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A lab based experimental study of a bioretention system to remove selected pharmaceuticals in stormwater

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In

Environmental and Water Science

Department of Earth Science, Faculty of Natural Sciences, University of the Western Cape.

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DECLARATION

I, Refiloe Maphiri, declare that the project titled "A lab-based experimental study of a bioretention system to remove selected pharmaceuticals in stormwater" is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full names: Refiloe Maphiri

Signature: 

Date: 04 February 2023

DEDICATION

I would like to dedicate this thesis to myself for believing in me and constantly pushing myself to do the work even when I did not know how, and to my late Granzo who always encouraged me to study further.

ABSTRACT

The use of treated stormwater as an alternative source of water supply in urban areas is receiving global recognition. This approach is being supported as an innovative initiative that improves urban water security and diversifies urban water demand management options. However, reported toxic pollutants from some land-use activities threaten the quality of stormwater thereby restricting the potential for using stormwater to augment the water supply system. Such reported risks entail the treatment of the stormwater before its use for any purpose. Therefore, this study reports the findings of the research which investigated the effectiveness of a bioretention system in removing pharmaceuticals from stormwater and the influence of retention time and stormwater volumes on the removal efficiency of the pharmaceuticals by the bioretention system. To achieve the objectives of the study, there was a need to identify and measure the concentration levels of the pharmaceuticals found in stormwater. It was also necessary to determine the removal efficiency of the identified pharmaceuticals by using the *Turf Grass* and *Pennisetum* plants in a bioretention system with soil media control.

In this study, first, the LC-MS was used to identify and quantify pharmaceuticals present in stormwater. Secondly, the synthetic stormwater in a laboratory-based bioretention experiment was used to test the ability of the bioretention remediation technique in removing detected pharmaceuticals from stormwater over a period of four weeks. Results showed that Carbamazepine (42.4ng/l), Naproxen (61.4ng/l), Caffeine (49.4ng/l), Progesterone (18.7ng/l), Diclofenac(0.22ng/l) and sulfamethoxazole (21.9ng/l) were the pharmaceuticals that were detected in stormwater in the field set up. The results of the lab-based experiment showed that the removal efficiencies of the selected pharmaceuticals followed the order of progesterone > naproxen > caffeine > diclofenac > carbamazepine > sulfamethoxazole with average removal efficiencies of 98.7%, 98.5%, 98%, 96.2%, 67.8% and 25.9%, respectively. This study showed that retention time plays a significant role in the treatment of pharmaceuticals when using a bioretention system. Considering the volumes used in the experiment, the 5 L volume was not an ideal volume to simulate a storm and to receive a significant outflow, whereas the 10 L volume saturated the system and yielded optimal results under the 12 and 24hr retention time. The 15L volume flooded the system which impacted the removal efficiency of the bioretention system. The 10L inflow volume had a very significant impact on the removal efficiency of the pharmaceuticals for comparatively all three different retention times.

The comparison of the removal efficiency of the selected pharmaceuticals by the different plants shows that different plants may remove different pharmaceuticals at varying removal rates. However, it is important to note that, apart from the effect of the plant type on the removal process, the initial concentration and retention time of the inflow may also affect the removal efficiency. The removal efficiency demonstrated that bioretention systems containing different media such as soil and plants have the potential to provide a combined effect to enhance the removal of pharmaceuticals for stormwater reuse. It was concluded that to remove pharmaceuticals from stormwater using a bioretention system, a mixture of soil types and plants is required. It is suggested that the use of the bioretention approach is a promising technology in stormwater remediation and hence it needs upscaling in various areas.

Abbreviations and acronyms

ACT	Acetaminophen
AOPs	Advanced Oxidation Processes
BMP	Best Management Practice
CBZ	Carbamazepine
DO	Dissolved Oxidants
E1	Estrone
ECs	Emerging contaminants
EDCs	Endocrine disrupting contaminants
GC	Gas Chromatography
GPS	Global Positioning System
IBP	Ibuprofen
KTP	Ketoprofen
LCMS	Liquid Chromatography Mass Spectrometry
LOD	Limit of Detection
LIP	Low Impact Development
Log K_{ow}	Octanol-Water partition coefficient
MBR	Membrane Bioreactor
MF	Microfiltration
NCI	Negative Chemical Ionisation
NF	Nanofiltration
NSAID	Non-steroidal anti-inflammatory drugs
PCPs	Personal Care Products
pKa	Acid strength/acid dissociation constant
RBF	Riverbank Filtration
RfD	Reference Dose
RO	Reverse Osmosis

RSD	Relative Standard Deviation
SAC	Saccharin
SAT	Soil-aquifer treatment
SD	Standard Deviation
SPE	Solid Phase Extraction
TDS	Total Dissolved Solids
TTC	Threshold of Toxic Concern
UF	Ultrafiltration
WSUD	Water Sensitive Urban Design
WWTPs	Wastewater Treatment Plants

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To the Student Enrolment Management Unit (SEMU) I am grateful for your financial support. To my supervisor, Dr S. Clark, and co-supervisor Dr J Okedi thank you for everything this would have not been possible without your help.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 BACKGROUND

South Africa is a semi-arid to an arid country with limited water resources. The dry conditions of the country are characterised by the average annual rainfall of ~ 450 mm, which is well below the world average of about 860 mm (Basson, 2011). South Africa is facing severe pressure on water security due to an increased water demand resulting from an increasing population, poor planning and management of water resources, limited investment into water reservoir infrastructure, and the recurring droughts over the past decade. Increased pressures on water resources resulted in the deterioration of water quality in rivers and the major sources/causes were identified as agricultural drainage and runoff, urban runoff and effluent return flows, industries, mining, and rural settlements with insufficient sanitation services. The most important of these currently is insufficiently treated urban effluent (Basson, 2011). The Western Cape Province is at present from 2014 to 2017 (hydrological years) experiencing the worst water shortages that are exacerbated by the drought conditions coupled with the increasing population, industrialisation, and urbanisation in the province (Botai et al., 2017). This prompted the City of Cape Town to develop alternative solutions to augment the existing water supply and prevent future droughts.

One of the response strategies that the City adopted was to maximize the re-use of wastewater in line with the National Water Resource Strategy (2013) to meet current and future water demand for different users. A very small volume of treated wastewater from industries is also used in combination with stormwater to recharge aquifers (National Water Resource Strategy, 2019). It was also reported that nearly 20% of the available surface water resources is water that was used and returned into the system (Basson, 2011). Due to the relative abundance of stormwater in urban areas, it is believed that stormwater can feasibly be harvested and treated to supply water demands. In the context of this study, stormwater is defined as water that comes from natural precipitation and the accumulation thereof, including groundwater, spring water, as well as seawater within estuaries, but excludes drinking water or wastewater reticulation system (City of Cape Town, 2005).

Stormwater treatment has become internationally accepted as an alternative to balance surface water supply for residential use. However, utilising stormwater has inherent difficulties due to the spatial and temporal variability of the population density, land use, percentage of

impervious area, waste disposal, sanitation practices, soil type, and climate (Mitchell et al. 2002). All these factors influence the physical, chemical, and biological characteristics of stormwater quality (Philp *et al.*, 2008). Stormwater treatment is currently being practised, although it is not effectively regulated (Hatt et al., 2006). This leaves stormwater treatment in a vulnerable position as the public could begin to doubt the adoption of stormwater re-uses (Fisher-Jeffes et al., 2017). Community acceptance is critical to the success of a stormwater harvesting and treatment system. However, community acceptance varies depending on the intended end-use of treated stormwater (Mitchell et al., 2006; Wu et al., 2012). Mitchell et al. (2006) state that the public is generally supportive of stormwater re-uses for non-potable purposes, but they are apprehensive of human contact with re-used stormwater due to perceived health risks. Previous research also highlighted that the acceptance of stormwater re-use by the public may be improved by increasing engagements and transparency about the quality of the water re-used and improve the knowledge about the systems in place to ensure that the re-used water is of better and/or improved quality (Fletcher et al., 2007; Wu et al., 2012).

Urban rainfall-runoff often carries high concentrations of nitrogen, phosphorus, nutrients, organic pollutants, and heavy metals. Studies have shown that half of the pollutants in urban rivers come from surface runoff formed during urban rainfall and their concentrations can exceed ten times the limitations for surface water quality (Luo et a., 2020). Responding to that, many countries have adopted different rainwater management systems based on the existing urban structure, such as the Low Impact Development and Best Management Practice (BMP) in the United States, the Water Sensitive Urban Design in Australia, and the Sponge City Concept in China (Luo et a., 2020). The idea of Sustainable Urban Drainage Systems (SUDS) was developed during the UN Earth summit at Rio de Janerio in 1992 and later adopted in the United Kingdom in 1999. The overall goal of SUDS is to get community involvement to increase the commitment of the community to take care of all the stormwater measures (Button, 2010).

Countries such as Australia and Singapore have successfully implemented the treatment and reuse of stormwater. The successful implementation of SUDS in these countries was attributed to publicising the standards to which stormwater was treated to offer public assurance. In South Africa, the approach was to consider stormwater as part of the urban water cycle, a strategy known as Water Sensitive Urban Design (WSUD) (Armitage et al., 2013). The aim of this was to design water quantity management, water quality treatment, improved amenity, and the

conservation of biodiversity. The current study aims to contribute to this knowledge by investigating the presence and treatment of selected pharmaceuticals in stormwater using a natural stormwater attenuation system known as the Bioretention system or Biofilter. The use of this system was also commended by Button (2010) as one of the effective interventions that The City of Cape Town has adopted to find a sustainable balance between environmental protection, the economic and social development of the growing population by means of treating and reusing stormwater to meet the demand.

It has been confirmed that pharmaceuticals are present all over the world in groundwater, surface water, wastewater, soils, and biota (Caban and Stepnowsk, 2021). The presence of pharmaceuticals in wastewater and natural water was first published in 1977–1978 (Caban and Stepnowsk, 2021). Pharmaceuticals are a class of emerging environmental contaminants that are extensively and increasingly being used in human and veterinary medicine (Fent, Weston and Caminada, 2006). They are specially designed medicines or drugs meant for the specific prevention of diseases in humans and animals (Fawell and Ong, 2012). For the past decades, stormwater has been tested and treated for the presence of nutrients, sediments, metals, bacteria, and viruses. Although the presence of pharmaceuticals in stormwater is acknowledged, there is still limited knowledge about the efficiency of using bioretention systems to eliminate and/or remove pharmaceuticals in stormwater (Caban and Stepnowsk, 2021). This has been of utmost concern due to their potential threat to ecosystems and human bodies (Fent, Weston and Caminada, 2006) as they are prevalent and persistent in aquatic environments and pose the risk of inducing toxic effects. The current study, therefore, seeks to assess the application of a bioretention system in removing pharmaceuticals in stormwater.

1.2 Problem statement and rationale of the study

The severe drought spell that The City of Cape Town experienced in the year 2016-2018 prompted the City of Cape Town municipality to investigate alternative water supply resources. In this regard, stormwater was identified as a potential water resource that can be used to augment and balance the City's water supply. However, the presence of pollutants in stormwater limits its utilisation. Previous studies have shown the benefits of removing contaminants in stormwater. However, little is known about the efficiency of removing pharmaceuticals. To contribute to this knowledge gap, this study seeks to investigate the effectiveness of a bioretention system in removing pharmaceuticals from stormwater.

1.3 Aim

This study aims to improve the understanding of using bioretention systems in removing or eliminating the presence of pharmaceuticals in stormwater. This is done to inform the City of Cape Town of interventions in improving the stormwater quality for potable use and making informed decisions about the effectiveness of bioretention systems in improving stormwater quality.

1.4 Objectives

1 Determine the concentration levels of pharmaceuticals found in the stormwater.

2 Investigate the influence of retention time and stormwater volume on the removal efficiency of the selected pharmaceuticals.

3 Assess the effectiveness of Turfgrass, *Pennisetum* plants, and soil media in removing pharmaceuticals from stormwater.

1.5 Research Question

To what extent can retention time, stormwater volume, and the different bioretention media influence the removal of pharmaceuticals (concentration difference)?

1.6 Study hypothesis

1. The hypothesis of this study is that the concentrations of pharmaceuticals will be reduced after treating runoff using the bioretention system.
2. The vegetation system will be more effective than bare soil or non-vegetated bioretention system.

1.7 Thesis Outline:

This thesis consists of 6 chapters. Chapter 1 presents the overview of the study, including the background, rationale, aims and objectives, and the research questions that will be addressed in the study.

Chapter 2 discusses previous research and reviews literature that covered stormwater management. This includes studies that discussed the physicochemical properties of the selected pharmaceuticals, the sources, and pathways of pharmaceuticals in aquatic environments. The fate and transport of pharmaceuticals in the environment, as well as the

potential health impacts, were also reviewed. This chapter also looked at the guidelines and standards of pharmaceuticals and the possible treatment methods.

Chapter 3 provides a detailed description of the study area. This includes the site selection criteria, the characteristics of the study site such as the climate of the area, geology, hydrology of the area, as well as the possible sources of the contaminants found in and around the Zeekoe catchment.

Chapter 4 outlines the materials and methodology that was followed to achieve the objectives of the study. Data collection and analyses methods are discussed in detail in this chapter.

Chapter 5 highlights and discusses the major results of the study.

Chapter 6 presents the conclusions and recommendations of the study.

References and appendices referred to and used in the study are included at the end of this thesis.

CHAPTER 2: LITERATURE REVIEW

This chapter provides background information on the relevant aspects of the study. A brief discussion and review of existing international and South African literature on the treatment of pharmaceuticals in stormwater for potable water use is provided. Topics covered include some background aspects of the treatment of pharmaceuticals in stormwater, a brief description and literature of the selected pharmaceuticals that were studied, tested, and treated. Selected pharmaceuticals that are said to be the most prevalent in stormwater or wastewater are discussed, their sources, pathways, and receptors, the potential risk of exposure to these chemicals, the ability of water reclamation and wastewater treatment plants to remove these chemicals, and the risk of potable water reuse.

2.1 Background and Introduction

Water scarcity has become an increasingly significant problem for most countries. Before stormwater was considered a valuable resource the primary objective of stormwater management was to dispose of stormwater instantly into receiving water bodies. Consequently, these receiving water bodies were adversely affected by water pollution (Akram *et al.*, 2014). Stormwater pollution includes litter, natural pollutants such as animal faeces, chemical pollutants, sediment pollutants etc. These pollutants are created by urbanisation, development and populating of an area and carried to inland water bodies such as streams, rivers, and lakes by stormwater and deteriorate their quality and endanger their ecosystems.

Treatment of stormwater for sustainable water supply demands the sustainable management of stormwater. Sustainable management of stormwater is defined as the management of stormwater that meets the needs of the present without hindering the ability of future generations to meet their own needs. It requires the management of stormwater quality and quantity, ensuring a balance between economic costs and environmental benefits (Department of Housing, 2000). Sustainable management of stormwater comes with benefits such as controlling runoff and flooding and preventing the direct discharge of contaminated stormwater into receiving surface water bodies or groundwater resources (Fisher-Jeffes *et al.*, 2017).

Many water-stressed countries are practising the treatment of stormwater for reuse such as Australia's Water Sensitive Urban Design (WSUD) approach which attempts to integrate stormwater management into urban planning, China launched the Sponge City program in 2015 which marked the beginning of the utilisation of stormwater in that Country.

Thousands of unregulated and regulated pharmaceuticals have been detected in aquatic environments due to the widespread use of pharmaceuticals on a daily basis. These include antibiotics, analgesics, anti-inflammatory drugs, hormones, beta-blockers, lipid regulators, and antiepileptics, in concentrations ranging from nanograms to micrograms per litre. (Ngqwala & Muchesa, 2020). Stormwater reuse contributes to the exposure of many emerging contaminants. These compounds may pass through conventional wastewater treatment systems without being removed and end up in potable water supplies. The risk associated with human exposure to emerging contaminants of concern found in stormwater that is treated for direct potable reuse is therefore uncertain. The potential health effects of these emerging contaminants in reused stormwater are a major cause for concern, especially the long-term health effects of ingesting these chemical contaminants, found in reclaimed stormwater(Swartz et al., 2016).

2.2 Pharmaceuticals detected in South African water bodies

Pharmaceuticals have been detected in South African water bodies in provinces such as KwaZulu Natal, Gauteng, Free State and the Western Cape. Patterton., *at el* (2011) investigated and identified the most important new substances in drinking water that could be a concern to human health in South Africa. The study concentrated on the detection of polar, water-soluble compounds. After careful consideration of the severity of the possible health effects of each of the identified contaminants three chemical determinants were identified as ones with the highest potential of having a negative health impact. These were the herbicides atrazine and terbuthylazine, and the anticonvulsant, carbamazepine. In this study, samples were collected from water purification plants in Bloemfontein, Johannesburg, Pretoria, Durban, Pietermaritzburg, Port Elizabeth, and Cape Town at points before the water entered the reticulation system. A combined total of 34 pharmaceuticals and pesticides from 618 tested, were detected in the water samples over a 4-season period. In line with the preliminary screen, atrazine, carbamazepine and terbuthylazine were detected in the highest number of water samples and with the greatest number of seasonal occurrences. Quantitation of the herbicide atrazine showed that it was present at elevated levels (approximately 12 ng/L) compared to the other cities, in each of the four seasons in Johannesburg. A similar elevated seasonal presence was observed in Johannesburg for the herbicide terbuthylazine, which was present at approximately 12 ng/L in each season. The anticonvulsant and mood-stabilising drug, carbamazepine, was present at elevated levels (approximately 200 ng/L) in all four seasons in

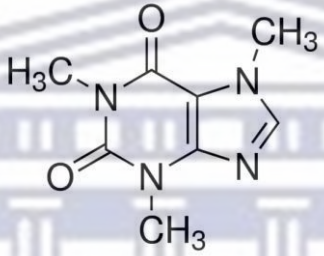
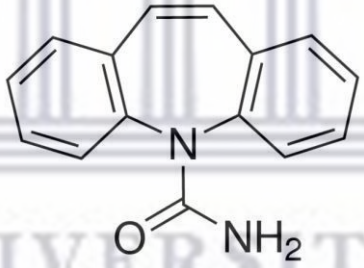
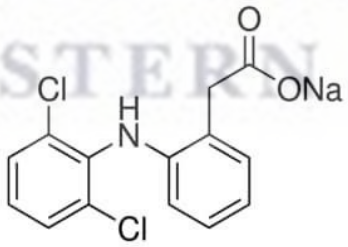
Bloemfontein. The highest level of atrazine (163 ng/L) and terbuthylazine (206 ng/L) determined, were in Pretoria in the autumn. The highest level of carbamazepine was 324 ng/L in Bloemfontein in the summer.

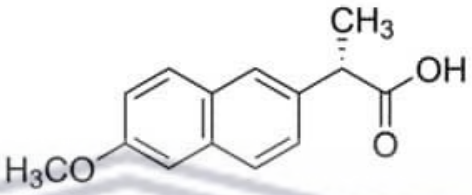
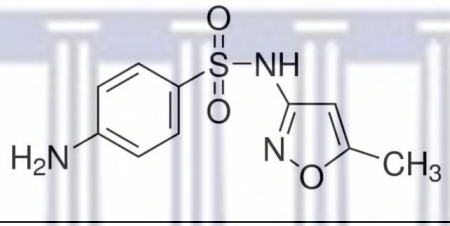
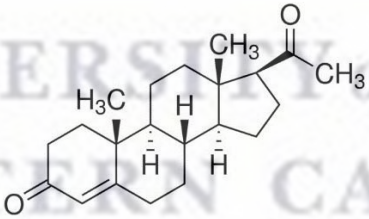
A study by Africa, Agunbiade and Moodley., (2014) investigated the occurrence of nine antibiotics, five antipyretics, atenolol, bezafibrate, and caffeine in wastewater from a domestic wastewater treatment plant, Umgeni surface water, and dams along the Umgeni River used for water supply in KwaZulu-Natal, South Africa. The water samples were extracted with solid-phase extraction using a hydrophilic-lipophilic balance (HLB) and C-18 cartridges for the acidic and neutral drugs, respectively. The wastewater had 100 % occurrence of the analytes studied, with caffeine having the highest concentration at 61 ±5 µg/L and nalidixic acid being the most observed antibiotic at 31±3 µg/L. The wastewater treatment process reduced the influent concentrations of all the studied pharmaceuticals by 43.0–94.2% before discharge except for atenolol which had lower removal (14.8 %) after treatment. The frequency of occurrences and concentrations in surface water were lower than in the influent. Blue Lagoon which is the mouth of the river and the discharge point into the ocean had the highest concentrations of some of the studied compounds in surface water which depicted the possibility of downstream load.

