# THE PRESENCE AND CONCENTRATION OF ANTIRETROVIRAL DRUGS IN DIFFERENT WATER BODIES AROUND THE WESTERN CAPE.


UNIVERSITY *of the*
WESTERN CAPE

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**By**

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**A thesis submitted in the fulfillment of the requirement for the degree of magister scientiae**

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# Table of Contents

## Declaration

I declare that *"the presence and concentration of antiretroviral drugs in different water bodies around the Western Cape"* 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 reference.

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**Date: January 2023**

## List of Tables



## List of Figures

## Abbreviations

HIV- Human immunodeficiency virus

AIDS- Acquired immunodeficiency syndrome

ARV- Aids related virus

ART-Antiretroviral treatment program

ARVD-Antiretroviral Drugs

NNRTI-Non-nucleoside reverse transcriptase inhibitors

NRTI-Nucleoside reverse transcriptase inhibitors

ADME-Absorption, Distribution, Metabolism and Excretion

WHO- World Health Organization

WWTP-Wastewater treatment plant

WTP-Water treatment plant

CAS-Activated sludge

MBr- Membrane bioreactors

DO-Conventional treatment

PPCPs-Pharmaceuticals and personal care products

PKa-Acid dissociation constant

SPE-Solid Phase Extraction

LC- Liquid chromatography

MS-Mass Spectrometry

LC-MS/MS-Tandem Chromatography

GC-MS- Gas Chromatography

## Abstract

Concerns regarding the prevalence of chemicals from medicines and personal care goods, such as cosmetics, in streams and rivers have grown in recent years. Water quality experts and environmentalists are becoming increasingly worried about contaminants found in prescription and over-the-counter pharmaceuticals that enter various water systems. Pharmaceuticals are significant contributors to water pollution in aquatic habitats such as surface and groundwater. These contaminants are produced not only by waste materials, but also by improperly disposed of pharmaceutical items. South Africa has the world's highest rate of HIV (Human immunodeficiency virus infection) and AIDS (Acquired immune deficiency syndrome), with 8.2 million South Africans infected with HIV/AIDS, and 68% are receiving antiretroviral therapy. These medications get deposited in the environment and might reach water bodies, contaminating them.

The purpose of this study is to determine the presence, distribution, and concentration of selected antiretroviral drugs in different water types in South Africa's Province of Western Cape. Samples will be collected from Mitchell's Plain, Bellville, Potsdam, Zeekoevlei, Athlone, Cape Flats, and Atlantis for this investigation. Wastewater treatment plants, water treatment plants, storm water, a vlei, rivers, and landfill boreholes are examples of sites. Wastewater, surface water, and groundwater samples (n=33) were collected on a seasonal basis during the summer (January 2021-February 2021), autumn (April 2021-May 2021), winter (June 2021-July 2021), and spring (August 2021-September 2021) for analysis to determine the presence of five antiretroviral drugs used in South Africa, which include Efavirenz, Lopinavir, Nevirapine, Ritonavir, and Tenofovir. Solid phase extraction (SPE) was used for the isolation and extraction of each sample, and liquid chromatography coupled with mass spectrometry was utilized to determine the quantities of each medication.

The results from the summer samples showed the presence of all the drugs that were analyzed, with Efavirenz being found in the least sample areas and Lopinavir being found in most of the samples. During summer concentrations of these drugs ranged from low concentrations of 0.0855 ng/ml (Nevirapine) to higher concentrations of 4.4389 ng/ml (Tenofovir). In autumn fewer sites had the presence of these drugs, with Ritonavir, Tenofovir, and Efavirenz not being

detected in any of the samples. During autumn concentrations of Nevirapine and Lopinavir ranged from low concentrations of 0.131ng/ml (Nevirapine) to higher concentrations of 1.41ng/ml (Lopinavir). In Winter 4 the drugs were detected, the only one that was not detected in any of the samples was Tenofovir and this is due to treatment methods used in the different plants being effective in removing this particular drug at this period and weather conditions. In spring 3 of the drugs were detected with Efavirenz and Tenofovir not being detected in any of the samples. In winter concentrations of the drugs detected ranged from low concentrations of 0.0241ng/ml (Ritonavir) to higher concentrations of 1.38ng/ml (Lopinavir).

These variations of concentrations in different seasons of the year are attributed to different factors such as temperature, rainfall, pH, and the number of effluents coming in and out of the different wastewater treatment plants. ARVs released into the aquatic environment via wastewater may cause environmental contamination and risks due to bioaccumulation and non-target exposures if they are not biodegraded or eliminated in WWTPs. This study concludes that there are different sources of emission that contribute to the presence of ARVs in different water bodies, these drugs are transported in different pathways and end up in water bodies and the environment.

Future recommendations include the amount of HIV-ARVs utilized in South Africa should be continually monitored and, if feasible, measured. Analyze the waters downstream of WWTPs to see if HIV-ARVs are transported far enough to possibly infect downstream towns' drinking water treatment facilities. Conduct investigations into the origins of HIV-ARVs in groundwater, plume investigations of the substances may point to possible origins. Existing extraction and analytical processes may be enhanced, which will aid in eventual relative risk judgments as well as provide information on better wastewater treatments, drinking water preparation, groundwater protection, and conservation.

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# Chapter 1 General information

## 1.1 Introduction

Water resources around the world are threatened by a wide variety of manmade chemicals, and South Africa is no exception. These contaminants can harm both the environment and the health of wildlife. The primary sources of freshwater pollutants are typically industry, domestic practices, and/or agriculture (Genthe et al., 2013). These practices introduce non-biodegradable and/or hazardous chemicals into water systems, putting both wildlife and humans at risk. Although the impact of water pollutants has primarily been linked to agricultural pesticides and industrial effluents, other pollutants such as pharmaceuticals and personal care products (PPCPs) go unnoticed but pose a potentially significant threat. The presence of pharmaceuticals in aquatic environments has sparked concerns about the possibility of unintended consequences, such as killing of aquatic species and contamination of water that is used for human consumption.

In South Africa, the primary treatment for Human immunodeficiency virus and Acquired immunodeficiency syndrome (HIV/AIDS) is antiretroviral therapy (ART). The goal of antiretroviral drugs (ARVs) is to keep the number of HIV viruses in the body low in order to prevent immune system weakness. It also helps the immune system recover somewhat if it has already been compromised by HIV, as it can quickly become resistant to one drug. Thus, combination therapy is becoming more popular in countries with high HIV prevalence (currently 29% in SA,(www.aids.org)). This entails a patient taking two or three antiretroviral medications concurrently. ARVs are also given alongside TB (Tuberculosis) treatment in developing countries such as South Africa. There are currently over 20 approved antiretroviral drugs, but not all of them are available in all countries. In South Africa, the most commonly used ARV drugs are: Lamivudine, Stavudine, Efavirenz, Nevirapine, Didanosine, Zidovudine, Lopinavir; Ritonavir, Abacavir, Tenofovir, Saquinavir, and Nelfinavir; (all generic names). These drugs are then used in a variety of combinations for either first-line (the combination of drugs given at the start of treatment) or second-line defense (a new combination of drugs given to a person when HIV has become resistant to the drugs first given).

South Africa is now the world's top user of HIV antiretroviral drugs (ARVs). Nevirapine (NVP), a first-line ARV, has been linked to significant liver damage in humans and has been found in South African surface water on several occasions due to its resistance to biodegradation (Nibamureke, 2019). Due to South Africa's high HIV prevalence and largescale manufacturing, HIV-ARV drugs have most certainly made their way into the aquatic ecosystem through prescription, administration, and usage.

With a global lack of information for the effective detection and removal of ARVs from wastewater, as well as the non-assessment of eco-toxicological concerns connected with ARV drugs in the environment, there is an urgent need to examine the existence, concentrations, removal, and detection methods of HIV-ARVs in water, as well as to identify drug sources, amounts released into wastewater, and their potential impacts on the environment and animal health. The presence of HIV-ARVs in water represents a hidden or latent concern. These dangers have not previously been identified or have existed for some time but are just now being investigated. Latent risk chemicals are expected to be in the environment or are unassessed compounds of historically low concern, but which may become a problem in the future (Daughton, Christian. (2005).

The excretion of HIV-ARVs varies depending on the molecule, however some, like Tipranivir, are removed in urine at 80% and Nevirapine at 2.7%. Assuming a 30% excretion rate to sewage via urine and faeces, its estimate that around 159 000 kg of HIV-ARV (www.uniaids.com, 2020) might reach South African aquatic systems each year. Another difficulty with increasing ARV use is that the country's prevalence rate will rise as more individuals live owing to treatment, while new infections increase the number of infected persons (WHO, 2020).



## 1.2 Background

In South Africa, the primary treatment for HIV/AIDS is antiretroviral therapy (ART). ARV (antiretroviral) drugs try to maintain the number of HIV viruses in the body low in order to prevent immune system weakness. It also aids in the recovery of the immune system if it has previously been damaged by HIV. Antiretroviral therapy is critical in South Africa, and with 4.5 million (UNAIDS, 2020) individuals on treatment, it raises the question: what will happen

if these ARV medications make their way into our environment and waterways? According to the literature, the presence of ARV drugs may cause difficulties for aquatic creatures, raising concerns and emphasizing the importance of removing them from wastewater and drinking water and ARV drugs are classified as emerging pollutants since they fall under the category of Pharmaceutical and Personal Care Products (PPCPs). When certain antiviral medicines are taken, they undergo biotransformation, whilst others are excreted unaltered (Galasso et al., 2002).

