incidents are recorded by the fixed testing scheme before the pathogens enter the distribution network. For water safety, the time delay by manual sampling and analysis, combined with testing frequency, can be crucial. An alternative to this, and as a supplement to the testing already required by water authorities, is a fully automated online instrument monitoring system. In this setup, a system is set up at the water source to automatically take samples and analyse them in much less time than traditional methods take.

The Vienna Water Monitoring Solutions (VWM) ColiMinder® is a relatively new technology based on an automated, online microbial analyser that allows the rapid and reliable measurement of bacterial load in liquid samples such as water. The ColiMinder® is an alternative method to detect microbial contamination. The technology is based on the direct measurement of the enzymatic activity of target organisms, giving a measurement of *E. coli*, coliform bacteria, enterococci and bioburden. The ColiMinder® uses the metabolic activity of target organisms (specific enzymatic activity) present in the sample as a measure for how many living *E. coli* are present per volume of sample to determine the level of contamination and risk.

The approximate measurement time for the ColiMinder® is 15 minutes, followed by a nine-minute cleaning cycle. Both continuous and interval working modes are available. Within the continuous mode, it can perform up to 84 measurements per day, depending on the cleaning program. The interval mode enables it to manually set the time between measurements. Furthermore, as there are two sample intakes (more by request), this makes it perfect for process monitoring applications. The interval mode can alternate between sample intakes.

The power here is in the "speed" of the process and the fact that it measures the actual "live activity", which makes this a great method for fast screening and process control. Its main advantage is that it offers fast and efficient analysis of the bacterial contamination of water. Where classic laboratory methods need up to 72 hours to detect strains of known *E. coli* that are indicators of faecal contamination, the ColiMinder® is fully automated and can directly analyse the water, letting operators know if there is bacterial contamination within 15 minutes. In addition to product safety, the economic benefits mean a savings potential of up to 50% of processing costs.

The main disadvantage is that the system can only be used for bacterial analysis, i.e. for faecal contamination, coliform bacteria and total bacteria. Its application has not been expanded to other pathogens. However, this technology is still very valuable as most pathogens that travel through water and cause diseases in humans are faecal in origin and the water industry often tests for a few groups of bacteria that act as indicators of faecal pollution. These should be sufficient to "raise the alarm" and be the basis of early warning monitoring systems. Some research has already shown the potential of such systems in monitoring environmental samples (Madrid et al., 1999).

5.5 EXPERIMENTAL PROCEDURES

5.5.1 DNA extraction and PCR from wastewater samples

Influent (16 samples) and effluent (eight samples) collected for metagenomic analysis were initially filtered using 1.6 μm pore-sized GF/A filters to remove solid impurities, followed by filtering through 0.22 μm pore-sized polyethersulphone membrane filters (Millipore, USA), using a peristaltic pump as required to concentrate the microbial cells. After filtration, the membrane filters were suspended in 50 mℓ of phosphate saline buffer (PBS) and centrifuged at 12,000 rpm for 5 minutes at 4 °C. Cell pellets were collected and resuspended in Tris-EDTA (TE) buffer (pH 8.0) and subjected to total DNA extraction using the Soil/Fecal Quick *g*-DNA Extraction Kit™ (Zymo Research Corporation, USA) according to the manufacturer's protocol. The eluted DNA was assessed for purity on 1.0% agarose gel and then quantified using a Qubit 2.0 Fluorometer (Thermo Scientific, USA). The PCR was performed on the extracted DNA samples using the universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) (Saiki et al., 1988) (5′-GTATTACCGCGGCTGCTG G-3′) (Muyzer and Stams, 2008), targeting the variable region V1-V3 of the 16S ribosomal DNA. The PCR reactions were prepared using 25 μℓ of one *Taq* 2X Master Mix, 22 μℓ of nuclease-free water and 1.5 μℓ of both forward and reverse primers at a concentration of 0.2 μM and 2 μℓ of extracted DNA (50-100 ng μℓ$^{-1}$). Following that, a thermal cycler program was used for the 16-second rRNA gene amplification, with an initial denaturation step at 95 °C for 10 minutes, followed by 32 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 1 minute, and final extension at 72 °C for 10 minutes. The PCR amplicons were purified using a DNA Clean and Concentrator Kit (Zymo Research Corporation, USA) according to the manufacturer's instructions.

5.5.2 Next-generation sequencing analysis

The resulting PCR product was cleaned following the manufacturer's instructions using AMPure XP beads (Beckman Coulter, Agencourt Bioscience Corporation, Massachusetts, USA). After purification, the Illumina sequencing adapters and dual-index barcodes were added to the amplicon targets using the full complement of Nextera XT indices (Illumina Inc., San Diego, California, USA) through a limited PCR cycle as follows: 95 °C for 3 minutes, eight cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes, then keeping it at 4 °C. The resulting PCR product was cleaned again following the manufacturer's instructions using AMPure XP beads. The PCR products were validated using the Bioanalyzer DNA 1000 chip (Agilent, Santa Clara, California, USA). The expected size of the final library is ~630 bp. The pooled final DNA library was sequenced on an Illumina MiSeq System using paired 300 bp reads to generate high-quality, full-length reads of the V3 and V4 region. Finally, the fastq files were obtained for further bioinformatics analysis.

5.5.3 Sequence data analysis

The obtained raw sequence datasets were analysed using the Mothur pipeline v.1.40.0 (Schloss et al., 2009). Sequence reads containing less than 50 nucleotides, reads with more than 2% of ambiguities or 7% of homopolymers were excluded during analysis. Likewise, sequences that belong to mitochondrial and chloroplast origins were also excluded from the analysis. Chimeric sequences were removed using the UCHIME algorithm according to the *de novo* method (Edgar et al., 2011). Non-chimeric 16S rRNA reads were later classified to the genus level using the Naïve Bayesian classifier algorithm (Wang et al., 2007; Cole et al., 2009) with a confidence threshold of 80% to assign the taxonomic identity of bacteria. Furthermore, the sequence datasets were aligned against the SILVA 16S rRNA database version 128 (Quast et al., 2013) and a pairwise distance matrix (Euclidean distance matrix) was created from the curated aligned datasets to group sequences into OTUs at a sequence similarity of 97% for genus level identification. The non-parametric diversity indices, including the Shannon-Weaver index and the Chao1 richness estimator, were calculated at the genetic distance of 0.03 to measure the diversity of bacterial species among the data sets.

The percentage of relative abundance of individual taxa within each community was estimated by comparing the number of sequences assigned to a specific taxon against the number of total sequences obtained for that sample. The identified dominant OTUs at genus level were used to generate a heat map to visualise the variations in influent and effluent bacterial community structure and their distribution. The sequence datasets were submitted to the Sequence Read Archive (SRA) library of the National Centre for Biotechnology Information (NCBI).

5.5.4   Biomarker analysis

The linear discriminant analysis (LDA) effect size (LEfSe) pipeline (http://huttenhower.sph.harvard.edu/galaxy) (Segata et al., 2011) was used to identify differentially abundant features among the influent and effluent samples. The differential features were identified on the OTU level (relative abundance >1%). The non-parametric factorial Kruskal-Wallis (KW) rank sum test was used to detect taxa with significant differential abundances. The LDA was used to evaluate the effect size of each differentially abundant trait. The LEfSe analysis performed under the alpha value for the KW test is <0.05, and the threshold on the logarithmic LDA score for discriminative features is >2.0 (Zhang et al., 2012).

5.5.5   Functional prediction and CCA analysis

To understand the potential genetic capabilities of the wastewater bacterial communities, the PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) software package was used, as described by Langille et al. (2013). Greengenes (May 2013 release) was used to classify OTUs, and their abundances across the samples were used to infer the functional profiles of the bacterial communities based on a constructed phylogenetic workflow of 16S rRNA marker gene sequences. The abundance of the classified OTUs was first normalised by copy number by dividing each OTU by the known 16S copy number abundance prior to functional predictions. Following the normalisation, prediction was performed by first removing the influence of the 16S marker gene copy numbers in the species genomes and obtaining KEGG Orthology (KO) information and KO abundance corresponding to the OTUs. The Nearest Sequenced Taxon Index (NSTI) value was used to validate the reliability of predicted functional and metabolic pathways. The predicted relative abundances of genes were plotted using a heat map. Canonical correspondence analysis was performed using PAST software (Hammer et al., 2001). Identified antibiotic concentrations and bacterial members were used for CCA analysis to identify the relationship between them.

5.6   RESULTS

A total of 260,291 quality filtered reads were obtained from the collected wastewater samples after the removal of PCR artifacts, and chimeric sequences were used further in the present investigation. As for bacterial diversity, the result showed 35 phyla and 566 genera across the collected wastewater samples. The quality reads of bacteria were distributed into 18,682 OTUs from all samples. With reference to the effluent samples, Effluent 3 recorded the highest number of OTUs (2,129). The lowest number of OTUs (104) was observed in Effluent 8. With reference to the influent samples, Influent 9 exhibited more OTUs (1,660) than any other sample (Figure 5.1). An Alpha Diversity Index such as the Chao1 index, used as an expected OTU richness estimator, showed the lowest OTU richness to be found in Effluent 8 wastewater, and the highest OUT richness to be found in Effluent 5. When compared to the influent samples, Influent 15 recorded the lowest OTU richness, and Influent 1 recorded the highest OUT richness. The overall results suggested that the effluent samples had a higher OTU richness than the influent samples (Figure 5.2).
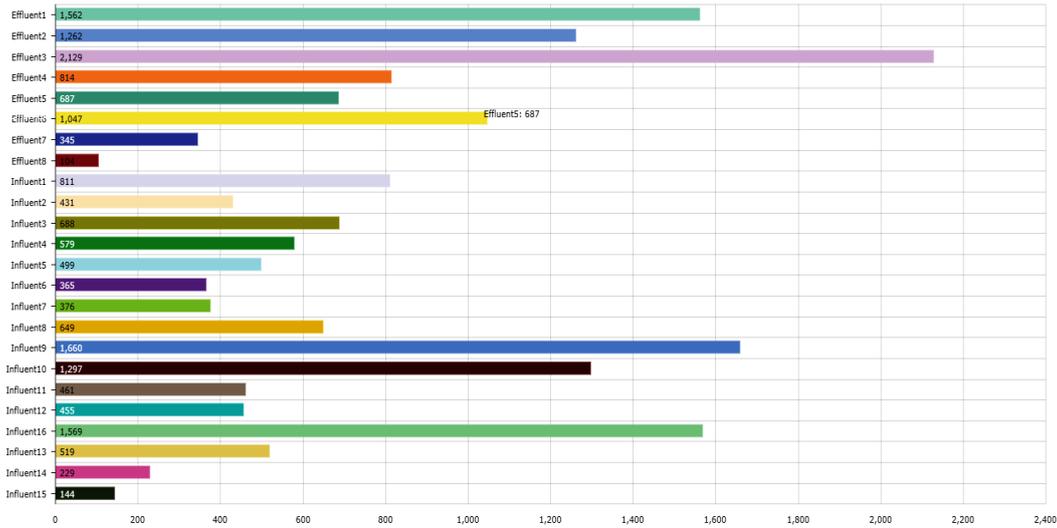
Figure 5.1: The OTUs observed across all collected wastewater samples
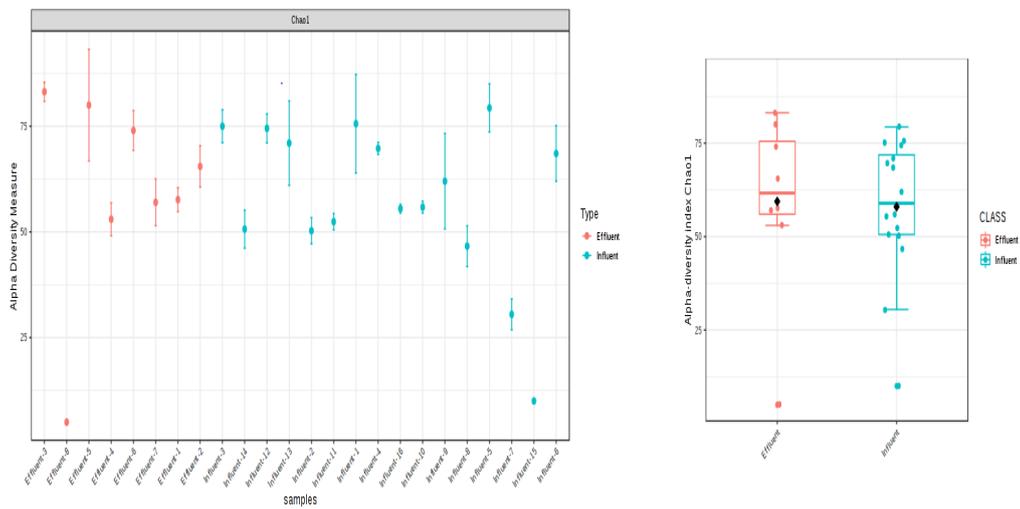
Figure 5.2: The OTU richness estimator (Chao1 index) across the collected water samples

Similar to Chao1, the diversity index, estimated by the Shannon-H index, showed the highest diversity index to be in the influent samples and the lowest diversity index to be in effluent wastewater. Individual results explained that Influent 3 had the highest diversity index (83.15) and Effluent 8 had the lowest diversity index (5.0) (Figure 5.3). To understand beta diversity more clearly and compare the bacterial communities, a phylogeny-based weighted Unifrac distance analysis and Principle Coordinate Analysis (PCoA) plot were used.
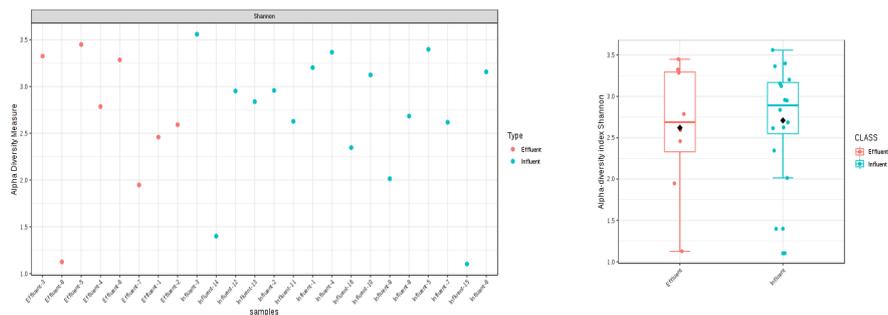
Figure 5.3: The diversity indices (Shannon-H index) of the collected water samples

The β-diversity analysis using UPGMA clustering revealed that the bacterial communities in the 12 samples could be clustered into two main groups. In the first group, Influent 13, Effluent 7 and Influent 14 were grouped together. In the second group, Influent 15 and Effluent 8 were grouped together (Figure 5.4a). In other words, bacterial communities of the samples collected on 1 and 8 November were similar to each other, while the remaining samples were clustered together in the second main group. Within the second main group, two subgroups were clustered together, for example, the bacterial communities of the samples collected in April and July. Influent samples collected in July and effluent samples collected in July and November were similar in bacterial diversity. Furthermore, the PCoA plot explained 58.6% of the observed variation, with the first axis explaining 38.5% and the second axis explaining 20.1% of the variation respectively (Figure 5.4b). Results of the PCoA based on Bray-Curtis similarities confirmed that Effluent 1, Influent 6, Influent 5, Effluent 3, Influent 1, Influent 3, Influent 11, Effluent 6, Effluent 5, Effluent 4, Influent 4 and Influent 10 were positively correlated to each other and clustered together.
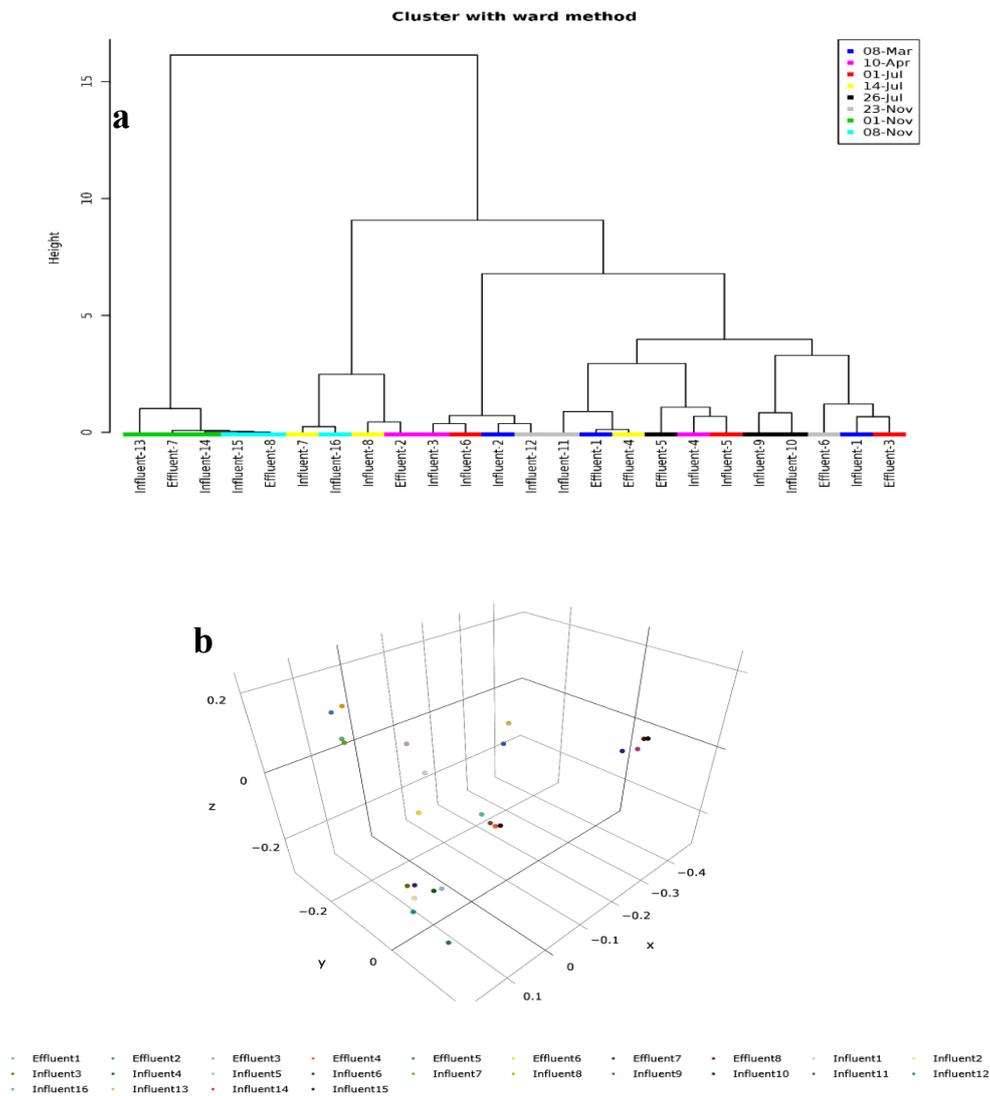
Figure 5.4: **(a) a hierarchical cluster analysis (UPGMA algorithm) dendrogram of 24 samples; and (b) principle coordinate analysis based on Bray-Curtis similarities**

Phylogenetic classification revealed the distribution of 35 phyla across all collected samples. Of these, the four most dominant phyla were, in order of magnitude of dominance, *Firmicutes,* whose relative abundance ranged from 0.83% in Influent 15 to 75.67% in Effluent 5; *Proteobacteria,* which ranged from 1.59% in Effluent 7 to 84.49% in Effluent 8; *Actinobacteria,* which ranged from 0.26% in Influent 13 and Effluent 7 to 37.16% in Influent 9; and *Verrucomicrobia,* which ranged from 0.1% in Influent 12 to 4.09% in Effluent 14. Furthermore, substantial reads belonging to the phyla *Bacteroidetes* (0.2-14.63%), *Fusobacteria* (0.02-3.12%) and *Cyanobacteria* (0.01-1.15%) were also identified among all wastewater samples. The distribution of the bacterial phyla obtained from different influent and effluent samples are given in Figure 5.5. Sequences belonging to some minor phyla with lower frequencies were also found and are given in Supplementary 1.
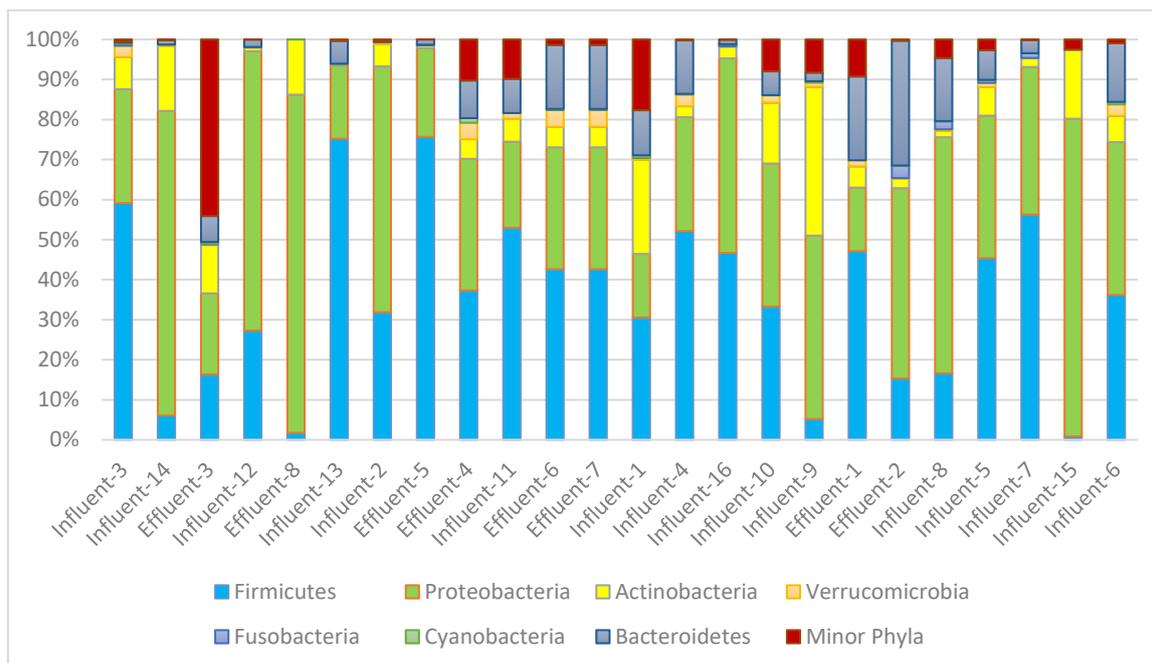
Figure 5.5: **The relative abundance of bacterial phyla obtained from collected water samples (members of minor phyla include other smaller phyla given in the supplementary)**