Africa, Agunbiade and Moodley *al et*., (2014) also detected the presence of diclofenac and sulfamethoxazole in surface water in Kwa-Zulu Natal with concentrations ranging between 0.3 – 15.6 µg/L for diclofenac and 3.68 µg/L for sulfamethoxazole. Whereas in the Gauteng province sulfamethoxazole ranged from 0.6 – 1.4 µg/L in surface water (Archer, Wolfaardt and van Wyk, 2017). Carbamazepine was detected in drinking water in the Free State province and Gauteng in surface water at concentrations ranging between 0.02 and 0.3 µg/L (Patterton, 2011; Archer, Wolfaardt and van Wyk, 2017). Caffeine was detected in Kwa-Zulu Natal and Gauteng surface water at concentrations of 0.1 – 6.6 µg/L (Matongo *et al.*, 2015; Archer, Wolfaardt and van Wyk, 2017) and lastly, Progesterone was detected in Kwa-Zulu Natal in various WWTW at concentrations ranging between 0.01 and 0.90 µg/L (Manickum and John, 2014). Madikizela and Chimuka *at el.*, (2017) studied the occurrence of naproxen, ibuprofen and diclofenac residues in wastewater and river water of Kwa-Zulu Natal in Mbokodweni River and wastewater treatment plants located around the City of Durban. This study used a high-performance liquid chromatography equipped with a photodiode array detector. Target compounds were detected in most wastewater and river water samples with ibuprofen being

the most frequently detected pharmaceutical. The maximum concentrations that were detected in river water for naproxen, ibuprofen, and diclofenac were 6.84, 19.2, and 9.69 µg/L, respectively. Pharmaceuticals were observed upstream of the Mbokodweni River, an indication that human activities contribute significantly to the contamination of water resources. The results of this study demonstrated that more research needs to be done on the occurrence of acidic pharmaceuticals in all South African water bodies including lakes and dams

Table 2.1: Physicochemical properties of the selected pharmaceuticals

Name	Use	Formula	Structure	Molecular weight (g mol^{-1})	Aqueous solubility (mg L^{-1})	Log K_{ow}	pK_a
Caffeine	Stimulant	$C_8H_{10}N_4O_2$		194.19	13.5	- 0.07 0.01	14.0
Carbamazepine	Sedative	$C_{15}H_{12}N_2O$		236.27	125.0 ± 2	2.45	14.0 (Tseng *et al.*, 2020)
Diclofenac	Sodium salt Anti-inflammatory Human & veterinary use	$C_{14}H_{10}Cl_2NNaO_2$		318.13	360.0 ± 10	4.51	4.15

Naproxen	NSAIDs	$C_{14}H_{14}O_3$		230.26	60.1 ± 2	3.18	4.15
Sulfamethoxazole	Antibiotic/Broad spectrum antibacterial/Human & veterinary application	$C_{10}H_{11}N_3O_3S$		253.28		0.9 (Schriks *et al.*, 2010)	3.92
Progesterone	Pregnancy hormone	$C_{21}H_{30}O_2$		314.46	23	3.32 (Vymazal, Březinová and Koželuh, 2015)	

2.3 Reason for the selected pharmaceuticals

The selected pharmaceuticals in this study include those listed in volume II: A prioritization framework for monitoring contaminants of emerging concern in reclaimed water. Swartz *et al.*, (2016) identified these pharmaceuticals as part of the priority list of pharmaceuticals to be monitored in wastewater that is treated for direct potable water use. Others were selected based on:

(i) Pharmaceuticals that have previously been detected in South African water bodies,
(ii) Pharmaceuticals that are prescribed by medical practitioners in large volumes,
(iii) Those that are used the most and are most likely to be detected in water bodies located near residential areas,
(iv) Pharmaceuticals that are persistent in water and are usually not removed by the water treatment processes,
(v) Those that have a potential health impact on humans.

2.4 Sources and pathways of pharmaceuticals in aquatic environments

Pharmaceuticals such as antibiotics, analgesics, anti-inflammatories, hormones, non-steroidal anti-inflammatory drugs, beta-blockers, blood lipid regulators, and antiepileptics have been detected in aqueous environments in concentrations ranging from nanograms to micrograms per litre. More than 80 distinct types of PCPs, EDCs, and pharmaceuticals were found in both treated and untreated sewage, streams, lake, oceans, sediments, and even tap water, according to the results of Jiang et al.,2013.

Some pharmaceuticals are completely degraded by the body, while some are partially excreted by the body through waste (Ngqwala & Muchesa, 2020). These compounds become part of human waste, and they are excreted into the environment as un-metabolised parent compounds and metabolites (Koutsouba *et al.*, 2003). Ultimately, they end up in the sewage system, where they are subsequently released into the environment, either through sewage leaks or wastewater being discharged from sewage treatment plants into aquatic environments (Archer et al., 2017). The primary sources of pharmaceuticals include the inappropriate disposal of medicines at home, hospital discharges, aquaculture facilities, animal farming activities, and municipal and industrial wastewater treatment plants. Contamination of water sources by these pharmaceuticals can occur via various pathways, which include surface run-off or leaching of human and other animal waste, and wastewater effluent discharges (Swartz et al., 2016). In

addition, veterinary pharmaceuticals can also enter aquatic systems through manure application to fields and subsequent runoff, as well as through direct application in aquaculture (e.g., fish farming). Veterinary pharmaceutical products are primarily released into the environment through the deposition of manure on the soil. Compounds with a low sorption efficiency and high-water solubility have a high potential for transport into groundwater. Moreover, groundwater can be contaminated by bank filtration or artificial recharge of loaded surface waters, leaks from sewer systems in urban areas, and, occasionally it can also be contaminated by production residues (Zwiener, 2007). Landfill sites also serve as a significant source of ECs (poly-chlorinated compounds) particularly prevalent in groundwater due to their toxicity. Many nations, including Croatia, Denmark, and the United States, have reported groundwater pollution with pharmaceutical chemicals in landfill regions (Kumar *et al*., 2022)

A high concentration of pharmaceutical compounds enters wastewater treatment plants (WWTPs) daily through urinary or faecal excretion and from pharmaceutical manufacturing facilities as their wastewater is directly discharged into sewage treatment plants (Farre et al. 200). Due to the polar nature of some pharmaceuticals such as naproxen, ibuprofen, and diclofenac, they escape the wastewater treatment process easily and contaminate the river water. The general environmental concern with pharmaceuticals is not necessarily their high production volumes, but their persistence in the environment and their critical biological activity (e.g., high toxicity, high potency for effects in biological key functions such as reproduction) (Fent et al., 2006).

The transport of pharmaceuticals between different environmental media depends on the sorption behaviour of the compound in treatment plants, soil, and the water-sediment system. Several pharmaceuticals can be found in sludge samples of sewage treatment plants through adsorption. This creates a potential pathway for these compounds to land into the environment by direct release or application of sludge to agricultural land as fertilizer. These pharmaceuticals can then be transported into groundwater, for instance when biosolids are applied onto agricultural land or when fields are irrigated with treated wastewater. This then results in the uptake of these compounds by crops which may create a potential pathway of human exposure through dietary intake. Runoff from biosolids containing pharmaceuticals either from landfills or fertilisers applied on agricultural land or veterinary pharmaceuticals used to treat livestock may be transported into the surrounding surface water or leach into the groundwater thereby posing a risk to aquatic life and public health. Osenbruck et al. identified

local river water infiltration, sewer exfiltration, and urban stormwater recharge as the major sources of pharmaceuticals such as carbamazepine, in groundwater underlying the city of Halle (Saale), Germany.

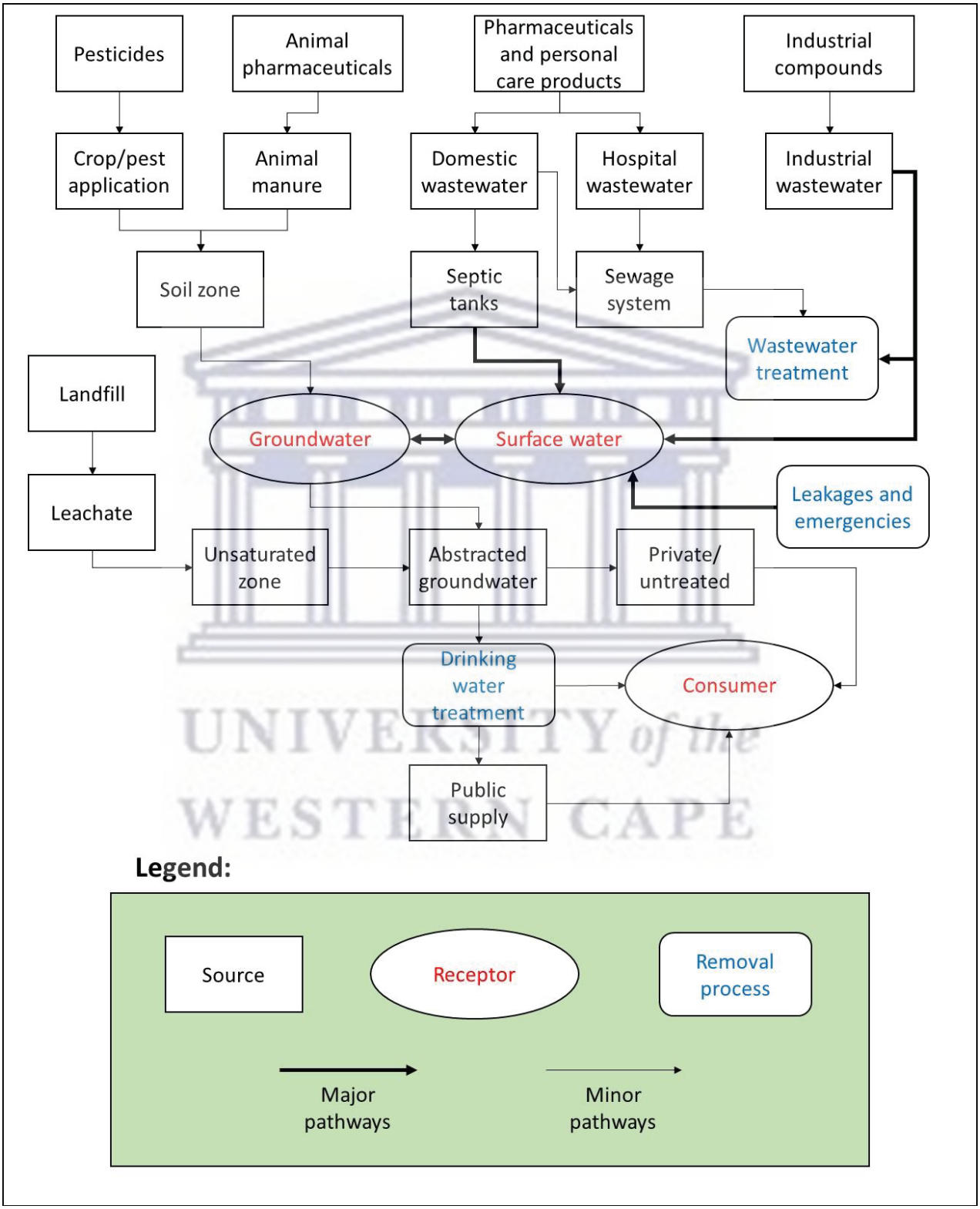


Figure 2.1: Sources and pathways of emerging contaminants to receptors and aquatic environment (Swartz et al., 2016)

2.5 Fate and transport of Pharmaceuticals in the environment/ Removal Processes

Adsorption

Adsorption is defined as the adhesion of atoms, ions, biomolecules or molecules of gas, liquid, or dissolved solids to a surface because of chemical or electrical attraction - typically accomplished with granular activated carbon to remove dissolved organics and chlorine. Adsorption implies the transference and accumulation of adsorptive molecules from the fluid phase to the interfacial layer and can involve physical and/or chemical interactions. Adsorption offers several advantages, such as low energy consumption, mild operation conditions, and lack of by-products added to the system; therefore, this technology can be potentially used for pharmaceutical removal (De Andrade *et al.*, 2018). Other studies on the effects of *Kow* on the adsorption of chemicals have suggested that lower values of log *Kow* show higher leaching of antibiotics (John et al., 2000; Xia et al., 2005). In wastewater treatment, adsorption is dependent on both hydrophobic and electrostatic interactions of the pharmaceutical with particulates and microorganisms. Acidic pharmaceuticals such as the NSAID acetylsalicylic acid, ibuprofen, fenoprofen, ketoprofen, naproxen, diclofenac and indomethacin having p*K*a values ranging from 4.9 to 4.1, as well as clofibric acid, bezafibrate (p*K*a 3.6) occur as ion at neutral pH and have little tendency of adsorption to the sludge (Fent, Weston and Caminada, 2006).

Sorption

Removal of a compound from solution by solid phase constituents. This term is often used when the mechanism of removal (adsorption, absorption, or precipitation) is unknown. Sorption is one of the main factors affecting the fate and transport of ECs in soil. For hydrophobic compounds, with a log*K*ow of 4.0 sorption to sludge is likely to play a role in the removal of pharmaceuticals from wastewater. Laboratory batch studies to characterize the sorption behaviour of carbamazepine, diclofenac and ibuprofen in sandy sediments revealed that sorption coefficients were generally quite low (Scheytt et al., 2005). No significant removal was observed in batch experiments with sand, indicating low sorption properties and persistence.

Biodegradation

Biodegradation of pharmaceuticals involves the conversion of the parent compound to metabolites by the action of microorganisms in WWTP or in the environment both in aerobic and anaerobic conditions. Biodegradation can occur under aerobic or anaerobic conditions (Haritash and Kaushik, 2009; LeFevre et al., 2012), both of which may favour specific

compounds. For instance, it was reported that aerobic conditions favoured the removal of ibuprofen and naproxen, while diclofenac was degraded under anaerobic conditions (Zwiener and Frimmel, 2003; Quintana et al., 2005). Biodegradation by microbial activity plays a minor role in more recalcitrant compounds such as clofibric acid (Dordio et al., 2009). In another study, Foolad et al. (2015) showed carbamazepine and crotamiton as recalcitrant compounds to microbial biodegradation. In general, biological decomposition of micro-pollutants including pharmaceuticals increases with an increase in hydraulic retention time (Fent, Weston and Caminada, 2006). For example, diclofenac was shown to be significantly biodegraded only when the sludge retention time was at least 8 days (Kreuzinger et al., 2004). In contrast, data from Metcalfe et al. (2003a,b) indicate that the neutral drug carbamazepine, which is hardly biodegradable, is only poorly eliminated (normally less than 10%), independent from hydraulic retention times.

Plant uptake

The removal of ECs can be improved by introducing plants to the system, as the system will benefit from the effect of plant uptake as well as microbial biodegradation (Scheytt et al., 2007; Xu et al., 2009a; LeFevre et al., 2011; Matamoros et al., 2012b)

Microbial Activity

Microbial activity is an important factor that influences the transformation of ECs in soil (Thiele-Bruhn, 2003) as microorganisms can directly use selected types of ECs as a growth substrate (Benotti and Snyder, 2009). Studies by (Hijosa-Valsero et al., 2010a; Matamoros et al., 2012b) suggested that PPCPs, such as caffeine and naproxen, were removed mainly by microbial biodegradation process. It was also found that microbial activity could play a significant role in the degradation process through soil (Xu et al., 2009a).

Soil

A plant-soil system is composed of a plant, soil, and, presumably, different microbial communities. Different chemical compounds have different behaviours in the soil column independent of the effects of the plant. Pharmaceuticals may be adsorbed to the soil, degraded, mineralised by microbial activities, or just leached out from the soil column (Scheytt et al., 2007; Xu et al., 2009a; LeFevre et al., 2011). Therefore, some compounds may be removed efficiently, such as caffeine; others may be removed moderately, such as ibuprofen; and some compounds such as diclofenac may be recalcitrant to removal via the soil column (Lee et al., 2011). Pharmaceuticals such as Carbamazepine, have been detected in soil irrigated with

reclaimed water, and the concentrations of detected compounds varied with the irrigation seasons (Kinney et al., 2006). Based on a study by Scheytt et al. (2007), clofibric acid was transported untransformed through an unsaturated soil column while ibuprofen and diclofenac were most likely transformed while passing through the soil column. The removal of ECs through soil can benefit from adsorption to soil particles, in addition to the positive role of microbial communities in degrading pollutants.

2.6 Potential health impacts of drinking water containing pharmaceuticals

Pharmaceuticals are chemicals that are designed to have a specific mode of action, and many of them for some persistence in the body, they are biologically active and hydrophilic so that the human body can absorb them easily. In the body, they are persistent to avoid degradation before they can have a curing effect. Depending on the composition of the drug, these pharmaceuticals can be completely degraded by the body, however, many are excreted as a mixture of metabolites, as unchanged substances, or conjugated with an inactivating compound attached to the molecule. These features among others make pharmaceuticals to be evaluated for potential effects on aquatic flora and fauna (Fent, Weston and Caminada, 2006). The health impacts of drinking water that is contaminated by pharmaceuticals have not yet been established, however, studies have been done on the health effects associated with exposure to contaminants of concern by aquatic organisms. These include low sperm count, high incidence of cancers, the incidence of intersex fish within the water system etc. The development of resistance to antimicrobial compounds is another risk that pharmaceuticals in aquatic environments can pose to public health (OECD, 2019). These compounds affect the body's hormonal balance by various mechanisms; they may disrupt hormone production, mimic hormones, influence the development of hormone receptors, function as hormone antagonists, or modify hormone binding (Kumar *et al.*, 2022).

In India and Pakistan, a high death rate among three vulture species was reported in 2004 to be caused by diclofenac, a widely used analgesic and anti-inflammatory drug. A direct correlation between residues of diclofenac and renal failure was reported both by experimental oral exposure and through feeding vultures of diclofenac-treated livestock. Hence, the residues of diclofenac were made responsible for the population decline (Fent, Weston and Caminada, 2006). The overuse and misuse of antibiotics may cause a risk to human health by promoting antibiotic-resistant bacteria and antibiotic-resistance genes in aquatic environments (NACWA, 2011). Inadequate management of wastewater may, therefore, release antibiotics, antibiotic-

resistant bacteria, and antibiotic resistance genes into the environment, thus, presenting a potential environmental health risk. Antibiotic resistance is a major health concern; the presence of antibiotics in treated wastewater is increasing and will lead to higher mortality and morbidity as untreatable infectious diseases increase. Evidence suggests that the presence of antibiotics in wastewater may be contributing to antibiotic resistance, and if these antibiotics are present in wastewater for a longer period, they may cause genetic effects on humans and marine life. Pharmacologically active compounds' ability to accumulate and have harmful effects on species other than those intended for use raises severe concerns(Kumar *et al.*, 2022).

Although current risk assessments indicate that the very low concentrations of pharmaceuticals found in drinking water are very unlikely to pose any appreciable risks to human health, knowledge gaps exist. These include the assessment of risks to human health associated with long-term exposure to low concentrations of pharmaceuticals and the possible combined effects of mixtures of pharmaceuticals (WHO 2011). Antibiotics used in food (milk, meat, eggs, fruits, vegetables, and fish) as growth promoters, therapeutics, and prophylactics can pose health risks.