As a result, there is an urgent need to eliminate ARV drugs from water since some have non-biodegradable metabolites (Mascolo et al., 2010, Prasse et al., 2010). Due to their toxicity, they have also been designated as a very hazardous therapeutic class (Sanderson et al., 2004). Many drugs are only partially eliminated during water purification (Prasse et al., 2010), and so reach waterways via WWTP (wastewater treatment plant) discharge; certain ARVs enter the WWTP in an inactive state, but due to the water treatment process, may enter the environment in an active form (Kummerer, 2008). This study shed more light on the concentration of these drugs in the ecosystem and possibly give solutions on how this can be prevented in the future. The world and millions of South Africans are on antiviral drugs, these drugs are deposited into the environment daily and there is no monitoring system put into place to determine their presence in the water. Due to the lack of proper sanitation around townships and rural areas these drugs are deposited into the water via faeces and urine, these drugs are not included as parameters of removal during the treatment process. This results in the possibility of the presence of these drugs in different water bodies, this project will highlight the distribution and concentrations of ARVs in water bodies around the Western Cape Province in South Africa and the effect they can have on the environment.



## 1.4 Research question and thesis statement

### 1.4.1 Research question

What are the contamination levels and possible implications of antiretroviral drugs in water bodies, and are there site-specific conditions that influence these changes in water sources?

### 1.4.2 Thesis statement

The central argument is that pharmaceuticals such as antiretroviral drugs make their way into aquatic ecosystems and the environment from different sources. Wastewater and water treatments are not effective in removing these drugs from their water, therefore, returning them to the environment.

## 1.5 Research aims and objectives

### 1.5.1 Research aim

This study is aimed to establish the distribution and concentration levels of antiretroviral drugs in water bodies around the Western Cape Province in South Africa.

### 1.5.2 Objectives

1. To identify and prioritize antiretroviral drugs in South Africa.
2. To identify the sources of emission of antiretroviral drugs into the different water types.
3. Sampling and analysis of identified sources to determine the distribution and concentration of antiretroviral drugs in water types.

## 1.6 Significant of the study

South Africa has the highest HIV/AIDS epidemic in the world with over 8.2 million people living with HIV/AIDS and over 4.5 million of those people taking antiretroviral therapy. Millions of these drugs are distributed to people all over the country and not enough research is being done on the possibility of them reaching and contaminating the environment via different sources. Understanding how these drugs react and behave in different ecosystems is essential for the protection of our resources, so this study is significant in determining the presence, concentration, and distribution of these drugs in the environment. Little research is being done on antiretroviral drugs in the environment and there is a gap that needs to be filled to understand the impact these pollutants can have on the environment and aquatic ecosystems. There are numerous sources of emissions that cause these drugs to be discharged or end up in the environment this is one of the many aspects of these pollutants that needs to be investigated. This study will provide the necessary insight into the distribution and concentration of these drugs in water systems.

## 1.7 Research framework

The study is aimed to determine the presence, concentration, and distribution of antiretroviral drugs in different water types around the Western Cape. To achieve the main aim of the study three objectives were considered. The first objective focused on identifying and prioritizing antiretroviral drugs in South Africa. The study considered the history and distribution timeline of ARVs in South Africa beginning from when there were introduced to the different therapies that were available in the beginning to the changes that have occurred over the years. The lack of laws that are implemented in South Africa that give WWTP and WTP guidelines to monitor and test for these drugs during treatment and after discharge to the public or the environment. The second objective of the study focused on the different sources of emissions of the antiretroviral drugs into different water types, these included landfill sites, wastewater treatment plants, solid waste disposed carelessly, and excretions from humans. The third objective focused on sampling and analysis of water samples to determine the presence and distribution of these drugs in different water types. These included wastewater and water treatment plants before and after treatment had occurred, discharge sources of these wastewater plants, landfill site boreholes, rivers, and lakes. This provided insight into the emission point's distribution and the quantity and concentration of these drugs in the different samples collected.

## 1.8 Scope and outline of the study.

In chapter 1 a general introduction to the research will be addressed, including the aim of the study and how the aim will be achieved. Chapter 2 provides a review of the key literature relevant to the research topic and guides which methods are applicable to achieve objectives, and addresses objective 1. Chapter 3 provides a detailed description of the study area and addresses object 2. Chapter 4 discusses the methodology and materials used to produce results. Chapter 5 illustrates and discusses the results of objective 3. Chapter 6 provides a conclusion on key findings and recommendations.

# Chapter 2: Literature review

## 2.1 Introduction

Ecologists and toxicologists began to express worry about the possible deleterious effects of medications on water supply in the mid-1960s, but it wasn't until the 1970s that the presence of pharmaceuticals was established (Snyder et al., 2003). Water pollution with medicines has been a serious environmental problem since the 1990s (Doeer-MacEwen and Haight, 2006). Pharmaceuticals in water systems had previously been largely disregarded due to their relative solubility and confinement in rivers compared to other traditional pollutants such as industrial chemicals, agrochemicals, and industrial wastes and by-products (Water Encyclopedia, 2009). Pharmaceuticals and personal care products (PPCPs) include thousands of compounds that are used as pain relievers, antibiotics, contraceptives, beta-blockers, lipid regulators, impotence drugs, dental care products, soaps, sunscreen agents, and hair care products. Pharmaceuticals, both intact and metabolized, are excreted by people and animals and released into the aquatic environment via municipal wastewater discharge and direct discharge, while personal care items reach the aquatic environment by showering and swimming in natural waterways.

In South Africa, the primary treatment for HIV/AIDS is antiretroviral therapy (ART). ARV (antiretroviral) medicines try to maintain the number of HIV viruses in the body low in order to prevent the immune system from deteriorating. It also aids in the recovery of the immune system if it has previously been damaged by HIV. South Africa has the biggest number of persons infected with HIV/AIDS, as well as the world's largest ART program. This raises the issue of the environmental impact of these medications.



## 2.2 Antiretroviral drugs in South Africa

HIV can quickly develop resistance to a single medication. Thus, combination treatment is becoming more popular in nations with high HIV prevalence (now 29% in SA) (Kharsany AB, Karim QA, 2016). This requires a patient taking two or three antiretroviral medications concurrently. ARVs are also provided alongside TB (tuberculosis) therapy in underdeveloped countries such as South Africa. There are now more than 20 antiretroviral medications licensed, although not all of them are available in every country (AVERT, 2014). The most often used ARV medicines in South Africa are: Lamivudine, Stavudine, Efavirenz, Nevirapine,

Didanosine, Zidovudine, Lopinavir; Ritonavir, Abacavir, Tenofovir, Saquinavir, and Nelfinavir; (all generic names). These medications are then employed in various combinations for first-line defense (the mix of drugs provided to a person).

### 2.2.1 HIV/AIDs cases in South Africa

South Africa has the highest rate of HIV/AIDS in the world. South Africa alone has a projected 8.2 million HIV infected people (UNAIDS 2021), accounting for 13.7% of the total population (Department of Statistics, 2021). South Africa has the largest antiretroviral therapy (ART) program in 2020, with an estimated 5.5 million individuals getting ARV (Department of Statistics, 2021). The presence of active pharmaceutical ingredients (API) such as antibiotics and antiretroviral medications in the environment has been recognized as an environmental problem in recent decades (Ngumba, Elijah & Gachanja, Anthony & Nyirenda, James & Maldonado, Johanna & Tuhkanen, Tuula. (2020)). in 2019, around 25.4 million people worldwide got antiretroviral medication (treatment for HIV infection), with 38.0 million people worldwide living with HIV (UNAIDS. org, 2019). Continuous release and persistence of antiretroviral medications in the environment, even at minimal amounts, has emerged as an emergent environmental hazard that may impose harm on the organisms there. In terms of the toxicity of Daphnis, fish, and algae, antiviral medications are said to be among the most dangerous treatment groups (AlRajab et al. 2010; Kummerer 2008).

*Table 1: Shows which provinces have the largest number of people living with HIV. (Sean MacDonell and Marcus Low, Spotlight article, 2019)*

| Province | Number of people living with HIV | Prevalence |
|---|---|---|
| Western Cape | 452210 | 6.76% |
| Northern Cape | 81778 | 7.13% |
| North West | 524593 | 13.59% |
| Mpumalanga | 705174 | 15.41% |
| Limpopo | 515091 | 8.99% |

| | | |
|---|---|---|
| KwaZulu-Natal | 2029470 | 18.23% |
| Gauteng | 1912590 | 13.05% |
| Free State | 419631 | 14.62% |
| Eastern Cape | 859329 | |

The United Nations Program on AIDS and HIV created the 90-90-90 objectives as a set of global goals. The objective is for "90% of persons living with HIV to know their HIV status, 90% of those who know their HIV-positive status to obtain therapy, and 90% of those on treatment to have suppressed viral levels" by 2020. These aims are a strong indicator of how well various provinces are achieving in crucial areas such as HIV testing availability and promotion, as well as assisting those who test positive to begin and stay on treatment. These aims inspire health care personnel to develop initiatives to urge individuals to be checked and know their status, which gives statistics to the government.