For comprehensive and detailed scrutiny, the researchers restricted in-depth analysis of the sequence data to the 10 OTUs displaying the highest richness in each sample. A total of 31 different OTUs were among the top 10, as shown in the heat map in Figure 5.6. The major OTUs that belong to genera in different influent and effluent samples are as follows: *Pseudomonas* (Influent 2, Influent 12 and Effluent 5), *Clostridium* (Effluent 1 and Influent 5), *Phenylobacterium* (Effluent 1 and Influent 9), *Mycobacterium* (Influent 9), *Polynucleobacter* (Influent 10), *Planctomyces* (Influent 11), *Turicibacter* (Effluent 5 and Effluent 6), *Sarcina* (Influent 4, Influent 10, Effluent 3, Effluent 4 and Effluent 6), SMB 53 group (Influent 1, Influent 4, Influent 11 and Effluent 3,4,5), *Roseomonas* and *Methylobacterium (*Influent 14, Influent 15, Effluent 7 and Effluent 8), *Bacteroides* (Influent 1, Influent 8, Effluent 2 and Effluent 3), Leptotrichia (Effluent 2), members such as *Acinetobacter, Enhydrobacter*, *Paracoccus, Blautia, Collinsella, Clostridium, Comamonas, Streptococcus, Enhydrobacter, Rumnococcus, Desulphovibrio,* which were high in Influent 16, Microbacterium (Effluent 7, Effluent 8, Influent 14, and Influent 15), *Rhodobacter* (Influent 11 and Effluent 1), *Akkermansia* (Influent 3 and Influent 14), *Janthinobacterium* and *Carnobacterium* (Influent 12) and *Paulidibacter* (Influent 6).
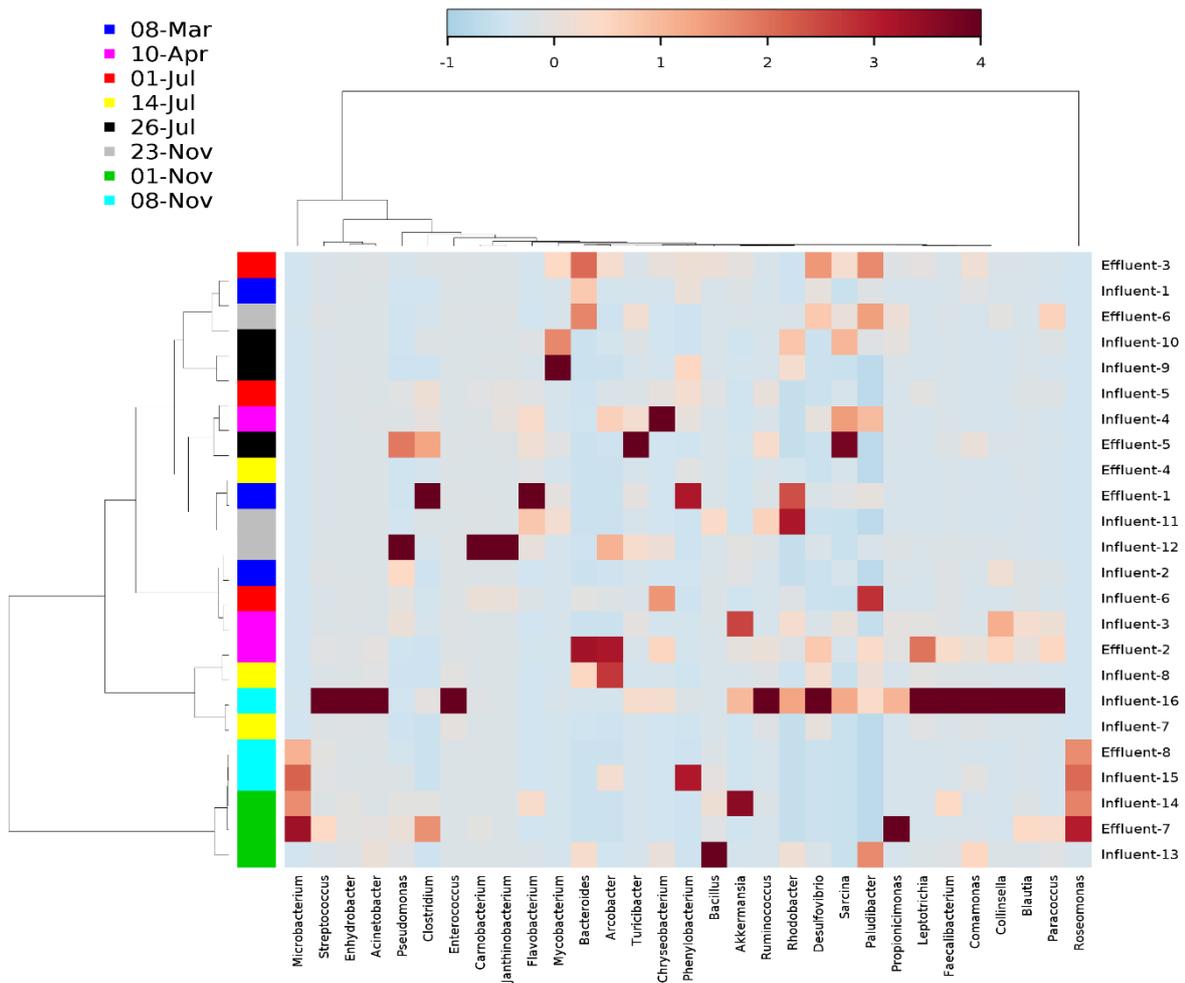
Figure 5.6: A heat map indicating the clustering of the top 10 OTUs representing genera from collected water samples. The colour indicates the relative abundance of OTUs in the samples.

To assess correlations among the dominant bacterial OTUs within wastewater samples across the different sampling sites, a Pearson correlation analysis was performed (Figure 5.7). Among the 31 dominant bacterial OTUs (based on 16S rRNA bacterials), varied positive and negative correlation patterns were observed despite random distribution at different sampling sites. For example, a significant and positive correlation was observed among *Enhydrobacter*, *Enterococcus*, *Actinobacter*, *Faecalibacterium*, *Comamonas, Collinsella*, *Bautia*, *Streptococcus*, *Paracoccus*, *Leptotrichia* and *Desulphovibrio* genera. In contrast, the same genera showed a moderate negative correlation with *Arcobater*, *Mycobacterium*, *Carnobacterium*, *Janthinobacterium*, *Clostridium*, *Pseudomonas*, *Clostridium*, *Flavobacterium*, *Microbacterium* and *Roseomonas*. However, the results showed that there were more negative than positive correlations between the different dominant OTUs across the different samples.
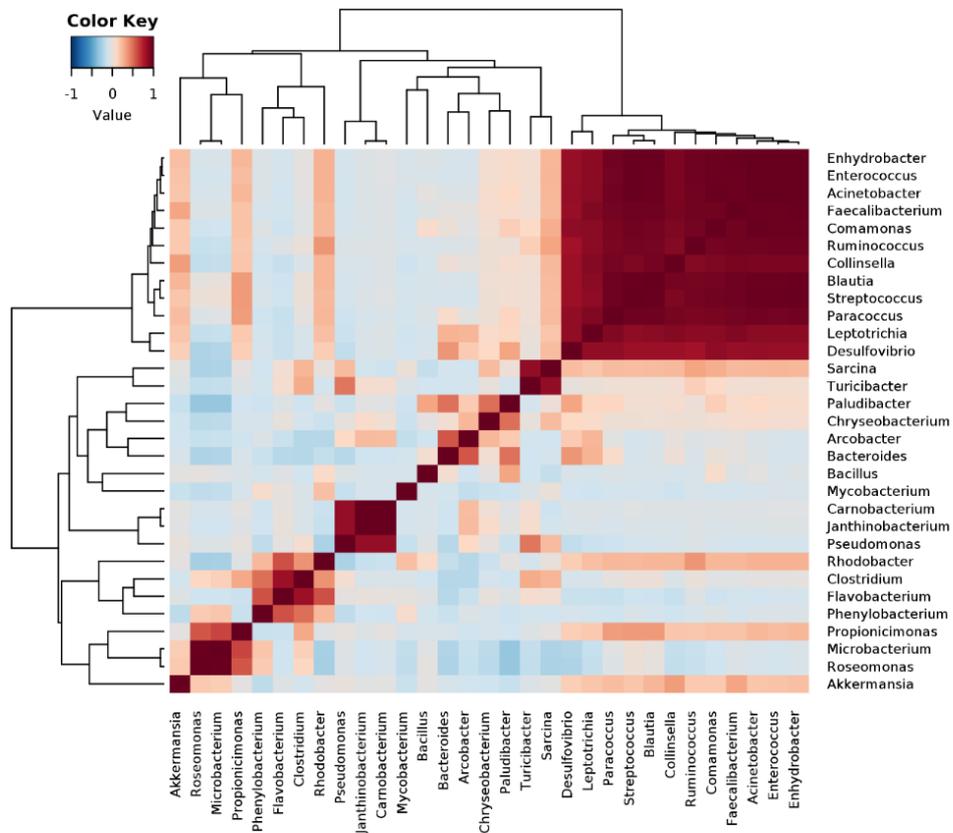
Figure 5.7: **A heat map indicating the Pearson correlation matrix of the top 10 OTUs representing genera from the collected water samples**

The differentially abundant features among the influent and effluent samples were identified by LEfSE analysis (Figure 5.8). The significantly differential abundant genera in influent samples are *Roseomonas* (LDA 5.45)*, Clostridium* (LDA 5.43)*, Methylobacterium* (LDA 5.14)*, Turicibacter* (LDA 4.27)*, Paracoccus* (LDA 3.74)*, Sarcina* (LDA 3.35) and *Bacteroidetes* (LDA 2.6), while the differential abundant genera in effluent samples are *Actinomyces* (LDA -2.73)*, Phenylobacterium* (LDA -3.13)*, Akkermansia* (LDA -3.99)*, Collinsella* (LDA -4.07)*, Neisseria* (LDA -4.4)*, Planctomyces* (LDA -4.67)*, Polynucleobacter* (LDA -4.86)*, Streptococcus* (LDA -4.96)*, Acinetobacter* (LDA -4.97)*, Enhydrobacter* (LDA -5.2)*, Mycobacterium* (LDA -5.29) and *Pseudomonas* (LDA -5.75).
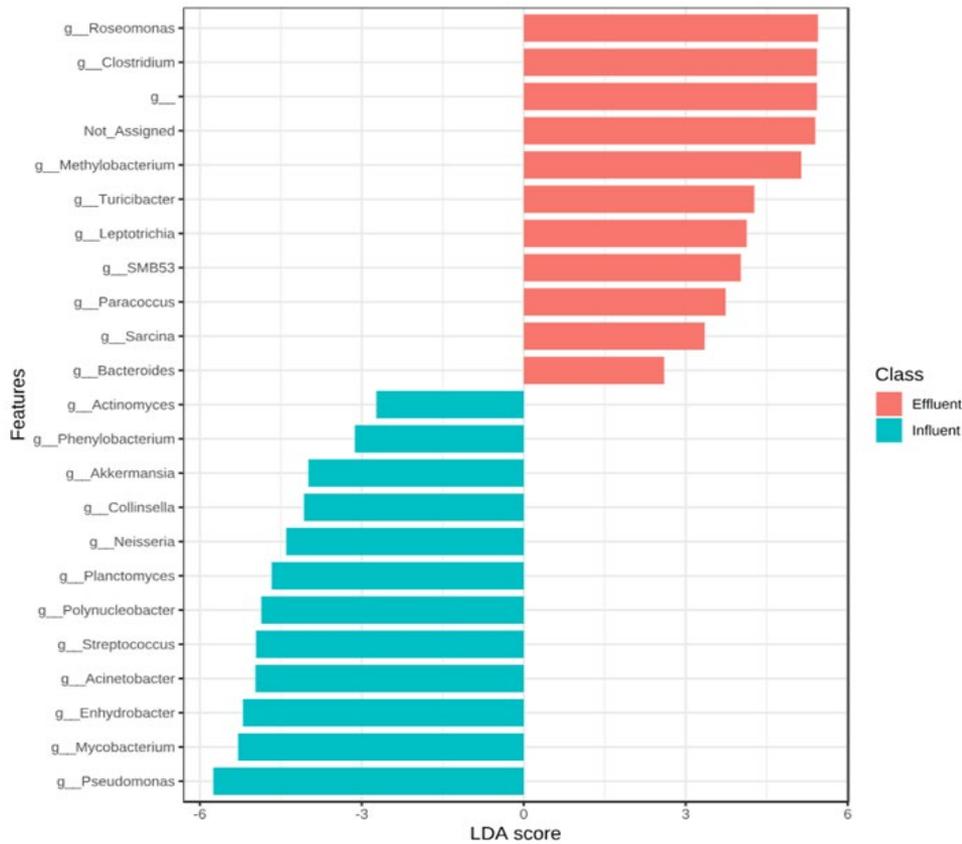
Figure 5.8: Linear discriminant analysis effect size analysis of influent and effluent samples

To identify the pathogenic bacteria present in the different effluent samples, the OTUs were automatically mapped from taxonomy to phenotype using approximately 20 different phenotypic categories in the METGENassist online tool. The results of phenotypic characterisation explained that most of the OTUs are not classified under pathogens and remain unknown. However, some of them are classified as pathogens, for example the effluents collected on 14 July 2018 carried a higher number of pathogens (33.7%) and the effluent sample collected in November recorded the lowest number of pathogens (15.4%). The complete characterisation of pathogenic abundance from the effluent samples is given in Figure 5.9.
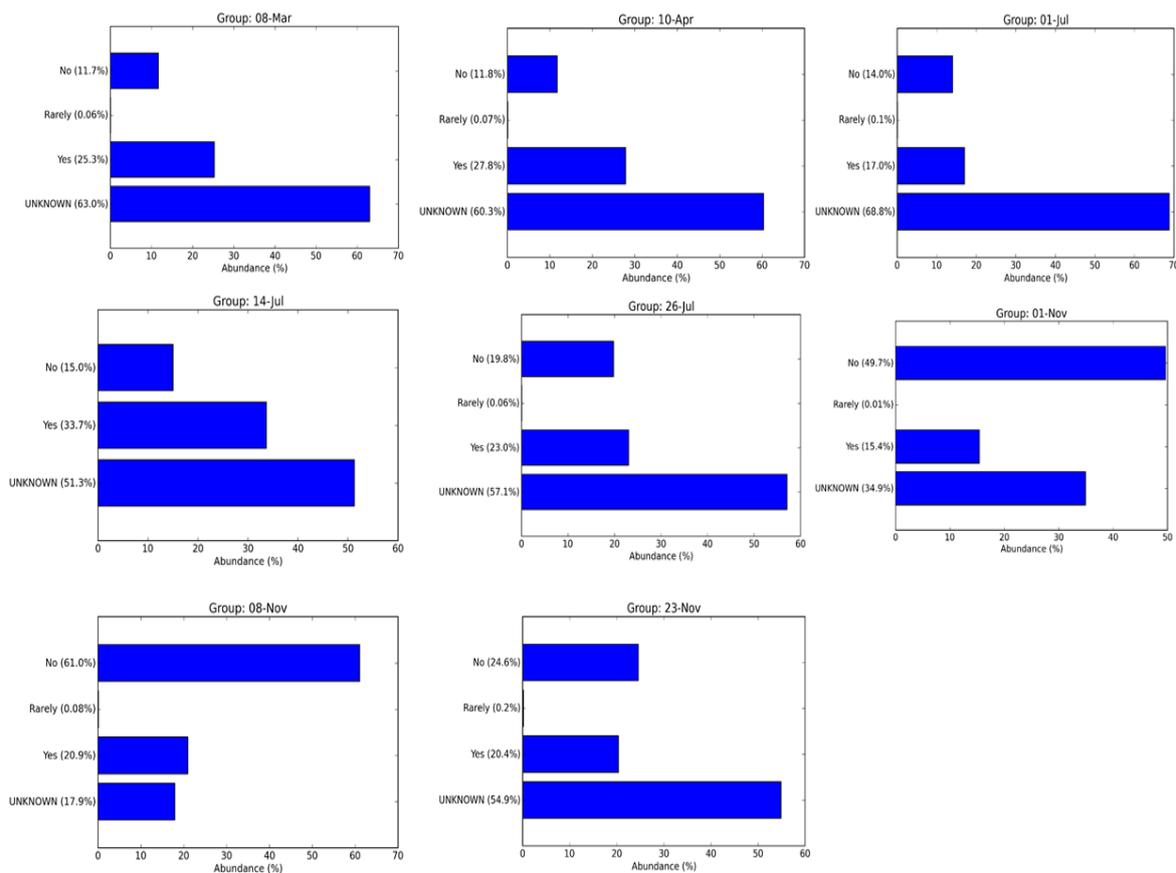
Figure 5.9: **The taxonomy to phenotype map of the pathogenic categories of collected effluent samples using METGENassist**

Functional capabilities of bacteria present in both influent and effluent samples were predicted using PICRUSt analysis. The obtained NSTI value was low (0.05-0.17), indicating that the prediction was accurate, as previously described by Hammer et al. (2001). A breakdown of all predicted metagenomes into KEGG pathways showed that Influent 12, Influent 13, Influent 14, Influent 15, Influent 16, Effluent 5, Effluent 7 and Effluent 8 had the highest number of KEGG pathways, while the other samples had the least KEGG pathways. The most abundant predicted pathways are presented in Figure 5.10. The genes most associated with amino acid metabolic pathways were for pyruvate metabolism, purine metabolism, histidine metabolism, alanine aspartate and glutamate metabolism, D glutamine and D glutamate metabolism, and arginine and proline acid metabolism. In addition, high relative abundance of carbohydrate metabolism was identified across all samples. Besides the metabolic pathways, the genes responsible for genetic information processing were identified, which includes ribosome biogenesis, transcription and translation factors. Other important identified functional interactions included ABC transporters, ion-coupled transporters, DNA repair and recombination proteins, as shown in Figure 5.10.

Canonical correspondence analysis was carried out to identify the relationship between antibiotics and bacterial communities identified in the wastewater samples. Twenty-two antibiotics belonging to the different groups were identified in both influent and effluent samples and were considered for the analysis. The major group of antibiotics was the sulphonamides, which included sulphabenzamide, sulphacetamide, sulphadimethoxine, sulphamethazine, sulphamethoxazole, sulphamonomethoxine, sulphanilamide, sulphapyridine, sulphaquinoxaline and sulphisoxazole. Figure 5.11a explains the CCA analysis between sulphonamides and the identified bacterial phylum.

The CCA axis 1 explains 59.44% of the variance, while the CCA axis 2 explains 23.68% of the variance in the bacterial-antibiotic (sulphonamides) relationship. Bacterial members such as *Proteobacteria* and *Actinobacteria* were strongly correlated with sulphadimethoxine, sulphamonomethoxine, sulphadimethoxine and sulphabenzamide compounds. While the compounds sulphanilamide, sulphisoxazole and sulphamethazine were permitting, the bacterial members belonged to *Firmicutes* in the wastewater system. Sulphapyridine has no effect on any of the bacterial members identified in the wastewater system.
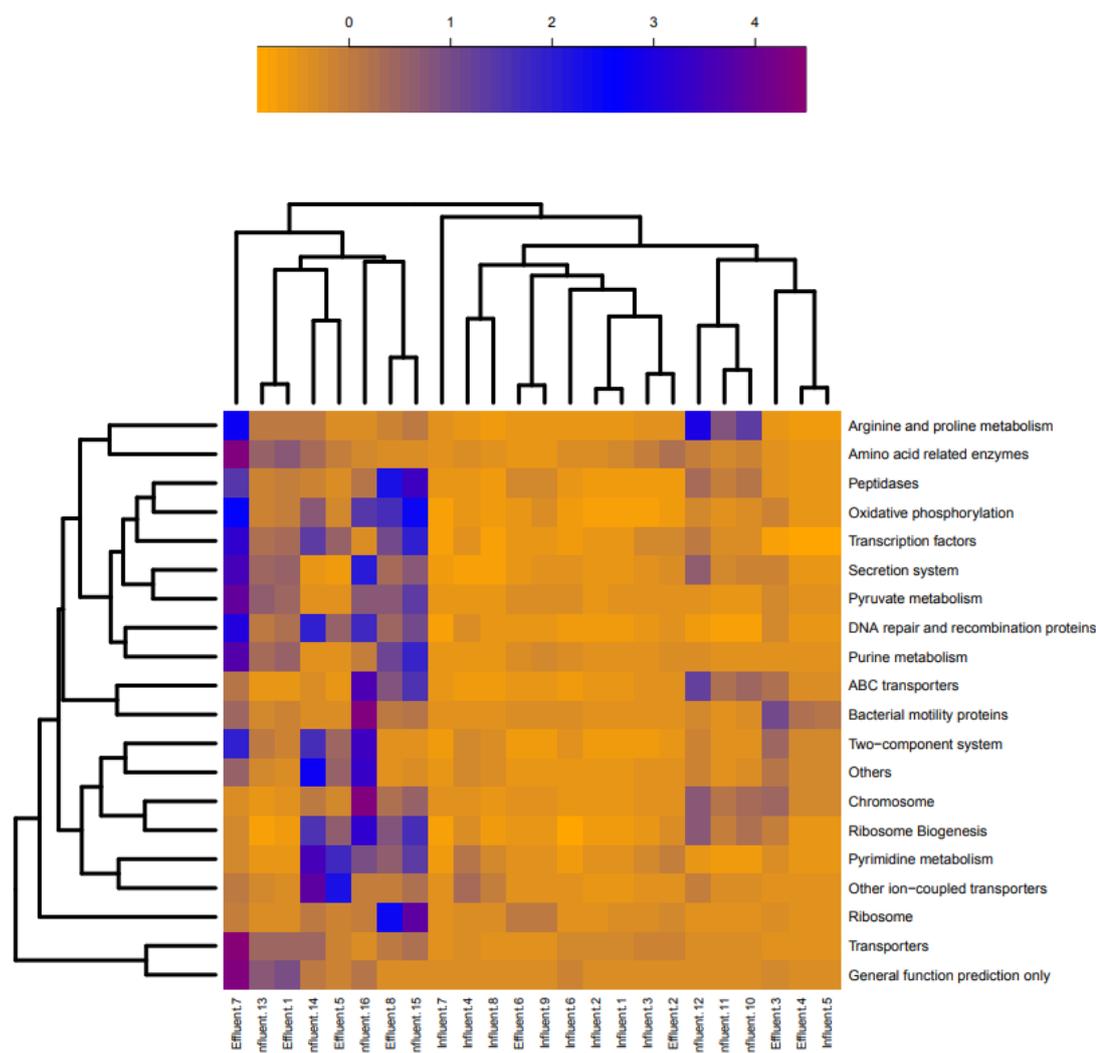
Figure 5.10: Functional predictions for bacterial populations of collected wastewater samples

In a similar way, the other groups of antibiotics, including quinolones, macrolides and pyrimidine inhibitor drugs, were also identified and further used for CCA analysis (Figure 5.11b). Compounds such as flumequine, norfloxacin, oxolinic acid and lincomycin displayed a strong converse relationship with *Proteobacterial* members. Bacterial members belonging to *Bacteroidetes, Acidobacteria* and *Planctomycetes* showed strong resistance to the macrolide members, i.e. tylosin. However, the antibiotic erythromycin that belonged to the same group had no effect on any bacterial members in the wastewater system.
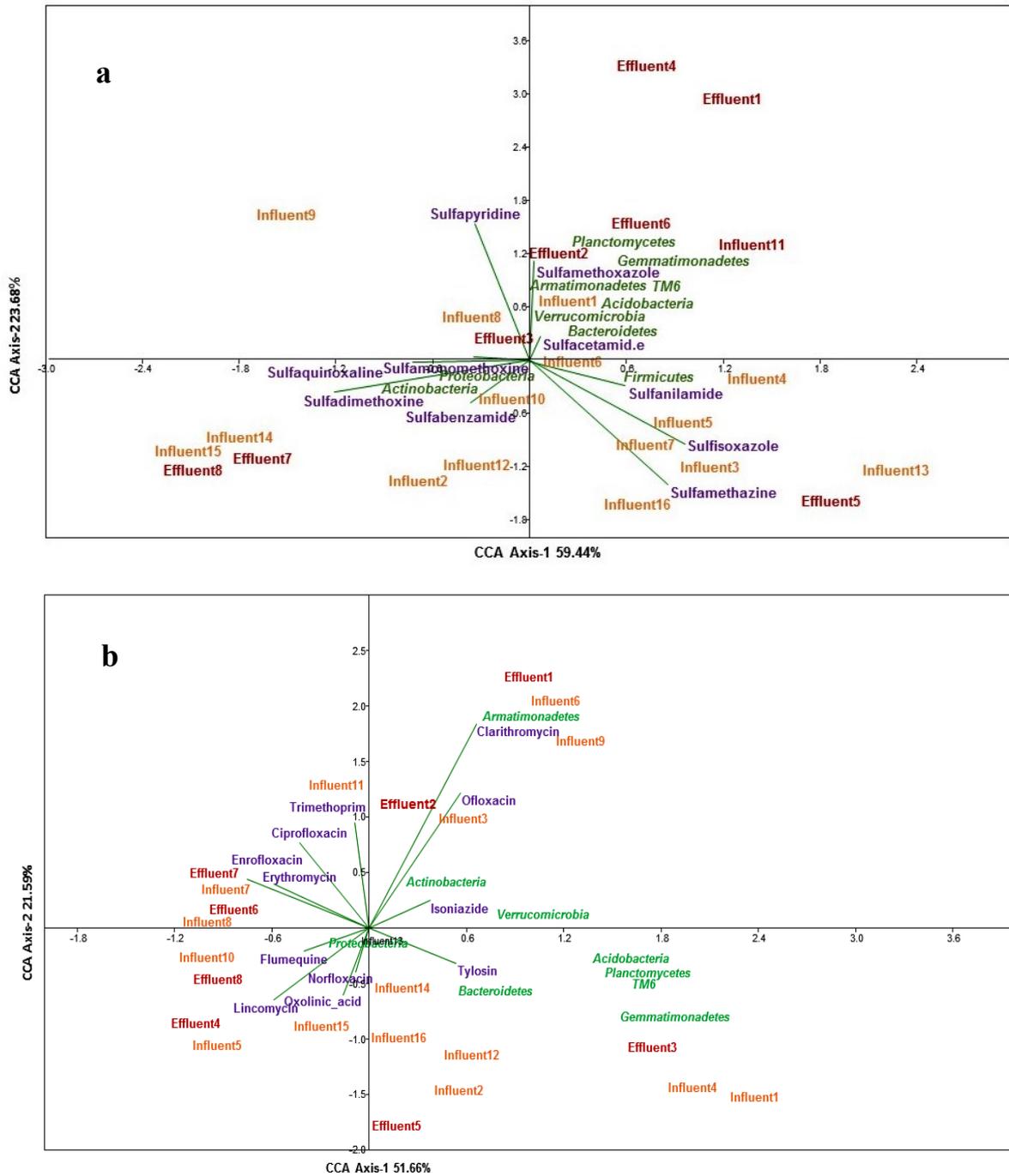
Figure 5.11: Canonical correspondence analysis showing the distribution and interrelationships of the bacterial phyla and identified antibiotics: (a) sulphonamides; and (b) other antibiotic groups in wastewater

5.7   DISCUSSION

Microbial pathogens, which can potentially be present in wastewater, can be divided into four separate groups: viruses, bacteria, fungi and protozoans/helminths. Bacteria are the most common microbial pathogens found in wastewater. A wide range of bacterial pathogens and opportunistic pathogens associated with wastewater are enteric in origin and have been reported in literature (Osuolale and Okoh, 2015; Osuolale and Okoh, 2017; Szekeres et al., 2017). Wastewater-associated infections generally include diarrhoea, dysentery, dysentery-like infections, *Leptospira interrogans* infections, typhoid, human enteritis, legionellosis, melioidosis, stomach ulcer and cancer (Yoder et al., 2008). In this study, the researchers collected influent and effluent wastewater samples from WWTPs, and analysed bacterial communities and possible emerging and opportunistic pathogens.