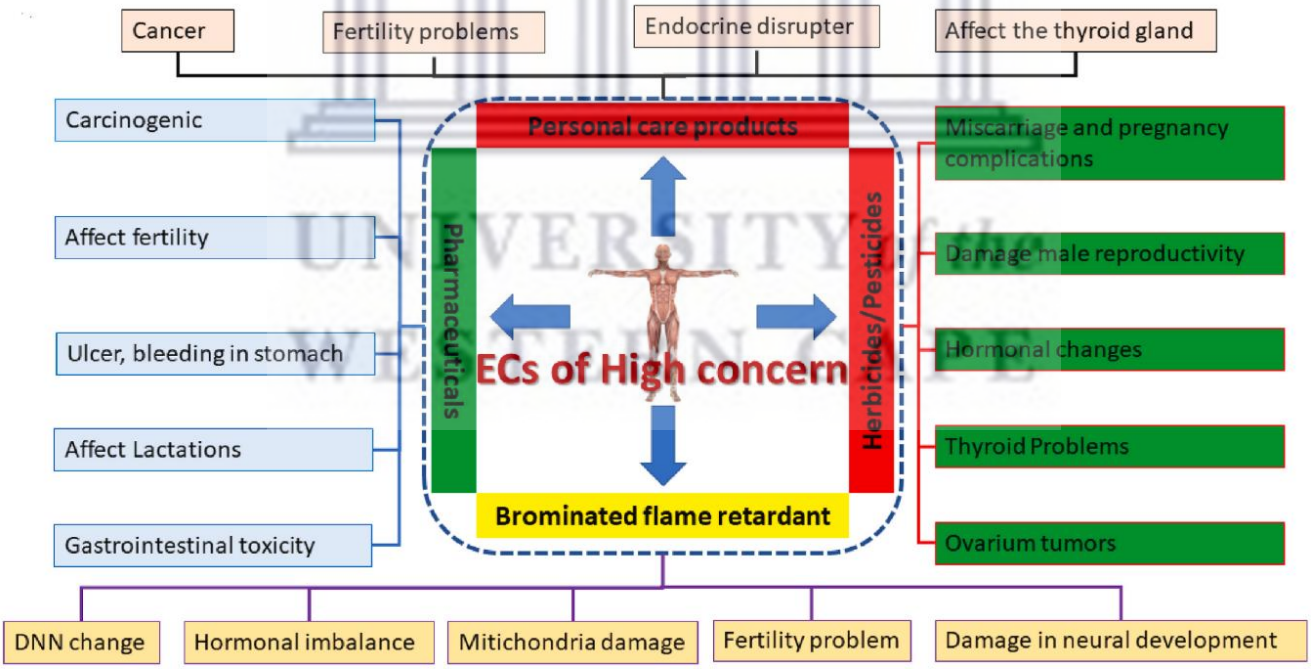


Figure 2.2: Major health effects of emerging contaminants on humans (Kumar *et al.*, 2022a)

2.7 Environmental impacts of Pharmaceuticals

In general, pharmaceuticals are biologically active, persistent, and bio-accumulative. Although being detected in low concentrations (ng/L to µg/L range), the incidence of a variety of

contaminants in the environment sharing the same mechanism of action may cause pronounced effects through additive exposures, including endocrine disruption, genotoxicity, aquatic toxicity, and development of resistant pathogenic bacteria.

2.8 Pharmaceutical found in stormwater and their guideline standards

Regulations on pharmaceutical standards are important to monitor concentrations and mitigate their adverse effects on the environment and human health. However, due to the lack of standard wastewater treatment procedures to eradicate pharmaceuticals, little is known about the significance of the potential presence of pharmaceuticals at trace concentrations in drinking water supplies.

Carbamazepine has been detected in South African drinking water, it is prescribed in abundant quantities, and it is persistent in the water (Swartz *et al.*, 2016). In South Africa, the calculated Reference Dose (RfD) for carbamazepine is 0.013 mg/kg/d, based on the human minimum therapeutic dose for children and accounting whereas the Australian Guidelines for Water Recycling Augmentation of Drinking Water Supplies (NRMMC, 2008) have a lower recommended reference dose of 2.8 µg/kg/d (or 0.0028 mg/kg/d).

Caffeine is a central nervous system and metabolic stimulant. The principal mode of action is as a nonselective antagonist of adenosine receptors. An adverse effect level of 3 mg/kg bw/day is based on observations of increased anxiety (NZFSA, 2012). No studies have reported the potential chronic effects of caffeine consumption by children. Toxic doses are found at greater than 10 grams for an average adult, which is greater than typically consumed doses of less than 500 milligrams. Ordinary consumption has low health risks, even when carried on for years. The Australian guideline value for caffeine has been recommended as 0.35 µg/L which was calculated based on a predicted Threshold of Toxicological Concern (TTC) of 1.5 µg/kg/d (NRMMC, 2008). The system used in deriving the predicted TTC assigns organic chemicals to one of three 'classes' based on their chemical structure, presence of structural alerts for toxicity and known metabolic pathways. Caffeine is most likely to persist in the water column largely because of its high solubility (13.5 g L^{-1}), low octanol-water partition coefficient (log Kow = 0.01) and negligible volatility.

Sulfamethoxazole A guideline for the antibiotic sulfamethoxazole in drinking water made from recycled water has been established at 35µg/l by applying the lowest acceptable daily intake for sulphonamides established by the NRA (namely 0.01 mg/kg bw/day [NRA 2000]).

Naproxen has been detected in all types of water including drinking water and groundwater with concentrations ranging from ng/l to ug/l. Although these concentrations are low, prolonged exposure to naproxen by nontarget organisms may cause a negative effect especially when naproxen is mixed with other pharmaceuticals. Recent investigations of European Union waters have indicated that concentrations of naproxen in wastewater treatment plants and surface waters exceed the concentration that is recommended by the European Medicines Agency by 10 to 500 folds (Wojcieszyńska and Guzik, 2020).

Diclofenac A prevalent anti-inflammatory drug is one of the most used pharmaceuticals in the world. The Australian guideline value for naproxen is 22 ug/l.

Progesterone is a hormone that is produced by both the female and male human body to help maintain pregnancy, regulate gamete maturation, organise reproductive behaviour, sperm capacitation and influence spermatogenesis (Kasambala, Rwiza and Mdegela, 2019). This hormone is commonly found in large quantities in aquatic environments because it is excreted through urine in great amounts by humans, administered to animals as a growth promoter and excreted by animals as endogenous hormones. The Australian guideline value for progesterone is 105ug/l.

Table 2.2: Australian guideline values of pharmaceuticals detected in the water adopted from Environment Protection and Heritage Council (2008)

Drug	**Guideline values (ug/l)**	**Guideline values (ng/l)**
Caffeine	0.35	350
Carbamazepine	100	100000
Diclofenac	1.8	1800
Naproxen	22	22000
Progesterone	105	105000
Sulfamethoxazole	35	35000

2.9 Possible Treatment methods of pharmaceuticals

Concentrations of most pharmaceuticals in the water environment can be reduced through natural processes (e.g., adsorption onto sediment, solar photodegradation and biological degradation) or during subsequent drinking-water and wastewater treatment processes (Bavumiragira, Ge and Yin, 2022). Whether these pharmaceuticals and other trace organic

chemicals are responsive to treatment depends on the physicochemical properties of the compound and the key underlying removal mechanisms of the particular treatment process (Chehrenegar, 2016). Given the wide range of properties represented by these chemicals, there is not a single treatment process that provides an absolute barrier to pharmaceuticals (Patel *et al.*, 2019). If the objective is to minimize the presence of pharmaceuticals in treated water, research studies have demonstrated that a sequence of diverse treatment processes is needed that is capable of tackling the wide range of physicochemical properties (Papagiannaki *et al.*, 2022). In most cases, this can be accomplished by combinations of different processes, for example, biological processes coupled with chemical oxidation or activated carbon adsorption, physical separation followed by chemical oxidation, or natural processes coupled with chemical oxidation or carbon adsorption. However, pharmaceutics are either transformed, separated, or mineralized (oxidized to carbon dioxide) during treatment.

2.9.1 Conventional Wastewater Treatment

Many pharmaceuticals are not completely mineralised in conventional WWTPs equipped with primary and secondary processes. Conventional WWTPs consist of mechanical and chemical processes followed by biological treatment to remove, precipitate, and biodegrade the organic compounds based on physicochemical characteristics (Ngqwala & Muchesa, 2020). Conventional WWTPs are usually designed for the removal of easily to moderately biodegradable compounds, while most pharmaceuticals are relatively persistent in biodegradation. Hence, their residues are found in water bodies (Chehrenegar, 2016b).

Pharmaceuticals in conventional treatment are either partially retained in the sludge or metabolised to a more hydrophilic but still persistent form and they, therefore, pass through the WWTP and enter surface water and groundwater. Tertiary treatment using technologies such as ozonation, reverse osmosis, photolysis, ultrafiltration and nanofiltration are more efficient in the removal of pharmaceuticals. However, their application in developing countries such as South Africa is relatively expensive. Hence this study proposes the use of natural treatment methods such as Bioretention/biofiltration systems to remove pharmaceuticals in stormwater through processes such as adsorption to the soil.

2.9.2 Conventional Drinking Water Treatment

The occurrence of pharmaceuticals in drinking water sources highly depends upon the degree of wastewater and non-point source impacts on the raw water supply. Conventional drinking water treatment consisting of coagulation/flocculation with ferric or alum followed by

sedimentation and filtration commonly employed for surface water treatment is not capable of removing pharmaceuticals.

Removal of some pharmaceuticals, however, can be expected during drinking water disinfection. Chlorine, chlorine dioxide and ozone disinfection are used in oxidation processes and thus have the potential to transform pharmaceuticals and other trace organic chemicals. Among the three oxidants, ozone is the most reactive. Previous studies reported that compounds with primary or secondary amines (i.e., diclofenac, sulfamethoxazole) and phenolic compounds were efficiently removed by chlorine (Alum et al. 2004, Westerhoff et al. 2005). Chlorine dioxide is generally a stronger oxidant than free chlorine. Huber et al. 2005 observed appreciable removals of sulfamethoxazole and diclofenac by chlorine dioxide. However, caffeine and naproxen were recalcitrant to chlorine dioxide oxidation. Ozonation is a strong oxidant and very effective in the transformation of many pharmaceuticals (i.e., sulfamethoxazole, diclofenac, and naproxen) that can be oxidized by more than 90-99 percent for ozone doses ≥ 2 mg/L (Ternes et al. 2002, Alum et al. 2004, Westerhoff et al. 2005, Huber et al. 2005). However, X-ray contrast media (i.e., iopromide) were only partially oxidized (Huber et al. 2005). Ultraviolet irradiation at typical disinfection doses of (5-30 mJ/cm2) is ineffective for the destructive treatment of pharmaceuticals (Cotruvo et al., n.d.).

Chlorination can remove approximately 50% of these compounds, whereas more advanced treatment processes, such as ozonation, advanced oxidation, activated carbon, nanofiltration and reverse osmosis, can achieve higher removal rates; reverse osmosis, for example, can remove more than 99% of large pharmaceutical molecules.

2.9.3 Advanced Water Treatment

Activated carbon adsorption can readily remove organic compounds from water, apart from some very polar water-soluble compounds, such as iodinated contrast agents and the antibiotic sulfamethoxazole (Adams et al. 2002, Westerhoff et al. 2005). Advanced oxidation processes (AOPs) are very effective treatment processes for oxidizing pharmaceuticals and other trace organic chemicals. However, compared to ozone, AOPs provide only a small increase in removal efficiency (Dickenson et al. 2009). Low-pressure membranes, such as microfiltration (MF) or ultrafiltration (UF), have pore sizes that are insufficient to retain pharmaceuticals based on their size. Some hydrophobic compounds can still adsorb onto MF and UF membrane surfaces providing some short-term attenuation. This also confirms the expectation that MF or UF utilised in a membrane bioreactor (MBR) process does not provide an additional benefit to

the removal of pharmaceuticals. However, high-pressure membranes, such as reverse osmosis (RO) and nanofiltration (NF), are very effective in the physical separation of a variety of pharmaceuticals from water (Bellona et al. 2008). Problematic for high-pressure membranes are low-molecular-weight organics, such as acetaminophen and the disposal of the concentrate (brine) with elevated levels of pharmaceuticals. Natural processes, such as riverbank filtration (RBF) and soil-aquifer treatment (SAT), can be employed either as an additional treatment step for wastewater reclamation or as a pre-treatment to subsequent drinking water treatment. These natural treatment processes act like a slow-sand filter with extended retention times. RBF and SAT are very effective in attenuating a wide range of pharmaceuticals and other trace organic chemicals by sorption and biotransformation processes in the subsurface but are limited in attenuating refractory compounds, such as antiepileptic drugs or chlorinated flame retardants (Drewes et al., 2003).

Advanced treatment techniques are costly to be implemented globally, especially in developing countries. Efforts into cost-effective treatments or complementary treatments could aid in restricting pharmaceuticals from infiltrating the environment. Natural remediation processes such as bioretention systems can be considered as a cost-effective and sustainable approach which has the potential to eradicate pharmaceuticals from water. Studies have shown the suitability of a bioretention system to remove pharmaceuticals in wastewater. A study by Chehrenegar et. al (2016), showed the removal efficiencies of selected ECs, namely acetaminophen (ACT), estrone (E1), ibuprofen (IBP), ketoprofen (KTP), saccharin (SAC) and carbamazepine (CBZ) using a lab-scale bioretention column. With removal efficiencies of 92.1%,83.1%,89.7%,51.4% and 30.8% respectively.

2.9.4 Bioretention General Description

Bioretention areas, also known as bioretention filters or rain gardens, are structural stormwater controls that capture and treat stormwater runoff from frequent rainfall events (Woods-Ballard *et al.*, 2007). A bioretention system consists of a soil bed planted with suitable non-invasive vegetation. Stormwater runoff entering the bioretention system is filtered through the soil planting bed before being either conveyed downstream by an underdrain system or infiltrated into the existing subsoil below the soil bed. Vegetation in the soil planting bed provides uptake of pollutants and runoff and helps to maintain the pores and associated infiltration rates of the soil in the bed.

In a plant-soil system for ECs removal, each of the two main components, namely plant and soil media, may have a role in the removal of ECs that would lead to enhanced total removals of ECs in the system. For instance, a persistent compound with a low removal rate by plants may be removed by adsorption onto the soil. However, high adsorption of ECs on the soil would reduce the availability of those compounds for plant uptake. Nevertheless, if the ECs removal efficiency of the plant-soil system is higher than the plant, using soil solely may provide a viable solution for the ECs removal. The importance of soil in the plant-soil system is the potential of soil sorption capability by choosing the proper soil characteristics. For instance, soils with high organic content (e.g., using compost) may increase the sorption of ECs onto soil media which may have a supporting role in the plant-soil system to decrease the ECs concentration in the system effluent (Chehrenegar, 2016).

2.10 Pharmaceutical detection methods

2.10.1 Gas chromatography

Gas Chromatography (GC) was first used for the analysis of environmental contaminants (Schollee, 2006). One of the disadvantages of GC-MS and GC-MS2 is that it requires derivatization steps, due to the low volatility of polar PPCPs. Additional derivatization steps make the sample preparation time-consuming, laborious and increases the possibility of contamination, which often results in sample loss. Furthermore, some compounds are thermolabile and decompose during GC analysis (e.g., carbamazepine forms iminostilbene as a degradation product). Derivatization is typically done after sample extraction and clean-up by using organic reactions (e.g., methylation, silylation, and acetylation) and the derivatization agents are carefully selected according to their reactivity with the analytes of interest or the stability of their products to avoid a high degree of hydrolysis. Derivatization with pentafluorobenzyl bromide was shown to be advantageous for more sensitive determinations. In a study conducted by Tauxe-Wuersch (2005), sample analysis was performed with GC-MS to investigate the occurrence of acidic drugs (Clofibric acid, Diclofenac, Ibuprofen, Ketoprofen and Mefenamic acid) in three different sewage plants. Extraction was done with SPE (C18) method followed by derivatization with pentafluorobenzyl bromide. The general recoveries after sample pre-treatments filtration, extraction, derivatization, and clean-up exceeded 70%. The relative standard deviation on reproducibility (RSD) and standard deviation (SD) on all recoveries varied from 2% to 16%. It was concluded that the precision was sufficient, and the analytical technique (GC-MS) was suitable for the analysis of the compounds. These methods

employ electron impact ionization (EI), which typically yields a limit of detections for the compounds in the higher ng/L concentration range. These detection limits may be suitable for many wastewaters after primary or secondary treatment but not be sufficiently sensitive to quantify all these compounds in the broad range of reclaimed wastewaters. GC-MS techniques have been used that employ negative chemical ionization (NCI), which allows the detection of these chemicals at concentrations in the low ng/L range, which is in the range of detection limits of enzyme-linked immunosorbent assay detection (ELISA) but has significantly better specificity than ELISA (Huang and Sedlak, 2001).

2.10.2 Liquid Chromatography - Mass Spectrometry (LC-MS)

LC-MS is the most commonly used method. The LC-MS-MS DuPont-11374 method can achieve a limit of quantitation of 0.01 mg/l (National health and medical research council, 2011). The use of LC-tandem MS (LC-MS/MS) for environmental analysis allows the determination of a wide range of compounds. LC-MS/MS can give a slightly higher limit of detection (LOD) than can be achieved with the GC-MS. LC-MS/MS analysis is more suitable for measuring target compounds that are more polar and highly soluble in water, whereas GCMS/ MS is better for more volatile target compounds (*Pharmaceuticals in Drinking-Water*, n.d.). Increased use of LC-MS has provided a new analytical tool that allows the identification of highly polar organic pollutants in the environment down to ng/l levels without derivatization. LC-MS techniques can be coupled with on-line devices for sample preparation and preconcentration methods, such as SPE. PPCPs can be analysed with LC-MS without derivatization. Acetonitrile with methanol is used as the organic mobile phase for the LC separation, and the use of a buffer in the eluent or acidification of the mobile phase is also recommended, to achieve sufficient retention for acidic drugs and reproducible retention times.

2.10.3 Summary

A review of the literature showed that stormwater reuse acts as a possible exposure pathway to several pharmaceuticals. Many of these pharmaceuticals pass through conventional wastewater treatment plants without being removed, some are partially removed and end up accumulating in the surface water bodies. The primary sources of pharmaceuticals include the inappropriate disposal of medicines at home, hospital discharges, aquaculture facilities, animal farming activities, and municipal and industrial wastewater treatment plants. Pharmaceuticals have been detected in water bodies all over the world and few studies have also been done in South Africa

to monitor the occurrence and distribution of these pharmaceuticals. The presence of these pharmaceuticals in reclaimed water is of critical concern because of the possible health effects on human beings.

Liquid chromatography linked to tandem mass spectrometry (LCMS/MS) was a modern technique that is mostly applied for the detection and quantitation of polar, water-soluble compounds including pesticides and pharmaceuticals. This technique was capable of detecting quantities in the ng/L range, with the exact lower limit depending on the instrument configuration. LC-MS/MS has also been successfully applied in most studies that were monitoring pharmaceuticals that are commonly found in water bodies. For these reasons, LCMS was found to be the best technique that can be used in this present study.

Conventional water treatment plants do not remove these pharmaceuticals hence finding alternative remediation techniques is important for water that will be reused. The literature review showed that concentrations of most pharmaceuticals in the water environment can be reduced through natural processes hence the use of a bioretention system to remove pharmaceuticals in stormwater was chosen as a remediation technique that is worth investigating.

CHAPTER 3: STUDY AREA DESCRIPTION

This is an experimental and quantitative study that included a combination of field sampling, laboratory analysis, and the design of an experimental bioretention stormwater treatment basin. The required field samples were collected during the dry month of April 2021 and the wet period of July 2021. These samples were sent to a pharmacological laboratory to determine the concentrations of pharmaceuticals in the stormwater. The study was conducted in the Zeekoe catchment located in the south-central part of the city of Cape Town, in the Western Cape Province of South Africa (Figure 3.1). Cape Town has a Mediterranean climate characterised by warm dry summers from December to February, and mild wet winters between June and August (Council, 2002). The average annual rainfall within the catchment ranges from 500 to 1100 mm, with 56 million m^3 of annual runoff (River Health Programme, 2005). Zeekoevlei is a 250-ha water body that is about 2.5 km in length and 1 km wide. The vlei is located in the Zeekoevlei Nature Reserve which forms part of the False Bay Nature Reserve. The area has a relatively flat terrain (less than 3% average slope) and is made up of pervious sandy soils.