*Table 2: Displays 90-90-90 targets for each province. (Sean MacDonell and Marcus Low, Spotlight article, 2019)*

| Province | % of HIV-positive individuals diagnosed | % of HIVdiagnosed individuals on ART | Fraction of ART patients virologically suppressed (RNA count <1000 copies/ml) | % of HIV-positive individuals on ART with VL <1000 |
|---|---|---|---|---|
| Eastern Cape | 90.14% | 61.60% | 87.19% | 48.41% |
| Free State | 89.24% | 73.40% | 91.48% | 59.92% |
| Gauteng | 88.93% | 60.89% | 87.29% | 47.27% |
| KwaZulu-Natal | 92.46% | 72.41% | 91.74% | 61.43% |

| | | | | |
|---|---|---|---|---|
| Limpopo | 91.66% | 73.26% | 85.23% | 57.24% |
| Mpumalanga | 90.45% | 73.53% | 88.09% | 58.60% |
| Northern Cape | 90.81% | 83.02% | 84.42% | 63.66% |
| North West | 89.21% | 57.65% | 87.87% | 45.20% |
| Western Cape | 88.73% | 65.53% | 89.79% | 52.24% |

## 2.3 Previous studies done on the presence of ARVs in South African water bodies

The presence of pharmaceuticals in the environment has been extensively examined, with a growing number of reports of drugs found in drinkable water, river water, ocean, or wastewater. Garrison and colleagues (1976) were the first to discover pharmaceutical residues in treated wastewater. Wastewater treatment plants are the initial recipients of drugs and, as a result, antiretroviral drug leftovers, which are partially or completely eliminated by different treatment processes such as adsorption on activated carbon, oxidation, chlorination, and reverse osmosis before being reintroduced into aquatic bodies as parent chemicals or biotic transformation products (metabolites, conjugates, and degradation products). (Madikizela, Lawrence, Nikita Tavengwa, and Luke Chimuka. (2017)). South Africa uses more ARVs per capita than any other country in the world (WHO, 2013), implying that large volumes of these chemicals would enter wastewater treatment facilities (WWTPs) that were not equipped to remove pharmaceuticals. In addition to inefficient WWTPs, insufficient sanitation and illegal sewage discharge should be taken into account. These considerations, together with the lower predicted dilution in a water-scarce country like South Africa, suggest that ARVs and their breakdown products should be ubiquitous in the environment.

Chlorination has long been used to treat wastewater and drinking water in South Africa since it is a cost-effective and broad-spectrum disinfection technology. Furthermore, chlorination of treated water guarantees that disinfection lasts longer since chloramines have a longer half-life than free residual chlorine (National Research Council (US), 1980). Several studies have been

conducted in South Africa to examine the concentrations of antiretroviral drugs in influents and effluents from wastewater treatment plants around the country. Chlorination has been demonstrated to result in the transformation of antiretroviral drugs, resulting in the development of a range of disinfection transformation products. (Wood, Tim, Basson, Adriaan, Duvenage, Cornelia & Basson, Adriaan & Duvenage, Cornelia & Rohwer, Egmont. (2016)).

Since ARVDs (Antiretroviral Drugs) may pose ecological dangers to aquatic creatures, a countrywide research of the existence, usage pattern, material flow analysis, and removal rate of ARVDs is required to determine the load of ARVDs discharged into the surrounding surface, ground, and freshwater bodies. Focus should be placed on locations without sufficient water and sanitation, as well as areas with a large number of persons infected with the virus and on the ART program. South Africa need improved monitoring methods to ensure that these medications do not cause significant harm to aquatic creatures.

## 2.4 Source emissions and pathways of ARVs into water bodies

Analyses of South African drinking water reveal the presence of a variety of medications and personal care items, including antibiotics, antidepressants, artificial sweeteners, illicit narcotics, and ARVs. Up to 90% of orally applied drugs are excreted (Halling-Sørensen et al., 1998) and end up in the sewage system. These drugs may occur unaltered or partially metabolized. Unused drugs once expired are disposed down drains and ultimately reach wastewaters. Human excretion is one mechanism through which these medications infiltrate our water supplies. Because the body does not absorb all components of ARVs, the portions that are not absorbed pass through urine and feces, making waste treatment facilities the largest contributor to water contamination with ARVs. ARVs are also found in our water as a result of inappropriate disposal of unwanted or expired medications. Medication washed down drains enters the water system immediately. Water-soluble components leak into ground and surface water if it is disposed of in landfills. Additionally, local ARV manufacturing was established to ensure the adequate and timely supply of the drugs. The production and distribution processes of ARVs could also see drugs or their components ending up in our water system. The path and fate of drugs once they are consumed by people may be determined by WWTPs. When released into the environment, these medications find their way into the food chain via

many methods and can impede or interfere with the normal biological processes of living species. ARVs enter surface water bodies primarily by the emission of untreated or inadequately treated effluents from WWTPs, hospitals, and manufacturing sites after excretion from the human body.

Another possible source of ARVs in the environment is faulty sanitation and unlawful dumping of both home and industrial trash. Antiretroviral drugs in the environment have gotten a lot of attention since they avoid degradation in wastewater treatment plants (WWTPs) and end up in surface and groundwater sources (De Clercq 2007; Kahn 2005; Osborn et al.2008). Antiretroviral medications in the aquatic environment have also sparked concern due to their role in the spread of antiviral drug resistance among influenza viruses (Tyring 2004). Antiretroviral medications are partially metabolized or excreted as active metabolites in urine and feces after delivery and then enter WWTPs (Wastewater Treatment Plants) where they are processed with other elements of wastewater. Unused drugs are disposed of in the sewage system, drains, and sometimes in the trash. There are five main sources for antiviral drugs to reach potable water sources through various pathways:

- Effluent from pharmaceutical industries
- Hospital wastes
- Disposal of out-of-date, unused, or unwanted medicine from residential households
- Leaking pipelines
- Landfills



These several mechanisms enhance the dispersion and concentration of antiretroviral medications in the environment, increasing the harm that these drugs pose to aquatic life. Large-scale research in local and national strata are thus necessary to explore the origins, overall fate, and impacts of pharmaceutical pollutants on flora and wildlife. Temporal investigations may reveal modest changes in the aquatic environment produced by these medications. As a result, the development of research networks and capability across

government and commercial organizations is critical for the building of a solid analytic methodology.

### 2.4.1 Hospitals and clinics

Pharmaceuticals are used at hospitals on a regular basis, from providing them to patients with various ailments to disposing of them when they expire. When a patient is diagnosed with HIV/AIDS, they must seek treatment from the hospital or clinic that is in charge of dispensing ARVs. As predicted, these institutions are responsible for disposing of expired drugs using various means; ARVs are present in hospital wastewater (Schuster et al., 2008). ARV concentrations are likely to be greater in hospital effluent than in municipal sewage. However, the overall material flow is substantially smaller due to the much lower proportion of hospital effluent in municipal wastewater in wealthy nations. (Swanepoel, C., Bouwman, Hindrik, Pieters, Rialet, and Carlos Bezuidenhout. (2015))

### 2.4.2 Private households

Expired drugs or their remnants are often disposed away in residences through the sewage system. In a survey conducted in Kuwait (Abahussain et al., 2006), over half of the respondents (45.4%) got prescription prescriptions more than three times per year, and nearly all had undesirable pharmaceuticals in their homes. The most common causes for having unused medicine were a change in recommended medication by the doctor (48.9%) or selfdiscontinuation (25.8%). Throwing unused drugs in the trash (76.5%) or flushing them down the drain (11.2%) was the most popular method of disposal. The findings of this study indicate that patient education on the correct disposal of unused and expired pharmaceuticals is vital in all nations. This form of disposal has resulted in these drugs being found in water sources from surface water to groundwater. In some countries, take-back systems are already in place and working towards decreasing the concentration of pharmaceuticals in water bodies. (Niquille and Bugnon, 2008).

### 2.4.3 Landfills

Antiretroviral drug incidence and fate in landfills have been generally ignored; when homes toss medicine in the garbage, it ends up in landfills. This eventually results in the drug getting into groundwater, which can then get into the water that people drink in their homes.

Antiretroviral medications abandoned in municipal solid waste may degrade, adsorb, or enter the leachate, eventually exiting the landfill and entering aquatic bodies (Musson et al., 2009).