Bacterial community analyses for both influents and effluents were achieved using an Illumina high-throughput sequencing platform. The quality reads of bacteria were distributed into 3,190 OTUs from all samples, which was higher than the average OTUs reported previously from gold and vanadium wastewater (1,315 OTUs) (Keshri et al., 2015), acid mine wastewater (960 OTUs) (Kamika et al., 2016), textile (196 OTUs) and municipal (297 OTUs) wastewater (Meerbergen et al., 2017), activated sludge treatment plants (1,063 OTUs) with different wastewaters (Shchegolkova et al., 2016), biofilm reactors (640 OTUs) treating chemical industrial effluents (Bassin et al., 2017) and lower than full-scale wastewater treatment plants (8,652 OTUs) of different industrial effluents (Shu et al., 2015). Furthermore, based on the number of OTUs, the community diversity (Shannon-Weaver) and OUT richness (Chao1) estimators were calculated. These values are in accordance with those reported from an anoxic-aerobic moving-bed biofilm reactor system treating a chemical industry wastewater in Brazil (Bassin et al., 2017).

Diverse bacterial communities were detected in all the influent and effluent wastewater samples. Overall, 35 bacterial phyla, together with 566 genera, were observed across all wastewater samples collected from WWTPs. Results of the phylum levels revealed that the four most dominant phyla were *Firmicutes*, *Proteobacteria, Actinobacteria* and *Verrucomicrobia*. A recent study of the bacterial community composition of an industrial wastewater reclamation plant in South Africa also confirms that the phyla *Proteobacteria, Firmicutes* and *Actinobacteria* were dominantly present in each treatment stage, whereas members of *Verrucomicrobia* were not recorded (Sekar et al., 2014). Furthermore, substantial reads belonging to the phyla *Bacteroidetes*, *Fusobacteria* and *Cyanobacteria* were identified among all wastewater samples. Members of these phyla have previously been reported to be widespread in different wastewater treatment systems, suggesting that these bacteria play key roles in nutrient removal processes (Ma et al., 2015). This study also suggests that bacterial distribution between the influents and effluents did not have the characteristic profile of high bacterial rank, which is commonly observed in domestic and municipal wastewater (Ibarbalz et al., 2013).

To simplify the results, the researchers selected the top 10 OTUs in each wastewater for comparison. In total, 31 OTUs were obtained across all the collected samples. The significantly differential abundant OTUs present in the influent samples are *Roseomonas, Clostridium, Methylobacterium, Turicibacter, Paracoccus, Sarcina* and *Bacteroidetes*. Members of *Roseomonas* are waterborne gram-negative coco bacilli, classified as opportunistic and emerging pathogens that can cause bacteremia in humans, especially in immunocompromised patients (De et al., 2004). In addition, these pathogens are resistant to ceftazidime and cefepime antibiotic groups. The species *Methylobacterium* can cause health care-associated infections, including infections in immunocompromised hosts. The ability of *Methylobacterium* species to form biofilms and develop resistance to high temperatures, drying and disinfecting agents may explain the colonisation of *Methylobacterium* in the hospital environment, e.g. in endoscopes (Kovaleva et al., 2014). These groups of bacteria were highly resistance to meropenem. However, they are susceptible to a wide range of antibiotics such as amikacin, gentamicin, ciprofloxacin, and trimethoprim-sulphamethoxazole and have various levels of susceptibility to the -lactam antibiotics.

Faecal microbiota such as *Clostridium difficile* can cause symptoms that range from diarrhoea to life-threatening inflammation of the colon. Currently available antibiotics for treating this pathogen are becoming limited due to their increasing resistance (Peng et al., 2017). *Sarcina* is a gram-positive organism that occurs in the soil and air, and has also been isolated from human faeces. However, the pathogenicity of *Sarcina* is not well established. Few case reports have documented its association with various gastric disorders (Radotra et al., 2015). Finally, the members of *Paracoccus* are best known for their nitrate-reducing properties (Rani et al., 2018). However, it can also cause some opportunistic infections like peritonitis, including symptoms like pain, tenderness, rigid abdominal muscles, fever, nausea and vomiting.

In contrast, effluent samples had significantly differential abundant OTUs compared to influent samples. The major abundant genera recorded in effluent samples were *Actinomyces, Phenylobacterium, Akkermansia, Collinsella, Neisseria, Planctomyces, Polynucleobacter, Streptococcus, Acinetobacter, Enhydrobacter, Mycobacterium* and *Pseudomonas*. Infections with *Pseudomonas* have become a real concern due to the fact that their high mortality lies in the appearance of drug-resistant strains, especially in critically ill and immunocompromised patients (Rani et al., 2018). These bacteria are classified as an emerging pathogen. They can develop many factors associated with antibiotic resistance involving almost all classes of antibiotics. Members of *Neisseria* that belong to the phyla *Proteobacteria* can cause the human disease gonorrhoea. This emerging pathogen recently attained cephalosporin resistance, particularly ceftriaxone resistance, and has greatly complicated the treatment of gonorrhoea, with the gonococcus now being classified as a "superbug" (Unemo and Nicholas, 2012). Similarly, the evolution of drug resistance in *Mycobacterium* also had to be considered. Recent studies confirmed that these bacteria acquired resistance to rifampicin or ethambutol, then resistance to pyrazinamide and finally resistance to second- and third-line drugs (Bassetti et al., 2018). Members of *Acinetobacter* are the most commonly encountered opportunistic pathogen in wastewater, which causes nosocomial infections with mortality. However, the antibiotic resistance rates of this genera increased from 32 to 100% against ciprofloxacin, 91 to 100% against cefepime, 90 to 92% against piperacillin-tazobactam, 24 to 94% against amikacin and 18 to 85% against gentamicin (Dookie et al., 2018).

Functional abilities involved in bacterial communities were identified using PICRUSt analysis. Genes involved in carbohydrate metabolism were predominantly identified in all samples (Figure 5.10), indicating that the degradation of organic pollutants was highly associated with those genes. It is also in accordance with previous studies, which showed that the basic metabolic functions are the same in predicted metagenomes (Hakyemez et al., 2013). In addition, the presence of genes associated with the metabolism of alanine, aspartate and glutamate were identified. This explains the bacteria's dependence on amino acids as an adaptive mechanism of bacteria in WWTPs. Furthermore, genes like ABC transporters and ion-coupled transporters were exhibited in the bacterial members, suggesting that these are signature genes for the transport of organic and inorganic molecules across bacterial cellular membranes and maintain the equilibrium state in the wastewater system (Gao et al., 2016). Besides the transporter genes, this study also identified the genes responsible for DNA repair and recombination protein, signifying the bacteria at WWTPs that are capable of repairing DNA when it is damaged during exposure to toxic heavy metals and antibiotics (Wilkens, 2015).

Understanding emerging antibiotics and their resistance in the wastewater system improves the management strategy and human health. In recent decades, antibiotic-resistant phenotypes have emerged significantly in wastewater treatment systems. Therefore, it is important to identify the relationship between antibiotics and bacterial members in WWTPs to improve our understanding of antibiotic-resistant bacteria. In this study, the researchers used CCA to identify the relationship between antibiotics and bacterial communities identified in the wastewater samples. Members of *Proteobacteria* were shown to have high resistant against a few sulphonamide members, including sulphadimethoxine, sulphamonomethoxine,

sulphadimethoxine and sulphabenzamide (Figure 5.11a), which agrees with the previous research on wastewater treatment systems (Zhou et al., 2008; Figueira et al., 2011; Ahn and Choi, 2016).

The antibiotic fluoroquinolones could bind strongly on soil, organic matter and sediments (Guo et al., 2017), which is easily carried to WWTPs. In this study, the antibiotics belonging to the class quinolones were identified, including flumequine, norfloxacin and ofloxacin. The results of CCA revealed that these antibiotics were not resistant to proteobacterial members. However, antibiotics such as ciprofloxacin and enrofloxacin, which belong to the same class, had no effect on any bacterial members. The presence of these antibiotics in WWTPs enhances the probability of transferring antibiotic resistance to bacteria, followed by human pathogens. This suggests that the acquisition of a specific antibiotic-resistant strains, either by horizontal gene transfer or by adaptive mutation, may take place preferentially in each habitat.

5.9   CONCLUSIONS

- Next-generation sequencing technology revealed that diverse bacterial communities were present in both influent and effluent samples, which is not possible in culture-dependent methods.
- Effluent samples recorded the highest bacterial richness compared to influent samples.
- *Proteobacteria* and *Firmicutes* were the two dominant phyla recorded across different wastewater samples.
- Significantly differential abundant OTUs showed that unique bacterial communities represent both influent and effluent samples.
- The CCA explained the interrelationship between bacterial members and identified antibiotics.
- Emerging and opportunistic pathogens with possible antibiotic resistance were recorded.

Future directions

- The investigation of fungal and viral communities in untreated sewage and treated effluents, especially targeting pathogens.
- The investigation of the available and emerging antibiotic-resistant genes in microbial communities present in WWTPs.
- The investigation of other microbial communities such as fungi, viruses and protozoans to identify the recurrent biomarkers and their toxigenic compounds.
- Developing and validating alternative molecular analysis like MALDI-ToF-MS for the identification of potential microbes as an indicator of pathogens.

CHAPTER 6: COST-BENEFIT ANALYSIS ON THE SELECTED METHODS

6.1   GENERAL INTRODUCTION

With the current water challenges, regulatory authorities have an increased responsibility to ensure safe water delivery for their populations. One way of doing this is by implementing improved monitoring technologies and management practices to safeguard populations and preserve the environment against a broad spectrum of chemical and microbiological contaminants. However, the pressure remains for the same authorities to remain financially sound in the face of increasing challenges.

Cost-benefit analysis (CBA) is one of the tools that assists regulators to decide on the feasibility of implementing various projects. The CBA is an economic tool for evaluating all relevant costs and benefits of an investment. It reflects the total impact of a project on society as a whole. Costs and benefits are measured and then weighed up against each other to generate criteria for decision making. The CBA can be used to guide a wide range of decisions, and contributes to good programme management as it is concerned with efficiency and is sensitive to the priorities of key stakeholders' needs. The purpose of the CBA is to provide information that can materially assist the decision-making process. It is used to evaluate the risks and rewards of projects under consideration.

Therefore, this section of the report provides a cost-benefit analysis to determine the optimal resourcing option that provides a feasible, affordable, yet sustainable solution that meet the needs of all stakeholders subject to the constraints mentioned above.

6.2   COST-BENEFIT ANALYSIS SCOPE

The CBA is a systematic approach to estimating the strengths and weaknesses of alternatives. The process flow of a CBA that was applied in this case included identifying and listing alternatives, identifying costs and benefits, quantifying costs and benefits, discounting future stream of benefits and costs to calculate NPV and a sensitivity analysis. Systems in this case include infrastructure, human resources, processes and procedures, and are the main constraints in achieving the intended results.

6.2.1   Alternatives and options

In order to provide an effective and efficient service for the analysis of water samples, i.e. wastewater, ground water and river water, the availability of adequate resources to procure the necessary infrastructure was identified as the main limiting factor in this project. A fully equipped laboratory to sample and analyse such samples requires an Orbitrap HRMS, with a current market value of R10 million, a LECO GCxGC-HRT-MS (with a market value of R9 million), a Q-ToF HRMS (with a market value of R5 million) and probably a LECO Pegasus 4D ToF-MS (with a market value of R4 million), and a Thermo Scientific™ Dionex™ AutoTrace™ 280 SPE instrument (with a market value of R250 000). Therefore, a total of R23 250 000 is required to purchase the abovementioned equipment.

Given that financial resources are often not readily available to resource a laboratory with the above-mentioned equipment, the following options were considered:

- Option 1: The "do nothing" approach (use the facility that is currently available at no extra cost).
- Option 2: Only buy an Orbitrap HRMS with a current market value of R10 million, a LECO GCxGC-HRT-MS (with a market value of R9 million) and a Dionex™ SPE (with a market value of R250 000).
- Option 3: Only buy a LECO GCxGC-HRT-MS (with a market value of R9 million) and Dionex™ SPE (with a market value of R250 000).

- Option 4: Only buy an Orbitrap HRMS with a current market value of R10 million and a Thermo Scientific™ Dionex™ AutoTrace™ 280 SPE instrument (with a market value of R250 000).
- Option 5: Only buy a Q-ToF-MS (with a market value of R4 million) and a Dionex™ SPE (with a market value of R250 000).

6.2.2 Costs and benefits

The costs considered in the CBA for this exercise are related to the laboratory operations for wastewater treatment and included the following:

- Chemical consumables
- Solvent consumables
- Column consumables
- Salaries (researcher, assistant)
- Transport

However, figures associated with utilities, rental space, communication, stationery and printing, and insurance and security were not readily available at this stage as bulk metering is used. An estimate of the above transactions, specifically for the laboratory unit, is possible with a bit more time.

The benefit streams that were considered in this exercise were grants (from WRC and the National Research Foundation (NRF)), as well as revenue generated from the analysis of samples. The revenue generated from the analysis of samples assumes that the laboratory is permitted to supplement its revenue base by charging commercial clients market rates for services rendered such as wastewater sample analysis.

6.2.3 Assumptions

The CBA provides a valuable means of determining if a project has generated a net benefit for the community. It is important to highlight the assumptions used in forecasting the costs and benefits of the project. The following assumptions were made in the evaluation of costs in this exercise:

- Costs were increased by an average of 10% per year.
- Prices for sample analysis were increased by an average of 10%.
- Discount rates were set at 9% per year.
- Other costs were 2% of the total of the hidden costs.
- Grants (from WRC and NRF) were available for the duration of the project (three years).
- The total amount of grants available were spread evenly across the three years.

6.3 RESULTS AND SENSITIVITY ANALYSIS

Once the costs and benefits of the project had been quantified, the data was used to determine the net benefit of the proposal. Note: the NPV results are in rand values as in 2019 (Year 0) and the project is assumed to operate from 2019 (Year 0) to 2021(Year 3). The results are presented in the Table 6.1. The results are based on a discount rate of 9% and a capacity to process 850 samples a month using one researcher and an assistant. Based on this, it can be observed in Table 6.1 that Option 1 is the most enviable position with the highest NPV, benefit cost ratio and return on investment, while Option 5 comes in second best. However, Option 2 is the worst option financially and is not economically beneficial, with a negative NPV and a benefit cost ratio that is less than 1.

Table 6.1: **Cost benefit analysis of the project (discount rate 9%, capacity of 850 samples a month)**

Indicator	Option 1	Option 2	Option 3	Option 4	Option 5
Net present value	R13,251,755	R-5,998,245	R4,001,755	R3,001,755	R9,001,755
Benefit cost ratio	1.55	0.86	1.12	1.09	1.32
Return on investment	0.55	-0.14	0.12	0.09	0.32
Internal rate of return	-	-0.11	0.29	0.22	-

As part of the CBA. a sensitivity analysis was done, as shown in Table 6.2. The sensitivity test involved changing the magnitude of key variables, such as the discount rate, number of researchers, number of samples processed in a month and the market price of a sample, and measuring the impact on the NPV, benefit cost ratio and return on investment.

Given that Option 2 and Option 4 were the least viable, these options were omitted from the sensitivity analysis. Table 6.2 shows the sensitivity analysis where the discount rate was varied from 9 to 7% and the capacity to process 850 samples a month was kept constant, still using one researcher and an assistant. All the options illustrated in Table 6.2 showed some improvements. The NPV for Option 1 improved from R13,251,755 to R13,662,111. Furthermore, the benefit cost ratios for all the options were favourable and greater than 1. Given the circumstances, a 7% discount rate was more appropriate. This rate is equal to the prevailing interest rate in South Africa and reflects the cost of capital.

Table 6.2: **Sensitivity analysis – varying discount rate from 9 to 7%**

Indicator	Option 1		Option 3		Option 5	
	9%	7%	9%	7%	9%	7%
Net present value	R13,251,755	R13,662,111	R4,001,755	R4,637,111	R9,001,755	R9,412,111
Benefit cost ratio	1.55	1.55	1.12	1.14	1.32	1.32
Return on investment	0.55	0.55	0.12	0.14	0.32	0.32
Internal rate of return	-	-	0.29	0.31	-	-

Table 6.3 shows the sensitivity analysis where the discount rate is kept constant at 7%, the capacity to process 850 samples a month is kept constant and the number of researchers is increased to two. Option 1 is the most enviable position with a higher NPV of R13,662,111 when one researcher is employed compared to an NPV of R12,746,053 when two researchers are employed. In all the options shown in Table 6.3, the project performs better when one researcher is employed, as indicated by the favourable NPV and benefit cost ratios.

The sensitivity analysis indicates the real risk posed by a failure to resource the laboratory with adequate yet lean staff numbers to perform the tasks required. The sensitivity analysis also indicates the risk posed by a failure to provide commercial services to supplement grants that are on offer. Further risk is inherent in failure to charge competitive prices that can absorb the cost of offering the service to commercial clients.

Table 6.3: **Sensitivity analysis – varying the number of researchers**

Indicator	Option 1		Option 3		Option 5	
	Two researchers	One researcher	Two researchers	One researcher	Two researchers	One researcher
Net present value	R12,746,053	R13,662,111	R3,721,053	R4,637,111	R8,721,053	R9,412,111
Benefit cost ratio	1.49	1.55	1.11	1.14	1.29	1.32
Return on investment	0.49	0.55	0.11	0.14	0.29	0.32
Internal rate of return		-		0.31	-	-

6.4 CONCLUSIONS

Based on the CBA, it can be concluded that Option 1 (the "do nothing" approach where the existing facilities and infrastructure are used at no additional cost) is the most beneficial option with a net benefit in excess of R13 million and a benefit cost ratio above 1.5. Furthermore, even with the sensitivity analysis scenarios that assumed more pessimistic costs and benefits, Option 1 results in a net benefit to the community.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

The review of work done in other parts of the world reveals that there is a need to expand the studies on emerging contaminants in Africa, including South Africa. While several examples of extensive work of multiclass emerging contaminant analysis has been done on other continents, Africa still lags behind in this research space. Therefore, there is a need to develop LC-MS/MS methods that can be validated and adopted by several monitoring laboratories.

This work focused on two methods based on Orbitrap high resolution LC-MS/MS using Water X-Bridge and Restek Bipheyl columns, which were successfully developed and validated for emerging contaminant compounds. The performance of the two columns was very similar, hence providing for flexibility. Using both methods, good linearity (0.9528 to 0.9997), LOD values (0.003 to 8.41 ng ℓ^{-1}) and LOQ values (0.01 to 28.0 ng ℓ^{-1}) were achieved. The methods were successfully applied to river and WWTP influent and effluent samples.

Using the developed and validated Orbitrap high-resolution LC-MS method, in which a Waters X-Bridge column was used, 71 and 73 PPCP compounds were quantified in influent and effluent samples, respectively. Both influent and effluent samples were heavily contaminated with emerging contaminants such as caffeine, paraxanthine, ibuprofen, paracetamol, estradiol and efavirenz, which were detected at higher concentrations of greater than 1,000 ng ℓ^{-1}. In general, the compounds detected in WWTP influent and effluent samples were antibiotics, ARVs, steroid hormones, NSAIDs, anti-inflammatories, antivirals, antifungals, antidepressants, anticonvulsants, cardiovascular agents, analgesics, anthelmintics, consumer product additives and bronchodilators. Antibiotics were the predominant class detected in the WWTP influent samples, accounting for about 28% of the compounds quantified.

All three rivers under study were contaminated with emerging contaminants, including Muldersdrift se Loop, which is not linked to the WWTP. For this particular river, it can be concluded that the source of the contaminants was the informal settlement where waste could have been discharged directly into the river. The two rivers linked to WWTPs were clearly highly contaminated, indicating the plant's limitations to completely remove the emerging contaminants. The contaminant load of the Juskei River (Northern WWTP), however, was much higher than for the Apies River (Daspoort WWTP), which had more compounds and at higher concentrations than the Jukskei River. Notably, our water systems seem to be contaminated with ARVs such as ritonavir, efivarenz and nevirapine, in addition to the usual frequently detected emerging contaminants that seem to be unique to the African context. This can be attributed to the high HIV burden experienced in several African countries, including South Africa, compared to other regions in the world.