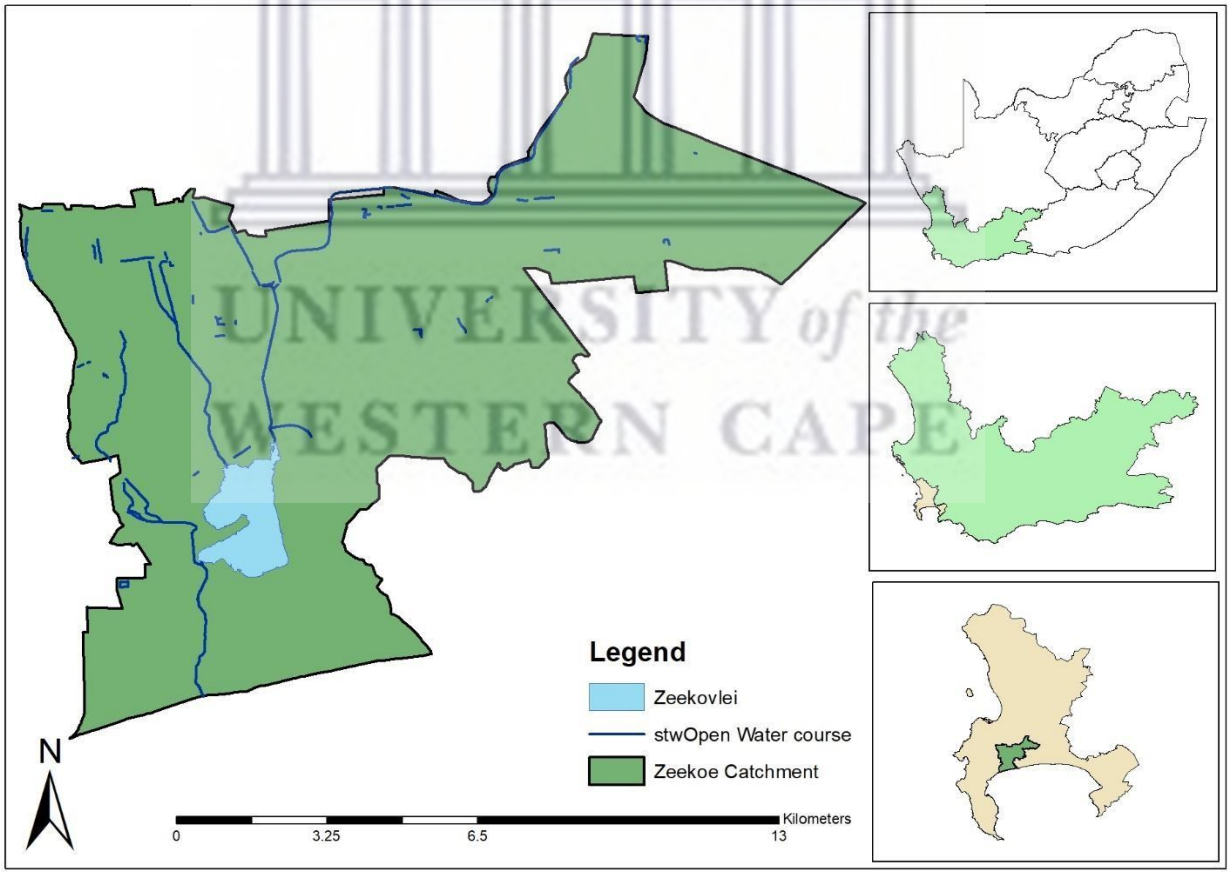


Figure 3.1: Location of the Zeekoe Catchment in Cape Town, in the Western Cape Province of South Africa.

Land use/cover

The Zeekoe catchment is surrounded by residential areas including Grassy Park, Lotus River, and Pelican Park. There are major activities such as agriculture, public parks, and residential areas where treated stormwater could be used as an alternative water supply.

Geology

Zeekoe catchment is located on the extensive Cape Flats Aquifer which is a large unconfined aquifer that ranges from 20 to 50 m in depth. The Cape Flats Aquifer comprises unconsolidated quaternary-aged sands of the Sandveld Group that overly Precambrian-aged rocks of the Cape Granite Suite, and argillaceous sedimentary rocks of the Malmesbury Group.

The management of the vlei

The lake is divided into three basins: the Northern Basin, near the mouths of the Great and Little Lotus River. Home Bay, the sheltered corner in the northeast; and the South Basin also known as Storm Bay. The Zeekoevlei Nature Reserve is managed by the City of Cape Town. However, much of the boundary of the Zeekoevei is open access and is shared with private homeowners. The Eastern shore of the vlei which was used to collect soil samples is a 2 km stretch of land bordering the vlei. It is a multipurpose open space and serves as a recreational site containing picnic facilities, ablution facilities and fishing space.

The Catchment is largely defined by stormwater drains consisting of stormwater ponds (about 61), and large shallow lakes where stormwater collects. Much of the stormwater that discharges into the Zeekoe Catchment is inflow from the Great Lotus River which receives the bulk of its water from the Boquinar Industrial Area, located close to the Cape Town International Airport. Around the airport industrial area, are densely populated informal settlements such as Gugulethu, Nyanga and Philippi, with poor sewage systems. As a result, the Great Lotus River carries the highest contamination load from the informal settlements that includes grey and black water as well as poor quality effluent discharging into the stormwater drains.

During periods of high flows, the runoff from highly urbanised areas as well as the contaminated runoff from the surrounding areas enters the vlei via the Big Lotus canal. Also, the agricultural runoff from the Philippi Horticultural Area, Cape Flats WWTW discharge into the Zeekoe outlet canal. Polluted flowing rivers in the northern portion of the vlei, Coastal Park

Landfill site, and the recreational site in the vlei are all major sources of pollution and/or contamination of stormwater in the catchment.

3.1 Site selection

A field visit was conducted to assess potential sampling sites in the Zeekoe catchment (Figure 3.2). The selection criteria for the suitable sites were based on the following:

1. Accessibility and permission to use the site,
2. Location near potential stormwater users such as residential areas.
3. Location with various land use activities such as agriculture, industrial and residential, which qualified the area for sampling.
4. Availability of stormwater drainages,
5. Availability of stormwater ponds,
6. Availability of stormwater quality data needed for validation,

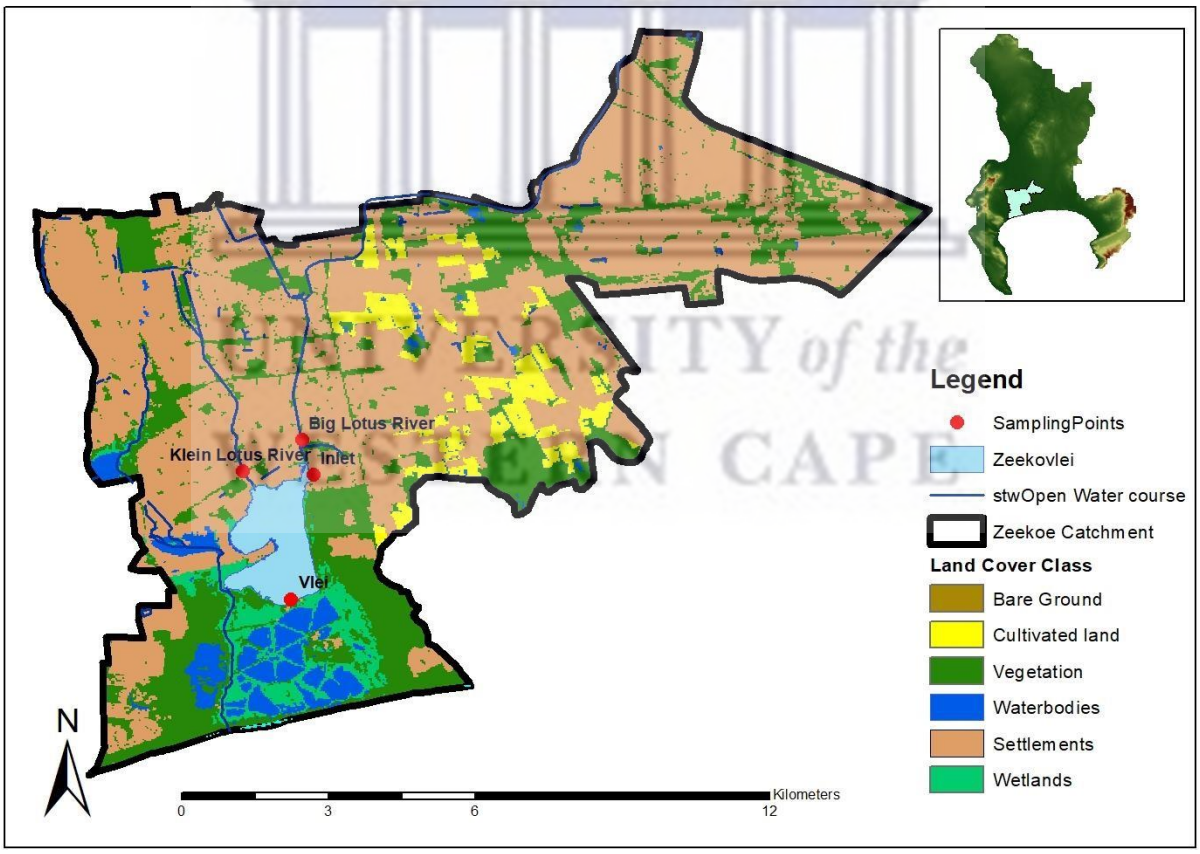


Figure 3.2: The locations of sampling points in the Zeekoe catchment.

CHAPTER 4: METHODS AND MATERIALS

To achieve the objectives of the study, there was a need to identify and measure the concentration levels of the pharmaceuticals found in stormwater. It was also necessary to determine the removal efficiency of the identified pharmaceuticals by using the *Buffalo Grass* and *Pennisetum* plants in a bioretention system with soil media control. The selected plants were identified by a study done by Milandri *et al.*, (2012) that tested their performance in treating stormwater nutrients such as TDS, PO_4, Nitrates etc. The selected plants were assessed to determine the removal efficiency of the detected pharmaceuticals. The method used in this study was adopted from previous studies such as Chehrenegar (2016a) and Liu Kimberly (2020). The removal efficiency was calculated to assess the performance of the bioretention columns, using the input concentrations and the output concentration of the drugs, the equation is expressed in percentage as shown below: Eq. 1

$$E\ (\%) = \frac{C_{input} - C_{output}}{C_{input}} \times 100 \qquad 4.1$$

Where E is removal efficiency in percentage (%)

C_{input} is influent concentration in ng/l

C_{output} is effluent concentration in ng/l

The results are presented in Chapter 5.

4.1 Methodology

4.1.1 Collecting field stormwater samples for analysis

The field samples were collected during the dry season in April 2021 and the wet season, in July 2021 in Cape Town, South Africa. A Global Positioning System (GPS) was used to take the coordinates/locations of the sampling points/ sites. Sterilised 100ml amber glass sampling bottles were used to collect stormwater samples from the inlets and outlets of the Zeekoevlei Lake. The water samples were collected and stored in a cooler bag with ice. A Hach HQ40D Multi Meter was used to measure in-situ water quality parameters such as temperature (°C), dissolved oxygen (mg/l), conductivity (µS/cm), and pH at each site. The precision of the instrument is claimed to be at 95% confidence interval (Malijani, 2020). The collected samples were stored in a fridge at -20 degrees Celsius until analysis.

4.1.2 Quality Assurance and quality control

Quality assurance and quality control measures were therefore to minimise errors:

1. Samples were collected using sterilised amber glass bottles,
2. The collected samples were stored in a cooler box with ice,
3. Thereafter, they were kept in the fridge at -20 degrees Celsius until analysis,

4.1.3 Analysing stormwater samples

Water samples were collected during the dry season (April 2021) and wet season (July 2021) to quantify the concentration levels of the selected pharmaceuticals. These samples were analysed for the presence of the most common pharmaceuticals in water, Carbamazepine, Naproxen, Caffeine, Progesterone and Sulfamethoxazole. The samples were analysed using Liquid Chromatography-Mass Spectrometry (Shimadzu 8040) to detect pharmaceuticals and their concentrations. While reliable methods have been established in laboratories worldwide, there is currently no internationally standardized analytical protocol for pharmaceuticals.

4.2 Laboratory Procedure:

Setting up standards for accuracy and method validation

The procedure before analysis using the LC-MS, was first to create 6 standard reference materials for the pharmaceuticals of interest that were obtained from Sigma-Aldrich, to set up a calibration line with known analyte concentration. This was done by weighing 10 mg of each standard reference material, placed into vials and dissolved with methanol. The vials were vortexed for 1 minute and then sonicated in a bath for 5 minutes to ensure adequate mixing of the solution.

Solid Phase Extraction

15ml of each sample was filtered using a syringe attached to a nylon syringe filter with 0.22um pore space and then transferred into 15 ml centrifuge tubes. Before the filtered sample containing the analyte was transferred into the Sep-Pak Vac 1cc (100 mg) using tc 18 cartridges; the cartridges were first conditioned and equilibrated to activate the sorbent. This was done by conditioning the sorbent with 1 ml of methanol and then equilibrating it with 2 ml of deionized water, repeated twice using a pipette. This process was done to allow the sorbent to retain the analyte during the SPE procedure.

After the above two steps were performed, the samples were then loaded into the cartridges using a 100 µl pipette with a new pipette tip for every sample. The loading of the cartridge was repeated three times to ensure that enough analyte was trapped by the sorbent. After the loading procedure, the cartridges were washed with 2 ml of deionised water to remove any other unwanted interference still present in the sorbent.

Lastly, elution was done to remove the analytes from the sorbent. This was done using 2ml of methanol. The analytes were then collected into glass test tubes. The eluates were then evaporated to dryness using a rotary evaporator in a miVAS DNA Concentrator for 60 minutes at 45 degrees Celsius. The analyte was then reconstituted with 2ml of a mobile phase solution (1:1 methanol and deionized water) and injected into vials for the LC-MS analysis.

Detecting pharmaceuticals using Liquid Chromatography- Mass Spectrometry

The LC-MS/MS method was developed on the Shimadzu Triple Quadrupole LCMS-8040 using LC-20ADXR binary pumps, a SIL-20ACXR autosampler, a CTO-20A column oven, and LabSolutions software (Shimadzu Corporation, Kyoto, Japan). Chromatographic separation on the LC-MS/MS was achieved using an Agilent Poroshell 120EC-C18 column (3.0 x 100 mm, 2.7µm: Agilent Technologies Inc., California, United States). The liquid chromatography system was used to push solvents through, these solvents included the mobile phases. The procedure used in the instrument included an injection of 3 ml of sample concentration into 0.2 ml (15-fold concentration) of the mobile solution. Blank samples were injected after the highest standard to establish carry-over to the lowest limit of quantification, the calibration range was set to 0.32-1000 ng/ml. A volume of 10 µl of each of the samples was injected into LC-MS, using two methods (the one in the negative ion mode and the other in the positive ion mode). The molecular weight and structure of the compound dictate whether it can either attract a proton or lose a proton, it has a preference on how the compound wants to ionise that is manipulated to encourage the ionization in the right way using certain combinations of mobile phases. Two separate methods were created for this analysis because the compounds that ionise in the negative ion mode require different mobile phases from those that ionise in the positive ion mode.

The first method is the negative ion mode which consists of two mobile phases, mobile phase A consists of 10 mm of ammonium acetate in water, and mobile phase B consists of acetonitrile. The column used is an infinity Lab Poroshell 120 EC-C18 (3.0 X 100 mm 2.7 m/z) with a

temperature of 30 degrees Celsius. The gradient is 60 to 95% B over 3 min, 95% up to 3.5 min, 95 to 60% up to 4 min, and equilibrate at 60% until 7 min. The flow rate was 0.5 ml/min.

The second method is the positive ion mode which consists of two mobile phases, mobile phase A consist of 5mm of ammonium formate with 0.1% formic acid in the water, ACN;95;5, and mobile phase B consists of 2 mm ammonium formate with 0.1 formic acids in ACN, water 95;5. The column used is infinity Lab Poroshell 120 EC-C18 (3.0X100mm, 2.7um) with a temperature of 30 degrees Celsius. The gradient is 10 to 95% B over 5 min, 95% up to 5.5 min, 95 to 10% up to 6 min, and equilibrate at 10% until 9 min. The flow gradient was 0.45 ml/min. The samples were then injected into the mass spectrometer using reversed-phase chromatography to separate the compounds. The flow went through 3 quadrupole mass analysers, the first quad looked for the precursor mass of the compounds and disregards any other compound present, the second quad had the presence of argon gas molecules, so the masses collide with the argon mass molecules and broken up into fragments ions, the third quad collected those fragment ions and used that to improve the specificity of the method so that each compound had a unique figure print. Then at the end stage, there was a detector that sends a signal to the software to generate peak graphs using the mass-to-charge ratio. This ratio of the molecular ion is equal to the molecular weight of the compound, which was used to detect how much of the compound is present in each sample.

4.3 Collecting Soil samples

Soil samples were collected following a zone-based strategy method as described by Ackerson *et al.,* (2018). In this method, the collected soil samples (composite sample) represent the average soil within each zone. 10 sampling points were selected adjacent to the Zeekoevlei in a zigzag pattern 20 meters apart to ensure that they were spread evenly. Bulk samples of 1700 kg were taken at 15 cm to 1 m depths (Figure 4.1.2) from the Eastern Shore of the vlei, which is a 2 km stretch of land bordering the vlei. From the bulk samples, 500g subsamples were taken to form composite samples for analysis. The samples were stored in a Ziplock bag and taken to the laboratory for soil classification.