### 2.4.4 Leaking pipelines

Hazardous waste items from sewage or septic systems are among the most well-known sources of water pollution. Human waste seeps out of broken sewage systems and ends up in surrounding groundwater. Toxic trash from the neighboring region might spill into your broken water lines. The pressure inside water pipes is usually considered to drive water out through leaks. Because this effluent includes undecomposed ARV metabolites, it is returned to the earth and enters groundwater and other water sources. (Swanepoel, C. (2015))

## 2.5 Fate of antiretroviral drugs

A substance's absence does not always imply biological or photochemical deterioration. The sorption of antiretroviral drugs is an essential elimination process that is dependent on the amount of neutral and ionic species present as well as the properties of the target particles (Arts EJ, Hazuda DJ, 2012). Sorption can affect the dissemination and (bio)availability of pharmaceuticals in the environment (particle-bound transport), as well as their removal during wastewater treatment (Thiele-Bruhn, 2003). Some antibiotics, such as tetracycline's, are known to attach to soil particles or form complexes with ions that are present (ter Laak et al., 2006). The number and kind of free and suspended particles in the aqueous phase, soil organic matter, and soil pH all have an impact on antibiotic sorption. (Thiele-Bruhn, 2003).

According to Halling-Srensen et al. (1998), the medications may be classified into three types of probable outcomes:

- The drug, such as aspirin, is mineralized to carbon dioxide and water (Richardson and Bowron, 1985).

- So because material is lipophilic and not easily degraded, some of it will be retained in the sludge.

- The substance is metabolized to a more hydrophilic form of the parent lipophilic one, but it is still persistent, and thus it will pass through the WWTP and end up in the receiving

waters (wastewater treatment effluents are frequently discharged to rivers), potentially affecting aquatic organisms if the metabolites are biologically active. Substances that have the ability to be kept in the sludge will be able to impact the environment since the sludge is scattered across fields. (Halling- Sørensen et al., (1998))

### 2.5.1 Sewage

Sewage discharge is known to be a major source of drugs and their metabolites into aquatic habitats. Medication removal rates in wastewater treatment plants (WWTPs) range from less than 10% to over 100%, depending on the physio-chemical properties of the pharmaceutical and the kind of treatment technology used (Chemosphere. 2009). Patient usage in the community, discharges from hospitals, and, in rare situations, effluent from pharmaceutical manufacture are all sources of human medicines in sewage (Daughton CG, Ternes TA Environ Health Perspect. 1999). Sewage can be released into marine ecosystems via coastal and ocean outfalls for combined sewer overflows from WWTPs, as well as rivers receiving WWTP effluents (Mar Pollute Bull. 2014).

Groundwater contaminated by sewage can potentially be a source of drugs entering coastal seas. Pharmaceuticals were found in a coastal aquifer on the Yucatan Peninsula, Mexico that had been injected with municipal sewage discharges (Reu Environ Pollute, 2011). Irrigation with treated household wastewater contributed to pharmaceutical contamination in groundwater on Mallorca (Arch Environ Contam Toxicol. 2013). Septic tanks or small decentralized systems are used for sewage treatment disposal in rural and urban regions across the world, including famous seaside vacation spots (Front. Ecol. Environ.). These systems are a possible source of pharmaceuticals in coastal waters via leakage to ground and surface waters, depending on their treatment effectiveness and the capacity of the surrounding soils (Dougherty JA, Swarzenski PW, Dinicola RS, Reinhard MJ Environ Qual. 2010).



### 2.5.2 Human excretion

Pharmaceutical residues can enter the environment through a variety of channels. Human excretion is the most common way for pharmaceutical chemicals to enter the aquatic environment. Many pharmacological drugs are eliminated in biologically active form through the urine because they are not digested in the body (Zhang Z, Tang W, 2018). Many

pharmacological drugs are not completely absorbed from the gut into the circulation and are expelled in the feces. As a result, pharmaceutical residues can be found in both urine and feces from treated individuals. The incorrect disposal of unwanted or expired medications is another cause of pollution in the pharmaceutical industry. Some pharmaceutical residues may adhere tightly to soil particles and have minimal potential to leach into groundwater or nearby surface waterways, Other more water-soluble wastes, on the other hand, can leach with rain and reach both groundwater and surface waterways ( Manvendra Patel,2019). Wastewater treatment facilities (WWTPs) are not intended to manage pharmaceutical products and remains deposited in water systems and the environment as a result of human intake and excretion, as well as incorrect disposal of these goods.

The mass manufacture of ARVDs has the potential to contaminate surface, ground, and drinking water throughout South Africa. The borehole water study reveals that the studied pharmaceuticals can pollute groundwater over broad areas, providing a direct pathway for human exposure. The use of polluted groundwater as a source of drinking water might thus provide a direct path for resistant bacteria reaching people. Some medications have been shown to flow through the floor. Some pharmaceuticals are known to pass through the floor of rivers and lakes and mix with groundwater (Reddersen et al., 2002; Heberer, 2002], and (Massmann et al.) showed that pharmaceuticals can persist in aquatic environments for decades (Massmann et al, 2008).

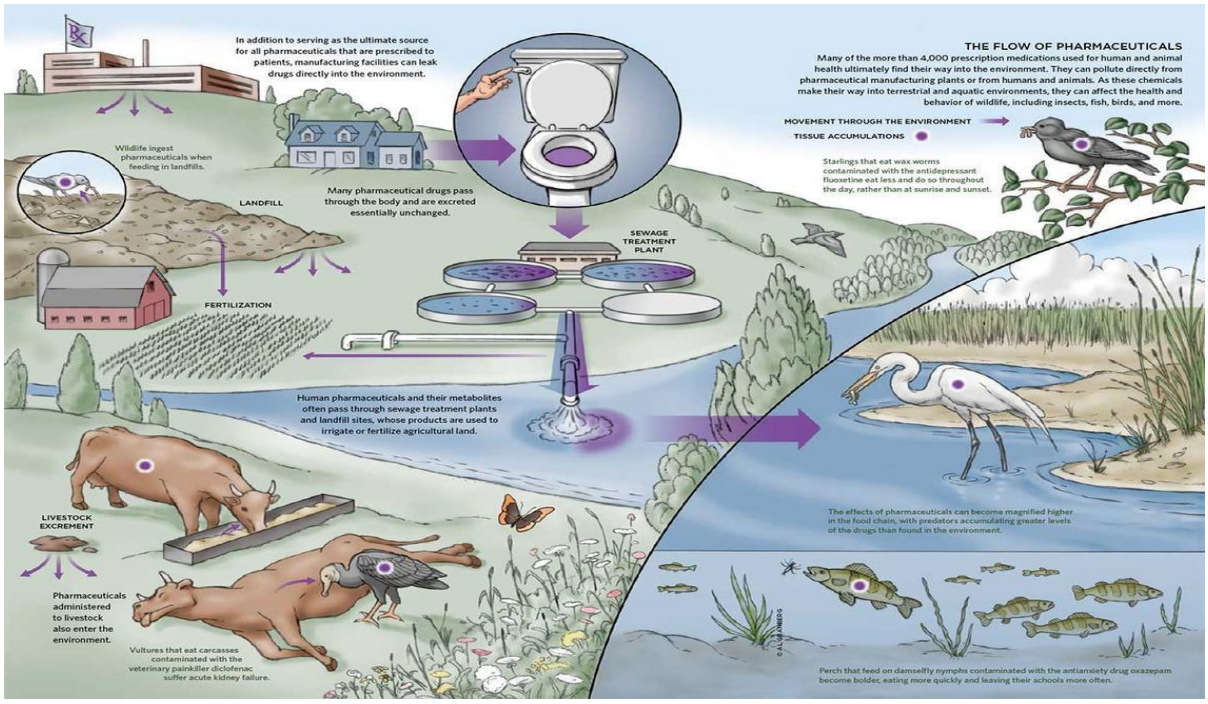


*Figure 1: The flow of pharmaceuticals (USGS, Al Granbreg, 2010)*

## 2.6 Behaviour of selected antiretroviral drugs in water

### 2.6.1 Nevirapine in water

Nevirapine is an anti-HIV medication that decreases viral levels in the body. Anti-HIV medications such as nevirapine decrease immune system degradation and prevent the onset of AIDS-defining diseases. Nevirapine belongs to the non-nucleoside reverse transcriptase inhibitor family of medicines (NNRTIs). NNRTIs inhibit the activity of the HIV reverse transcriptase enzyme, which is required for HIV to transcribe its genetic code into a form that can be introduced into human cells. Boehringer Ingelheim sells nevirapine under the brand name Viramune. In the European Union, nevirapine was approved in February 1998, and in the United States, it was approved in June 1996. There are several generic versions of Nevirapine available as a single medicine or as part of a three-drug fixed-dose combination (National Institutes of Health). Nevirapine was found to be resistant to degradation at relevant chlorination levels, which may partially explain its ubiquitous presence in South African water. (Wood, Tim & Basson, Adriaan & Duvenage, Cornelia & Rohwer, Egmont. (2016))

### 2.6.2 Lopinavir and ritonavir

Lopinavir/ritonavir is an HIV treatment drug. It is a combination of the antiretroviral medicine Lopinavir with a low dosage of the antiretroviral drug Ritonavir, which is intended to enhance the action of Lopinavir. These two pharmaceuticals are combined into a single pill that is used in conjunction with other antiretroviral medications to form a treatment regimen. Ritonavir is an antiretroviral protease inhibitor that is often used in the treatment and prevention of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS). For adults, the standard lopinavir/ritonavir dosage is 800mg lopinavir and 200mg ritonavir per day. This dosage consists of two yellow tablets containing 200mg Lopinavir and 50mg Ritonavir, which should be taken twice a day (National Center for Biotechnology Information, 2021). Lopinavir/ritonavir is occasionally branded as Kaletra, but generic versions are also available. Lopinavir is hygroscopic, meaning it is nearly insoluble in water but soluble in a variety of organic solvents. It is stable in normal storage circumstances but degrades when exposed to UV radiation.