Based on the Pearson correlation analysis, carbamazepine, fluconazole and ritonavir showed good correlation with other compounds. These may therefore constitute potential biomarkers. In addition, based on the frequent detection rate and the high concentration levels, caffeine, paraxanthine, ibuprofen, paracetamol, sulphamethoxazole, fluconazole and trimethoprim can also be considered compounds that contribute to the early warning system as possible biomarkers for contaminated water.

The non-targeted approach provides invaluable information about the status of the level of contamination. An average of 624 and 677 compounds were identified based on accurate mass in influent and effluent samples, respectively. Using additional qualifications with isotopic patterns (with at least 50% isotopes observed) and fragmentation patterns (with at least one fragment observed), these numbers were reduced to less than 50% identified using accurate mass alone. The sensitivity in full scan acquisition mode and high mass accuracy was well demonstrated when the method was applied to real wastewater and river water.

The non-targeted GCxGC-HRT-MS approach revealed additional environmentally related compounds such as plasticisers, flavouring agents, fire retardants, herbicides, surfactants and other compounds that were present together with the emerging contaminants.

Next-generation sequencing technology revealed that diverse bacterial communities were present in both influent and effluent samples, which is not possible in culture-dependent methods. Effluent samples recorded the highest bacterial richness compared to influent samples. *Proteobacteria* and *Firmicutes* were the two dominant phyla recorded across different wastewater samples. Significantly differential abundant OTUs showed that unique bacterial communities represent both influent and effluent samples. The CCA explained the interrelationship between bacterial members and some sulphonamide and fluoroquinolone antibiotics. Finally, emerging and opportunistic pathogens with possible antibiotic resistance were recorded.

The CBA revealed that Option 1 (the "do nothing" approach, where the existing facilities and infrastructure are used at no additional cost) is the most beneficial option with a net benefit in excess of R13 million and a benefit cost ratio above 1.5. Furthermore, even with the sensitivity analysis scenarios, which assumed more pessimistic costs and benefits, Option 1 results in a net benefit to the community.

Recommendations

- There is a need to expand the scope of the study to include several rivers that feed into drinking water treatment plants.
- The level and impact of emerging contaminants can be well understood by including sediments in the study.
- Available and emerging antibiotic resistance genes in microbial communities present in WWTPs should be investigated.
- Available and emerging antibiotic-resistant genes in microbial communities present in WWTPs should be investigated.
- Other microbial communities, such as fungi, viruses and protozoans, should be investigated to identify the recurrent biomarkers and their toxigenic compounds.
- Alternative molecular analysis like MALDI-ToF-MS should be developed and validated for the identification of potential microbes as an indicator of pathogens.
- A systematic approach that simultaneously determines parent compounds, transformation products and degradation products is long overdue. The non-targeted analysis using high-resolution LC-MS affords such as opportunity. The identification of transformation products would lead to the possible synthesis of transformation products that could be used for toxicological studies. The toxicology of emerging contaminants and/or transformation products should be periodised as regulations and polices are written
- A water reference laboratory should be established in South Africa to support the monitoring laboratories.
- Research on new technologies for the removal of emerging contaminants from wastewater should be promoted.

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APPENDIX A

Table A1: **Compound database information recorded for environmental contaminants from LC-HRMS**

Reference No.	Compound	Class	Polarity	Expected mass	Mass observed	Frag 1	Frag 2	Frag 3	RT
1	1,7 dimethylxanthine		+	181.0732	181.0731	124.0514	142.6200	-	0.86
2	17-α ethynylestradiol		+	279.1772	279.1760	133.0658	159.0815	105.0709	11.61
3	2 napthylamine		+	144.0813	144.0807	117.0704	115.0547	91.0448	7.05
4	2,4 diaminoanisole		+	139.0873	139.0867	108.0684	124.0632	80.0499	0.90
5	4 nitroaniline		+	139.0485	139.0490	122.0471	125.0471	93.0576	7.81
6	Acetaminophen		+	152.0710	152.0706	110.0602	111.0442	134.0600	0.88
7	Acetylsalicylic acid		+	179.035	179.0349	121.0287	65.03878	93.03382	0.85
8	Alachlor		+	270.1255	270.1249	238.0990	162.1274	90.0104	13.46
9	Albendazole		+	266.0959	266.0972	234.0692	191.0146	192.0224	9.34
10	Amitryptiline		+	278.1903	278.1892	233.1324	205.1012	105.0698	10.08
11	Amphotericin b		+	925.4951	925.5040	-	-	-	10.21
12	Ampicillin		+	350.1186	350.1169	106.0653	160.043	174.0553	4.24
13	Atenolol		+	267.1703	267.1722	145.0652	74.06022	190.0867	0.86
14	Atrazine		+	216.1012	216.1006	174.0541	132.0325	96.0557	10.58
15	Azithromycin		+	749.5158	749.5143 **375.2610**	**83.0495**	**116.1070**	**158.1172**	7.76
16	Benalaxyl		+	326.1754	326.1742	294.149	208.1334	266.1541	14.07
17	Benz[e]acephenanthrylene		+	253.0953	253.0964				17.64
18	Benzylbutylpthalate		+	313.1442	313.1431	149.0233	205.0859	91.0546	15.38
19	Betaxolol		+	308.225	308.2238	116.1070	72.08128	74.06053	8.92
20	Bisoprolol		+	326.2330	326.2342	116.1069	74.06051	72.08127	8.29
21	Bisphenol A		+	229.1223	229.1215	119.0495	214.0949	135.0808	18.59
22	Buspirone		+	386.2550	386.2566	122.0713	150.1024	148.0867	5.06
23	Caffeine		+	195.0875	195.0868	138.066	110.0714	69.04532	6.22

Reference No.	Compound	Class	Polarity	Expected mass	Mass observed	Frag 1	Frag 2	Frag 3	RT
24	Carbadox		+	263.0775	263.0775	231.051	229.0717	145.0396	1.01
25	Carbamazepine		+	237.1022	237.1031	194.0978	192.0821	179.0704	9.84
26	Carbazole		+	168.0813	168.0805	89.0158	151.1001	133.0013	12.86
27	Carbofuran		+	222.1124	222.1119	165.0980	123.7256	137.0253	10.22
28	Cefotaxime		+	456.0647	456.0666	396.0431	241.0389	216.0325	5.41
29	Cephalothin		+	397.0449	397.0459	216.0330	271.0388	56.0136	10.18
30	Chlorpyrifos		+	349.933	349.9336	114.9615	197.9278	171.0243	16.29
31	Ciprofloxacin		+	332.1405	332.1421	231.0559	288.1499	245.1078	3.10
32	Clarithromycin		+	748.4842	748.4825	158.1174	83.04967	116.1071	10.07
33	Cloxacillin		+	436.0765	436.0754	160.0432	56.0136	220.0164	11.16
34	Danofloxacin		+	358.1579	358.1562	82.0657	96.0812	255.0560	6.30
35	Dexamethasone		+	393.20655	393.2072	147.0802	237.1269	355.1895	10.01
36	Diclofenac		+	296.0240	296.0248	215.0493	180.0818	250.0181	13.08
37	Diethylstilbestrol		+	269.1546	269.1559	135.0802	173.0594	121.0645	12.29
38	Difloxacin		+	400.1467	400.1484	299.0988	356.1566	58.06589	10.71
39	Digoxin		+	781.4469	781.4418	97.06526	113.0607	69.0345	9.63
40	Efavirenz		+	316.0347	316.0342	244.0129	168.0805	224.0067	13.67
41	Enalapril		+	377.2076	377.2088	234.1492	117.0704	160.1122	14.93
42	Enrofloxacin		+	360.1732	360.1718	316.1816	245.1082	286.0983	0.88
43	Erythromycin		+	716.4580	716.4562	158.1175	83.04971	116.1072	6.25
44	Esbiothrin		+	303.1959	303.1947	135.0828	107.0856	93.0702	16.05
45	Estradiol		+	273.1744	273.1757	107.0502	159.0816	213.1289	11.03
46	Estriol		+	289.1789	289.1798	253.1583	133.0648	157.0647	11.24
47	Estrone		+	271.1681	271.1693	253.1605	133.0658	157.0660	11.79
48	Famciclovir		+	321.1537	322.1525	136.0627	202.1101	280.1422	6.49
49	Fenbendazole		+	300.0823	300.0818	268.0556	159.0438	190.0083	10.57
50	Fenoprofen		-	241.0863	241.0872	197.0966	93.0340	119.0496	12.52

Reference No.	Compound	Class	Polarity	Expected mass	Mass observed	Frag 1	Frag 2	Frag 3	RT
51	Fluconazole		+	307.1113	307.1108	220.0676	238.0781	169.0457	7.51
52	Flumequine		+	262.0879	262.0891	244.0763	220.0407	238.0506	10.06
53	Fluoxetine		+	310.1413	310.1409	259.0944	64.3338	231.0630	10.35
54	Furazolidone		+	226.0457	226.0458	67.0422	122.0112	95.0369	7.22
55	Gabapentin		+	172.1331	172.1332	154.1224	137.0959	95.08587	5.37
56	Ibuprofen		+	207.1380	207.1385	161.1328	119.0858	105.0701	13.39
57	Ifosfamide		+	261.0328	261.0337	92.0272	153.9829	78.0115	8.38
58	Indometacin		+	358.0841	358.0838	138.9954	129.0107	174.0918	13.06
59	Isoniazide		+	138.0651	138.0660	121.0396	93.0449	78.0342	0.94
60	Ketoprofen		+	253.0870	253.0873	105.034	209.0962	177.0548	11.42
61	Lamivudine		+	230.0581	230.0591	112.0625	130.1485	95.0237	1.11
62	Levofloxacin		+	362.1527	362.1511	261.1033	318.1613	221.0721	10.32
63	Lidocaine		+	235.1801	235.1805	86.0967	58.06582	-	6.65
64	Lincomycin		+	407.2210	407.2230	126.1287	359.2185	-	5.96
65	Lomefloxacin		+	352.1467	352.1489	252.0472	72.0813	-	6.87
66	Lopinavir		+	629.3669	629.3688	447.3104	183.1377	155.1172	13.42
67	Malathion		+	331.0427	331.0433	284.8	67.0298	84.02118	13.47
68	Marbofloxacin		+	363.1463	363.1481	72.08145	320.1039	70.06583	5.49
69	Mebendazole		+	296.1051	296.1048	264.0784	105.0345	95.0502	9.24
70	Mefenamic acid		+	240.1037	240.1031	224.1067	209.0833	192.0822	20.29
71	Metoprolol		+	268.1916	268.1924	116.1080	74.0601	191.1081	7.37
72	Miconazole		+	414.9933	414.9943	158.9773	69.0447	227.0145	11.95
73	Naproxen		+	231.1016	231.1028	185.0961	170.0727	154.0777	11.50
74	Neomycin		+	615.4856	615.4865 **124.0869**	**83.0608**	**107.0606**	-	17.17
75	Nevirapine		+	267.1236	267.124	226.0842	227.092	107.0604	7.92
76	Nitrofurantoin		-	257.0453	257.0464	77.0021	152.0094	124.0031	6.88

Reference No.	Compound	Class	Polarity	Expected mass	Mass observed	Frag 1	Frag 2	Frag 3	RT
77	Nitrofurazone		-	197.0319	197.0312	53.9979	72.0085	56.0136	6.55
78	Nitrophenol		-	138.0194	138.0184	108.0205	92.9187	94.9158	8.64
79	Norgestrel		+	313.2189	313.2183	109.0659	245.1918	83.0502	12.34
80	Norfloxacin		+	320.1405	320.1414	276.1502	233.1081	300.1338	
81	Oxacillin		+	402.1118	402.1131	160.0432	215.0490	144.0449	10.83
82	Ofloxacin		+	362.1511	362.1527	261.1041	318.1619	221.0729	1.99
83	Oxibendazole		+	250.1190	250.1200	148.0511	132.0562	188.0823	8.18
84	Oxolinic acid		+	262.0710	262.0715	216.0654	158.0600	234.0396	
85	Oxytetracycline		+	461.1569	461.1585	426.1181	201.0546	444.1288	0.86
86	Penciclovir		+	254.1261	254.1254	152.0577	135.0311	67.0553	0.87
87	Penicillin G		+	335.1060	335.1076	217.0645	220.0429	91.05434	8.86
88	Phenacetin		+	180.1028	180.1019	138.0912	110.0602	152.0705	4.45
89	Pindolol		+	249.1605	249.1598	116.1080	172.0768	74.06011	0.88
90	Prednisolone		+	361.2004	361.2010	147.0809	171.0812	173.0967	9.13
91	Progesterone		+	315.2328	315.2340	97.06584	109.06583	123.0815	13.64
92	Propranolol		+	260.1645	260.1660	116.1079	74.0611	183.0817	8.75
93	Reserpine		+	607.2669	607.2661	211.0614	153.0559	181.0145	14.03
94	Ribavirin		+	245.0885	245.0894	113.0459	114.0299	133.0494	
95	Rifabutin		+	847.4488	847.4507	95.0867	124.0804	158.0795	12.11
96	Rifampicin		+	823.4124	823.4110	95.0857	123.0804	151.0751	11.76
97	Rifapentine		- +	875.4467 877.4632	875.4484 877.4647	197.8081	257.8200	423.2228	12.42
98	Ritonavir		+	721.3195	721.3188 **361.1627**	**98.0061**	**140.0526**	**197.0739**	13.08
99	Roxithromycin		+	837.5355	837.5318	158.1172	83.04956	116.1069	7.52
100	Sarafloxacin		+	386.1311	386.1327	299.0989	342.141	366.1247	3.79
101	Simazine		+	202.0851	202.0854	132.0324	124.0871	96.05608	9.33
102	Sotalol		+	273.1271	273.1283	255.1157	133.0759	213.0689	0.87

Reference No.	Compound	Class	Polarity	Expected mass	Mass observed	Frag 1	Frag 2	Frag 3	RT
103	Spiramycin		+	843.5238	843.5265	422.2671	87.3665	103.3663	7.73
104	Sulphacetamide		+	215.0491	215.0500	108.0454	156.0125	92.0505	3.40
105	Sulphabenzamide		+	277.0648	277.0661	156.0125	108.0455	92.0505	8.87
106	Sulphachloropyridazine		+	285.0219	285.0228	156.0113	108.0447	92.04996	7.74
107	Sulphadiazine		+	251.0605	251.0614	156.0113	108.0447	92.04995	4.65
108	Sulphadimethoxine		+	311.0836	311.0830	156.0768	108.0448	156.0114	9.07
109	Sulphadimidin		+	279.0921	279.0930	204.0437	124.0871	108.0447	6.76
110	Sulphadoxin		+	311.0816	311.0829	140.0460	94.0656	156.0773	8.02
1111	Sulphaguanidin		+	215.0602	215.0612	156.0125	60.0567	108.0454	1.17
112	Sulphamerazine		+	265.0780	265.0771	156.0113	108.0447	110.0715	6.04
113	Sulphameter		+	281.0703	281.0699	156.2	188.2	215.4	7.00
114	Sulphamethoxazole		+	254.0594	254.0591	156.0115	108.0445	92.0496	8.21
115	Sulphamethizole		+	271.0325	271.0336	80.0500	94.0657	225.9996	7.02
116	Sulphamethoxypyridazine		+	281.0713	281.0721	156.0113	108.0448	126.0664	7.07
117	Sulphamonomethoxine		+	281.0713	281.0722	156.0112	108.0446	126.0662	7.59
118	Sulphamoxol		+	268.0767	268.0768	156.0125	108.0454	113.0720	6.72
119	Sulphanitran		+	336.0659	336.0671	134.0611	108.0454	198.0234	10.29
120	Sulphapyridine		+	250.0650	250.0645	156.0115	108.0445	184.0872	8.52
121	Sulphaquinoxaline		+	301.0754	301.0747	92.05002	119.0609	146.0718	9.11
122	Sulphasalazine		+	399.0764	399.0751	95.06092	183.0558	243.0769	10.11
123	Sulphathiazole		+	256.0213	256.0225	156.0113	108.0447	92.04998	5.78
124	Sulphisoxazole		+	268.0761	268.0768	156.0113	113.0711	108.0447	8.47
125	Temephos		+	466.9962	466.997	142.9926	341.0062	437.0038	16.05
126	Terbutryn		+	242.1429	242.1434	186.0808	91.03296	71.06106	10.2
127	Testosterone		+	289.2172	289.2183	96.0659	109.0659	123.0815	11.22
128	Thiabendazole		+	202.0438	202.0446	175.0336	131.0603	92.0492	5.83
129	Tilmicosin		+	869.5753	869.5783	435.2930	154.9912	-	8.43

Reference No.	Compound	Class	Polarity	Expected mass	Mass observed	Frag 1	Frag 2	Frag 3	RT
130	Triclabendazole		+	360.9572	360.9566	273.9978	345.9330	198.9740	13.72
131	Trimethoprim		+	291.1452	291.1446	123.0664	261.0975	230.1157	6.52
132	Tylosin		+	916.5309	916.5325	174.1137	88.0767	101.0607	7.04
133	Valacyclovir		+	325.1652	325.1641	152.0578	72.0818	84.0818	1.15
134	Zalcitabine		+	212.1030	212.1027	112.0507	101.0600	-	1.09
135	Zidovudine		+	268.1037	268.104	127.0579	110.0237	142.0608	6.58

Table A2:   Data on the X-Bridge C18 column method validation

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Albendazole	266.0958	1-100	0.9986	0.009	0.027
Amitriptyline	278.1903	1-100	0.9993	0.003	0.01
Atazanavir	705.3970	1-100	0.9964	0.095	0.289
Azithromycin	749.5158	1-1000	0.9528	7.56	22.9
Bufexamac	224.1281	2.5-500	0.9995	0.530	1.607
Cafeine	195.0877	1-500	0.9983	0.099	0.299
Carbamazepine	237.1022	1-100	0.9989	0.015	0.046
Cefotaxime	456.0642	5-500	0.9909	0.536	1.625
Ciprofloxacin	332.1405	10-500	0.9902	3.22	10.73
Clarithromycin	748.4842	1-100	0.9984	0.033	0.099
Cloxacillin	436.0729	10-1000	0.9903	4.19	12.7
Danofloxacin	358.1562	25-1000	0.944	8.41	28.0
Desipramine	267.1856	1-100	0.9985	0.009	0.027
Dexamethasone	393.2072	1-100	0.9983	0.062	0.189
Diclofenac	296.0240	1-250	0.9993	0.061	0.184
Diethylbestrol	269.1536	5-250	0.9948	1.333	4.04
Digoxigenin	391.2480	2.5-100	0.9984	0.029	0.088
Difloxacin	400.1467	2.5-250	0.9973	0.065	0.196
Efavirenz	316.0347	1-250	0.9992	0.059	0.179
Enalapril	377.2071	2.5-500	0.9995	0.023	0.071
Enrofloxacin	360.1718	2.5-500	0.9943	0.08	0.241
Estradiol	273.1849	10-500	0.9886	2.97	9.01
Estriol	289.1798	25-1000	0.9867	7.89	23.9
Estrone	271.1693	2.5-250	0.9979	0.113	0.345
Erythromycin	734.4685	1-100	0.9966	0.341	1.032
Famciclovir	322.1510	2.5-500	0.9980	0.05	0.152
Fenbendazole	300.0801	1-100	0.999	0.048	0.148

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Fenoprofen	241.0870	2.5-250	0.9963	0.701	2.125
Fluconazole	307.1113	1-500	0.9993	0.049	0.148
Flumequine	262.0874	1.500	0.9995	0.035	0.107
Gabapentin	172.1332	5-100	0.9877	0.105	0.317
Gemfibrozil	251.1642	2.5-500	0.9971	0.717	2.17
Ibuprofen	207.1380	25-500	0.9922	5.178	15.69
Indometacin	358.0841	2.5-500	0.9982	0.067	0.201
Ifosfamide	261.0321	1-100	0.9993	0.012	0.026
Ketoprofen	255.1016	1-500	0.9994	0.026	0.078
Lamivudine	230.0590	5-500	0.987	4.91	14.9
Lidocaine	235.1805	1-500	0.9973	0.008	0.025
Lincomycin	407.2210	1-100	0.9886	0.054	0.163
Marbofloxacin	363.1463	2.5-100	0.9885	0.2	0.606
Mebendazole	296.1030	1-500	0.9994	0.010	0.031
Medroxyprogesterone	345.2424	1-500	0.9994	0.020	0.062
Mefenamic acid	242.1176	1-500	0.9993	0.017	0.052
Mestranol	311.2006	10-500	0.9917	6.436	19.50
Methylparaben	153.0546	1-100	0.9916	0.018	0.055
Metoprolol	268.1907	1-100	0.9947	0.025	0.075
Miconazole	414.9933	1-100	0.9991	0.016	0.047
Naproxen	231.1016	2.5-100	0.9916	0.565	1.711
Nevirapine	267.1240	1-100	0.9986	0.011	0.033
Norfloxacin	320.1405	10-500	0.9856	5.28	17.6
(-)Norgestrel	313.2162	1-1000	0.9916	2.35	7.13
Ofloxacin	362.1511	10-500	0.9884	4.92	14.9
oxibendazole	250.1186	1-100	0.9984	0.003	0.009
Oxolinic acid	262.0710	1-100	0.9958	0.02	0.06
Oxytetracycline	461.1555	10-500	0.993	2.678	8.117
Paracetamol	152.0706	1-500	0.9911	0.291	0.882

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Paraxanthine	181.0720	1-250	0.9938	0.33	1.00
Penicilline G	335.1060	10-1000	0.9867	4.13	12.5
Phenacetin	180.1019	1-100	0.9959	0.003	0.01
Pindolol	249.1598	1-100	0.9871	0.012	0.037
Prednisolone	361.2010	2.5-100	0.9973	0.031	0.094
Procaine	237.1598	1-100	0.9915	0.019	0.055
progesterone	315.2319	1-100	0.9983	0.017	0.05
Ractopamine	302.1751	1-100	0.9876	0.012	0.035
Rifapentine	877.4594	5-500	0.9916	0.094	2.74
Rifampicin	823.4124	5-500	0.9939	0.737	2.234
Ritonavir	721.3200	1-250	0.9944	0.098	0.297
Roxithromycin	837.5319	2.5-100	0.9977	0.130	0.328
Salbutamol	240.1594	1-100	0.9933	0.043	0.13
Salicylamide	138.0550	1-500	0.9983	0.045	0.135
Sarafloxacin	386.1311	2.5-500	0.9906	0.898	2.723
Spiramycin	843.5213	25-1000	0.9613	10.1	30.7
Sulphacetamide	215.0485	5-1000	0.9848	4.16	12.6
Sulphabenzamide	277.0641	1-500	0.9994	0.043	0.132
Sulphadiazine	251.0597	1-500	0.9913	0.197	0.598
Sulphadimethoxine	311.0809	1-500	0.9987	0.031	0.095
Sulphachloropyridazine	285.0208	1-500	0.9992	0.185	0.561
Sulphadoxin	311.0809	1-500	0.9993	0.03	0.092
Sulphaguanadin	215.0597	1-1000	0.9926	1.52	4.61
Sulphamerazine	265.0754	1-1000	0.9935	0.139	0.421
Sulphamethazine	279.0910	1-500	0.9885	0.024	0.071
Sulphamethizole	271.0318	1-500	0.9978	0.175	0.531
Sulphamethoxazole	254.0594	1-500	0.9994	0.035	0.106
Sulphathiazole	256.0209	2.5-500	0.9963	0.112	0.339
Sulphamoxol	268.0750	2.5-500	0.9970	0.088	0.268