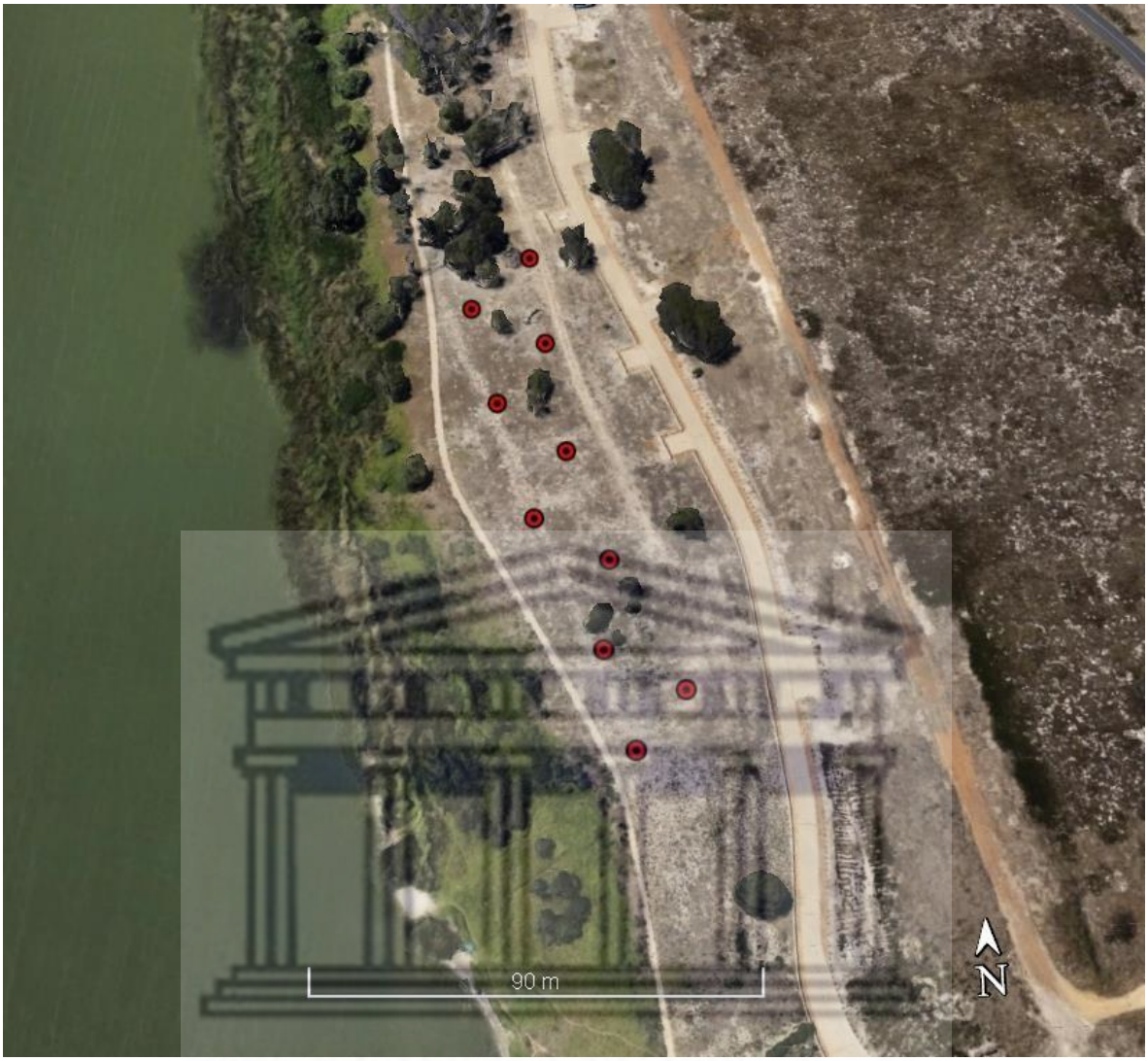


Figure 4.1: Soil sampling points (red dots)

4.4 Classification of soil samples

Soil texture

The settling method which is based on Stokes' law settling velocity of soil particles was used to determine the distribution of particle sizes. Disturbed soil samples were acquired from auguring at different depths (0 - 10 cm, 11 - 30 cm, 31 - 60 cm, and 61 - 90 cm) on each site. The soil aggregates of the samples were grinded and sieved to remove the large organic fragments that were greater than 2 mm. About 100 g of soil samples from each depth were sieved. The sieved samples were then transferred to four different beakers (600 ml) and labelled for each depth. Distilled water was then added to dampen the samples. 20 ml of hydrogen peroxide (H2O2) was added to the samples and heated over a hot plate. The beakers were removed from the hot plate and 10 ml of HCL (hydrochloric acid) was added to each beaker.

One hundred millilitres of distilled water was then added to the mixtures and the contents in the 600 ml beakers were poured into the funnel containing a filter paper. Once all the liquids filtered through, the filter papers were then placed into clean 600 ml beakers and dried in the oven at 105 ºC for 24 hours. The dried samples were then grinded with a pestle to disperse the samples. One hundred (100) ml of the dispersing agent sodium hexametaphosphate was added to the grinded samples, and the mixtures were transferred to 1000 ml cylinders which were labelled for different depths and sites. Three aliquots were taken at different settling times on each cylinder, the settling times depended on the temperature of the sample in the cylinder. The aliquots were then dried for 24 hours and then weighed. The results were then plotted on the USDA Soil Texture Triangle to describe the soil textures at different depths.

Figure 4.2: Subsamples collected for the analysis of soil properties.

4.5 Bioretention system setup

4.5.1 Soil samples

Bulk soil samples were collected as described in Chapter 4, section 4.3. The samples were taken and used to design/construct a bioretention system used in this study.

Figure 4.3 Pit dug to 1m depth for bulk soil samples.

4.5.2 Vegetation

200g specimen pack of Turf Grass tray/ Buffalo grass and 4 kg of *Pennisetum Rubrum* were used in this study. *Bouteloua Dactyloides* (Buffalo Grass) is indigenous to coastal areas of South Africa with a coarse, broad texture. It requires some time to establish itself and adapt to new areas but once an area is covered, it is a strong, low-maintenance grass. It can withstand extreme temperatures, droughts, and cold conditions. It grows in shaded and semi-shaded areas but does not flourish as it would in sunny areas. *Pennisetum Rubrum* (Purple fountain grass) is a popular ornamental plant and has been planted widely in areas with warm, arid climates. It is a sparsely branching, tussock-forming perennial grass with feathery, spike-like inflorescences. The bristles are long and detach with the spikelet. The leaves are up to 40cm long, the inflorescence is 10-25cm long and is usually purple or rose-coloured. The vegetation specimens were bought from a Garden Centre (Stodels, Western Cape).

The plant roots were washed several times with tap water to remove the fertilizers and any other substances that may be contained. After washing, the plants were then planted in the bioretention columns and allowed to grow for 4 months and were watered every 3rd day with 5 litres of tap water.

4.5.3 Construction of Columns

The columns were constructed using Perspex also known as Plexiglas, with an inner diameter of 490 mm and a height of 1m. The columns were then sprayed with spray paint to minimise the influence of external light sources during the experiment, as sunlight may affect the chemical composition of the compounds (the pharmaceuticals may degenerate). Each Bioretention system was made up of four layers with different depths that mimic the selected study location soil profile:

Top layer: Consisted of organic soil from the Zeekoevlei nature reserve. This layer was characterized as dark grey organic sand, fine-grained. The top layer was 110 mm and required 65 kg of sand. This layer was covered by two different plant types, namely: Turf grass, and P*ennisetum*. A 150 mm transparent ponding zone was left above the sand to provide light for the plants. The second layer: Had a depth of 100 mm. This was filled with reddish firm calcrete sand of about 50 kg in weight. The third layer: was 250 mm, composed of light grey to yellowish sand (130 kg). The Fourth layer: was 390 mm deep and consisted of fine-grained dark grey sand of approximately 180 kg in weight.

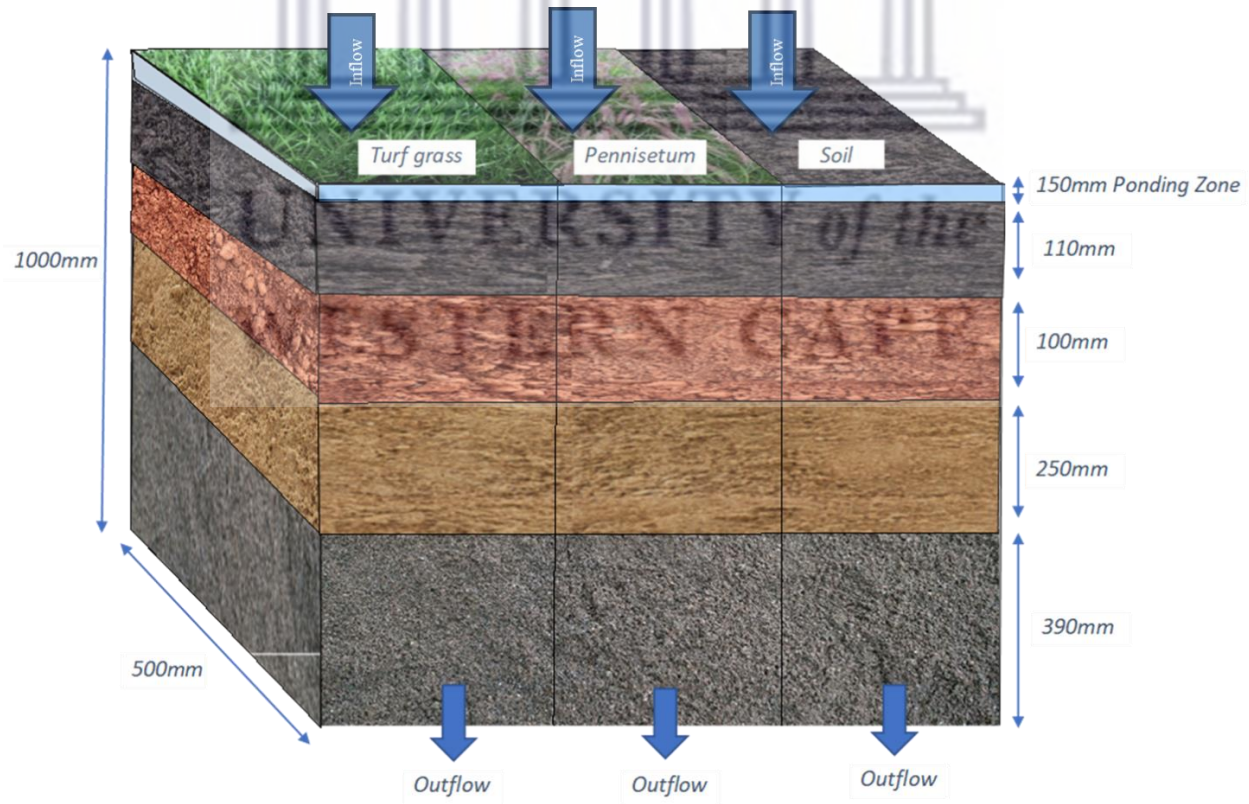


Figure 4.4: Schematic diagram of a bioretention system designed for this study.

4.5.4 Rainfall data

Rainfall data for the period January 2016 to December 2020, collected at Mitchells Plain Wolfgat ARS station (weather station closest to Zeekoevlei) was acquired from the South African Weather Services. The data was used to estimate the runoff volume (mm/day) received in Zeekoevlei per day using the Rational equation which is expressed as:

$$Q = CiA \qquad 4.2$$

Where:

Q = Peak runoff in cubic feet per second

C = Runoff coefficient

i = Average intensity of rainfall in inches per hour

A = The watershed area in acres

4.6 Procedure:

Laboratory Bioretention columns were used to study the efficiency of a bioretention system in removing commonly found pharmaceuticals in stormwater such as Carbamazepine, Naproxen, Caffeine, Progesterone, Diclofenac and Sulfamethoxazole (Swartz *et al.*, 2016). Three bioretention columns were designed for the removal of these pharmaceuticals. In each column, about 425 kg of soil was poured and flushed with tap water. Of the three systems, two were planted with Buffalo grass and Pennisetum, while one contained bare soil (Figure 4.5). All the columns were watered with 5 litres of tap water every third day for 4 months (June - September). This was done to allow the plants to grow and the roots to anchor into the soil before the actual experiment took place.

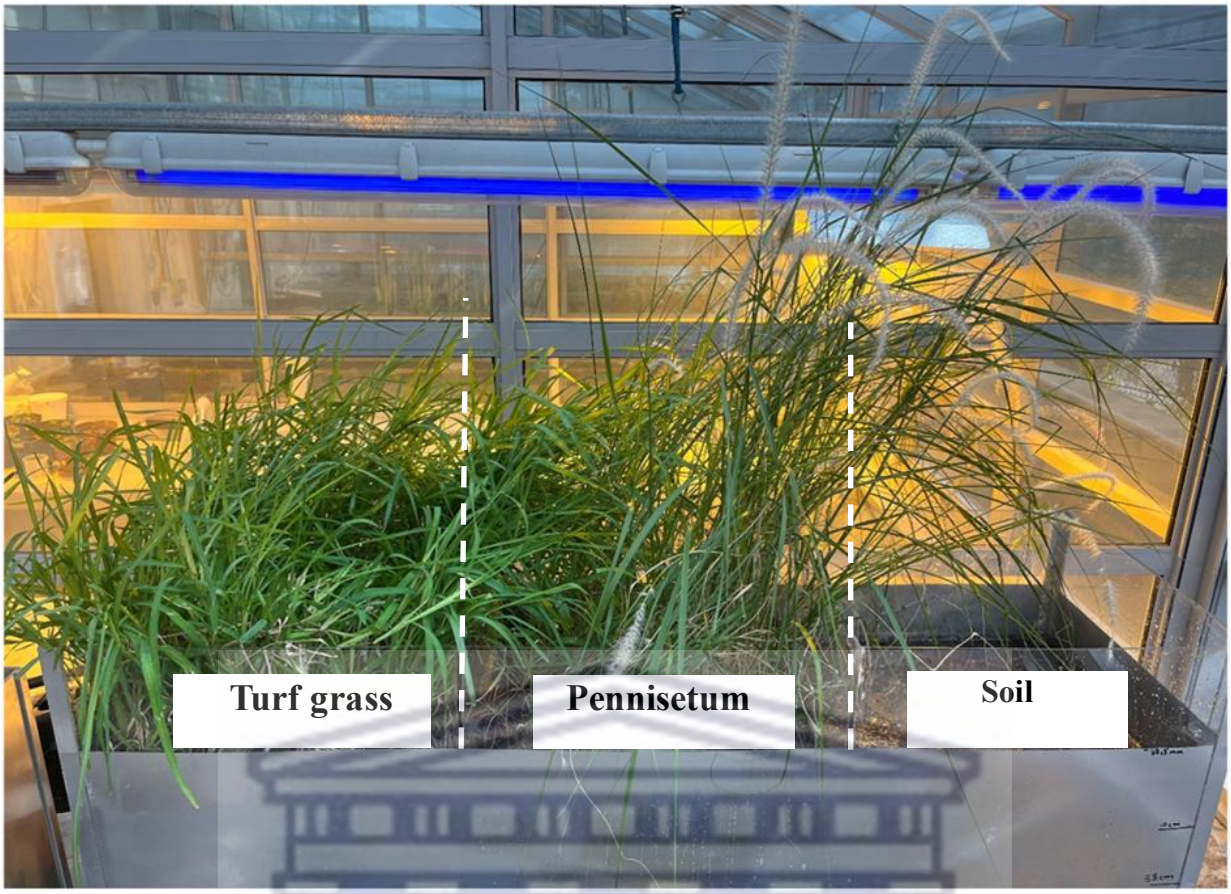


Figure 4.5: Photo of the built lab-based bioretention system showing the three different treatments of the experiment.

4.6.1 Analytical reagents and Stock solution

For the feasibility of the study, stock solutions were prepared in 2ml Eppendorf tubes at an initial concentration of 10mg/ml for each of the selected pharmaceuticals and dissolved in methanol according to the ratios of the field detected concentrations (as displayed in figure 5.1.1 and 5.1.2) and stored in -20 degrees Celsius. The 10mg/ml was selected so that the pharmaceuticals may still be detected when they are diluted in the big water volumes when running the experiment. The purity of the standards was ≥ 98% for Carbamazepine, sulfamethoxazole, and Naproxen, ≥ 99% for progesterone and Caffeine, and ≥ 98.5% for Diclofenac. The stock solutions were then diluted in 5L, 10L and 15L of deionised water. The stock solution dilutions were equivalent to the mentioned number of litres.

For the feasibility of the study, stock solutions were prepared in 2ml Eppendorf tubes at an initial concentration of 10mg/ml for each of the selected pharmaceuticals and dissolved in methanol according to the ratios of the field detected concentrations (as shown in figure 5.1.1 and 5.1.2) and stored at -20 degrees Celsius. The 10mg/ml concentration was chosen so that pharmaceuticals could still be detected even when diluted in water. The stock solutions were then diluted in 5L, 10L and 15L of deionised water. The stock solution dilutions were

equivalent to the mentioned number of litres. The purity of the standards was ≥ 98% for Carbamazepine, sulfamethoxazole, and Naproxen, ≥ 99% for progesterone and Caffeine, and ≥ 98.5% for Diclofenac.

4.6.2 Synthetic stormwater test

Synthetic stormwater: deionised water spiked with stock solutions of the selected pharmaceuticals, was used to simulate pharmaceutical concentrations that were found present in the Zeekoevlei (April 2021) stormwater samples. The synthetic stormwater was then poured into each bioretention column, and the outflows were regularly analysed for the selected pharmaceuticals (Carbamazepine, Naproxen, Caffeine, Progesterone, Diclofenac and Sulfamethoxazole) to determine the effectiveness of the bioretention system in removing these pharmaceuticals.

Samples of the water emanating from the taps of the bioretention columns were collected every 24 hours in 15ml centrifuge tubes. These grab samples represented the outflow of water from a respective storm event. The pH, EC, DO and temperature of the samples was tested, and the samples were labelled and taken to the Stellenbosch clinical pharmacology laboratory (Tygerberg campus) for pharmaceutical analysis. All the water samples were analysed using the Shimadzu 8040 LC-MS instrument and following the laboratory procedure as explained in chapter 4.1.2b.

4.6.3 The synthetic stormwater test:

The test was performed in three weeks. The synthetic stormwater was used to recharge the columns and the effluent emanating from the columns was analysed using LC-MS and the concentrations were measured in ng/l. The experiment was run over three weeks using 3 different stormwater volumes (5 L, 10 L and 15 L). In week one the columns were recharged with 5 L, in week two with 10 L, and in week three with 15 L volumes. The columns were watered for 3 days a week with synthetic stormwater. To ensure that the columns had dried completely two dry days were left in between the inflow days. The first inflow for all 3 volumes had 0 hrs retention time (the taps were left open for 24hrs). The second inflows had a 12hr retention time and the third inflows had 24hr retention time.

Table 4.1: September – October 2021, lab-bioretention stormwater treatment schedule

			September - October				
	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
week 1	26	27	28	**29) 5L**	30	1	**2) 5L**
week 2	3	4	**5) 5L**	6	7	**8) 10L**	9
week 3	10	**11) 10L**	12	13	**14) 10L**	15	16
week 4	**17) 15L**	18	19	**20) 15L**	21	22	**23) 15L**

Key

	Retention Time
	leave taps open 24hrs
	Closed taps for 12hrs
	Closed taps for 24hrs

4.7 Data acquisition (data sources)

Stormwater samples were taken from the field and sent to the laboratory for examination, land use and historical stormwater quality data were obtained from the reviewed literature. Field tests or measurements were done to develop field data and chemical data was obtained from the laboratory test results. Rainfall data obtained from the South African Weather Services was used to estimate the volume of runoff received in Zeekoevlei per day to simulate the runoff volumes in the lab experiment.

4.8 Data analysis

T-test analysis was used to determine the significance of the concentration differences between the influent and effluent of the stormwater, and the removal efficiency of the treatment method.

4.9 Treatment method selection (selection criteria of treatment method)

Determining treatment requirements is based on comparing the incoming stormwater quality to the required end-use quality for specific contaminants, and determining their required removal rates (Goonrey *et al.*, 2009). The method was selected based on its capacity to remove

pollutants from previous studies. The chosen method had an effective water quality treatment and met the required end-use quality for certain emerging contaminants and their required removal rates as recommended by the national drinking water guidelines. The method was more cost-effective and used minimal energy compared to most treatments. Implementing the remediation method was operationally and financially feasible for the current project. It was easy to maintain and had an average to long lifespan.

4.10 Study Limitations

Limited access to the LC-MS instrument, a backlog in the Stellenbosch laboratory was a challenge that limited how soon and how often the samples could go to the lab for analysis. However, the samples were analysed every week. High charging rate to analyse the samples (R300 per sample).

CHAPTER 5: RESULTS AND DISCUSSION

This chapter presents the results and discussions of the study. The results of the field samples that were collected before running the experiment are presented for both the dry and wet seasons. The removal efficiency of the selected pharmaceuticals by the three bioretention columns is presented in graphs. The influence of retention time and stormwater volumes on the removal efficiency of the pharmaceuticals by the bioretention columns is discussed. Influent and effluent concentrations of the experiment are demonstrated in the appendices.