### 2.6.3 Tenofovir in water

Tenofovir is an antiviral medication used to treat HIV, a virus that can lead to acquired immunodeficiency syndrome (AIDS). Tenofovir is not an HIV or AIDS cure. Tenofovir is also employed in the treatment of chronic hepatitis B. Tenofovir is intended for use in adults and children above the age of two and weighing at least 22 pounds (10 kilograms). Tenofovir belongs to a family of drugs known as nucleoside reverse transcriptase inhibitors (NRTIs). It works by lowering the concentration of HIV and HBV in the blood. Although tenofovir will not cure HIV, it may reduce your chances of acquiring AIDS and HIV-related disorders such as severe infections or cancer. Tenofovir is somewhat soluble in water, dichloromethane, and methanol (National Center for Biotechnology Information (2021)). The hydrolysis rate of Tenofovir is affected by the pH and water content in the environment (Pharmaceuticals, 2020)



### 2.6.4 Efavirenz in water

Efavirenz is an anti-HIV medication that decreases viral levels in the body. Anti-HIV medications such as Efavirenz decrease immune system destruction and avoid the onset of AIDS-defining diseases. Efavirenz belongs to the non-nucleoside reverse transcriptase inhibitor family of medicines (NNRTIs). Non-nucleoside reverse transcriptase inhibitors block

the activity of the HIV reverse transcriptase enzyme, which is required for HIV to transcribe its genetic code into a form that can be injected into human cells. Efavirenz, formerly known as DMP 266, was created by Du Pont Pharma. It was licensed for HIV therapy in the United States in 1998, and it received European approval in May 1999. (2021 National Center for Biotechnology Information)

## 2.7 Methodology used to determine the concentration of ARVs in water samples

Environmental fate and Eco toxicity effects, worldwide status, and future prospects have all been studied. Most sample procedures employed do not account for the probability of shortterm ARV changes. Most articles report on grab sampling within a treatment facility or at various locations along a river system and dams. ARVs are administered at specified times of the day throughout the duration of the person's life, meaning that there are periods when ARV levels in water systems are quite high. Furthermore, Ort et al. (2010) recognized sample mistakes caused by lengthy sampling intervals and insufficient sampling modes as contributing factors to the misinterpretation of pharmaceutical eco-toxicological data (Ort et al., 2010). The design of prediction models, as well as the consideration of passive sampling methodologies, automated samplers, and online sensors, can be used to address these mistakes and biases. Wooding et al. (2017) have created a low-cost disposable polydimethylsiloxane sorptive sampling loop for selective extraction of medicines, including ARVs, from water samples (Wooding et al., 2017).



Understanding the fate of pharmaceutical pharmaceuticals necessitates suitably validated analytical methods capable of successfully extracting low concentration analytes from high matrix samples that also exist as mixes with other organics with comparable physicochemical features. Because environmental materials are complex, and pharmaceutical contaminants occur in trace amounts, pre-concentration and isolation are always required before chromatographic quantification. The present methods for analyzing ARVs in aqueous materials rely mostly on solid phase extraction (SPE).

### 2.7.1 Solid phase extraction

Solid phase extraction (SPE) is a sample preparation process that uses a solid adsorbent, often enclosed in a cartridge device or on a disk, to adsorb certain species from a solution. SPE is used to isolate a species from a sample or to clean it up before analysis. The analyte and some of the sample matrix chemicals may be maintained on the SPE material when the sample is slowly processed through the SPE cartridge or disk (Anon, 2020). Depending on the analyte and SPE sorbent qualities, a wash solvent can be used to selectively remove (elute) components from the SPE sorbent while preserving others. The ultimate objective is to eliminate matrix interferences from the analyte, resulting in a solution. (Anon, 2020)

SPE materials are classified into numerous varieties, including reversed phase, normal phase, ion exchange (anion/cation), and mixed-mode phases, which have properties of more than one type of SPE material. (Anon, 2020)

•Phase reversal SPE procedures are best for moderate to low polarity analytes, and they separate analytes based on hydrophobicity, with the more polar molecules eluting first. SPE with reversed-phase sorbents is frequently used in many studies involving analytes dissolved in an aqueous sample (e.g., surface waters, wastewater, urine, or plasma).

•Normal stage is when the analyte of interest has a low to high polarity or is neutral, SPE methods are widely utilized. A polar adsorbent, such as silica, is used in the cartridge. This separation will be based on polarity, with the least polar components being separated first.



•Ion exchange SPE lets us separate compounds based on charge, there are two types of ion exchange SPE, cation exchange and anion exchange.

### 2.7.2 Liquid chromatography

Liquid chromatography (LC) is a separation method in which sample ions or molecules are dispersed in a liquid mobile phase. It is done in either a column or a plane. The mobile liquid sample will travel through a column or plane filled with a stationary phase made up of irregularly or spherically shaped particles. Because of changes in ion exchange, adsorption, partitioning, or size, various solutes will interact with the stationary phase to varying degrees,

allowing separation of the compounds and determination of the transit time of the solutes through the column. 2020 (Linde.com)

### 2.7.3 Mass spectrometry

Using mass-to-charge ratios, an analytical approach for determining the atomic and molecular composition of inorganic and organic materials. MS uses the mobility of ions in electric and magnetic fields to sort them according to their mass-to-charge ratios. Thus, MS is an analytic method that identifies chemical compounds by sorting gaseous ions in electric and magnetic fields. These sensors work on the premise that moving ions can be deflected by electric and magnetic fields. A mass spectrometer is a device that conducts this action and employs electrical methods to detect the sorted ions. 2020 (Linde.com) MS offers qualitative and quantitative data on the atomic and molecular composition of inorganic and organic compounds.

Mass spectrometers are made up of four basic parts: a handling system for introducing the unknown sample into the equipment; an ion source that produces a beam of particles characteristic of the sample; an analyzer that separates the particles according to mass; and a detector that collects and characterizes the separated ion components. To monitor drugs, including ARVs, and their transformation products in complicated environmental samples, fast equipment with sensitive detectors is required (Linde. com, 2020). The most often used method in pharmaceutical analysis is liquid chromatography linked to mass spectrometry (LC-MS).

## 2.8 Sampling methods used in previous studies

Most studies had the goal of assessing the extent of antiretroviral analysis in environmental samples as well as looking at strategies such as instrumentation and predictive models that have been reported in the literature on the fate and Eco toxicological effects of antiretroviral in different environmental compartments. The high dose levels for ARVs necessitate high manufacturing, suggesting that large quantities of ARVs are generated, all of which have the potential to end up in environmental water bodies. The research indicates that ARVs are only partially converted and are eliminated from the human body in their original form and/or as metabolites in urine and feces. ARV, like any other medicine, might have side effects. ARV

can be viewed as pseudo-persistent pollutants in the environment because of their continuous release (Daouk et al., 2015).

The possible generation of resistant strains of HIV in the body as a result of misinformed exposure to contaminated water is a severe problem associated with the presence of ARVs in drinking water and food sources. ARVs vary from other medications in that they fight against a virus that can readily mutate into resistant strains if medication is not taken as prescribed. Most sample procedures employed do not account for the probability of short-term ARV changes. Previous research revealed that water samples were gathered at several places around South Africa, with distinct techniques used for each area from where the samples were collected.

Several research investigations have found HIV/AIDS in South African rivers. Currently, project k5/2594, a three-year WRC-funded initiative led by the University of the Northwest, investigates the presence, concentration, and risk implications of HIV/ARVS in treated wastewater, groundwater, tap water, and aquatic life, including fish. The long-term consequences of exposure to ARVs in water, which are mainly unknown, are presently being studied. The magnitude and durability of South Africa's HIV/AIDS treatment program, the world's biggest, necessitates an awareness of HIV/ARV in the environment, since this might jeopardize the health of both HIV-positive and HIV-negative persons.

Numerous studies throughout the world have found medicines and personal care items in water sources, but there is still no clear proof or awareness of the influence on human health. The increase in reported detections of extremely low concentrations of pharmaceuticals in many environmental matrices, including the water cycle (e.g., surface water, groundwater, treated wastewater effluent, and drinking water), is mostly due to technical breakthroughs in the sensitivity and accuracy of detection equipment and analytical procedures. Gas chromatography with mass spectrometry (GC-MS) or tandem mass spectrometry (GCMS/MS) and liquid chromatography with mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) are sophisticated technologies that can identify target substances to the Nano gram per liter level and are routinely used for pharmaceutical identification. The methods used

are determined by the physical and chemical characteristics of the target substance. LC-MS/MS analysis is more suited to measuring target compounds that are more polar and highly soluble in water, whereas GC-MS/MS is better suited to measuring target compounds that are more volatile. While improved detection and analytical capabilities will allow us to learn more about the fate and occurrence of pharmaceutical chemicals in the environment, including the water cycle, it is critical to recognize that detection of these compounds does not directly correlate to human health risks that can be validated using available human risk assessment methods. Furthermore, there is presently no established method or protocol for sampling and analyzing drugs in water or other environmental media that assures accuracy.