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Sulphamethoxypyridazine	281.0703	1-500	0.9981	0.054	0.163
Sulphamonomethoxine	281.0703	1-500	0.9987	0.059	0.179
Sulphanilamide	172.0307	1-500	0.9952	0.081	0.245
Sulphanitran	336.0649	1-500	0.9989	0.076	0.23
Sulphasalazine	399.0758	1.500	0.9983	0.084	0.255
Sulphapyridine	250.0645	1-500	0.9913	0.063	0.192
Sulphaquinoxaline	301.0754	1-500	0.9993	0.049	0.148
Sulphisoxazole	268.0750	1-500	0.9994	0.028	0.084
Terbutaline	226.1438	1-100	0.9903	0.017	0.053
Testosterone	289.2162	1-100	0.9991	0.017	0.052
Thiabendazole	202.0433	1-250	0.9982	0.009	0.027
Tonalid	259.2058	1-250	0.9933	0.016	0.048
Tramadol	264.1958	1-500	0.9985	0.01	0.032
Triclocarban	314.9853	1-250	0.9992	0.080	0.244
Triclosan	286.9439	1-500	0.9984	0.038	0.122
Trimethoprim	291.1452	1-100	0.9925	0.006	0.019
Tylosin	916.5264	2.5-100	0.9957	0.219	0.883
Valsartan	436.2343	2.5-100	0.9962	0.478	1.448
Venlafaxine	278.2115	1-100	0.9991	0.005	0.016
Verapamil	455.2904	1-100	0.9982	0.010	0.029

Table A3: **Biphenyl C18 column method validation data**

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Albendazole	266.0958	0.5-100	0.9994	0.005	0.014
Amitriptyline	278.1903	1-500	0.999	0.007	0.020
Ampicillin	350.1169	2.5-500	0.9984	0.801	2.429
Atazanavir	705.3970	1-500	0.9992	0.032	0.096
Azithromycin	749.5158	1-500	0.9528		
Bufexamac	224.1281	5-100	0.9949	0.022	0.066
Buspirone	386.2551	0.5-500	0.9998	0.009	0.027
Cafeine	195.0877	1-250	0.9941	0.007	0.021
Carbamazepine	237.1022	0.5-250	0.9961	0.004	0.013
Cefotaxime	456.0642	0.5-100	0.9961	0.075	0.226
Chlorpheniramine	275.1310	1-250	0.9993	0.024	0.072
Ciprofloxacin	332.1405	10-500	0.999	0.127	0.385
Clarithromycin	748.4842	1-500	0.9991	0.043	0.131
Cloxacillin	436.0729	5-250	0.9931	0.474	1.437
Danofloxacin	358.1562	10-500	0.9991	0.093	0.281
Desipramine	267.1856	0.5-500	0.9984	0.007	0.020
Dexamethasone	393.2072	1-100	0.9979	0.020	0.060
Diclofenac	296.0240	2.5-100	0.9916		
Digoxigenin	391.2480	1-100	0.9973	0.02	0.06
Digoxin	781.4369	5-500	0.9974	1.487	4.505
Difloxacin	400.1467	10-500	0.9995	0.051	0.155
Efavirenz	316.0347	2.5-100	0.9958		
Enalapril	377.2071	1-500	0.9994	0.01	0.031
Enrofloxacin	360.1718	10-500	0.9983	0.041	0.123
Estradiol	273.1849	1-1000	0.9886		
Estriol	289.1798	2.5-100	0.9969	0.325	0.985
Estrone	271.1693	fail	0.9916		
Etilefrine	182.1176	0.5-250	0.9953	0.007	0.022

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Erythromycin	734.4685	1-100	0.9986		
Famciclovir	322.1510	1-100	0.9900	0.008	0.025
Fenbendazole	300.0801	1-100	0.9992		
Fluconazole	307.1113	1-100	0.9988	0.005	0.016
Flumequine	262.0874	2.5-500	0.9996		
Gabapentin	172.1332	5-100	0.9943	0.005	0.016
Gemfibrozil	251.1642	2.5-100	0.9952		
Hyoscyamine	290.1751	2.5-500	0.9986	0.009	0.028
Indometacin	358.0841	1-100	0.9959	0.025	0.075
Ifosfamide	261.0321	0.5-100	0.9993	0.008	0.023
Isoniazide	138.0662	0.5-75	0.9943	0.008	0.025
Ketoprofen	255.1016	0.1-75	0.9993	0.006	0.018
Lamivudine	230.0590	0.5-250	0.9938	0.015	0.046
Lidocaine	235.1805	0.5-500	0.9996	0.006	0.017
Lincomycin	407.2210	1-500	0.9994	0.022	0.068
Lopinavir	629.3697	1-500	0.9991	0.022	0.067
Marbofloxacin	363.1463	5-500	0.9989	0.065	0.196
Mebendazole	296.1030	1-100	0.9965	0.005	0.015
Medroxyprogesterone	345.2424	0.5-100	0.9980	0.008	0.024
Mefenamic acid	242.1176	1-100	0.9913	0.007	0.022
Mestranol	311.2006	10-250	0.9930	1.855	5.617
Metformin	130.1087	0.5-500	0.9958	0.005	0.016
Metoprolol	268.1907	0.5-500	0.9986	0.007	0.021
Miconazole	414.9933	1-500	0.9997		
Naproxen	231.1016	2.5-100	0.9957	0.015	0.045
Nevirapine	267.1240	0.5-100	0.9987	0.005	0.015
Norfloxacin	320.1405	10-500	0.9993	0.120	0.365
(-) Norgestrel	313.2162	0.5-500	0.9985	0.009	0.027
Ofloxacin	362.1511	10-500	0.9991	0.076	0.232
oxibendazole	250.1186	0.5-100	0.9995	0.004	0.013

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Oxytetracycline	461.1555	0.5-500	0.9989	0.058	0.177
Paracetamol	152.0706	0.5-250	0.9927	0.008	0.023
Paraxanthine	181.0720	1-500	0.9995	0.021	0.063
Penciclovir	254.1248	0.5-75	0.9943	0.070	0.211
Penicilline G	335.1060	1-100	0.9977	0.072	0.218
Phenacetin	180.1019	0.1-100	0.9981	0.002	0.007
Pindolol	249.1598	0.5-500	0.9992	0.008	0.025
Prednisolone	361.2010	1-100	0.9961	0.010	0.031
Procaine	237.1598	0.5-250	0.9981	0.007	0.021
progesterone	315.2319	1-100	0.9985	0.004	0.013
Ractopamine	302.1751	0.5-500	0.9969	0.009	0.027
Ribavirin	245.0881	0.5-75	0.9933	0.075	0.227
Rifabutin	847.4488	1-1000	0.9928		
Rifapentine	877.4594	5-1000	0.9914		
Rifampicin	823.4124	1-500	0.9992		
Ritonavir	721.3200	1-500	0.992		
Roxithromycin	837.5319	2.5-500	0.9957	0.195	1.183
Salbutamol	240.1594	0.5-500	0.9977	0.011	0.033
Salicylamide	138.0550	1-100	0.9978	0.015	0.045
Sarafloxacin	386.1311	10-500	0.9972	0.097	0.294
Stavudine	225.0870	5-500	0.9991	1.607	4.501
Sulphacetamide	215.0485	5-500	0.9994	0.014	0.042
Sulphabenzamide	277.0641	1-100	0.9981	0.007	0.022
Sulphadiazine	251.0597	0.5-250	0.9959	0.006	0.020
Sulphadimethoxine	311.0809	0.5-100	0.9989	0.006	0.017
Sulphachloropyridazine	285.0208	1-100	0.9947	0.010	0.029
Sulphadoxin	311.0809	1-250	0.9988	0.007	0.022
Sulphaguanadin	215.0597	0.1-75	0.9914	0.009	0.026
Sulphamerazine	265.0754	2.5-100	0.9989	0.002	0.007
Sulphamethazine	279.0910	1-250	0.9972	0.009	0.027

Compound	Quantification ion (*m/z*)	Linearity range (ppb)	r²	LOD (ppb)	LOQ (ppb)
Sulphamethizole	271.0318	1-100	0.9972	0.009	0.027
Sulphathiazole	256.0209	0.5-100	0.9947	0.008	0.025
Sulphamoxol	268.0750	1-500	0.9978	0.024	0.073
Sulphamethoxypyridazine	281.0703	1-100	0.9882	0.008	0.025
Sulphamonomethoxine	281.0703	1-100	0.9898	0.007	0.022
Sulphamethoxazole	254.0594	1-100	0.9947	0.006	0.018
Sulphanilamide	172.0307	0.5-100	0.9913	0.042	0.128
Sulphanitran	336.0649	1-100	0.9964	0.292	0.886
Sulphasalazine	399.0758	1-100	0.9984	0.015	0.045
Sulphapyridine	250.0650	0.5-100	0.9989	0.006	0.019
Sulphaquinoxaline	301.0754	1-100	0.9971	0.007	0.023
Sulphisoxazole	268.0750	1-1000	0.9947	0.007	0.022
Telmisartan	515.2442	0.5-250	0.9995	0.009	0.029
Terbutaline	226.1438	0.1-100	0.9990	0.006	0.019
Testosterone	289.2162	10-250	0.9953	0.006	0.017
Thiabendazole	202.0433	0.5-500	0.9983	0.005	0.015
Tilmicosin	869.5733	10-250	0.9978	2.055	6228
Tramadol	264.1958	2.5-500	0.999	0.007	0.022
Triclocarban	314.9853	2.5-500	0.9971		
Triclosan	286.9439	2.5-250	0.9990	0.301	0.913
Trimethoprim	291.1452	1-500	0.9965	0.007	0.022
Tylosin	916.5264	10-250	0.9983	0.583	1.765
Valsartan	436.2343	1-100	0.9953		
Venlafaxine	278.2115	0.5-500	0.999	0.006	0.018
Verapamil	455.2904	1-500	0.9997	0.005	0.015
Zalcitabine		0.5-500	0.9952	0.139	0.421
Zidovudine	268.1040	2.5-100	0.9976	0.325	0.984

Table A4: **GCxGC-HRToF-MS method validation data**

Analyte	Name	Quantification ion	Correlation coefficients	Linear range (µg ℓ⁻¹)	LODs (µg ℓ⁻¹)	LOQs (µg ℓ⁻¹)
1	Phenol, 2-chloro-	128.0025	0.9954	0.01-1	0.090	0.301
2	Benzene, 1,3-dichloro-	145.9685	0.9983	0.01-1	0.035	0.117
3	Benzene, 1,4-dichloro-	145.9685	0.9997	0.01-1	0.043	0.145
4	Acetylpyrazine	80.0369	0.9996	0.025-1	0.038	0.127
5	Benzene, 1,2-dichloro-	145.9684	0.9986	0.001-1	0.029	0.099
6	Bis(2-chloro-1-methylethyl) ether	121.0414	0.9994	0.01-1	0.047	0.157
7	Phenol, 2-methyl-	108.0570	0.9993	0.05-1	0.062	0.209
8	p-Cresol	107.0491	0.9997	0.05-1	0.019	0.064
9	Ethane, hexachloro-	200.8408	0.9991	0.01-1	0.043	0.146
10	1-Propanamine, N-nitroso-N-propyl-	70.0651	0.9959	0.01-1	0.200	0.669
11	Isophorone	82.0414	0.9990	0.01-1	0.052	0.175
12	Phenol, 2-nitro-	139.0266	0.9967	0.025-1	0.062	0.207
13	Phenol, 2,4-dimethyl-	107.0491	0.9996	0.05-1	0.056	0.186
14	Phenol, 2,4-dichloro-	161.9633	0.9993	0.05-1	0.062	0.206
15	Benzene, 1,3,5-trichloro-	179.9295	0.9997	0.005-1	0.042	0.141
16	Naphthalene-D8	136.1123				
17	Naphthalene	128.0621	0.9991	0.001-1	0.039	0.132
18	p-Chloroaniline	127.0183	0.9997	0.025-1	0.026	0.088
19	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-	224.8408	0.9993	0.005-1	0.022	0.076
20	Phenol, 4-chloro-3-methyl-	107.0491	0.9995	0.05-1	0.019	0.066
21	Indole	117.0573	0.9981	0.025-1	0.054	0.180
22	4-Chloroaniline, N-isopropylidene	152.0264	0.9989	0.025-1	0.046	0.153
23	Naphthalene, 2-methyl-	142.0776	0.9985	0.005-1	0.046	0.153
24	Hexachlorocyclopentadiene	236.8409	0.9905	0.2-1	0.372	1.24
25	Phenol, 2,4,6-trichloro-	195.9245	0.9987	0.025-1	0.057	0.193
26	Naphthalene, 1-chloro-	162.0231	0.9979	0.01-1	0.098	0.328
27	o-Nitroaniline	138.0425	0.9992	0.075-1	0.034	0.116

Analyte	Name	Quantification ion	Correlation coefficients	Linear range (µg ℓ$^{-1}$)	LODs (µg ℓ$^{-1}$)	LOQs (µg ℓ$^{-1}$)
28	Dimethyl phthalate	163.0390	0.9989	0.005-1	0.060	0.201
29	Etridiazole	182.9181	0.9992	0.1-1	0.123	0.411
30	Acenaphthylene	152.0621	0.9991	0.01-1	0.035	0.117
31	Benzene, 2-methyl-1,3-dinitro-	165.0293	0.9986	0.025-1	0.085	0.286
32	Acenaphthene-d10	162.1264				
33	Acenaphthene	153.0698	0.9994	0.005-1	0.027	0.091
34	Chloroneb	190.9661	0.9995	0.01-1	0.031	0.105
35	Dibenzofuran	168.0570	0.9995	0.005-1	0.021	0.071
36	Benzene, 1-methyl-2,4-dinitro-	165.0295	0.9993	0.05-1	0.045	0.152
37	Methiocarb	168.0603	0.9996	0.025-1	0.041	0.138
38	2-Naphthalenamine	143.0730	0.9986	0.05-1	0.043	0.145
39	Fluorene	165.0670	0.9993	0.005-1	0.033	0.113
40	Diethyl Phthalate	149.0233	0.9996	0.05-1	0.022	0.074
41	p-Nitroaniline	65.0386	0.9984	0.075-1	0.116	0.389
42	Azobenzene	77.0386	0.9992	0.005-1	0.055	0.184
43	Phenol, 4-heptyl-	107.0491	0.9996	0.075-1	0.039	0.132
44	Benzene, hexachloro-	283.8096	0.9996	0.005-1	0.016	0.053
45	Simazine	201.0777	0.9998	0.05-1	0.036	0.121
46	Carbofuran	164.0831	0.9994	0.075-1	0.097	0.326
47	Atrazine	200.0670	0.9991	0.05-1	0.062	0.209
48	[1,1'-Biphenyl]-4-amine	169.0888	0.9999	0.075-1	0.023	0.078
49	Dibenzothiophene	184.0341	0.9995	0.075-1	0.032	0.107
50	Phenanthrene-D10	188.1405				
51	Phenanthrene	178.0778	0.9992	0.001-1	0.036	0.122
52	Anthracene-D10-	188.1404				
53	Anthracene	178.0778	0.9997	0.005-1	0.017	0.056
54	Tetrachloroisophthalonitrile	265.8780	0.9992	0.01-1	0.026	0.089
55	Carbazole	167.0730	0.9992	0.05-1	0.043	0.144
56	Endosulphan ether	69.0335	0.9991	0.05-1	0.069	0.232

Analyte	Name	Quantification ion	Correlation coefficients	Linear range (µg ℓ⁻¹)	LODs (µg ℓ⁻¹)	LOQs (µg ℓ⁻¹)
57	Galaxolide 1	243.1745	0.9982	0.025-1	0.046	0.153
59	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	243.1744	0.9981	0.01-1	0.030	0.101
60	Heptachlor	100.0074	0.9961	0.1-1	0.286	0.956
61	Alachlor	160.1121	0.9989	0.05-1	0.070	0.234
62	Metalaxyl	160.1122	0.9986	0.025-1	0.033	0.110
63	Terbutryn	185.0731	0.9993	0.01-1	0.075	0.251
65	Dibutyl phthalate	149.0234	0.9993	0.001-1	0.014	0.049
66	Malathion	127.0391	0.9991	0.05-1	0.061	0.205
67	Aldrin	66.0464	0.9984	0.01-1	0.031	0.104
68	Chlorpyrifos	196.9196	0.9995	0.01-1	0.075	0.250
69	4,4'-Dichlorobenzophenone	138.9947	0.9990	0.05-1	0.078	0.262
70	Heptachlor epoxide	352.8436	0.9995	0.075-1	0.079	0.264
71	Bioallethrin	123.1168	0.9990	0.025-1	0.135	0.453
72	Fluoranthene	202.0777	0.9998	0.025-1	0.034	0.116
73	trans-Chlordane	372.8253	0.9996	0.075-1	0.067	0.225
74	Pyrene	202.0777	0.9995	0.005-1	0.044	0.149
75	α-Endosulphan	236.8409	0.9988	0.025-1	0.084	0.282
76	Dibenzothiophene sulphone	216.0240	0.9986	0.025-1	0.069	0.230
77	cis-Chlordane	372.8253	0.9990	0.01-1	0.024	0.080
78	trans-Nonachlor	408.7830	0.9994	0.05-1	0.043	0.144
79	p,p'-DDE	245.9998	0.9995	0.01-1	0.026	0.089
80	Dieldrin	79.0543	0.9989	0.075-1	0.104	0.349
81	Dicofol	138.9947	0.9982	0.05-1	0.101	0.339
82	β-Endosulphan	236.8408	0.9998	0.025-1	0.028	0.093
83	m,p'-DDD	235.0076	0.9995	0.05-1	0.064	0.216
84	o-Aminoazotoluene	106.0652	0.9991	0.075-1	0.038	0.128
85	Endrin ketone	67.0545	0.9995	0.05-1	0.056	0.189
86	Benalaxyl	148.1123	0.9984	0.01-1	0.073	0.243

Analyte	Name	Quantification ion	Correlation coefficients	Linear range (µg ℓ⁻¹)	LODs (µg ℓ⁻¹)	LOQs (µg ℓ⁻¹)
87	Benzyl butyl phthalate	149.0232	0.9993	0.005-1	0.055	0.185
88	p,p'-DDT	235.0078	0.9991	0.075-1	0.106	0.353
89	Endosulphan sulphate	271.8094	0.9996	0.05-1	0.052	0.176
90	Methoxychlor	227.1068	0.9997	0.05-1	0.028	0.093
91	Bifenthrin	181.1011	0.9984	0.01-1	0.058	0.195
92	Tetramethrin	164.0706	0.9991	0.05-1	0.057	0.190
93	Naphthacene	228.0935	0.9994	0.05-1	0.054	0.182
94	1,6-Dimethoxyphenazine	240.0240	0.9987	0.05-1	0.081	0.273
96	Chrysene-D12	240.1686				
97	Benz[a]anthracene	228.0933	0.9992	0.005-1	0.056	0.188
98	Bis(2-ethylhexyl) phthalate	149.02337	0.9992	0.01-1	0.044	0.147
99	Di-n-octyl phthalate	149.0233	0.9989	0.01-1	0.044	0.147
100	Permethrine	183.0803	0.9988	0.05-1	0.108	0.363
101	Benzo[k]fluoranthene	252.0934	0.9992	0.01-1	0.062	0.208
102	Perylene	252.0936	0.9990	0.01-1	0.100	0.334
103	Benzo[a]pyrene	252.0932	0.9990	0.075-1	0.069	0.232
104	Dinaphtho(1,2-b:2',1'-d)thiophene	284.0654	0.9995	0.075-1	0.026	0.089
105	Benzo[ghi]perylene	276.0932	0.9982	0.075-1	0.117	0.391
106	Dibenz[a,j]anthracene	278.1091	0.9959	0.05-1	0.284	0.949
107	Indeno[1,2,3-cd]pyrene	276.0932	0.9972	0.05-1	0.175	0.584

APPENDIX B

OCCURRENCE OF EMERGING CONTAMINANTS

Table B1: **Summary of wastewater influent-1 samples and concentrations (ng ℓ$^{-1}$)**

Compound	Samples													
	March 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Albendazole	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Amitriptyline	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.304	nd
Bufexamac	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.296	0.538
Caffeine	42229	43293	32475	33703	41900	29734	13363	15723	16302	24572	20685	11869	2990	7233
Carbamazepine	34.91	32.15	46.00	115.7	38.39	84.34	6.284	15.74	17.46	15.17	25.59	8.938	2.416	7.073
Ciprofloxacin	77.04	72.24	5.470	33.55	13.29	14.11	9.092	16.54	21.34	9.693	33.96	<loq	9.191	12.79
Clarithromycin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.125	nd
Diclofenac	129.79	135.6	127.2	185.9	172.7	146.3	31.42	44.46	51.14	42.48	71.78	44.60	14.51	30.36
Diethylbestrol	nd	nd	nd	nd	nd	21.35	nd	nd	nd	nd	nd	nd	nd	nd
Digoxigenin	2.219	nd	nd	nd	1.737	nd	nd	nd	nd	nd	nd	nd	nd	nd
Efavirenz	2112	2169	1517	1026	1098	1009	313.0	577.1	572.9	524.4	688.8	316.2	74.43	181.8
Enalapril	13.07	0.752	nd	1.230	0.745	nd	5.331	nd	0.833	nd	0.550	nd	3.925	5.214
Enrofloxacin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Estradiol	2206	1406	1167	1754	2126	1660	1373	404.8	628.2	1288	666.4	893.3	209.8	66.45
Estriol	63.94	966.3	1313	551.1	406.8	381.0	613.4	56.57	310.8	99.52	528.0	53.23	96.82	129.2
Estrone	11.24	nd	11.07	nd	nd	nd	nd	1.161	nd	nd	10.99	10.64	0.927	1.437
Famciclovir	nd	2.859	nd	7.213	10.71	17.67	2.222	nd	nd	3.798	nd	nd	nd	nd
Fenoprofen	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Compound	Samples													
	March 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Fluconazole	333.4	168.8	187.2	192.8	192.8	157.3	75.49	52.41	38.88	134.9	107.6	43.57	19.52	31.72
Flumequine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Gabapentin	73.18	5.787	45.01	45.47	67.46	37.59	146.4	36.65	22.44	56.19	10.27	20.62	45.97	122.4
Gemfibrozil	330.0	230.5	119.8	192.8	233.9	nd	156.1	220.2	190.7	84.43	233.5	357.8	86.33	320.3
Ibuprofen	19936	76377	14291	24695	26930	23327	2576	7491	8643	13535	13924	4579	568.1	1292
Ifosfamide	nd	nd	nd	1.689	nd	2.122	nd	0.992	1.635	nd	nd	nd	nd	nd
Indometacin	42.52	37.36	31.95	30.26	18.84	24.31	2.978	7.187	5.509	7.912	9.613	nd	1.305	3.708
Isoniazide	nd	nd	nd	nd	0.576	11.85	9.663	5.246	6.022	8.494	14.04	7.017	nd	13.17
Ketoprofen	nd	6.797	8.723	11.46	7.000	10.84	4.454	nd	nd	nd	nd	4.379	5.212	3.932
Lamivudine	nd	nd	nd	9.379	8.176	75.82	226.2	nd	169.5	67.82	56.15	378.6	65.78	237.2
Lidocaine	12.08	nd	nd	2.437	nd	nd	1.696	1.309	nd	nd	nd	0.951	0.899	1.018
Lincomycin	nd	nd	nd	nd	2.801	nd	nd	nd	nd	nd	nd	nd	nd	nd
Marbofloxacin	nd	<loq	nd	<loq	<loq	nd	nd	nd	nd	nd	nd	nd	nd	nd
Mebendazole	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Medroxyprogesterone	3.148	4.831	3.013	2.540	5.309	3.517	2.878	3.335	2.234	2.366	4.160	5.345	2.741	2.648
Mefenamic acid	59.98	59.27	58.23	88.76	85.18	48.41	15.95	32.15	31.64	40.67	52.66	11.30	12.64	27.87
Methylparaben	139.1	110.6	67.91	123.6	166.5	286.7	600.4	1.649	530.6	11.07	151.1	4.472	139.9	1.570
Naproxen	190.3	288.8	120.2	104.6	546.1	103.4	128.1	59.87	55.33	283.5	110.5	35.03	20.17	45.62
Nevirapine	1.062	0.430	0.310	24.84	17.91	12.57	2.290	3.514	3.714	5.967	6.327	26.34	0.714	2.010
Norfloxacin	25.83	27.71	nd	31.70	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ofloxacin	57.60	43.67	30.63	66.06	67.50	34.25	24.15	37.03	32.46	44.44	30.93	47.13	27.36	27.49
Oxolinic acid	0.125	nd	nd	nd	nd	nd	nd	nd	nd	0.079	nd	nd	nd	0.145
Oxytetracycline	nd	nd	3.067	nd	nd	2.810	20.70	nd	nd	nd	4.312	21.01	nd	nd
Paracetamol	22889	12125	nd	7043	5037	4684	5468	5337	1850	1797	3364	1552	1123	1849