5.1. Concentrations from field samples

Field samples were collected in April 2021, and the detected pharmaceuticals and their concentrations are presented in (figure 5.1.1). Five drugs were detected out of the commonly found pharmaceuticals that were tested. Samples were again collected in July 2022, during the rainy season of the Western Cape South Africa (figure. 5.1.2). Only carbamazepine was detected out of the five pharmaceuticals that were detected in April. In addition to the ones detected in April, Diclofenac which is also a very common drug was detected in July the winter, wet season of Cape Town. There are quite a few possible reasons for the non-detection of the other four samples in July, one being dilution and /or decreased consumption. These lead to available concentrations being below the limit of detection which the sensitivity of LC-MS can detect. The concentrations detected were very low compared to the Australian guideline values recommended for these pharmaceuticals in reclaimed water: Carbamazepine 42.4 ng/l/ $10x10^4$ ng/l, Naproxen 61.4 ng/l / $22x10^3$ ng/l, Caffeine 49.4 ng/l / 350 ng/l, Sulfamethoxazole 21.9 ng/l / $35x10^3$ ng/l, Progesterone 18.7 ng/l / $10.5x10^4$ ng/l, Diclofenac 0.221 ng/l / $1.8x10^4$ ng/l. However, their presence in stormwater still raises concerns about the use of harvested stormwater considering that their long-term consumption may have adverse health effects on humans. These pharmaceuticals have also been detected in other provinces in the country such as Kwa-Zulu Natal, Free State and Gauteng and they were detected in concentrations higher than the ones that were detected in the field in Zeekovlei. Madikizela and Chimuka *al et*., (2017) monitored the occurrence of naproxen and diclofenac residues in wastewater and river water in Kwa-Zulu Natal Province. These target compounds were detected in most wastewater and river water with maximum concentrations of 6.84 and 9.69 µg/L being detected in river water for naproxen and diclofenac respectively. Africa, Agunbiade and Moodley *al et*., (2014) detected the presence of diclofenac and sulfamethoxazole in surface water Kwa-Zulu Natal with concentrations ranging between 0.3 – 15.6 µg/L for diclofenac and 3.68 µg/L for

sulfamethoxazole. Whereas in the Gauteng province sulfamethoxazole ranged from 0.6 – 1.4 µg/L in surface water (Archer, Wolfaardt and van Wyk, 2017). Carbamazepine was detected in drinking water in the Free State province and Gauteng in surface water at concentrations ranging between 0.02 and 0.3 µg/L (Patterton, 2011; Archer, Wolfaardt and van Wyk, 2017). Caffeine was detected in Kwa-Zulu Natal and Gauteng surface water at concentrations of 0.1 – 6.6 µg/L (Matongo *et al.*, 2015; Archer, Wolfaardt and van Wyk, 2017) and lastly, Progesterone was detected in Kwa-Zulu Natal in various WWTW at concentrations ranging between 0.01 and 0.90 µg/L (Manickum and John, 2014).

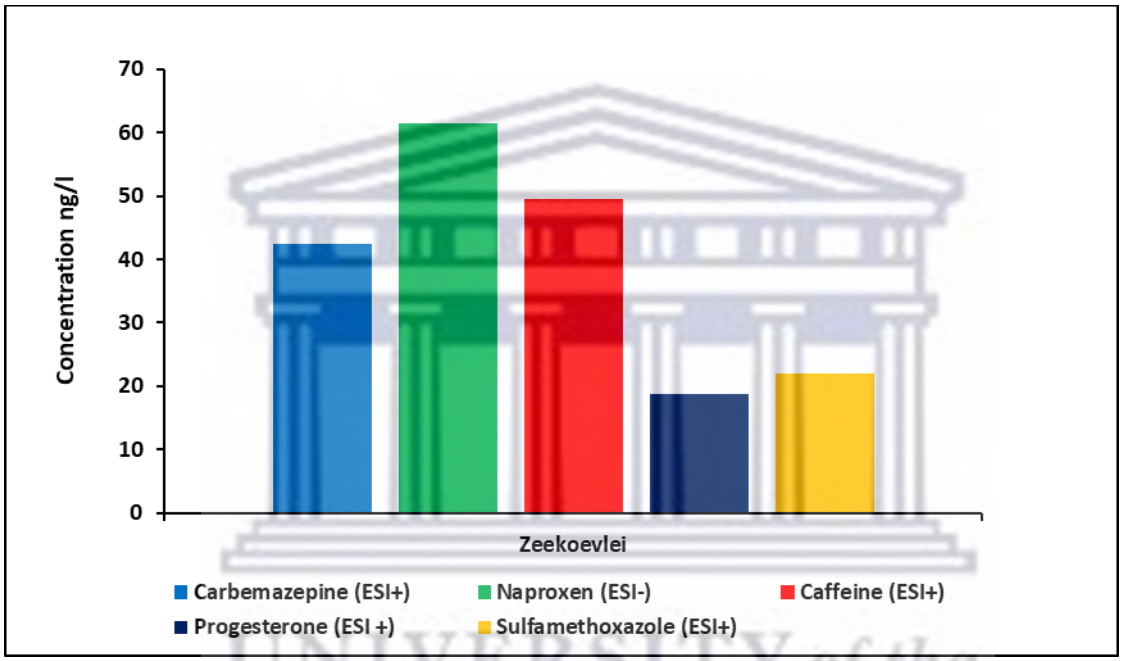


Figure 5.1.1: Pharmaceutical concentrations detected in Zeekoevlei April 2021(dry season).

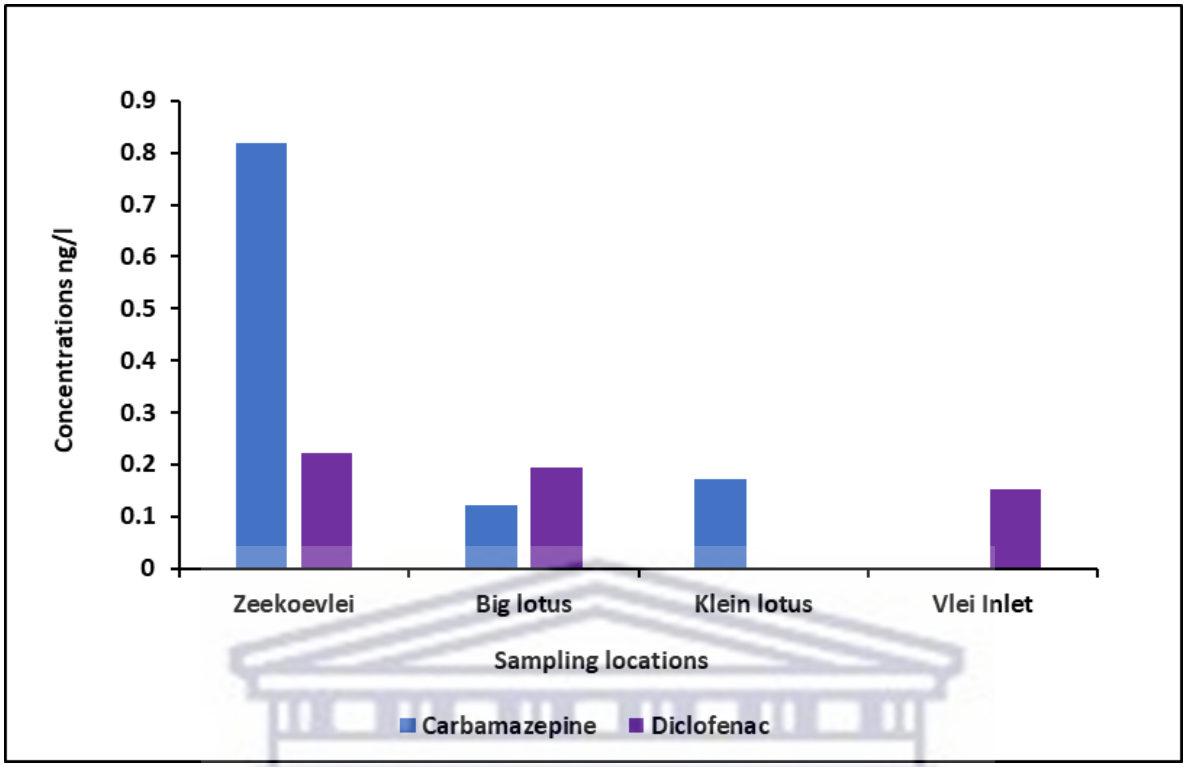


Figure 5.1.2: Pharmaceutical concentrations of field stormwater samples taken from Zeekoevlei, Big lotus and Klein lotus river and an inlet of the vlei in July 2021.

5.2 Soil texture analysis

Soil classification revealed that the soil was mostly sandy (figure 5.2). The first layer was an organic soil layer with a slight percentage of clay. The second layer had 1.23 % of clay which was a bigger percentage compared to the rest of the soil profile layers. It was compacted red soil that had larger granules that degraded to fine sandy soil, this is shown in figure 4.2 and 4.3. The third layer and bottom layer were sandier than the other two layers with a very small percentage of clay, 0.26 and 0.23 respectively. Sandy soil has poor water retention properties, high permeability, and high sensitivity to compaction. Poor water retention could be a possible reason for some of the pharmaceuticals being transported through the soil profile untransformed or not completely degraded hence being detected in the effluent (Xu, Wu and Chang, 2009).

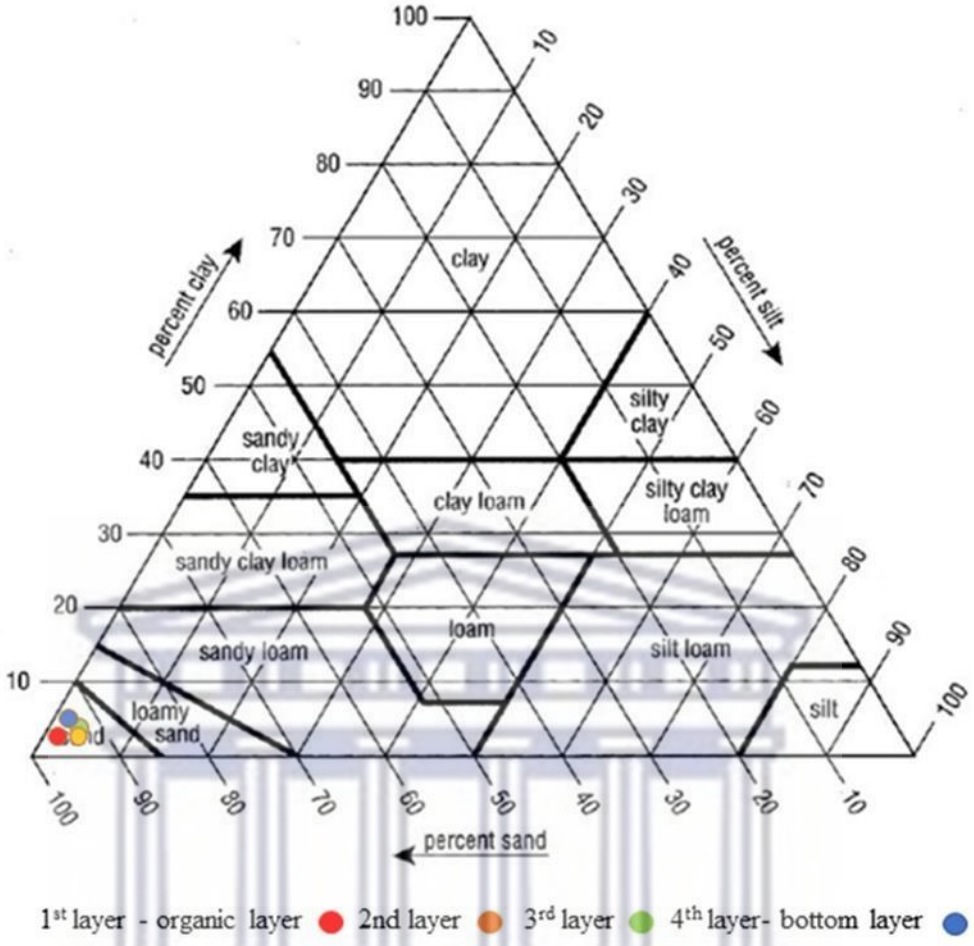


Figure 5.2 Soil texture at different depths

5.3 The Removal efficiency of selected pharmaceuticals by bioretention columns with common soil media; two planted columns and a soil control

Soil Removal Efficiency

The soil type and properties of the chemical compound determine whether the pharmaceuti cals will be eradicated from the stormwater. The 5 L soil treatment had varying removal efficiencies during the first effluent with Carbamazepine and Diclofenac being the less removed with percentages of 70.7 % and 73.98 % respectively, refer to Fig 5.3.1. Gworek *et al.*, (2021) showed that carbamazepine in the soil tends to have poor sorption and high persistence which may lead to lesser removal efficiency by a soil medium. The percentages of the removal efficiency of these compounds by the bioretention columns increased with the increase of inflows and retention time, this is evident in figures 5.3.1, 5.3.2 and 5.3.3. The fluctuating removal efficiencies stabilised during the second and third 5 L effluent (figure 5.3.1) with

removal percentages ranging between 96% for diclofenac and 99% to 100% for the rest of the drugs (see figure 5.3.1), while carbamazepine had removal of 84.1 % during the second 10L inflow/ 12hr retention time (figure 5.3.2). The first effluent of the 10 L inflow (figure 5.3.2) had a 100% removal efficiency for all the drugs except diclofenac which had a removal percentage of 97. During the second 10L inflow (figure 5.3.2) sulfamethoxazole had a negative removal of 405.69% as shown in figure 5.3.2b, diclofenac and progesterone had 94.58% and 97% respectively, and the rest of the drugs had 100% removal efficiency (figure 5.3.2). The removal efficiency was more constant and stable during the 15 L inflows (figure 5.3.3a) with sulfamethoxazole being the least removed and showing negative removal efficiency of 23.52% and 77.47% during the second and third 15 L effluent respectively (figure 5.3.3). However, this was an improvement from the -405.69 that was previously detected in figure 5.3.2. The negative removal percentage improved by 94.2% during the second 15 L inflow/ 12hr retention time and by 80% during the 3rd 15L inflow/24hr rt (figure 5.3.3). Literature has demonstrated that Sulfamethoxazole is a persistent organic compound with negligible sorption properties which increase the mobility of this compound in the soil, hence, it is poorly treated by the soil column, this is also evident in this study. Grossberger et al. (2014) showed that non-ionic drugs, such as carbamazepine, lamotrigine, sulfamethoxazole, and sildenafil, were resistant to breakdown and accumulated in the soil after being irrigated with treated wastewater containing residues of the drugs. The resistance of sulfamethoxazole to breakdown has also been demonstrated in this study with an average of -135% of negative removal efficiency for the soil column. The average removal efficiency for this column was 78.3%.

Turf Grass Removal Efficiency

Turf grass had the highest average removal efficiency among the columns, with the highest removal of Carbamazepine at 96.2% and Sulfamethoxazole at 60.8%. The average removal efficiency for Caffeine was 95.3%, Naproxen 97.5%, Progesterone 97.1% and 86.1% for Diclofenac. However, the 5L influent (figure 5.3.1) had more unstable and fluctuating removal efficiencies compared to the other two treatment columns. Nonetheless, turf grass had the smallest negative removal of 4.14% for sulfamethoxazole (figure 5.3.1). The 10L influent had an improved stable removal efficiency (figure 5.3.2) for all the drugs including sulfamethoxazole. More 100% removal efficiencies were observed with a drastic decrease of 47% for sulfamethoxazole during the 24hr retention time. The removal efficiency was constant during the 15L inflows (figure 5.3.3) with a steady removal increase for sulfamethoxazole. Removal efficiency increased with retention time from 47%, 60.8 % and 66.6% during the 24hr

retention time. The average removal efficiency for this column was 91.2%. This shows that Turf grass has a very promising potential for being one of the plants that should be used in bioretention systems for the treatment of pharmaceuticals.

Pennisetum Removal Efficiency

Pennisetum had the least overall removal percentage of 67.4% with the highest removal of Caffeine at 99.1%, Naproxen at 99.4% and Progesterone at 99.6% compared to the other columns. Carbamazepine and Sulfamethoxazole were not significantly removed by Pennisetum, this is shown by the significant negative removal efficiency of -710% and -168.8% respectively (fig 5.3.1 and fig 5.3.3). Negative removal efficiency for carbamazepine was also detected in a study by Ejhed et al., (2018). The high negative removal efficiency for carbamazepine that was observed in this column was for the very first inflow of the experiment which was also the lowest inflow volume. A study by Chehrenegar et al. (2016) showed that there was no significant difference in removal rate that was observed for carbamazepine between planted autoclaved columns and unplanted columns, however, differences in removal rate were observed when the flow rate was reduced from 20mL h^{-1} to 4mL h^{-1}. These results suggest that the parent compound may have metabolised. Studies also show that concentrations of some pharmaceuticals get increased in the effluent than the concentrations in the influent, leading to negative removal efficiency of the treatment plants (Kumar et al., 2020). Negative removal efficiency is a common problem that is encountered in most water treatment plants, irrespective of the pollutants, amount of water, plant capacity, or region. There is always a chance that even the most efficient pollutant separation technique will have a negative removal efficiency for more than one pollutant (M. Kumar *et al.*, 2022b). Negative removal efficiency is a phenomenon in which the influent concentration of a contaminant is lower than the effluent concentration detected. This can be due to metabolised pharmaceuticals that may be present in the influent and the deconjugation of these metabolites in the treatment process which leads to higher measurable concentrations of the pharmaceuticals in the effluent than in the influent. This phenomenon is also seen in WWTPs, where there are higher concentrations of these pharmaceuticals detected in effluent than in the influent and this is mostly due to a change in the adsorption behaviour of the compounds to particles during treatment processes, which may influence their ratio in influent or effluent (Lindberg et al., 2005). For example, carbamazepine has mostly been reported with poor removal efficiency and is described as a recalcitrant compound which leads to negative removal efficiency in most cases. The low removal

efficiency can also be explained by the persistence in water and the water-soluble nature of the compound (M. Kumar *et al.*, 2022b). The removal efficiency results of this study were in correspondence and even had higher removal than some studies done on the removal of pharmaceuticals by plants. The results of the study done by Matamoros *et al.*, (2012) showed the removal of some pharmaceuticals using four types of aquatic plants during 38 days of incubation in planted reactors. They reported the removal of diclofenac, naproxen, and caffeine in a range of 99%, (40-53%) and (81-99%) respectively.