### 2.8.1 Continental research

Other African countries have taken the effort to test their waterways for ARVs. In Machakos, Kenya, some effluent surface water grab and river silt were collected upstream and downstream of the effluent discharge point from the wastewater treatment facility (WWTP). Machakos Town is the administrative town of the greater Machakos County, and the WWTP in Machakos uses waste stabilization ponds for wastewater treatment. Two sample operations were conducted in January and September of 2019. September is typically a particularly dry month, with most arid and semi-arid areas experiencing drought, impacting flow rates into the treatment plant and in the rivers. The river Matheu, which collects wastewater from the WWTP, was nearly dry, and the flowing waters were choking. In general, river water quantities were much lower during the September sampling compared to the January sampling. A prior research conducted by Northwest University's Research Unit for Environmental Sciences and Management in Potchefstroom. This study investigates the presence, concentration, and possible consequences of HIV-Antiretroviral in selected South African water resources. PPCPs (Pharmaceuticals and personal care products) can be found in South African natural waters. As a result, fish and other aquatic biota are exposed to them and may potentially acquire HIVARVs directly from the water via their gills, diet, or both.

The presence of numerous additional substances in the waterways that were examined suggests that other PPCPs, pesticides, and industrial pollutants may be present. If mostly unstable HIVARVs were detected in the studied samples, it is expected that comparable unstable

compounds would be present, indicating that more stable compounds, whether PPCPs or others, will also survive intact in waters for varied durations of time. The unsubstantiated existence of several substances in our waterways requires immediate attention in order to convey the need for greater protection of the sources, as well as the ecosystem and consumers.

In the US, pharmaceuticals are considered as chemicals first and then therapeutic agents by the EPA. The EPA and the DEA recommend the incineration of medical waste [Environmental Protection Agency, 2022]. However, there are no official, uniform guidelines on managing PPCPs in the nation. Each state has the authority to choose how they dispose of waste. Often, pharmaceuticals in households are disposed of in the trash or in sewage. It has been thought that PPCP pollution may be reduced through their proper disposal [Hossain, M.S.; Santhanam,2011]. However, it may be important to consider the broader usage of pharmaceuticals and limiting their use through stewardship programs along with enforcement of manufacture–usage–disposal practices that limit waste and pollution. In 2008, the EPA proposed to add pharmaceuticals to the kinds of hazardous wastes that could be managed as Universal Wastes. The EPA currently has a rule geared toward one type of PPCP, nicotine, titled "Management Standards for Hazardous Waste Pharmaceuticals and Amendment to the P075 Listing for Nicotine," signed in 2018, and published in 2019. The rule supports the nation's move toward returning unused PPCPs [Environmental Protection Agency; 2019].

Several jurisdictions in Canada do not have official policies or regulations governing PPCPs. There is less regulation for pharmaceutical and medical waste when compared to the US. Similar to the US, existing guidelines and recommendations for waste disposal are enforced at the discretion of each province or municipality [Walkinshaw, E,2011]. The country's goal is to reach the minimum national standards for managing medical waste set by the Canadian Council of Ministers of the Environment in 1992 [Walkinshaw, E,2011]. To minimize pharmaceutical waste, Canada also partakes in drug take-back programs for people to return unused pharmaceutical products. The Guidelines for the Management of Biomedical Waste in the Northwest Territories (2005) specify that pharmaceutical products must be segregated from general waste and handled by incineration or chemical neutralization [Environment and Natural

Resources Canada, 2022]. This is still widely followed and serves as the major guideline for pharmaceutical waste in the country.

In 2013, the European Commission resolved to minimize water pollution from pharmaceutical products. In response, a 12-week consultation period was conducted to develop an approach for limiting pharmaceutical waste in the environment [European Commission. Pharmaceuticals and the Environment, 2022]. The deadline for implementing this approach was in 2018. However, there is little to no evidence of a follow-up on the matter. The European Union subsequently developed a document named “The European Green Deal”. This follows the European Commission’s attempt to manage environmental pollution. The Green Deal aimed to adopt a “zero pollution action plan” by 2021 [European Commission. Strategic Approach to Pharmaceuticals in the Environment,2021]. Notably, within this document, the discussion of pharmaceutical products appeared only once. An example of the recommendations to be included in the new approach include the European Medicines Agency working along with the Commission to reduce pharmaceutical waste. This includes developing policies to reduce the packaging size of pharmaceuticals that would amount to reductions in disposal [European Commission. Strategic Approach to Pharmaceuticals in the Environment,2021].

## 2.9 South African standards of wastewater discharged in different water bodies.

Pharmaceutical drugs can be found in effluent from WWTPs, hospitals, and pharmaceutical manufacturing facilities, as well as in the improper disposal of old and expired medicines. In South Africa, there are currently no monitoring programs or legislative guidelines for their restrictions. Antiretroviral drugs, for example, are not regulated and may pollute water bodies, damaging the aquatic habitat.

The NWA was established in 1998 to provide legal force to the White Paper on a National Water Policy for South Africa (April 1997), as well as the DWA's water quality management plans and strategies. The National Water Resource Strategy (created under the NWA) establishes the foundation for the overall protection, use, development, conservation, management, and control of South Africa's water resources. The strategy emphasizes, in particular, that while "most water utilized in a non-consumptive way is immediately recycled

for re-use or returned to rivers for re-use elsewhere," "there is greater potential for re-use of water, particularly in coastal regions" (RSA DWAF, 2002b). South Africans are responsible for ensuring water quality control. The Department is responsible for developing and implementing water quality policies and documents, such as the South African Water Quality Guidelines (DWAF). The recommendations serve as a foundation for designing materials to educate water consumers about the physiochemical, aesthetic, and biological aspects of water.

The essential goal of producing uniform wastewater final effluent in this nation remains unmet due to issues such as the badly operating state and inadequate maintenance of some wastewater treatment plants, as well as a lack of equipment to monitor the micro pollutant content of effluent, particularly in impoverished neighborhoods. Numerous studies have found that several wastewater treatment plants continue to discharge effluent containing considerable levels of enteric pathogens, resulting in the contamination of receiving water bodies and posing a major hazard to human health. Figure 2 displays the general water discharge standards that are used in South African wastewater treatment plants and the concentrations theses set standards should be.

| Variables and Substances | General Standards |
|---|---|
| Chemical oxygen demand | 75 mg/L |
| Colour, odour or taste | No substance capable of producing the variables listed |
| Ionised and unionised ammonia (free and saline ammonia) | 3 mg/L |
| Nitrate | 15 mg/L |
| pH | 5.5–9.5 |
| Phenol index | 0.1 mg/L |
| Residual chlorine (Cl) | 0.25 mg/L |
| Suspended solids | 25 mg/L |
| Total Aluminium (Al) | - |
| Total Cyanide (Cn) | 0.02 mg/L |
| Total Arsenic (As) | 0.02 mg/L |
| Total Boron (B) | 1 mg/L |
| Total Cadmium (Cd) | 0.005 mg/L |
| Total Chromium III (CrIII) | - |
| Total Chromium VI (CrVI) | 0.05 mg/L |
| Total Copper (Cu) | 0.01 mg/L |
| Total Iron (Fe) | 0.3 mg/L |
| Total Lead (Pb) | 0.01 mg/L |
| Total Mercury (Hg) | 0.005 mg/L |
| Total Selenium (Se) | 0.02 mg/L |
| Total Zinc (Zn) | 0.1 mg/L |
| Faecal Coliform | 1000 cfu/100 mL |

Source: [38].



*Figure 2**: South African National Water Act waste discharge standard guidelines. (DWAF, 1997)***

These wastewaters must be monitored once they are released into various bodies of water. The necessity for direct investigation of particular pathogens of concern, in addition to frequently monitored traditional microbiological markers, is obvious in the context of wastewater

management. Many of the outbreak-related infections found in wastewater are not frequently checked indicators, causing contamination in the aquatic ecosystem and the death of aquatic creatures.

## 2.10 Theoretical concepts

### 2.10.1 Antiretroviral drugs in the environment

Pharmaceuticals (PCs), particularly antiretroviral (ARV) drugs, have been widely observed in diverse aquatic environments, with wastewater treatment facilities (WWTPs) recognized as the principal point source. (Karen Reddy, Nirmal Renuka, Sheena Kumari, Faizal Bux,2021) According to studies, given drugs are not completely digested in the human body, resulting in residues and metabolites. The drug residue and metabolite are released into the environment via sewage, resulting in antiviral drug increases in wastewater and ambient waterways (Race et al., 2020; Funke et al., 2016; Khetan and Collins, 2007). Furthermore, studies have confirmed that antiviral drugs are only partially removed in wastewater treatment plants (WWTPs) (Nannou et al., 2020; Mlunguza et al., 2020; Ngumba et al., 2020). These expelled compounds, together with urine and feces, enter the sewage network in cities.