Compound	Samples													
	March 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Paraxanthine	21314	18503	14485	16056	17389	16945	11495	4963	9555	6153	7262	6338	1134	5438
Penciclovir	17.02	nd	nd	nd	nd	nd	nd	18.02	21.01	17.30	22.94	19.13	15.41	22.28
Phenacetin	18.28	6.505	33.07	3.084	3.533	4.629	9.791	4.239	5.235	21.52	0.188	19.30	0.679	2.982
Pindolol	nd	nd	nd	0.561	0.187	nd	0.117	nd	nd	nd	nd	0.205	0.124	0.115
Prednisolone	2.556	0.868	nd	2.089	0.411	0.411	nd	nd	nd	2.529	nd	nd	1.592	5.828
Procaine	0.596	7.782	nd	10.24	7.926	14.16	1.101	0.185	nd	6.067	1.430	0.265	0.603	0.265
progesterone	4.079	nd	5.891	6.454	3.910	3.947	0.288	0.670	nd	1.450	0.423	nd	nd	nd
Ractopamine	nd	nd	nd	nd	0.544	nd	0.551	0.449	0.241	0.993	nd	0.610	<loq	0.121
Ritonavir	172.4	400.5	187.6	196.9	159.9	117.0	232.3	32.12	32.77	41.75	43.66	30.88	13.55	5.918
Salbutamol	nd	5.171	nd	nd	nd	0.431	nd	nd	0.345	0.498	0.679	nd	0.307	nd
Salicylamide	228.3	117.0	229.2	215.2	125.3	206.3	5.472	96.99	5.512	67.39	58.92	99.63	10.53	6.223
Sarafloxacin	nd	nd	8.33	nd	nd	<lod	nd	<lod	8.14	nd	nd	<loq	nd	nd
Sulphadimethoxine	0.225	0.223	0.643	nd	0.236	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphadoxin	6.750	nd	1.876	0.660	0.269	0.576	<loq	1.037	nd	0.250	1.125	0.254	<loq	0.452
Sulphaguanadin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphamethazine	nd	nd	0.357	0.165	0.116	21.33	nd	2.439	2.163	nd	nd	nd	nd	nd
Sulphamethoxazole	361.4	937.7	1200	981.9	250.8	755.6	2405	445.1	476.3	485.4	817.3	433.1	143.0	569.5
Sulphanilamide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.342	0.745	nd
Sulphapyridine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphaquinoxaline	nd	nd	nd	nd	nd	nd	nd	0.295	nd	nd	nd	nd	nd	nd
Terbutaline	0.216	0.322	0.486	1.444	1.296	0.501	nd	0.054	nd	nd	nd	nd	nd	nd
Testosterone	34.06	25.35	nd	18.41	17.35	22.32	3.513	nd	1.357	14.67	10.53	nd	2.003	4.868
Thiabendazole	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Tonalid	46.02	70.19	80.16	43.45	29.07	28.37	6.592	12.27	8.354	33.57	25.84	3.682	0.597	1.337

Compound	Samples													
	March 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Tramadol	nd	1.806	nd	1.123	12.26	1.49	3.056	4.350	5.340	nd	1.255	1.092	8.122	11.00
Triclocarban	129.4	116.9	133.3	79.99	44.08	99.18	11.39	49.43	20.05	48.45	47.31	17.86	11.41	14.45
Triclosan	93.61	97.78	77.99	51.36	44.72	58.47	nd	12.08	5.130	19.88	19.23	1.644	2.362	nd
Trimethoprim	577.6	337.9	198.3	385.2	248.4	220.2	21.86	62.43	61.65	51.50	172.1	24.85	33.63	42.22
Valsartan	248.6	273.7	181.6	187.4	194.3	190.5	441.5	254.4	318.8	213.8	256.5	293.7	105.3	279.8
Venlafaxine	nd	nd	nd	0.386	nd	0.179	nd	nd	nd	nd	nd	nd	0.414	1.385
Verapamil	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.174	nd

Table B2: **Summary of wastewater influent-2 samples and concentrations (ng ℓ⁻¹)**

Compound	Samples													
	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Albendazole	nd	nd	nd	17.58	0.188	0.288	nd	nd	0.309	0.476	0.118	0.040	0.242	0.228
Amitryptiline	0.304	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.614	nd
Atazanavir	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	11.16	nd
Bufexamac	0.296	nd	nd	3.196	1.747	nd	1.377	nd	nd	nd	1.592	nd	nd	nd
Caffeine	1770	11869	14968	43398	36618	36472	43156	60136	42564	33776	43238	35001	7528	42208
Carbamazepine	1.775	8.938	15.29	38.52	52.35	14.61	39.34	69.48	40.16	50.54	42.59	38.11	22.31	26.92
Ciprofloxacin	8.595	15.33	17.89	81.80	24.63	49.30	30.39	105.6	44.32	67.48	30.02	25.25	11.00	44.26
Clarithromycin	2.125	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	10.06	nd
Diclofenac	12.16	44.60	70.31	212.2	204.4	197.8	212.1	246.3	190.9	205.5	221.9	162.2	46.50	171.5
Diethylbestrol	nd	nd	nd	nd	77.89	nd	91.11	18.98	nd	18.95	60.14	25.28	30.91	43.29
Digoxigenin	nd	nd	nd	nd	3.124	nd	3.532	3.223	nd	nd	nd	nd	nd	nd
Efavirenz	50.98	316.2	811.5	1181	1443	1779	1375	1484	1813	1264	1311	1142	330.4	1292
Enalapril	4.728	nd	nd	0.611	0.714	1.255	30.94	32.53	23.58	0.459	0.772	14.46	4.738	nd
Enrofloxacin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Estradiol	122.2	782.1	381.3	1071	1335	1351	1103	1335	1363	974.5	1096	1102	222.3	1280
Estriol	72.99	56.31	97.44	276.9	176.5	196.7	119.4	143.1	236.4	100.7	393.1	252.7	138.7	180.6
Estrone	0.371	11.64	nd	5.400	35.96	13.72	7.088	9.802	7.909	17.70	26.56	23.73	1.295	12.81
Famciclovir	nd	nd	nd	4.836	8.269	nd	nd	nd	nd	nd	5.879	nd	nd	nd
Fenoprofen	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Fluconazole	13.54	43.52	86.04	351.4	208.1	154.4	169.0	315.4	185.4	189.3	396.4	167.5	26.53	162.3
Flumequine	nd	nd	nd	3.125	3.077	0.217	nd	nd	nd	nd	3.079	3.341	2.884	nd
Gabapentin	49.73	20.62	9.633	7.277	nd	69.93	nd	nd	nd	21.59	nd	24.92	14.31	12.45

Compound	Samples													
	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Gemfibrozil	78.79	357.8	169.7	344.9	598.6	578.5	360.8	207.6	224.5	159.0	177.7	338.9	233.9	216.7
Ibuprofen	568.7	5900	9181	19 147	14 063	21 053	14 837	20 679	14 736	16 867	22 219	12 144	5228	13 217
Indometacin	1.122	nd	12.39	12.54	17.85	15.78	9.916	nd	29.93	30.17	15.85	21.43	7.212	14.01
Isoniazide	6.663			15.64	31.55	nd	nd	nd	nd	nd	nd	23.15	10.66	23.65
Ketoprofen	4.039	5.503	4.282	14.42	12.96	6.669	13.91	20.62	9.133	13.51	23.10	8.477	12.56	6.642
Lamivudine	56.87	nd	nd	145.9	481.3	750.3	1001	474.1	573.6	271.3	492.9	239.5	51.81	478.6
Lidocaine	0.930	0.950	nd	6.685	0.757	4.624	4.580	38.94	26.02	14.32	11.05	nd	3.641	93.29
Marbofloxacin	nd	nd	nd	nd	nd	nd	nd	nd	nd	<lod	nd	nd	nd	nd
Mebendazole	nd	nd	nd	30.72	15.70	20.93	10.98	61.83	24.13	22.19	18.87	24.50	10.19	38.34
Medroxyprogesterone	1.714	5.345	2.294	nd	nd	7.984	nd	16.83	16.85	nd	nd	4.380	2.510	nd
Mefenamic acid	10.32	11.30	47.10	20.78	32.44	91.15	24.78	19.40	54.50	28.57	47.51	33.13	13.24	32.45
Mestranol	nd	nd	nd	nd	106.2	nd	102.6	nd	nd	123.4	68.10	nd	nd	nd
Methylparaben	148.6	4.472	157.4	483.9	357.2	617.8	418.7	425.2	275.7	234.7	319.4	332.5	79.45	513.8
Metoprolol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.091	nd
Naproxen	16.85	35.06	140.2	89.49	84.51	66.71	61.02	89.55	98.76	50.44	44.97	54.25	37.46	71.57
Nevirapine	0.491	26.34	3.030	4.733	9.957	10.99	11.02	17.10	19.65	8.345	4.825	6.210	2.576	14.77
Norfloxacin	nd	nd	nd	25.86	nd	nd	nd	nd	nd	26.13	25.91	nd	nd	nd
Ofloxacin	26.554	24.901	24.659	44.002	36.905	42.602	43.354	38.029	42.121	42.732	46.385	29.98	26.87	40.299
Oxolinic acid	nd	nd	nd	0.187	nd	nd	0.123	nd	nd	nd	nd	0.142	nd	nd
Oxytetracycline	nd	nd	nd	5.569	nd	3.645	20.373	9.314	nd	6.161	nd	2.426	nd	20.21
Paracetamol	347.3	427.2	3960	10 412	10 866	11 564	7719	11 367	10 523	12 271	8491	4427	1894	5330
Paraxanthine	1134	6338	9875	27817	31805	32393	25544	35286	30519	27840	32110	19254	5431	27750
penciclovir	15.57		nd	nd	18.26	nd	16.88	nd	18.70	nd	nd	nd	nd	nd
Phenacetin	0.769	19.30	0.315	19.81	15.75	21.08	68.58	18.12	2.283	28.84	7.463	10.79	3.661	8.315

Compound	Samples													
	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Pindolol	0.069	nd	nd	0.417	0.183	0.357	0.375	2.725	0.574	0.322	0.425	2.757	0.224	0.403
Prednisolone	1.283	nd	nd	7.383	1.865	4.565	3.602	nd	5.244	nd	2.717	3.256	6.453	3.016
Procaine	0.495	0.265	0.883	8.108	11.93	14.36	8.172	10.95	9.503	7.379	15.47	8.596	1.005	12.20
progesterone	nd	0.205	0.556	4.626	14.52	9.121	10.04	4.384	5.757	8.477	4.233	2.449	1.477	4.657
Ractopamine	<loq	0.610	0.698	nd	0.475	nd	nd	0.448	nd	0.955	nd	0.614	0.747	2.294
Reserpine	nd	nd	nd	nd	nd	43.13	nd	nd	35.93	nd	nd	nd	nd	nd
Ritonavir	4.084	59.39	27.18	48.95	79.91	62.93	95.80	58.56	96.55	61.72	112.9	145.6	18.57	146.7
Salbutamol	0.134	nd	0.169	0.750	nd	1.564	0.445	nd	2.287	0.639	0.544	0.214	0.872	nd
Salicylamide	6.340	99.63	128.6	182.3	211.0	293.0	563.5	276.4	139.5	354.2	276.9	142.1	59.46	233.8
Sarafloxacin	nd	<lod	<loq	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphadiazine	nd	nd	nd	nd	nd	nd	nd	0.396	0.376	nd	nd	0.416	nd	0.218
Sulphadimethoxine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.261	nd	0.228
Sulphadoxin	<loq	0.253	0.171	1.052	0.245	1.657	2.351	2.108	0.906	0.923	0.459	0.286	<loq	2.923
Sulphaguanadin	nd	nd	nd	11.06	5.178	7.100	nd	nd	11.47	5.328	nd	nd	<lod	nd
Sulphamerazine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.335
Sulphamethazine	nd	nd	nd	nd	3.937	nd	nd	nd	26.72	nd	nd	<loq	nd	0.111
Sulphamethoxazole	122.7	433.1	635.6	238.6	342.0	391.5	328.2	285.7	574.6	196.2	370.9	602.7	52.92	388.4
Sulphanilamide	0.300	1.342	nd	nd	1.711	nd	4.003	nd	nd	nd	1.689	1.395	nd	nd
Sulphapyridine	nd	nd	nd	110.2	55.89	88.21	77.64	29.82	73.60	27.93	101.2	20.36	12.76	21.87
Terbutaline	nd	nd	nd	nd	0.262	nd	nd	0.981	0.462	nd	0.461	0.340	nd	0.603
Testosterone	1.579	nd	7.789	27.96	nd	33.61	26.82	31.65	36.17	37.07	44.09	38.82	17.60	22.28
Thiabendazole	nd	nd	nd	0.557	1.521	0.971	0.329	nd	1.684	nd	0.556	0.913	1.468	0.747
Tonalid	0.211	3.682	18.35	72.48	57.14	60.82	420.7	78.38	66.47	50.09	90.20	76.28	13.36	60.77
Tramadol	6.057	1.092	1.104	nd	1.367	nd	73.14	nd	nd	nd	nd	nd	27.79	77.16

Compound	Samples													
	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Triclocarban	8.973	17.86	21.84	257.6	178.8	191.0	185.7	214.9	276.1	144.2	229.8	284.0	119.0	257.5
Triclosan	1.172	1.644	8.756	9.509	12.27	9.378	5.162	10.97	15.32	4.478	22.37	13.67	2.552	15.43
Trimethoprim	16.61	24.85	52.64	123.9	133.7	153.6	99.46	201.9	177.1	194.5	122.3	89.76	39.91	92.15
Valsartan	99.37	293.7	232.8	920.5	883.8	818.6	1088	605.2	872.6	602.5	1289	1075	259.1	591.1
Venlafaxine	0.275	nd	nd	1.542	7.585	0.504	6.506	4.642	1.450	4.519	5.256	4.948	3.650	5.737
Verapamil	0.148	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.472	nd

Table B3:   **Summary of wastewater effluent samples and concentrations (ng ℓ^{-1})**

Compound	Samples															
	March 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Albendazole	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<loq	0.157	nd	nd	nd
Amitriptyline	2.337	19.55	nd	4.620	0.429	0.285	0.215	0.129	0.361	nd	0.470	nd	nd	nd	nd	nd
Atazanavir	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	220.9	278.8	308.2	111.7	282.4
Bufexamac	1.187	0.470	1.303	3.432	3.826	2.160	0.962	0.245	1.189	nd	3.513	2.246	nd	4.557	7.467	10.69
Caffeine	1282	1951	1342	2250	4878	4277	279.1	163.0	312.7	6565	365.8	202.4	85.76	284.2	123.6	180.9
Carbamazepine	416.3	394.6	326.9	232.5	19.16	16.39	167.7	112.1	139.4	19.60	142.6	234.9	199.5	264.9	126.1	284.3
Ciprofloxacin	<loq	<loq	<loq	<loq	5.459	<loq	<loq	<lod	nd	5.433	nd	<loq	<loq	5.590	nd	nd
Clarithromycin	27.53	75.44	nd	21.54	1.300	1.195	1.785	2.799	5.832	nd	7.173	nd	nd	nd	nd	12.79
Dexamethasone	nd	0.924	nd	nd	0.342	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Diclofenac	29.93	68.15	80.53	71.59	23.11	19.82	8.424	5.561	8.949	44.65	10.70	114.5	148.8	243.6	116.8	195.9
Diethylbestrol	80.47	22.95	85.88	290.4	90.13	32.21	22.59	73.13	75.91	nd	43.58	547.7	259.8	325.7	139.4	199.2
Difloxacin	nd	nd	nd	nd	<loq	<lod	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Efavirenz	2042	868.7	942.4	2109	227.5	210.1	1100	708.5	1403	566.8	1463	1030	1133	1445	590.6	737.6
Enalapril	0.336	1.016	1.650	3.100	2.746	2.257	0.107	nd	nd	nd	0.253	0.440	0.618	0.197	0.177	0.292
Enrofloxacin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.737	0.383	0.374	nd	0.499
Erythromycin	8.045	11.66	nd	8.228	nd	nd	nd	nd	nd	nd	nd	11.89	10.54	7.230	0.949	5.551
Estradiol	6593	2310	2664	7133	303.9	278.0	2600	1576	3481	154.1	3940	1646	1697	2027	895.6	1335
Estriol	659.4	779.1	298.7	64.83	90.44	195.0	107.9	56.53	122.6	68.43	174.6	101.7	435.1	363.4	347.15	537.5
Estrone	32.58	nd	60.83	1.358	18.92	9.317	10.66	12.82	3.581	nd	12.51	126.2	nd	nd	nd	31.80
Famciclovir	nd	nd	7.165	2.392	nd	nd	nd	nd	nd	nd	1.379	nd	2.193	1.982	nd	4.267
Fenoprofen	nd	207.6	195.2	7.270	nd	nd	18.31	7.326	nd	nd	nd	19.45	89.20	100.5	49.57	54.38
Fluconazole	299.9	307.6	243.7	261.9	15.65	14.78	111.2	50.59	119.1	32.74	120.2	170.9	204.0	270.9	118.8	212.1

Compound	Samples															
	March 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Flumequine	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.175	nd	nd	nd	nd	nd	nd
Gabapentin	9.077	28.65	32.23	20.67	11.81	14.96	3.134	2.910	5.006	26.11	5.743	25.36	41.79	23.26	18.93	29.63
Gemfibrozil	66.91	396.1	300.2	181.2	3.776	5.292	151.7	94.66	51.22	283.7	70.35	309.3	479.4	169.5	101.6	363.9
Ibuprofen	2459	5730	4433	3551	7582	5995	709.0	57.45	748.4	7652	410.2	nd	178.9	287.1	92.76	190.5
Ifosfamide	2.045	0.808	1.747	5.246	nd	nd	0.282	0.135	0.712	nd	0.648	2.192	1.735	nd	nd	1.002
Indometacin	13.41	10.81	15.71	18.70	3.544	2.577	1.463	0.273	1.009	4.144	1.160	12.02	12.02	16.88	9.158	9.087
Isoniazide	1.587	2.391	nd	3.316	2.595	2.766	14.87	17.10	27.77	10.34	25.16	16.70	19.42	25.90	12.68	19.40
Ketoprofen	3.837	11.85	16.67	3.785	8.098	4.063	4.138	3.627	4.272	nd	4.347	16.23	34.78	49.48	19.66	29.17
Lamivudine	nd	nd	nd	3.286	25.58	33.41	0.476	0.204	0.787	323.4	2.055	12.91	15.52	14.99	3.791	12.68
Lidocaine	0.187	0.431	424.6	26.16	1.605	1.439	2.175	1.299	2.671	nd	3.000	25.08	20.57	64.35	11.24	20.88
Lincomycin	nd	nd	nd	20.65	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	3.760
Marbofloxacin	<loq	nd	nd	<loq	nd	nd	nd	nd	nd	nd	nd	nd	<loq	nd	nd	nd
Mebendazole	1.776	nd	nd	1.622	1.597	nd	nd	0.077	1.604	nd	1.586	16.34	20.43	23.66	8.760	29.36
Medroxyprogesterone	nd	2.343	nd	4.788	3.378	3.114	3.053	0.359	1.983	4.016	3.008	nd	nd	nd	nd	nd
Mefenamic acid	11.09	4.789	19.15	14.13	17.83	11.14	55.05	21.41	9.903	8.944	10.02	23.55	27.60	32.85	20.98	31.43
Mestranol	nd	nd	nd	nd	110.0	29.67	nd	nd	nd	nd	nd	nd	nd	86.41	nd	nd
Methylparaben	21.76	49.38	56.90	33.53	6.561	0.742	nd	nd	12.08	12.08	11.13	27.42	86.63	66.12	82.85	110.0
Metoprolol	1.361	1.387	nd	<loq	nd	nd	nd	nd	1.155	nd	1.170	1.526	nd	0.694	0.139	2.215
Naproxen	254.6	99.86	22.92	349.6	105.7	126.5	16.33	13.09	142.4	60.65	85.70	231.4	93.17	144.9	44.01	166.5
Nevirapine	0.801	0.389	0.864	0.621	2.879	2.594	0.814	0.449	1.113	0.352	1.028	15.17	24.06	80.53	11.49	20.03
Norfloxacin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<lod	<lod	<lod	9.833
Ofloxacin	36.74	85.71	86.51	50.45	26.60	24.39	25.05	22.47	24.02	11.54	24.81	47.66	53.74	52.89	42.21	57.95
Oxolinic acid	<loq	nd	nd	<loq	<loq	<loq	nd	<loq	nd	0.087	0.045	<lod	0.107	0.205	0.032	0.071
Oxytetracycline	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<lod	nd	1.198	1.248	1.050	1.365

Compound	Samples														
	March 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Paracetamol	23.70	71.31	53.25	17.72	25.56	nd	106.8	nd	102.2	41.77	10.15	nd	3.467	33.84	7.041	4.268
Paraxanthine	1704	2182	1706	2715	708.4	990.6	9.704	159.4	145.3	8452	162.6	151.3	130.9	433.0	146.3	335.0
penciclovir	59.96	94.69	91.14	58.07	16.31	19.71	28.43	28.34	37.99	18.03	36.54	88.47	77.91	84.32	52.12	104.8
Phenacetin	2.620	1.079	1.700	2.519	0.621	0.466	1.524	0.908	1.121	25.81	1.421	3.661	2.688	1.623	0.931	3.410
Pindolol	1.243	0.793	0.633	nd	0.106	0.458	0.069	<loq	0.477	0.108	0.711	nd	nd	18.41	11.45	nd
Prednisolone	36.17	8.902	9.586	2.280	1.505	7.809	2.589	5.015	4.382	nd	3.655	30.69	nd	nd	23.57	nd
Procaine	0.484	1.825	0.827	1.729	nd	nd	nd	nd	0.303	0.202	<loq	nd	0.381	0.258	nd	0.439
progesterone	1.221	1.069	1.063	2.600	1.273	0.833	0.288	0.244	1.667	0.349	1.119	2.700	1.600	4.025	0.862	1.618
Ractopamine	0.199	0.141	0.164	0.129	nd	nd	nd	nd	nd	0.542	nd	0.938	0.394	0.261	0.140	nd
Ritonavir	278.8	685.5	335.4	206.3	14.43	16.43	53.08	31.29	93.28	26.68	96.96	39.18	48.56	82.29	24.49	48.22
Salbutamol	nd	nd	8.599	4.613	<loq	0.208	nd	nd	nd	nd	0.122	3.150	3.883	3.960	1.758	3.656
Salicylamide	39.38	4.864	23.72	35.07	17.71	17.00	10.92	9.065	10.16	112.9	9.192	42.11	39.28	26.89	18.52	39.31
Sarafloxacin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphadimethoxine	<loq	0.347	0.409	<loq	<loq	nd	nd	nd	nd	nd	nd	nd	nd	0.139	nd	nd
Sulphadoxin	0.554	0.625	1.256	<loq	0.189	0.240	<loq	nd	<loq	nd	<loq	1.094	1.073	0.635	0.576	0.829
Sulphaguanadin	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphamerazine	0.402	0.696	0.881	<loq	1.643	nd	nd	<lod	nd	nd	nd	<loq	<loq	0.533	<lod	0.404
Sulphamethazine	nd	nd	41.88	2.023	1.710	1.527	nd	nd	nd	nd	<loq	0.208	0.822	12.37	0.217	0.635
Sulphamethoxazole	268.5	240.6	504.4	238.8	147.9	135.2	35.13	34.93	50.06	584.0	56.16	229.0	220.1	219.8	108.0	196.7
Sulphanilamide	3.799	0.321	10.00	0.823	nd	nd	nd	nd	0.229	1.175	0.280	nd	nd	nd	nd	10.01
Sulphapyridine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	18.51	23.22	18.99	9.552	15.92
Sulphaquinoxaline	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<loq	nd
Terbutaline	0.275	0.424	0.125	0.448	<loq	nd	nd	nd	<loq	nd	0.128	0.102	0.076	0.351	0.171	0.225
Testosterone	nd	nd	nd	nd	nd	nd	nd	0.247	nd	5.826	nd	1.304	nd	nd	nd	2.08