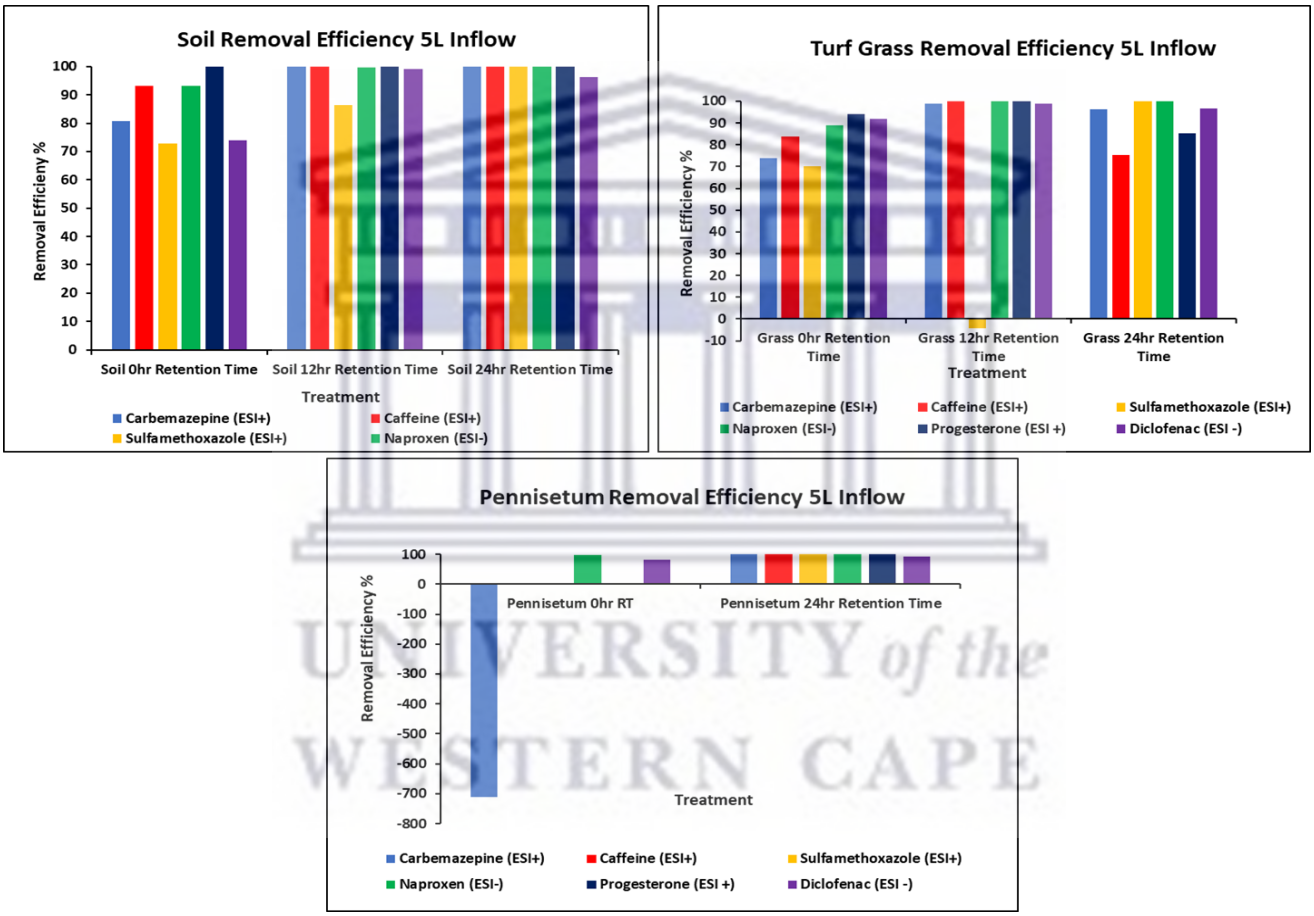
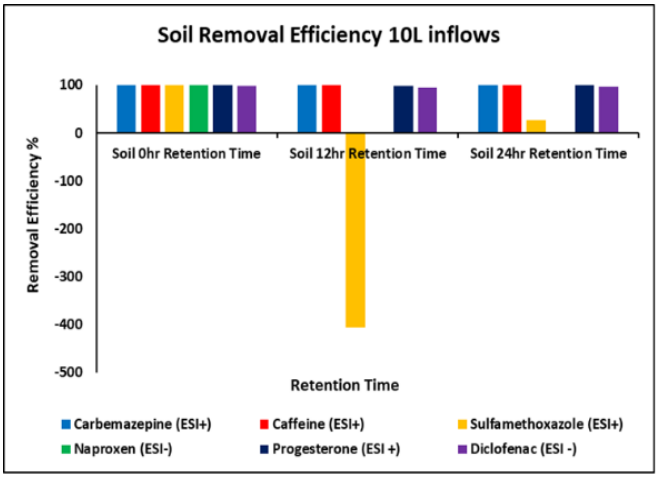
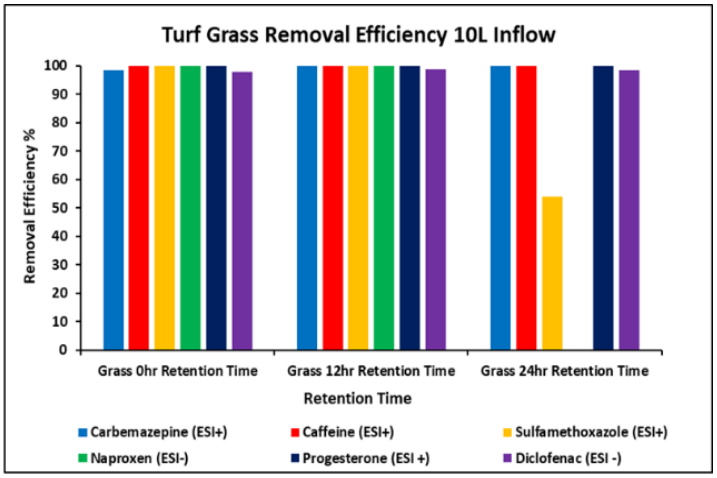


Figure 5.3.1: Removal efficiency of pharmaceuticals by soil and two planted lab-based bioretention columns with three different retention times (5L influent: 0hr,12hr and 24hrs RT).





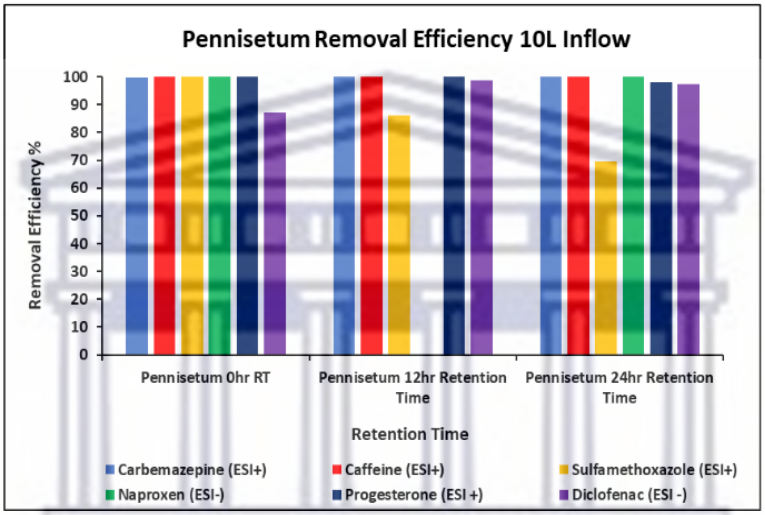


Figure 5.3.2: Removal efficiency of pharmaceuticals by a lab-based soil and two planted bioretention columns under 3 different retention times (10 L influent: 0hr,12hr and 24hrs RT).

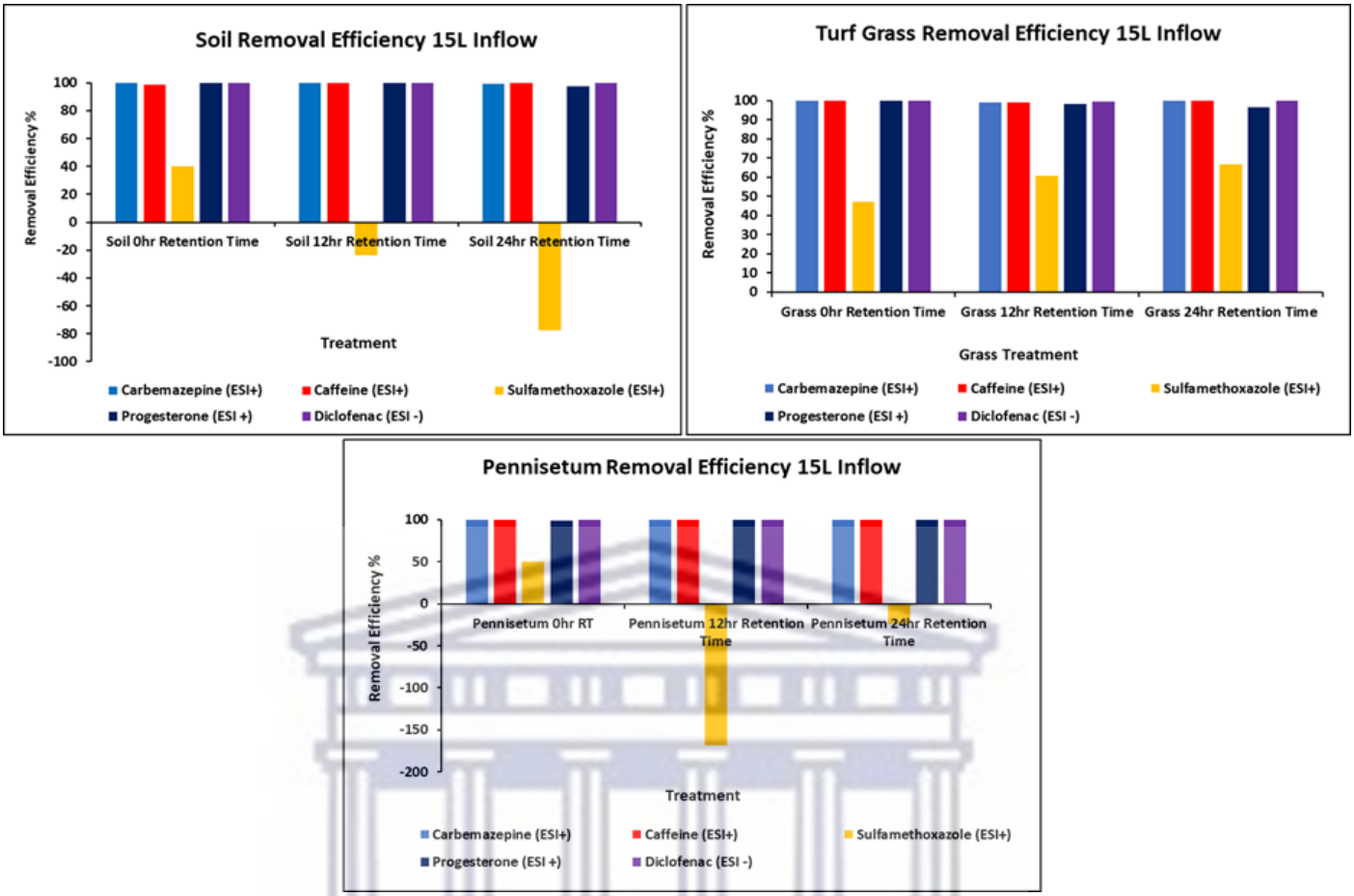


Figure 5.3.3: Removal efficiency of selected pharmaceuticals by soil and two planted lab-based bioretention Systems under 3 retention times (15L Influent 0hr,12hr and 24hr RT).

5.4 The influence of retention time and stormwater volume on removal efficiency of pharmaceuticals by the different bioretention columns

Hydraulic retention time and varying inflow volumes were used to control the flow and the quantity of water during the experiment. Retention and inflow rate are two variables that are known to affect the efficiency of treatment systems. These two variables were therefore included to test how they would affect the removal efficiency of the bioretention columns, with the assumption that longer retention time will increase removal.

Retention time had a significant impact on the removal efficiency of the columns under the 10L inflow (figure. 5.4.2), with the highest removal concentrations being observed in the Turf grass column and the lowest removal in the Pennisetum column. During the 0-hour retention time of the 5L inflow volume caffeine, carbamazepine, and naproxen were not removed efficiently. The most significant removal was observed during the 12-hr retention period. In the 10L inflow

(Figure. 5.4.2), most removals were recorded in the 0-hour retention samples, particularly under the soil column treatments. Progesterone was the least removed drug in both the 0- and 24-hour retention periods. This could be due to the high initial concentrations of progesterone which might have influenced the removal efficiency. The 12-hr RT had the least significant impact on the removal efficiency during the 15L inflow volumes (figure. 5.4.3). Progesterone and caffeine were again the least removed for all three treatments (figure 5.4.3). During the 15L inflow volumes, 0-hr RT had more removal efficiency compared to the 12 and 24hr retention times. Similar results were also observed in a study by El-Bestawy et al., 2005 where greater removal efficiencies were seen with higher volumes and shorter hydraulic retention time. The 10L inflow volume had a very significant impact on the removal efficiency of the pharmaceuticals for comparatively all three different retention times.

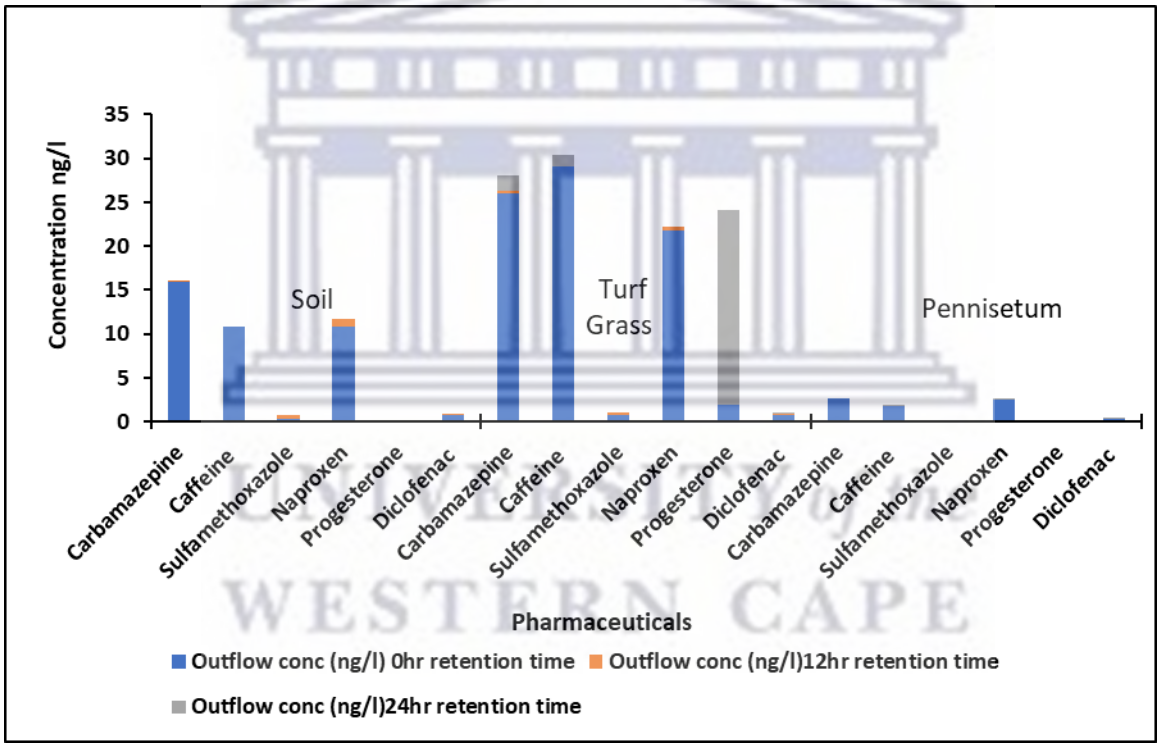


Figure 5.4.1: Effluent concentrations of the selected pharmaceuticals per retention time for the 5L inflows

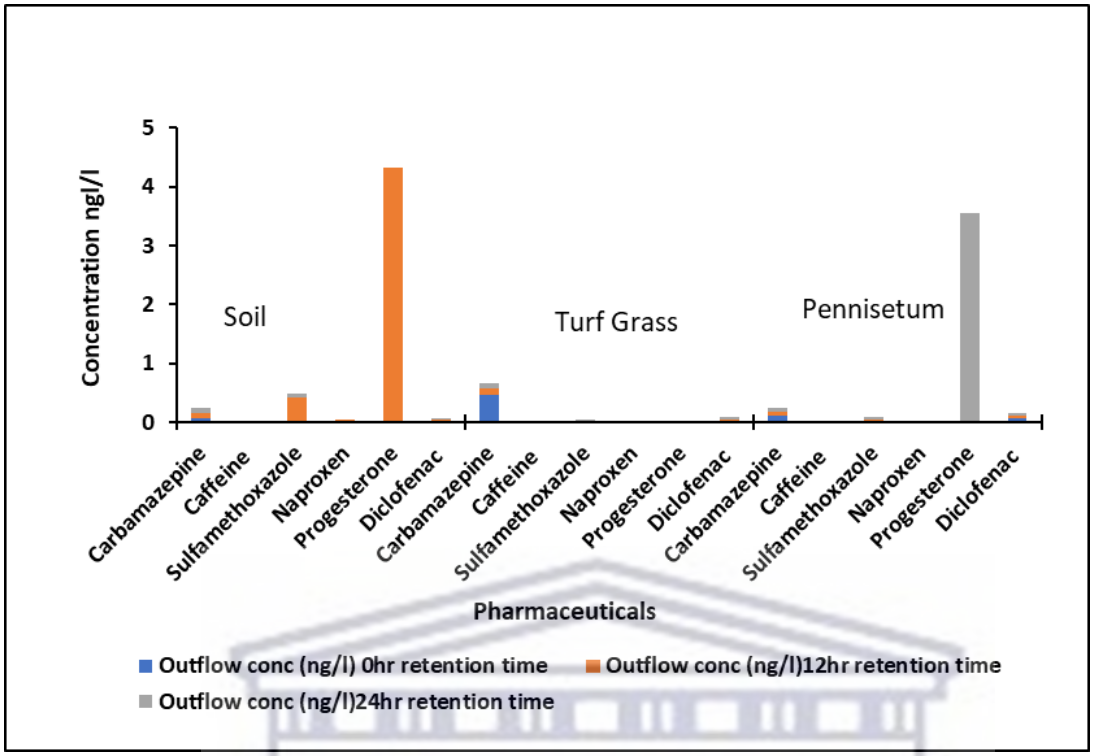


Figure 5.4.2: Effluent concentrations of pharmaceuticals per different retention times for the 10L inflow test.

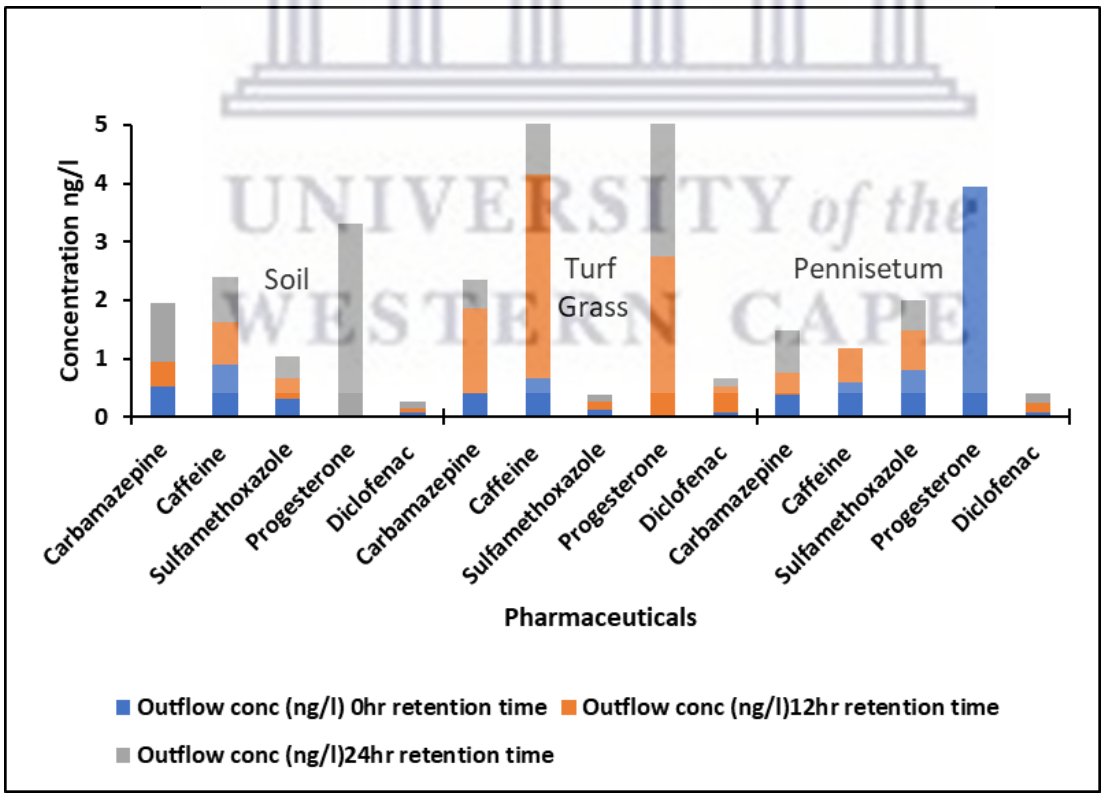


Figure 5.4.3: Effluent concentrations of pharmaceuticals per different retention times for the 15L inflow test.

Table 5.1: Presents the P-values obtained from the T-test statistical analysis for the significance of the difference between the unplanted and the planted columns.