### 2.10.2 Reactions and behavior of ARVs in the environment

TDF (Tenofovir disoproxil), an ARV medication, has a low bioavailability, with a median terminal elimination half-life of around 17 hours (Vaidya et al., 2016). According to Quercia et al. (2018), the bioavailability of 3TC and TDF was 86% and 70-80%, respectively. ARV drug metabolism in the human body may also result in a variety of metabolites (Gopalan et al., 2017; Sinxadi et al., 2015). Several of these metabolites have been found in urine (Rakhmanina and van den Anker, 2010; Ray et al., 2016). These ARV medications and their metabolized metabolites may degrade and/or change into chemicals with potential long-term biological action in the environment (Tak et al., 2020; Wood et al., 2016). Recent research has found significant amounts of ARV drugs in a variety of environmental matrices, including wastewater, surface water, groundwater, and drinking water (Abafe et al., 2018; Boulard et al., 2018; Funke et al., 2016; Peng et al., 2014b; Rimayi et al., 2018; Wood et al., 2015). It should be emphasized that the vast majority of investigations on ARV drug detection have concentrated on WWTPs and surface waters (Boulard et al., 2018; Fisher et al., 2016; Rimayi

et al., 2018). However, evidence on the presence of ARV medicines in groundwater and drinking water is inadequate and only covers a small geographic area. (Isaac Kudu, Vishalan Pillay, Brenda Moodley, 2022)

Several research on the detection and occurrence of ARV drugs in WWTPs and surface water indicated greater amounts in underdeveloped nations, particularly in Africa, than in industrialized countries (Ngumba et al., 2016a; Prasse et al., 2010; Vergeynst et al., 2015; Wood et al., 2015). Given the possible negative effects of ARV medications on aquatic creatures (Robson et al., 2017), there is a need for data from undiscovered locations across the world where ARV therapies are commonly used. Major problems include a lack of consistent testing techniques and access to high-end equipment for detecting low amounts of ARV medicines in the environment. The majority of research on ARV drugs in the aquatic environment has been on detecting the original parent chemical rather than their behavior or transformation products (TPs) (Abafe et al., 2018; Azuma et al., 2019; Mosekiemang et al., 2019). This is mostly due to the difficulty in comprehending the behavior of these chemicals under environmental circumstances. More thorough research on the detection of ARV drugs and associated TPs are therefore required to understand their incidence, fate, and accumulation in environmental matrices. Because of their toxicity to living beings, antiretroviral drugs and their metabolites are considered one of the most dangerous therapeutic families of pharmaceuticals (Castiglioni et al., 2006). Some ARV drugs are known to persist in the environment can lead to drug resistance in microbes and can also directly impact aquatic organisms. (´Alvarez-Mu˜noz et al., 2015; Zhou et al., 2015).



## 2.11 Certain parameters effect on the concentration of ARVs in different water bodies.

Climate change and other global environmental changes may have a considerable impact on the usage, transport, fate, and dose-response relationship of environmental pollutants. Such changes in chemical exposure and toxicity profiles may have a major impact on ecosystem health (Schiedek et al. 2007). Environmental factors in water, such as temperature, precipitation, pH, salinity, and UV light irradiation, are changing as a result of global climate

change. These environmental characteristics are significant because they may interact with toxicants and impact the pollutants' dose-response relationship.

### 2.11.1 Potential of Hydrogen (pH)

Approximately 78-95% of currently available pharmaceuticals are ionizable. Mallanack (2013) (Bhal 2007). The degree of ionization of a particular molecule at a certain pH is governed by its acid dissociation constant (pKa), and this influences the biopharmaceutical properties of a medicine, impacting solubility, lipophilicity, permeability, and protein binding, which in turn underlies the drug ADME (Absorption, Distribution, Metabolism, and Excretion) features. 2013 (Mallanck) These mechanisms, when combined, provide a specific internal dosage, the dynamic intracellular speciation of which is the driver for possible toxicity. The amount to which a particular medication gets ionized will vary depending on the ambient pH, potentially influencing bioaccumulation and effects significantly.

### 2.11.2 Temperature

Water temperature can affect chemical accumulation kinetics or metabolism, and hence the sensitivity of test organisms (Heugens et al. 2003). Higher temperature normally signifies increased metabolism, although this does not always imply increased toxicity. It should be determined if temperature-induced metabolic alterations result in greater bio-activation or detoxification (Noyes et al. 2009).

### 2.11.3 Electro conductivity

Conductivity measurements are a useful indicator of the number of dissolved ions present in a water sample and can serve as a measure of water quality. Conductivity can affect the rate of dissolution of a drug due to electrolytes that might have common ions with the drugs.

# Chapter 3 study area

## 3.1 Introduction

South Africa's ART programs have expanded dramatically in recent years, in accordance with the World Health Organization's (WHO) evolving recommendations. South Africa launched the 'test and treat' method in 2016, making everyone with a positive HIV diagnosis eligible for treatment regardless of how far the virus has progressed in their body. The number of persons eligible for treatment has more than doubled in recent years, rising from 3.39 million in 2015 to 8.2 million in 2021. (UNAIDS, 2021). Many people were initially concerned about the rapid expansion of ART. Many people were afraid that the rapid expansion of ART would strain clinics and services, lowering the quality of care. However, research shows that the increased availability of ART has had no discernible impact on patient outcomes, either in terms of AIDS-related fatalities or illnesses. 2020 (Department of Statistics)

The Western Cape has one of the lowest HIV/AIDS rates in South Africa, however it does contain places with a big ART program, such as Khayelitsha. The first government facilities in South Africa to consistently give antiretroviral therapy were maternity services in Khayelitsha, which began giving Zidovudine in 1999 for the prevention of HIV transmission from mother to child. Khayelitsha, which began delivering Zidovudine for the prevention of HIV transmission from mother to child in 1999. MSF began assisting the government in running HIV services in Khayelitsha in 1999, with the intention of developing a pilot program for the provision of antiretroviral therapy (ART), collaborating to create HIV care services in 2000 and ART in May 2001. The initial cohort study was established with the assistance of the University of Cape Town to demonstrate the feasibility of ART in a high HIV-burden African setting, employing a public health approach to ART provision, generic antiretroviral, and an adherence model based on patient education and empowerment. The initial initiative was undertaken against global and national criticism about the efficacy and safety of ART in African women.

By 2004, ART had become the standard of therapy for clinically qualified individuals in South Africa. Since then, the Khayelitsha program has grown dramatically, with over 40 000 patients

receiving ART by the end of 2014. (UNAIDS, 2021). In that it is one of South Africa's longestrunning and largest treatment programs, and one in which extensive innovation around models of service delivery continues, there is a great deal of interest in the continued tracking of clinical outcomes and the effectiveness of health service interventions.

## 3.2 Study area and sample areas.

Water samples will be collected from 6 areas around the Western Cape which are Atlantis, Mitchell's Plain, Cape Flats, Potsdam, Athlone, and Bellville because these are some of the areas that are most affected by HIV/AIDS and have wastewater treatment plants present, so they are prone to have a concentration of ARVs in their waters. Some of these areas also do not have proper water and sanitation systems which result in waste getting into the water and contaminating it. The water treatment plants that are responsible for purifying water for human consumption and wastewater being deposited back into the environment do not take into account pharmaceuticals during the purification process these chemicals end up in the water that people drink and also back into the environment. Samples will be collected at wastewater treatment plants, water treatment plants, boreholes, landfill site boreholes, and storm water vlei.

The study will take place at Bellville wastewater treatment plant, Mitchell's plain wastewater treatment plant, Athlone wastewater treatment plant, Cape Flats wastewater treatment plant, Potsdam wastewater treatment plant, Atlantis wastewater treatment plant, and Atlantis water treatment plant. Water bodies that are used for discharging wastewater after treatment has occurred will also be assessed these include the Kuilsriver, Black river, lagoon beach, Atlantis recharge pond 6, Salt River, Zeekoe Canal, Sonwabe Beach, and Zeekoevlei. Landfill site boreholes at the Coastal Park landfill site, Bellville South industrial landfill site, and Visserhok landfill site will also be assessed as solid waste is deposited in these areas. The purpose of assessing these areas is because there is a system on how these treatment plants work and therefore to have a broader knowledge of where antiretroviral drugs are present in the water bodies it is important to assess the flow pattern of the wastewater from beginning to the end. These treatment plants are responsible for treating residential and industrial wastewater, therefore, pre and post-treatment will be assessed, also considering the outflow of water after treatment had occurred into the water bodies (rivers, vlei, canals, beaches, and lakes). The City

of Cape Town uses landfills to dispose of their solid waste which can include ARVs that are either expired or not being used anymore these drugs can end up in groundwater due to rain and can then flow to other areas such as landfills nearby. Table 3 illustrates each sample areas coordinates and figure 3 displays the map of all the sample areas selected throughout the City of Cape Town.