Compound	Samples															
	March 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Dec 2016	Dec 2016	Dec 2016	Dec 2016	Dec 2016
Thiabendazole	nd	nd	nd	nd	nd	0.066	nd	nd	nd	nd	nd	3.739	5.542	6.017	3.101	10.01
Tonalid	23.94	6.467	8.004	28.57	0.429	0.519	0.438	6.997	1.658	12.43	2.406	5.271	7.515	5.707	nd	5.598
Tramadol	134.4	224.5	289.8	195.7	12.63	13.04	21.50	12.70	26.17	0.718	27.63	136.3	191.7	168.3	74.57	1.246
Triclocarban	29.50	10.77	16.82	44.89	21.54	13.05	19.87	4.566	24.92	13.07	29.69	24.56	23.86	42.48	10.44	14.91
Triclosan	20.07	8.742	9.061	26.96	5.383	4.195	4.042	1.828	4.162	3.467	4.462	7.831	8.455	14.31	5.202	6.968
Trimethoprim	23.79	39.38	108.5	51.40	81.69	63.74	7.881	3.609	9.346	36.02	nd	32.89	121.1	57.16	33.32	136.6
Valsartan	762.4	603.5	570.4	567.8	121.5	106.2	232.3	149.1	294.1	206.2	293.7	131.9	231.0	336.7	150.1	357.7
Venlafaxine	13.33	28.01	39.60	28.45	2.298	2.289	3.061	0.292	3.565	nd	4.408	27.69	25.06	34.40	0.292	29.52
Verapamil	0.643	0.527	nd	1.209	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table B4:   Summary of Apies river samples and concentrations (ng ℓ$^{-1}$)

Compound	Apies River upstream							Apies River downstream						
	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017
Amitriptyline	1.158	nd	nd	0.254	0.083	0.134	0.858	0.966	0.737	1.452	2.272	0.202	0.074	nd
Bufexamac	1.553	1.152	0.155	0.365	0.316	0.954	3.188	0.786	2.389	0.487	0.797	1.047	1.577	2.181
Cafeine	2785	830.7	4.098	1570	1305	1015.3	2464	7718	2612	1218	823.8	880.6	942.1	6660.5
Carbamazepine	32.02	176.0	19.14	36.56	15.36	8.774	121.7	103.4	240.7	228.6	90.28	35.07	32.08	23.42
Ciprofloxacin	<loq	nd	<lod	<lod	nd	nd	nd	5.053	nd	<loq	<lod	<lod	<lod	10.78
Clarithromycin	5.480	nd	4.777	9.425	0.408	0.921	1.346	10.427	2.687	12.33	1.209	1.226	1.869	0.982
Desipramine	nd	0.620	nd	nd	nd	nd	0.741	nd	nd	nd	nd	nd	nd	nd
Dexamethasone	nd	nd	0.365	nd	nd	nd	nd	nd	nd	0.707	nd	nd	nd	nd
Diclofenac	13.42	12.66	11.32	10.58	5.642	7.066	81.98	15.28	24.20	22.93	20.26	9.488	19.45	13.07
Diethylbestrol	249.1	91.75	nd	41.32	22.18	29.40	82.80	25.80	221.1	53.91	242.2	41.01	57.41	368.4
Efavirenz	345.3	143.0	193.3	344.9	155.1	116.7	163.7	514.6	227.1	269.1	577.9	247.9	294.7	170.9
Enalapril	2.453	1.733	2.891	2.609	0.517	0.874	5.872	0.277	1.025	0.561	0.418	1.533	0.598	0.348
Enrofloxacin	nd	nd	nd	nd	nd	nd	ND	nd	nd	1.835	nd	nd	nd	ND
Erythromycin	4.400	nd	nd	6.589	nd	nd	ND	2.668	nd	3.619	9.713	nd	nd	ND
Estradiol	161.5	644.0	639.7	415.6	134.7	215.9	323.7	748.7	931.1	691.0	627.7	134.7	239.1	168.7
Estriol	135.1	244.5	105.3	114.9	83.3	81.3	178.7	98.07	533.7	546.0	544.3	83.3	182.5	89.26
Estrone	51.51	23.13	33.46	63.04	7.124	12.55	21.93	46.95	24.36	38.84	35.15	11.9	nd	nd
Famciclovir	nd	8.693	6.975	nd	nd	1.883	nd	nd	3.107	2.896	nd	ND	0.996	nd
Fenoprofen	nd	67.98	nd	nd	31.22	11.84	nd	nd	418.1	285.6	nd	16.25	10.1	nd
Fluconazole	26.49	81.87	45.80	20.00	10.67	13.16	144.9	76.73	84.69	200.8	87.01	35.30	36.79	26.52
Flumequine	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.929	0.932
Gabapentin	18.62	9.830	7.502	2.061	2.719	6.997	16.24	14.77	11.32	17.86	8.887	4.703	12.96	10.44
Gemfibrozil	62.63	173.9	35.64	45.19	30.62	85.54	8.505	41.98	545.2	51.67	91.24	66.39	93.66	8.505

Compound	Apies River upstream							Apies River downstream						
	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017
Ibuprofen	8651	1977	nd	4482	2688	2910	1637	1548	12812	5962	1414	2018	2693	2530
Ifosfamide	nd	nd	nd	nd	nd	nd	0.106	0.546	0.418	0.462	1.149	0.109	0.284	0.237
Indometacin	2.486	nd	1.800	4.403	0.812	1.213	2.997	8.555	nd	nd	4.385	1.946	2.611	1.253
Isoniazide	2.392	0.557	3.638	2.958			1.965	1.042	nd	1.132	3.396	3.575	5.873	0.422
Ketoprofen	3.950	8.853	3.767	1.456	4.388	4.032	8.801	5.362	39.49	16.50	nd	nd	3.8	0.561
Lamivudine	2.144	6.048	nd	0.592	nd	nd	8.912	nd	nd	nd	0.354	6.218	9.923	10.32
Lidocaine	1.292	49.59	3.573	1.497	1.372	0.732	36.93	5.624	22.93	112.4	10.11	3.125	7.060	5.110
Marbofloxacin	nd	<lod	<lod	nd	nd	nd	nd	<lod	<lod	<lod	<lod	nd	<lod	nd
Medroxyprogesterone	nd	nd	6.711	nd	2.358	2.791	2.275	2.776	4.810	nd	9.822	2.158	4.053	2.628
Mefenamic acid	15.79	10.84	8.454	14.27	6.412	32.113	2.239	5.861	9.572	7.142	8.769	19.60	16.54	6.848
Mestranol	nd	nd	nd	19.55	<loq	<loq	nd	nd	nd	nd	81.59	nd	<lod	nd
Methylparaben	5.990	12.70	16.04	14.01	8.513	8.493	4.376	11.75	32.99	16.72	41.94	13.62	9.461	9.724
Metoprolol	0.121	<loq	<loq	0.217	nd	<loq	ND	<loq	0.114	<loq	0.101	<loq	<loq	ND
Naproxen	132.3	30.33	89.07	130.6	89.47	87.49	137.9	75.58	486.9	234.4	436.8	186.8	136.4	64.61
Nevirapine	0.589	7.260	0.389	7.332	1.586	1.571	2.879	0.274	10.99	2.642	1.278	1.600	3.724	2.621
Norfloxacin	nd	nd	nd	nd	nd	nd	nd	nd	9.675	nd	nd	nd	nd	nd
Ofloxacin	<loq	<loq	4.654	<loq	<loq	<loq	<loq	5.6	8.581	<lod	3.402	22.4	22.7	30.7
Paracetamol	1.221	33.44	17.65	323.0	nd	nd	3.884	288.1	4.647	892.7	9.917	114.3	37.08	1683
Paraxanthine	856.9	1245	1064	842.1	453.2	525.0	789.8	2343	2907	817	204.7	262.9	808.9	994.6
Penciclovir	18.66	nd	nd	nd	nd	nd	4.703	33.94	17.48	26.25	nd	20.80	31.16	26.74
Phenacetin	2.174	2.157	1.635	1.059	0.337	0.570	2.144	0.372	2.746	0.775	0.452	0.433	0.322	0.674
Pindolol	0.204	0.244	0.326	0.083	<loq	0.421	0.082	0.077	0.349	0.657	0.701	0.057	0.062	0.206
Prednisolone	nd	7.862	25.27	1.385	16.65	2.361	13.31	nd	16.66	15.49	36.12	4.052	5.788	8.130
Procaine	nd	0.206	0.058	0.253	nd	nd	0.296	0.070	0.261	0.098	0.116	nd	<loq	nd
Progesterone	4.694	0.345	0.741	2.204	0.161	0.565	0.936	0.908	1.973	0.206	3.588	0.666	0.468	0.639

Compound	Apies River upstream							Apies River downstream						
	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017
Ractopamine	0.097	0.211	0.392	0.095	nd	<loq	nd	0.150	0.311	nd	0.654	<loq	nd	<loq
Ritonavir	58.84	9.084	36.57	nd	5.0	12.3	57.0	44.07	32.97	52.57	47.28	5.0	6.4	57.0
Salbutamol	<loq	0.939	0.056	0.225	0.149	<loq	<loq	0.184	0.204	0.172	1.326	nd	0.379	0.456
Salicylamide	26.37	23.88	4.905	10.14	19.19	10.83	nd	10.50	40.81	20.33	21.70	19.49	3.290	nd
Sulphadimethoxine	0.859	0.689	0.353	nd	nd	<loq	nd	0.608	1.830	0.332	0.718	nd	nd	nd
Sulphadoxin	<loq	<lod	<loq	nd	nd	nd	0.351	0.721	0.621	0.121	0.376	<loq	0.389	0.341
Sulphamethazine	nd	nd	<lod	nd	nd	nd	1.768	2.803	4.891	0.381	4.323	nd	4.517	3.728
Sulphamethoxazole	185.3	109.4	98.35	nd	54.45	39.37	237.4	185.3	270.5	123.7	252.3	52.97	67.2	297.4
Sulphanilamide	nd	nd	nd	nd	nd	nd	0.300	nd	0.418	nd	<loq	nd	0.601	nd
Sulphapyridine	nd	nd	nd	nd	nd	nd	nd	nd	1.151	nd	nd	nd	nd	nd
Terbutaline	0.073	0.090	nd	<loq	nd	nd	<loq	0.089	0.283	nd	0.070	nd	nd	nd
Testosterone	nd	nd	nd	nd	nd	nd	nd	nd	<loq	nd	nd	nd	nd	2.381
Thiabendazole	nd	nd	nd	nd	nd	nd	nd	nd	<loq	nd	nd	nd	<loq	<loq
Tonalid	2.767	1.574	2.563	3.535	0.198	0.133	1.164	4.639	2.155	0.707	7.445	0.369	0.235	0.158
Tramadol	20.59	25.26	15.73	16.92	6.056	6.846	8.815	32.81	38.27	59.21	40.38	8.361	18. 50	12.14
Triclocarban	17.96	6.529	7.028	28.31	3.494	9.915	28.99	6.690	nd	nd	9.351	11.35	8.378	0.618
Triclosan	7.235	nd	1.489	11.52	1.611	1.999	4.405	6.528	2.609	2.903	8.975	2.384	3.805	0.587
Trimethoprim	24.22	102.4	57.73	33.22	9.043	6.901	114.8	110	164.5	47.29	67.48	17.76	18.93	171.3
Valsartan	123.5	92.47	143.0	128.3	83.65	81.61	86.44	171.5	143.2	75.60	160.5	73.90	54.01	322.1
Venlafaxine	2.035	1.761	1.900	1.407	0.354	0.775	0.167	4.298	5.142	4.227	2.579	1.357	1.890	0.972

Table B5: Juskei river samples and concentrations (ng ℓ⁻¹)

Compound	Juskei River													
	Mar 2018	Mar 2018	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Albendazole	nd	nd	nd	nd	nd	nd	nd	nd	<lod	nd	nd	nd	nd	nd
Amitriptyline	9.727	0.444	6.596	6.234	6.784	0.105	1.517	0.363	0.025	nd	nd	0.923	0.026	0.062
Bufexamac	7.532	7.099	7.622	3.923	5.278	1.190	1.197	6.175	5.071	5.219	0.170	6.206	1.448	5.796
Cafeine	3688	3007	4074	2835	3357	3892	4188	2912	3757	4176	2788	5005	3389	5040
Carbamazepine	139.1	166.8	143.1	194.2	266.4	215.1	217.9	147.9	21.44	27.81	20.44	36.63	29.61	45.54
Ciprofloxacin	<loq	<lod	<loq	<lod	<loq	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod
Clarithromycin	14.42	nd	14.88	11.03	15.84	nd	15.51	nd	nd	nd	4.358	1.343	nd	nd
Desipramine	nd	8.143	nd	nd	nd	4.692	nd	nd	nd	1.074	nd	nd	1.420	nd
Diclofenac	121.1	149.9	116.9	82.65	94.69	94.66	115.8	144.1	40.76	50.86	35.66	95.77	55.21	101.1
Diethylbestrol	150.2	241.5	212.5	291.1	262.7	134.6	234.4	170.2	20.82	35.56	27.33	90.41	62.87	88.01
Efavirenz	1948	1903	1968	1131	1095	855.5	916.2	1755	396.6	520.9	140.9	928.0	567.6	1202
Enalapril	0.525	0.299	8.363	4.273	0.448	6.626	6.206	5.892	2.223	1.535	2.801	7.941	2.631	2.858
Erythromycin	0.854	nd	3.740	2.794	1.869	nd	7.075	nd	nd	nd	ND	nd	nd	nd
Estradiol	1132	1854	902.6	1149	545.2	1235.6	381.1	838.1	732.7	1018.1	150.8	283.817	281.2	2096
Estriol	121.7	151.8	125.6	563.6	186.6	103.6	281.0	86.26	316.1	156.8	221.9	190.0	95.2	98.9
Estrone	23.60	nd	20.41	nd	nd	25.97	55.87	5.698	11.11	12.0	1.225	22.05	4.490	nd
Famciclovir	nd	nd	nd	nd	4.920	6.112	nd	nd	4.493	nd	nd	nd	nd	nd
Fenoprofen	nd	nd	nd	98.80	86.16	387.7	117.2	nd	8.1	nd	7.5	6.8	nd	nd

Compound	Juskei River													
	Mar 2018	Mar 2018	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Fluconazole	159.2	158.4	165.1	129.7	150.3	164.3	175.6	141.9	38.17	48.05	36.21	57.35	46.01	58.35
Gabapentin	53.53	143.2	52.44	108.3	48.07	54.65	65.82	52.50	57.53	53.47	151.8	64.63	48.98	144.2
Gemfibrozil	533.3	122.8	nd	246.6	328.7	589.3	660.1	308.2	126.1	90.64	140.8	54.80	10.77	109.2
Ibuprofen	3398	3186	4530	4671	5688	10978	10042	3195	1352	1910	1942	4848	2723	4350
Ifosfamide	0.733	0.571	0.759	0.313	0.536	0.413	0.407	0.355	nd	nd	nd	nd	ND	nd
Indometacin	22.50	18.72	19.63	10.38	12.59	5.187	16.26	19.22	4.189	4.973	1.830	9.008	7.013	5.789
Isoniazide	nd	3.600	0.996	nd	nd	nd	1.705	nd	7.984	6.796	7.612	6.867	4.331	9.253
Ketoprofen	4.167	4.925	4.376	12.25	8.691	35.57	11.70	nd	3.794	4.316	3.923	5.948	6.001	8.269
Lamivudine	28.54	31.62	45.47	27.61	18.44	3.112	24.43	15.45	56.02	52.91	110.2	56.86	47.31	106.6
Lidocaine	nd	nd	0.263	79.28	21.07	0.219	nd	nd	5.958	0.534	6.133	0.143	7.009	0.874
Mebendazole	nd	0.215	0.125	0.091	nd	nd	nd	0.107	nd	nd	nd	0.123	nd	0.090
Medroxyprogesterone	4.936	4.823	4.796	3.938	2.881	nd	3.017	4.409	3.178	2.255	2.658	2.178	2.977	2.808
Mefenamic acid	37.25	36.66	29.79	18.93	31.97	26.44	34.15	29.07	33.23	19.63	24.76	30.79	32.72	14.93
Mestranol	nd	nd	51.48	49.39	nd	nd	48.18	nd	nd	nd	nd	nd	nd	nd
Methylparaben	4.015	26.73	24.59	36.53	57.54	73.90	37.82	41.51	32.60	33.79	57.76	17.47	1.612	23.07
Metoprolol	0.377	0.789	0.250	0.430	0.134	0.143	0.382	0.087	nd	<loq	<loq	<loq	nd	0.075
Naproxen	170.9	328.8	90.09	219.4	52.19	271.9	317.4	355.8	120.6	93.51	120.3	276.6	113.9	71.70
Nevirapine	1.907	0.957	0.659	0.416	31.92	0.518	0.884	2.628	4.218	5.379	3.459	6.965	5.710	7.600
Norfloxacin	nd	nd	nd	<lod	9.744	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ofloxacin	7.216	7.209	7.503	5.651	7.238	25.6	24.3	7.580	25.2	25.6	24.4	23.8	<lod	24.1

Compound	Juskei River													
	Mar 2018	Mar 2018	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Oxytetracycline	nd	nd	nd	nd	nd	nd	ND	<loq	nd	nd	<loq	nd	nd	ND
Paracetamol	5.951	654.6	7.429	nd	nd	2441	718.0	1093	336.2	296.0	650.0	2.252	399.9	5.217
Paraxanthine	5295	3425	4498	2567	2991	3576	4393	3139	1267	1312	1958	1327	890.5	2242
penciclovir	28.77	30.59	42.27	46.01	31.26	36.84	35.77	32.38	31.47	30.38	28.44	40.42	31.27	47.68
Phenacetin	3.877	2.500	3.165	2.502	0.847	0.976	1.938	1.187	1.213	0.992	0.435	0.746	1.496	0.826
Pindolol	1.382	1.156	0.576	0.378	0.545	0.839	1.391	0.199	0.176	0.071	0.344	0.111	0.838	0.563
Prednisolone	nd	11.83	3.357	1.808	12.30	7.587	13.19	14.12	11.29	16.92	15.54	nd	3.864	12.47
Procaine	1.452	3.387	1.908	0.745	3.735	14.51	7.543	0.836	0.167	0.386	0.161	0.132	0.084	0.647
Progesterone	1.174	2.398	0.956	1.531	1.191	0.360	0.628	5.827	0.115	0.605	0.504	0.144	0.494	0.539
Ractopamine	0.780	0.439	0.770	<loq	0.238	0.241	0.166	0.290	0.149	nd	nd	0.662	0.104	0.128
Rifampicin	nd	24.46	nd	nd	nd	2.940	nd	15.22	16.4	16.4	nd	nd	1.507	16.4
Ritonavir	236.6	191.3	235.7	256.0	473.4	454.2	325.8	178.3	22.3	31.5	14.3	14.5	16.6	53.1
Salbutamol	nd	nd	nd	nd	1.546	nd	nd	nd	<loq	nd	0.106	nd	nd	<lod
Salicylamide	6.366	29.59	26.78	21.17	26.20	nd	32.31	39.39	4.104	4.867	3.585	2.936	5.203	32.89
Sulphadoxin	10.29	7.744	14.22	6.581	10.63	5.301	6.212	5.690	0.143	0.455	0.181	0.616	0.326	0.226
Sulphamerazine	0.185	0.573	0.296	<loq	<loq	0.471	0.488	0.861	nd	nd	nd	nd	nd	nd
Sulphamethazine	1.525	2.478	2.330	0.916	1.638	0.461	0.362	1.064	nd	0.362	<loq	0.469	0.259	0.181
Sulphamethizole	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphamethoxazole	744.6	836.6	1082	524.4	788.9	404.7	499.6	539.4	293.7	364.1	267.3	406.9	303.5	365.7
Sulphanilamide	nd	nd	0.340	0.316	nd	nd	0.489	0.706	0.939	0.755	nd	2.821	3.602	nd

Compound	Juskei River													
	Mar 2018	Mar 2018	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017	Oct 2017
Terbutaline	0.368	0.155	0.350	0.414	0.976	0.431	0.315	0.112	nd	<loq	<loq	0.084	nd	nd
Testosterone	nd	nd	nd	nd	nd	nd	nd	nd	1.935	2.471	nd	nd	0.617	nd
Tonalid	24.27	23.10	24.98	6.754	15.41	7.472	7.937	21.14	2.666	3.475	0.125	2.500	3.764	1.404
Tramadol	161.0	162.2	176.4	140.9	168.7	174.3	196.9	140.5	21.71	37.75	23.03	45.13	40.49	80.05
Triclocarban	28.71	22.25	23.28	20.30	19.15	14.64	17.64	20.96	8.548	16.77	nd	17.03	17.97	7.653
Triclosan	38.81	30.78	31.85	34.46	21.62	9.266	14.48	27.21	1.153	1.839	1.227	11.73	2.788	5.427
Trimethoprim	152.6	133.6	157.4	87.78	128.7	141.3	149.1	107.3	19.24	12.60	15.56	10.11	27.39	1.936
Valsartan	754.6	966.6	774.3	744.7	840.3	402.2	661.1	845.5	236.1	296.3	289.4	370.8	271.6	377.4
Venlafaxine	82.97	nd	88.15	79.44	91.85	21.47	90.72	nd	nd	10.69	nd	17.61	8.646	26.68
Verapamil	0.510	nd	0.319	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table B6: **Mulderift Se Loop river samples and concentrations (ng ℓ$^{-1}$)**