Treatment/ Volume	Pharmaceuticals	P-Values (5 L concentrations)	10 L P-Values (10 L concentrations)	15 L P-Values (15 concentrations)
Soil	Carbamazepine	0.061	-	-
	Caffeine	0.205	-	0.118
	Sulfamethoxazole	0.153	0.554	0.955
	Naproxen	0.215	0.459	
	Progesterone	0.264	-	-
	Diclofenac	0.351	-	-
grass	Carbamazepine	0.062	-	-
	Caffeine	0.205	-	-
	Sulfamethoxazole	0.153	0.088	-
	Naproxen	0.215	0.423	
	Progesterone	0.264	-	-
	Diclofenac	0.264	0.119	0.064
Pennisetum	Carbamazepine	0.54	-	0.107
	Caffeine	0.700	-	0.128
	Sulfamethoxazole	0.500	0.067	0.823
	Naproxen	0.497	0.358	
	Progesterone	0.500	-	0.173
	Diclofenac	0.355	0.177	0.070

*Pharmaceutical concentrations with P-values < 0.05 are represented in – and blank spaces represent the missing data (naproxen analyte powder was finished)

P < 0.05 = Significant difference between inflow and outflow concentrations

P > 0.05 = No significant difference between inflow and outflow concentrations

The 5L volume showed P-values > 0.05 for all the different treatment columns, which means that there was no significant statistical difference between the outflow and inflow concentrations, which means the removal efficiency was poor for this inflow. The inflow stock solution concentrations for the 5L test were very low as the concentrations were calculated relative to the water volume. The low pharmaceutical concentrations and small effluent volume could have possibly led to the detected concentrations not being significantly different. The 10L and 15 L inflow volumes showed more P-values < 0.05 which proves that there was a significant statistical difference between the outflow and inflow concentrations of the selected pharmaceuticals for these volumes. This could be attributed to the increase in water volumes and the pharmaceutical concentrations were two times more in the 10 L and three times more

in the 15 L compared to the 5 L volume, as a result, the pharmaceuticals could be detected by the instrument of analysis (LC-MS). P-values also show that the Turf-grass treatment had more removal efficiency than the unplanted and Pennisetum column. This could be due to the physical properties of the Turf-grass, smaller roots and more compacted leaves that cover the whole surface of the treatment column.

5.5 The effectiveness of the Turf Grass, Pennisetum plant and unplanted soil media in the bioretention system for removing pharmaceuticals in stormwater.

The Turf grass treatment had a higher overall removal percentage compared to the Pennisetum and the unplanted soil bioretention system, with the highest removal of 97.9% for diclofenac compared to 95.3 % and 95.4 % for Pennisetum and Soil respectively. Turf grass also had a higher average removal for Sulfamethoxazole which was 66.0 % compared to 20.5% for Pennisetum and -8.9 % for unplanted soil treatment. Pennisetum had the highest removal for Caffeine with a removal average of 99.8 % compared to 99 % for Soil and 95.3 % for Turf grass. Naproxen was also well removed by the system with an average removal efficiency of 99.4 % for Pennisetum, followed by 98.2 % for the unplanted soil media and 97.8 % for the Turf grass bioretention system. The unplanted soil media had a higher average removal efficiency for Carbamazepine which was 97.6 %, Turf grass had the second highest average at 96.2 % and Pennisetum had the least removal efficiency average which was 9.7 %, Pennisetum had the most negative removal efficiency for carbamazepine. Progesterone was significantly well removed by the bioretention systems with the Soil media having the highest average of 99.5 % and 99.4 % for Pennisetum and 97.1% for Turf grass.

The comparison of the removal efficiency of the selected pharmaceuticals by the different plants shows that different plants may remove different pharmaceuticals at varying removal rates. However, it is important to note that, apart from the effect of the plant type on the removal process, the initial concentration may also affect the removal efficiency. Turf grass showed relatively good removal efficiency for all the drugs and had the smallest negative removal percentage for sulfamethoxazole which was -4.1 %. Soil media had the second highest removal efficiency with the highest removal of carbamazepine and progesterone. Pennisetum had the least average removal efficiency among the columns, with the highest removal of Carbamazepine at 96.2% and Sulfamethoxazole at 60.8%.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

Conclusion

The study investigated the presence and treatment of pharmaceuticals in stormwater. The presence of pharmaceuticals was confirmed at all sampled sites, except during Winter sampling, where only a limited number was present. The results of the study demonstrated that bioretention systems containing different media such as soil and plants have the potential to provide a combined effect to enhance the removal of pharmaceuticals for stormwater reuse. This study showed that retention time plays a significant role in the treatment of pharmaceuticals when using a bioretention system. Considering the volumes used in the experiment, the 5 L volume was not an ideal volume to simulate a storm and to receive a significant outflow, whereas the 10 L volume saturated the system and yielded optimal results under the 12 and 24hr retention time. The 15L volume flooded the system which impacted the removal efficiency of the bioretention system. Moreover, it also confirmed that the selected vegetation has a great potential of performing well in a bioretention system for the treatment of pharmaceuticals in stormwater combined with other plants that may enhance the treatment of the least removed pharmaceuticals. There was no significant difference between the removal efficiency of the planted and the unplanted bioretention treatment media, which shows that a soil profile plays a big role in the absorption and degradation of the pharmaceuticals, however, the accumulation of the pharmaceuticals in the soil profile needs to be tested in the long run and how it contributes to the negative removal of some of the pharmaceuticals. The hypothesis of the study was proven to be correct for the treatment of most of the selected pharmaceuticals with only two out of the six selected pharmaceuticals not being eliminated from the synthetic stormwater and showing negative removal. Considering the results of the study it is suggested that the use of the bioretention approach is a promising technology in stormwater remediation and it needs upscaling in various areas. Bioretention systems can be implemented post a stormwater treatment plant to further treat the remaining pharmaceuticals and store the stormwater as groundwater.

Recommendations

The removal of pharmaceuticals by a bioretention system is influenced by a lot of factors such as treatment processes in the soil, physical properties of the plants and the physiochemical

properties of the pharmaceuticals which were not investigated in detail in this study. It is therefore recommended that future studies investigate how the properties of the plant influence the removal efficiency of the pharmaceuticals and test many other plants. This study was restricted to a lab-based experiment, it is recommended that an analysis of a pilot study be done to help with the understanding of the removal efficiency in the real world.

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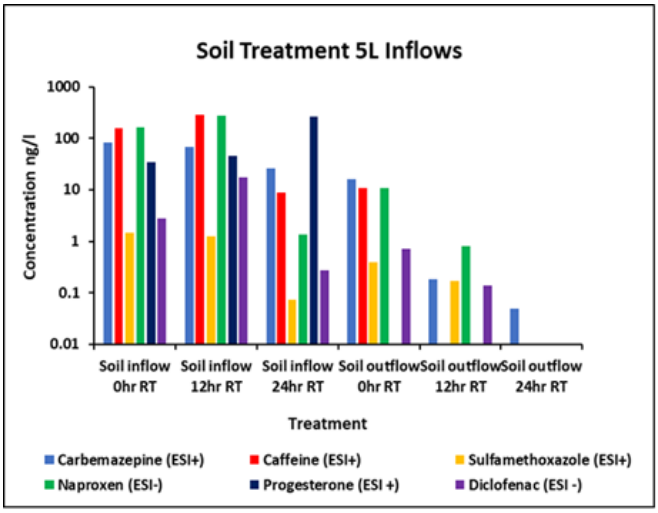
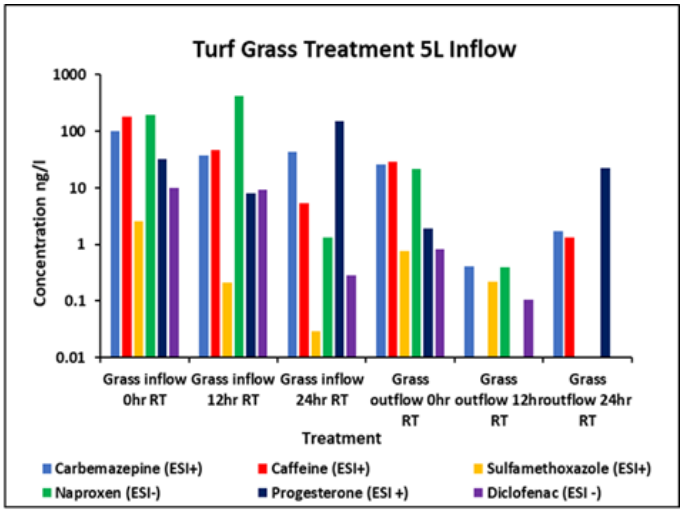
APPENDICES

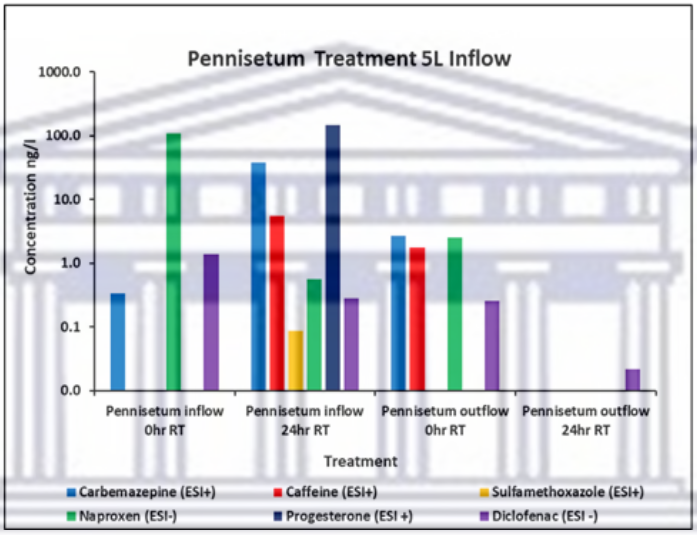
APPENDIX A

Table 1: Removal efficiency of the selected pharmaceuticals by lab-based bioretention system

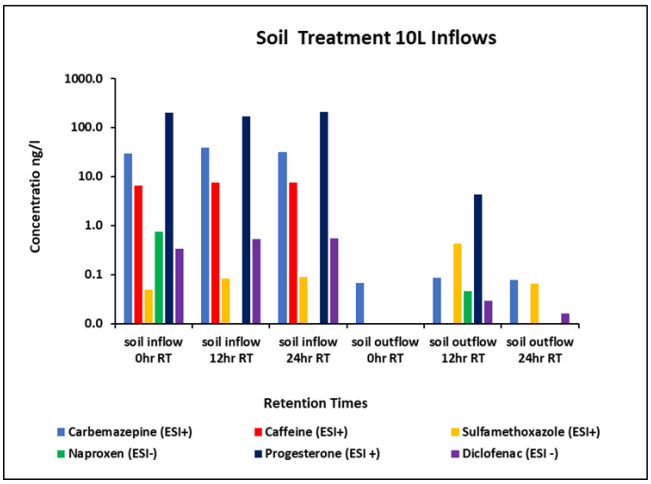
Treatment/retention time	Carbemazepine (ESI+)	Caffeine (ESI+)	Sulfamethoxazole (ESI+)	Naproxen (ESI-)	Progesterone (ESI +)	Diclofenac (ESI -)
5L Soil 0hr	80.7	93.2	72.7	93.2	100.0	74.0
10L Soil 0hr	99.8	100.0	100.0	100.0	100.0	98.0
15L Soil 0hr	99.8	98.7	40.3	BLQ	100.0	99.9
5 L Soil 12hr	99.8	100.0	86.4	99.7	100.0	99.2
10L Soil 12hr	99.8	100.0	-405.7	BLQ	97.4	94.6
15L Soil 12hr	99.8	99.8	-23.5	BLQ	100.0	99.9
5L Soil 24hr	99.8	100.0	100.0	100.0	100.0	96.4
10L Soil 24hr	99.8	100.0	27.2	BLQ	100.0	97.1
15L Soil 24hr	99.5	99.8	-77.5	BLQ	97.7	99.9
Treatment/retention time	**Carbemazepine (ESI+)**	**Caffeine (ESI+)**	**Sulfamethoxazole (ESI+)**	**Naproxen (ESI-)**	**Progesterone (ESI +)**	**Diclofenac (ESI -)**
5L Grass 0hr	73.9	83.7	70.1	89.0	94.0	91.8
10L Grass 0hr	98.5	100.0	100.0	100.0	100.0	97.7
15L Grass 0hr	99.8	99.8	47.1	BLQ	100.0	99.7
5L Grass 12hr	98.9	100.0	-4.1	99.9	100.0	98.8
10L Grass 12hr	99.7	100.0	100.0	100.0	100.0	98.7
15L Grass 12hr	99.2	99.0	60.8	BLQ	98.2	99.4
5L Grass 24hr	96.1	75.1	100.0	100.0	85.1	96.5
10L Grass 24hr	99.8	100.0	53.9	BLQ	100.0	98.3
15L Grass 24hr	99.7	99.7	66.6	BLQ	96.4	99.8
Treatment/retention time	**Carbemazepine (ESI+)**	**Caffeine (ESI+)**	**Sulfamethoxazole (ESI+)**	**Naproxen (ESI-)**	**Progesterone (ESI +)**	**Diclofenac (ESI -)**
5L Pennisetum 0hr RT	-710.4	BLQ	BLQ	97.7	BLQ	81.8
10L Pennisetum 0hr RT	99.7	100.0	100.0	100.0	100.0	87.2
15 Pennisetum 0hr RT	99.9	99.9	49.9	BLQ	99.3	100.0
Soil 12hr Retention Time	99.8	99.8	-23.5	BLQ	100.0	99.9
Grass 12hr Retention Time	99.2	99.0	60.8	BLQ	98.2	99.4
Pennisetum 12hr Retention T	99.8	99.8	-168.8	BLQ	100.0	99.8
5L Pennisetum 24hr	100.0	100.0	100.0	100.0	100.0	92.4
10L Pennisetum 24hr	99.9	100.0	69.6	100.0	97.9	97.2
15L Pennisetum 24hr	99.6	100.0	-24.2	BLQ	100.0	99.8

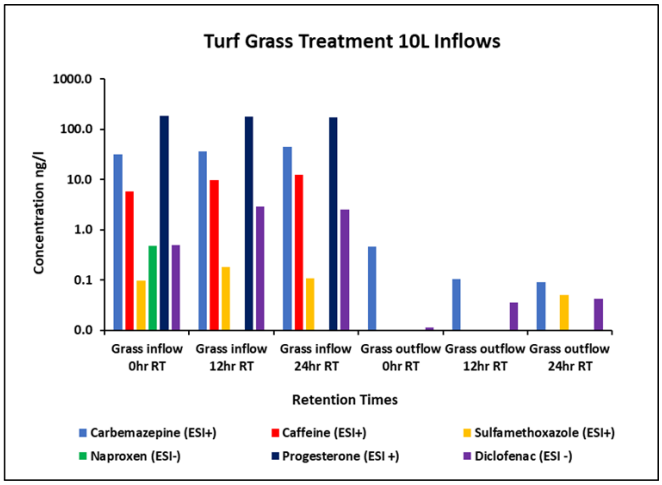


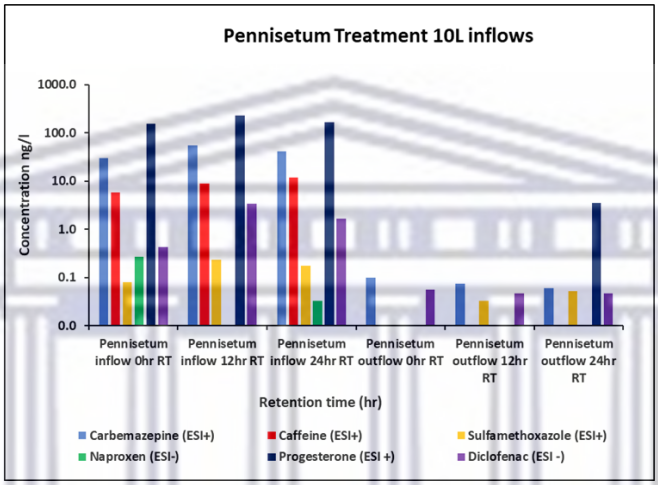




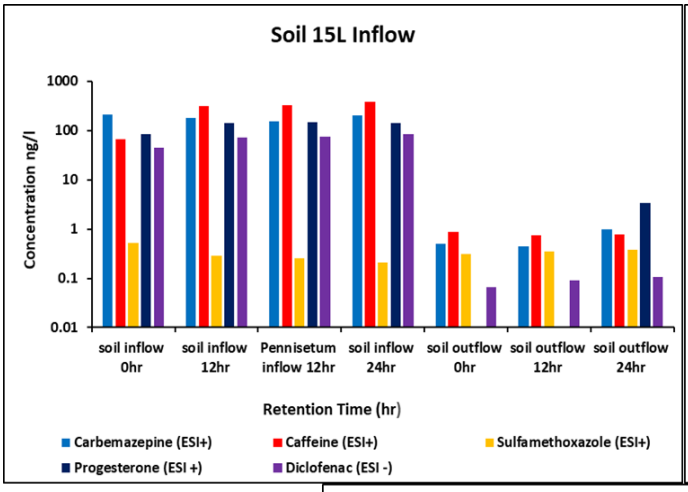
Appendix B: Influent and effluent concentrations of the 5L synthetic stormwater of the selected pharmaceuticals treated by a soil and two planted lab-based bioretention columns.

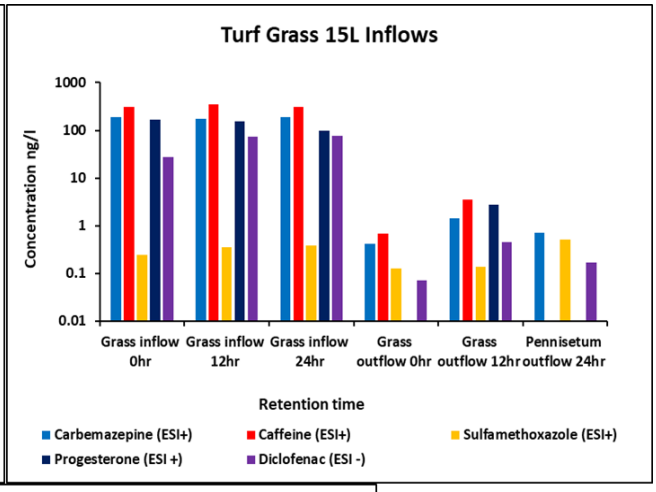


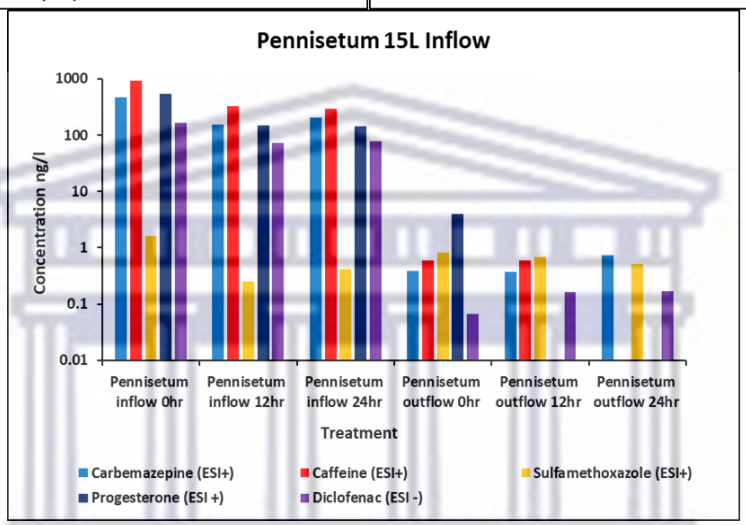




Appendix C: Influent and effluent concentrations of the 10 L synthetic stormwater of the selected pharmaceuticals treated by a lab-based soil and two planted bioretention column.







Appendix D: Influent and effluent concentrations of the 15 L synthetic stormwater of the selected pharmaceuticals treated by a lab-based soil and two planted bioretention column under 3 different retention times (0hr,12hr and 24hrs).