Compound	Mulderift se Loop							
	Mar 2018	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017
Cafeine	128.2	154.7	403.4	169.2	253.7	355.3	277.0	231.6
Carbamazepine	16.09	17.25	166.3	51.46	15.2.4	23.37	13.08	21.02
Dexamethasone	0.204	0.231	nd	0.535	nd	nd	0.276	nd
Diclofenac	nd	4.732	8.233	5.321	10.44	9.508	5.254	8.550
Diethylbestrol	21.30	26.39	50.83	68.13	38.95	nd	55.84	27.40
Efavirenz	46.96	30.61	42.93	29.27	88.77	70.94	88.57	29.50
Estradiol	521.1	377.3	249.4	232.4	388.8	632.4	414.9	71.55
Estriol	91.70	53.67	182.5	98.71	85.03	100.1	269.2	45.94
Estrone	3.554	11.48	8.755	13.65	9.110	4.113	1.413	15.27
Famciclovir	1.097	1.374	2.470	1.107	3.354	3.153	nd	1.109
Fenoprofen	nd	nd	nd	nd	nd	nd	nd	6.500
Fluconazole	7.066	8.347	9.704	28.47	15.36	10.43	7.346	7.681
Gabapentin	10.49	6.367	7.193	4.801	2.195	8.04	7.377	7.275
Gemfibrozil	17.85	50.81	89.43	91.40	101.9	177.7	34.22	14.84
Ibuprofen	1477	1825	4598	2092	2599	4912	423.0	156.5
Ketoprofen	nd	4.009	17.72	7.192	16.71	21.04	3.806	3.634
Medroxyprogesterone	nd	4.238	nd	nd	nd	nd	3.386	3.710
Mefenamic acid	4.726	5.629	6.010	5.183	5.356	5.465	11.52	7.718
Methylparaben	10.38	9.030	26.19	44.63	10.15	20.64	16.15	6.834
Naproxen	35.11	67.72	49.10	33.45	77.72	47.58	26.31	96.68
Nevirapine	0.244	0.147	0.695	0.997	0.222	0.429	0.321	0.531
Paracetamol	62.93	94.55	161.3	128.8	366.6	185.3	414.5	41.39
Paraxanthine	284.7	294.6	440.2	97.08	356.3	508.6	125.8	335.2
Penciclovir	15.50	14.95	16.11	15.72	nd	nd	nd	14.91

Compound	Mulderift se Loop							
	Mar 2018	Mar 2018	Feb 2018	Feb 2018	Feb 2018	Feb 2018	Oct 2017	Oct 2017
Phenacetin	<loq	<loq	0.780	0.338	0.609	0.468	0.201	<loq
Pindolol	0.163	0.083	0.080	0.219	0.109	0.273	0.107	0.293
Prednisolone	1.991	4.332	1.569	2.149	0.267	5.023	3.828	5.273
Progesterone	nd	0.420	0.542	0.665	0.286	0.543	nd	0.383
Ritonavir	0.759	2.357	nd	7.659	19.14	13.83	3.208	1.728
Salbutamol	0.153	<loq	<loq	0.139	<loq	0.334	<loq	<loq
Salicylamide	9.958	11.55	3.568	9.22	10.27	3.332	12.92	11.35
Sulphamethoxazole	36.58	24.90	21.73	12.904	26.36	22.44	18.32	12.48
Testosterone	0.073	0.094	1.651	1.087	nd	nd	nd	0.149
Tonalid	1.696	1.110	0.337	0.237	0.484	0.583	1.032	0.545
Tramadol	11.81	10.71	12.64	11.19	17.27	17.69	7.496	13.28
Triclocarban	21.33	9.029	nd	nd	11.49	nd	2.998	9.655
Triclosan	2.486	2.064	2.341	nd	nd	nd	3.969	nd
Trimethoprim	3.315	2.762	3.007	2.130	4.201	3.703	2.205	1.116
Valsartan	32.10	39.08	54.33	34.13	37.26	35.446	42.38	41.90
Venlafaxine	3.790	3.175	0.464	3.891	0.636	1.025	1.728	0.439

Table B7: **Supplementary data obtained NSTI values for collected wastewater samples**

| Sample	Metric	Value
Influent-3	Weighted NSTI	0.125051
Influent-14	Weighted NSTI	0.074708
Effluent-3	Weighted NSTI	0.14331
Influent-12	Weighted NSTI	0.087437
Effluent-8	Weighted NSTI	0.073126
Influent-13	Weighted NSTI	0.063356
Influent-2	Weighted NSTI	0.090422
Effluent-5	Weighted NSTI	0.161955
Effluent-4	Weighted NSTI	0.158275
Influent-11	Weighted NSTI	0.162249
Effluent-6	Weighted NSTI	0.152936
Effluent-7	Weighted NSTI	0.084706
Influent-1	Weighted NSTI	0.170387
Influent-4	Weighted NSTI	0.161043
Influent-16	Weighted NSTI	0.054208
Influent-10	Weighted NSTI	0.15592
Influent-9	Weighted NSTI	0.128032
Effluent-1	Weighted NSTI	0.125694
Effluent-2	Weighted NSTI	0.106537
Influent-8	Weighted NSTI	0.111243
Influent-5	Weighted NSTI	0.14714
Influent-7	Weighted NSTI	0.058453
Influent-15	Weighted NSTI	0.064372
Influent-6	Weighted NSTI	0.147584

APPENDIX C
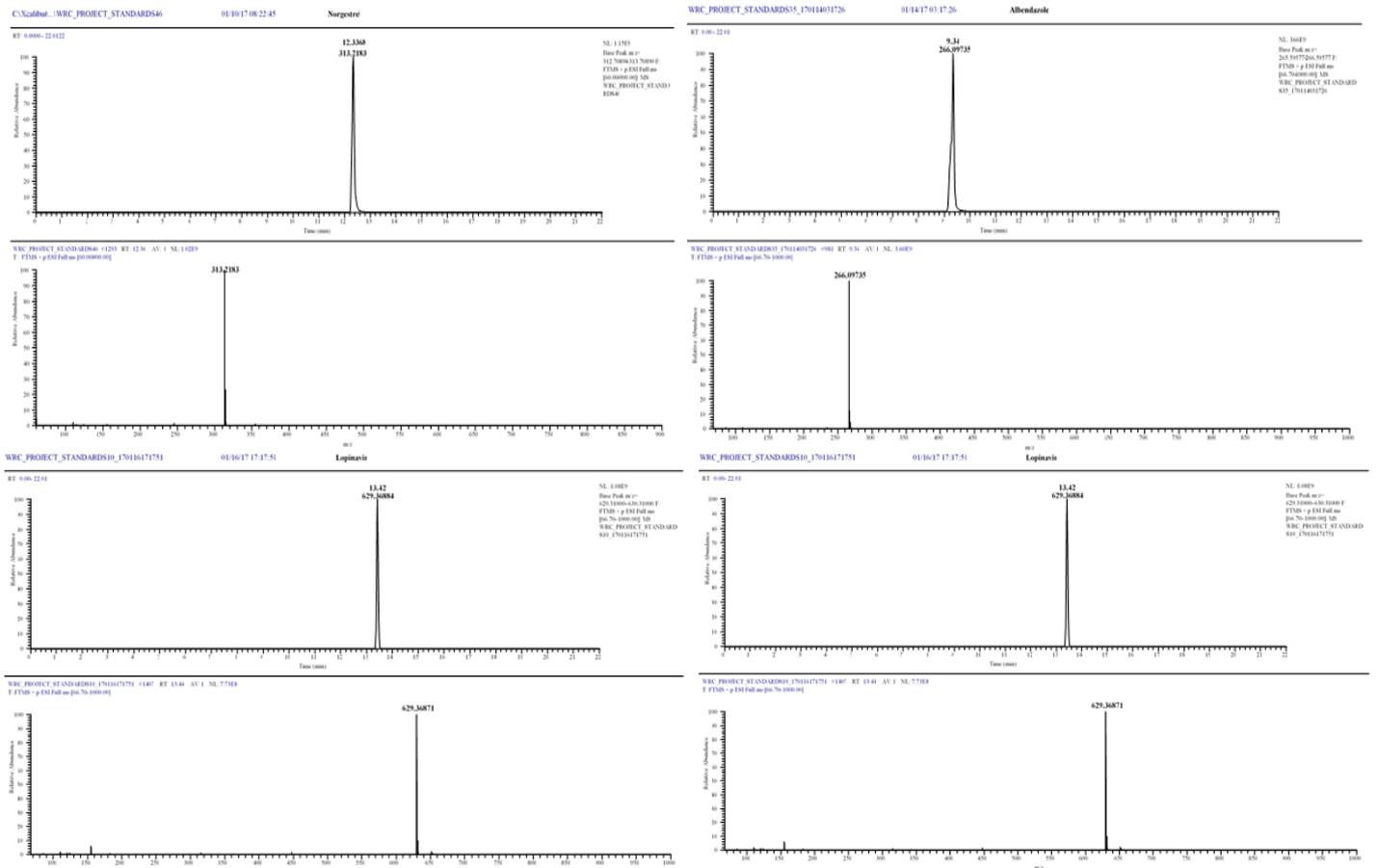
Figure C1:   A typical selected ion chromatogram, showing exact mass and retention time

Google maps for the sampling points

Figure C2:   **Apies river upstream**
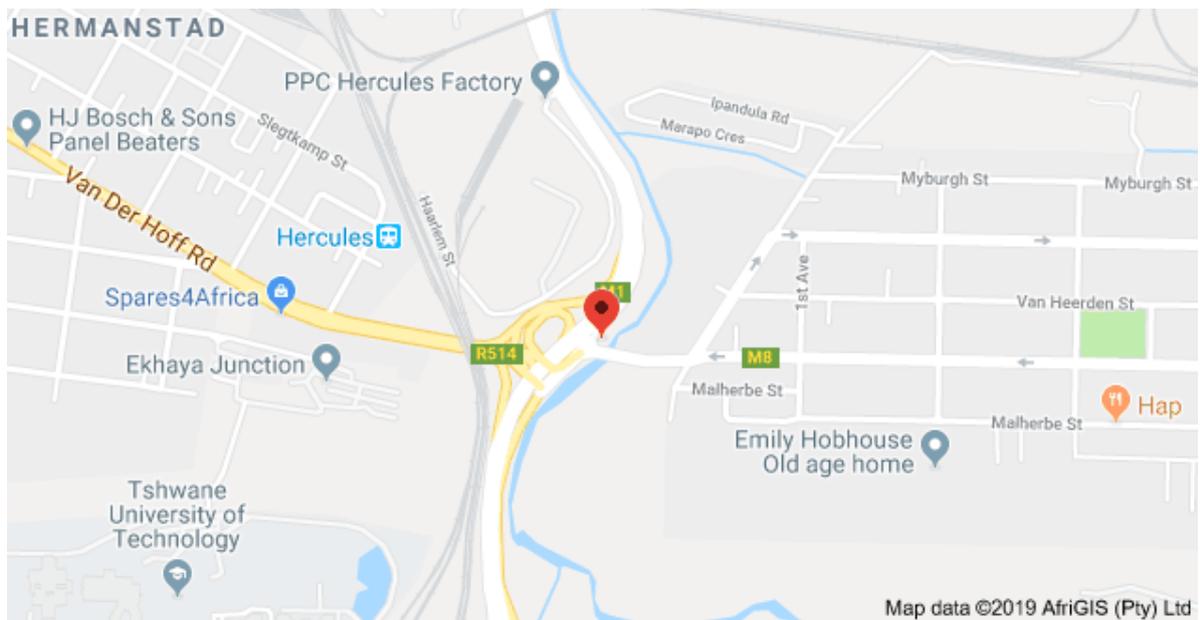

Figure C3:   **Apies river downstream**

Figure C4:   Juskei River (Heron Bridge) downstream

Figure C5:   Muldersdrift se Loop

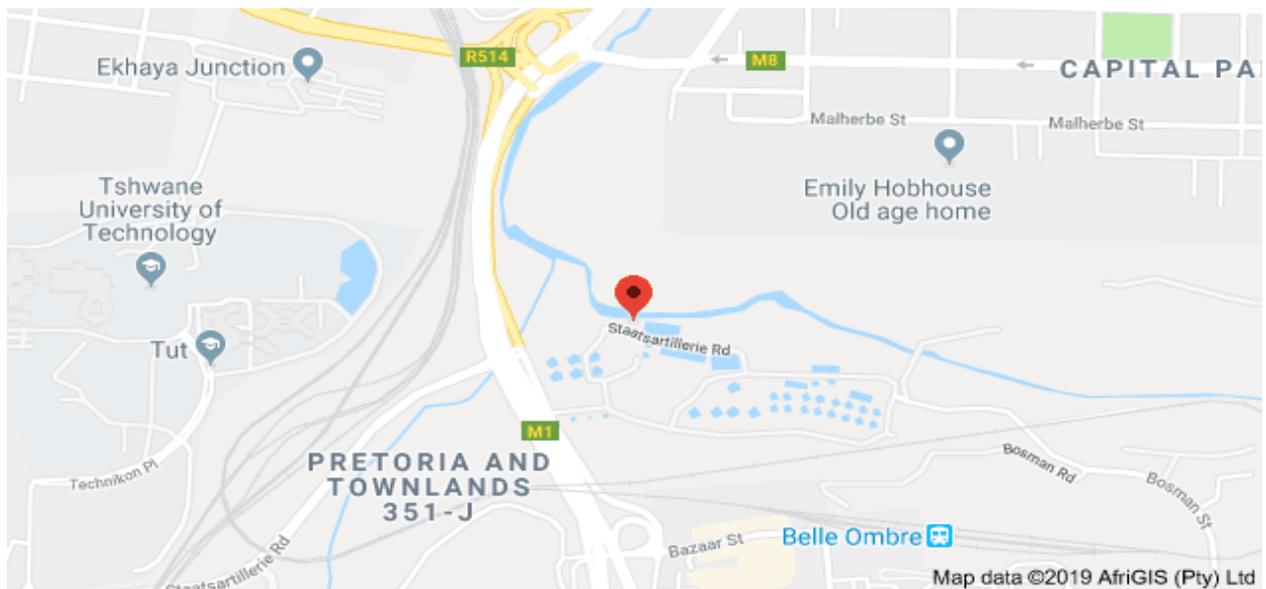**Figure C6:   Daspoort WWTW influent**

Figure C7: Daspoort WWTW effluent

APPENDIX D

A. STANDARD OPERATIONAL PROCEDURE (SOP) FOR THE METHODS

DETERMINING CHEMICAL EMERGING CONTAMINANTS USING SOLID PHASE EXTRACTION

1. SCOPE AND APPLICATION

1.1 This is the procedure used for isolating target organic analytes from aqueous samples using the Dionex® Auto Trace 280 SPE instrument (Dionex®, Thermo Fischer®) and/or SPE Manifold.

1.2 It describes conditions for extracting a variety of organic compounds from aqueous matrices that include groundwater and wastewater.

2. SAFETY CONSIDERATIONS

The Dionex® AutoTrace 280 contains warnings and precautionary statements that can prevent personal injury and/or damage to the instrument. Safety messages appear in bold type and are accompanied by icons. One should follow these safety messages before operating the instrument.

3. REAGENTS AND EQUIPMENT

3.1 Equipment

- Dionex® Auto Trace 280 SPE instrument and/or SPE Manifold
- Waters Oasis® HLB cartridges (6 cc, 500 mg)
- Vials

3.2 Chemicals and reagents

- Methanol (HPLC or LC-MS grade)
- Distilled water
- Nitrogen gas

4. PROCEDURE FOR SAMPLE EXTRACTION

4.1 The treated wastewater sample was extracted using Dionex® Auto Trace 280 SPE instrument.

4.2 The Waters Oasis® HLB cartridges (12 cc, 500 mg) were used for all sample preparation

4.3 Before extraction, each Waters Oasis® HLB cartridge was pre-conditioned with 3 mℓ of methanol, and then rinsed with 3 mℓ deionised water on a Dionex® Auto Trace 280 SPE instrument and/or SPE Manifold

4.4 Some 1,000 mℓ of the water sample was then passed through the HLB cartridge.

4.5 After extraction, the cartridge was washed with 1 mℓ of 5% methanol in water, subsequently air-dried under vacuum for at least 20 minutes.

4.6 The residues were then eluted from the cartridge with two portions of 5 mℓ methanol (HPLC or LC-MS grade).

4.7 All the extracts were completely evaporated to dryness by a gentle stream of nitrogen.

4.8 The dried sample under a gentle stream of nitrogen was followed by reconstitution in 1,000 µℓ methanol.

4.9 The …µℓ reconstituted sample directly injected to LC-MS.

B.   DETERMINING ORGANIC ENVIRONMENTAL CONTAMINANTS USING GCxGC-HRT-MS

1. SCOPE AND APPLICATION

This method specifies a procedure for the determination of organic environmental contaminants in water using gas chromatography time of flight mass spectrometry.

PRINCIPLE

a.   The principle steps involve the extraction of emerging contaminants from water using the Waters Oasis® HLB SPE.

b.   Interference: This will depend on your matrix.

2. SAFETY CONSIDERATIONS

a.   The TFDA/DLS/SOP/021 should be adhered when using this method.

b.   Suitable gloves must be worn.

c.   Do not eat or drink in the laboratory.

d.   Organic and mineral acids are highly corrosive, and cause severe burns on contact with the skin and eyes.

3. REAGENTS AND EQUIPMENT

a.   Equipment

A gas chromatography high-resolution time of flight mass spectrometer system	2 mℓ amber vials
Microbalance	Freezer
Volumetric flasks: 1 mℓ and 3 mℓ	Micropipettes
Beakers 100 mℓ and 250 mℓ	Sonicator

b.   Chemicals and reagents

- Methanol
- n-Hexane
- Acetone
- Dichloromethane
- Diethyl ether
- Benzene
- Dimethylformamide
- Ethanol
- Chloroform

Analyte	Name	Main category	Mol wt. (g/mol)
1	Phenol, 2-chloro-		128.55
2	Benzene, 1,3-dichloro-		147.00
3	Benzene, 1,4-dichloro-		147.00
4	Acetylpyrazine		122.13
5	Benzene, 1,2-dichloro-		147.00
6	Bis(2-chloro-1-methylethyl) ether		171.06
7	Phenol, 2-methyl-		108.13
8	p-Cresol	Flavouring agents	108.14
9	Ethane, hexachloro-	Volatile organic compounds	236.72
10	1-Propanamine, N-nitroso-N-propyl-		130.19
11	Isophorone		138.21
12	Phenol, 2-nitro-		139.11
13	Phenol, 2,4-dimethyl-		122.16
14	Phenol, 2,4-dichloro-		163.00
15	Benzene, 1,2,4-trichloro-		181.45
16	Naphthalene-D8		136.22
17	Naphthalene		128.17
18	p-Chloroaniline		127.57
19	1,3-Butadiene, 1,1,2,3,4,4-hexachloro-		260.76
20	Phenol, 4-chloro-3-methyl-		142.58
21	Indole	Flavouring agents	117.15
22	4-Chloro-2-methylaniline		141.59
23	Naphthalene, 2-methyl-		142.20
24	Hexachlorocyclopentadiene	Intermediates	272.75
25	Phenol, 2,4,5-trichloro-		197.43
26	Naphthalene, 2-chloro-		162.61
27	o-Nitroaniline		138.12
28	Dimethyl phthalate	Plasticizers	194.18
29	Etridiazole	Fungicide	247.51
30	Acenaphthylene	PAH	152.19
31	Benzene, 2-methyl-1,3-dinitro-		182.13
32	Acenaphthene-d10		164.27
33	Acenaphthene	PAH	154.21
34	Chloroneb	Fungicide	207.05
35	Dibenzofuran		168.19
36	Benzene, 1-methyl-2,4-dinitro-		182.13
37	Methiocarb	Insecticide	225.31
38	2-Naphthalenamine		143.19
39	Fluorene	PAH	166.22
40	Diethyl Phthalate	Plasticizers	222.24
41	p-Nitroaniline	Intermediates	138.12
42	Azobenzene		182.22
43	Phenol, 4-heptyl-		192.30
44	Benzene, hexachloro-	Pesticides	284.78
45	Simazine	Herbicide	201.66
46	Carbofuran	Insecticide	221.25
47	Atrazine	Herbicide	215.68
48	[1,1'-Biphenyl]-4-amine		169.22
49	Dibenzothiophene		184.26
50	Phenanthrene-D10		188.29
51	Phenanthrene		178.23
52	Anthracene-D10-		188.29

Analyte	Name	Main category	Mol wt. (g/mol)
53	Anthracene		178.23
54	Tetrachloroisophthalonitrile	Fungicide	265.90
55	Carbazole		167.21
56	Endosulphan ether		342.84
57	Galaxolide 1	Synthetic musk	258.40
58	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	Synthetic musk	258.40
59	Heptachlor	Insecticide	373.32
60	Alachlor	Herbicide	269.7
61	Metalaxyl	Fungicide	279.33
62	Terbutryn	Herbicide	241.36
63	Dibutyl phthalate	Plasticizer	278.34
64	Malathion	Insecticide	330.36
65	Aldrin	Insecticide	364.91
66	Chlorpyrifos	Insecticide	350.57
67	4,4'-Dichlorobenzophenone		251.10
68	Heptachlor epoxide	Metabolite-heptachlor	389.29
69	Bioallethrin	Insecticide	302.41
70	Fluoranthene	Sealant chemicals	202.25
71	trans-Chlordane	Insecticide	409.75
72	Pyrene	PAH	202.25
73	α-Endosulphan	Insecticide	406.90
74	Dibenzothiophene sulphone		216.25
75	cis-Chlordane	Insecticide	409.75
76	trans-Nonachlor	Organochlorine	444.2
77	p,p'-DDE	Insecticide	318.03
78	Dieldrin	Insecticide	380.91
79	Dicofol	Pesticide	370.47
80	β-Endosulphan	Insecticide	406.90
81	p,p'-DDD	Insecticide	320.04
82	o-Aminoazotoluene	Dye	225.29
83	Endrin aldehyde		380.89
84	Benalaxyl	Fungicide	325.40
85	Benzyl butyl phthalate	Plasticizers	312.36
86	p,p'-DDT	Pesticide	354.49
87	Endosulphan sulphate	Insecticide	422.92
88	Methoxychlor	Insecticide	345.64
89	Bifenthrin	Insecticide	422.87
90	Tetramethrin	Insecticide	331.41
91	Naphthacene	PAH	228.29
92	1,6-Dimethoxyphenazine		240.26
93	Chrysene-D12		240.36
94	Benz[a]anthracene	PAH	228.29
95	Bis(2-ethylhexyl) phthalate	Plasticizers	390.56
96	Di-n-octyl phthalate	Plasticizers	390.56
97	Permethrine	Insecticide	391.28
98	Benzo[k]fluoranthene	PAH	252.31
99	Perylene	PAH	252.32
100	Benzo[a]pyrene	PAH	252.31
101	Dinaphtho(1,2-b:2',1'-d)thiophene		284.37
102	Benzo[ghi]perylene	PAH	276.33
103	Dibenz[a,j]anthracene	PAH	278.35
104	Indeno[1,2,3-cd]pyrene	PAH	276.33

    c.   Preparation of standard stock solution (1,000 mg/mℓ)

        Weigh 1 mg of the selected environmental contaminants in a 1 mℓ amber volumetric flask. Dilute to the mark with an appropriate solvent. Store the solutions in the dark at -5 °C for later use.

    d.   Preparation of environmental contaminants working standard (10 mg/ℓ)

        Pipette 10 µL of stock solution above to 1 mℓ in a volumetric flask. Dilute to the mark with n-Hexane.

    e.   Standard curve

        A working calibration standard mixture of all environmental contaminants was prepared by diluting appropriate volumes of individual stock solutions with n-Hexane to give a concentration range of 0.001 to 1 µg ℓ$^{-1}$.

4. QUALITY ASSURANCE

Analyse a quality control sample or spiked, known sample in each batch of samples. Acceptance of results is based on the appropriate determined tolerance in the quality control chart between the upper warning limit and the lower warning limit and then determined by percentage recovery. The laboratory code number and date should be recorded in the quality control chart.

NOTE: The spiked sample should theoretically have the intermediate concentration of calibration standard solutions.