*Table 3: GPS coordinates of sampling sites in different locations.*

| Sampling area | GPS Coordinates (South) | GPS Coordinates (East) |
|---|---|---|
| Mitchells plain WWTP pre | S 34 04,01 | E 018 35,38 |
| Mitchells plain WWTP post | S 34 04,23 | E 018 35,48 |
| Mitchells plain GW | S 34 40,00 | E 018 36,03 |
| Sonwabe beach | S 34 04,39 | E 018 35,52 |
| Witzand WTP Pre | S 33 37,34 | E 018 26,32 |
| Witzand WTP Post | S 33 37,33 | E 018 26,34 |
| Wesfleur WWTP Domestic pre | S 33 36,23 | E 018 28,57 |
| Wesfleur WWTP Domestic post | S 33 36,41 | E 018 28,42 |
| Wesfleur WWTP Industrial pre | S 33 36,21 | E 018 28,51 |
| Wesfleur WWTP Industrial post | S 33 36,35 | E 018 28,414 |
| Recharge pond 6 | S 33 36,48 | E 018 28,21 |

| | | |
|---|---|---|
| Potsdam WWTP Pre | S 33 50,27 | E 018 31,16 |
| Potsdam WWTP Post | S 33 50,29 | E 018 31,14 |
| Milnerton residential Borehole GW | S 33 51,220 | E 018 30,102 |
| Lagoon Beach | S 33 53,15 | E 018 29,15 |
| Bellville WWTP MBr pre | S 33 55,52 | E 018 39,09 |
| Bellville WWTP MBr post | S 33 55,55 | E 018 39,18 |
| Bellville WWTP DO pre | S 33 55,51 | E 018 39,11 |
| Bellville WWTP DO Post | S 33 55,52 | E 018 39, 22 |
| Bellville WWTP Outflow point | S 33 55,51 | E 018 39,22 |
| Kuilsriver | S 33 56,00 | E 018 40,22 |
| Bellville South Landfill site Borehole GW | S 33 56,19 | E 018 39,30 |
| Athlone WWTP Pre | S 33 57,11 | E 018 30,51 |
| Athlone WWTP Post | S 33 57,096 | E 018 31,00 |
| Ronderbosch golf course borehole GW | S 33 57,11 | E 018 29,32 |
| Blackriver | S 33 57,08 | E 018 29,38 |
| Saltriver | S 33 54,31 | E 018 28,23 |

| | | |
|---|---|---|
| Cape Flats WWTP Pre | S 34 04,50 | E 018 31,14 |
| Cape Flats WWTP Post | S 34 05,37 | E 018 30,13 |
| Zeekoevlei | S 34 04,22 | E 018 30,57 |
| Coastal park landfill borehole GW | S 34 05,35 | E 018 30,08 |
| Outflow Zeekoe canal | S 34 05,44 | E 018 30,14 |

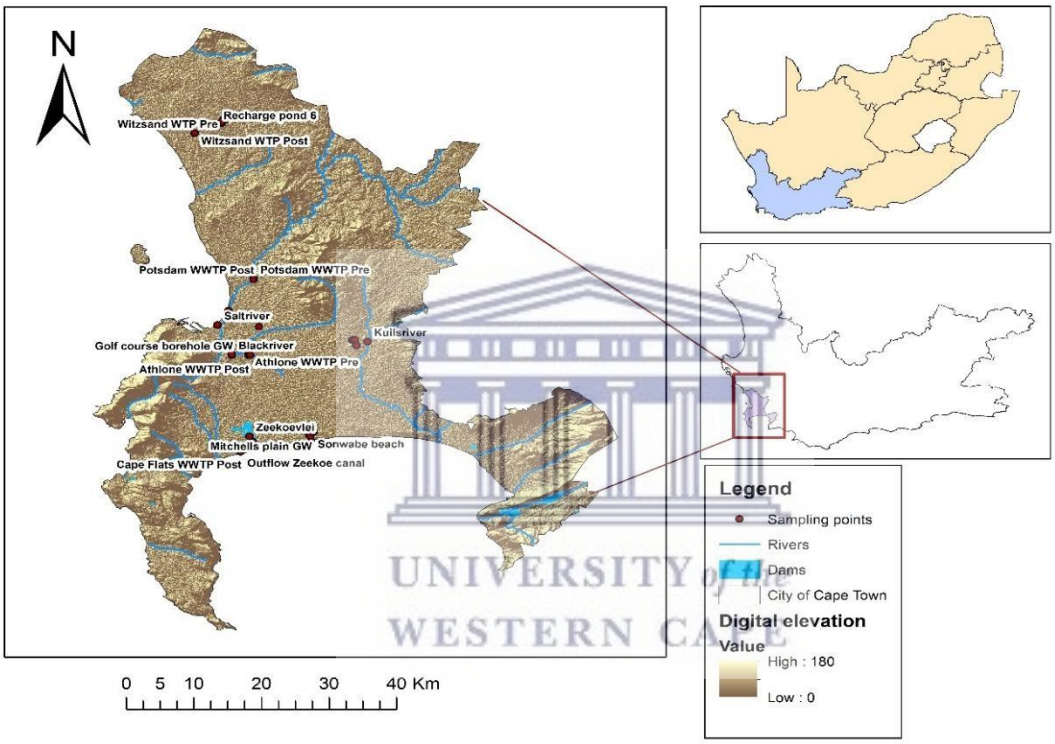


*Figure 3: Map displaying sampling locations through the City of Cape Town.*

## 3.3 Description of treatment process in the WWTPs around the City of Cape Town

The major aim of South African wastewater and fresh water treatment is to minimize contaminants (solids, organic matter, nutrients, and microorganisms) to fulfil the Department of Water and Sanitation's standard standards (DWS). That is, the released effluent should be of

a quality that does not pollute the environment or pose a safety concern. Wastewater is water given by communities after it has been used in different ways (showering, bathing, washing dishes and clothing, and using the toilet) that render it unfit for use prior to treatment (Tchobanoglous et al., 2003; Metcalf and Eddy, 2014). Pollutant levels differ based on the kind of wastewater (black water, organic waste, grey water, and rainwater). Wastewater in the form of sewage (black water) has an unpleasant stench, which is usually caused by hydrogen sulfide (Riffat, 2013). Wastewater includes dangerous bacteria, viruses, and worms that dwell in human intestines, as well as nutrients (phosphorus, nitrogen, ammonium, and so on) that stimulate aquatic flora growth. Treatment is required to protect the environment and public health for these reasons. From the early 1900s until the early 1970s, the key concerns were:

(i)the removal of suspended particles and floatable debris,

(ii) the removal of pathogenic organisms, and the removal of harmful organisms.

(iii) Handling biodegradable organics. Since 1980, the emphasis has also shifted to the elimination of elements that can be hazardous to public health and the environment (Tchobanoglous et al., 2003; Riffat, 2013). The standards defined by the Department of Water and Sanitation (DWS) for releasing wastewater into the environment after treatment are South African National Standard (SANS 241) (SANS, 2011) and National Water Act (NWA) No 36 (NWA, 2008). (Parrett et al., 2000)



The City of Cape Town runs 17 wastewater treatment plants and six smaller facilities. Before being safely released into rivers, canals, vleis, and subterranean water sources known as aquifers, or the sea, wastewater is subjected to a stringent treatment procedure. Water from bathtubs, showers, sinks, toilets, and other drains is referred to as wastewater. It may also include industrial liquid waste, particularly if it is draining from the city's industrial zones. Wastewater treatment plants are responsible for cleansing filthy water and making it safe for discharge into the environment.

### 3.3.1 City of Cape Town wastewater treatment methods

To clean the city, Cape Town's wastewater treatment facility employs a variety of methods. Most treatment works, on the other hand, contain the following treatment phases (City of Cape Town website, 2020):

- Pre-treatment and screening to eliminate big foreign matter such as rags, paper, plastic, and leaves, among other things.

- Grit, sand, tiny stones, and broken glass filtration It is critical to remove them as soon as possible so that they do not harm pumps and other equipment later in the treatment process.

- Primary sedimentation to remove solids, which are then treated as sludge separately. This cycle also removes fats, greases, and oils.

- Activated sludge, bio-filtration, and rotating biological contactors are examples of secondary treatment techniques utilized in Cape Town works. Some small rural works also make use of oxidation ponds. In this treatment phase, microscopic organisms are used to process the wastewater to remove organic matter and various chemicals such as ammonia, nitrogen, and phosphorous.

- Disinfection using chlorine, ozone, or ultraviolet light to kill bacteria, viruses, and other pathogens. In some works, the final treated effluent is allowed to remain in maturation ponds, which improves the quality further before it is discharged.



### 3.3.2 Activated Sludge

The effluent from the main clarifier chamber is poured into an aeration basin during the activated sludge process. The initial section of the aeration chamber is an anoxic zone, which is populated with microorganisms that survive in low-oxygen environments. The oxic zone is the second half of the aeration chamber, where diffusers split up the air to overdose the microorganisms with oxygen. It is critical to consider how long the wastewater remains in the aeration basin, a period known as the solids retention time (SRT), as well as to assure continuous electricity for the diffusers, including backup generators in the event of a power outage (LA Boyd & Mbelu, 2009).

### 3.3.3 Bio-filter

A bio-filter is another typical form of secondary treatment (also known as trickling filters and rotating biological contactors). According to Boyd and Mbelu (2009), these procedures, "To remove organic debris from wastewater, use microorganisms that grow on a media, such as stones and discs. They may also be utilized to produce nitrification, which is the process of converting ammonia to nitrate/nitrite." The bio-filters, like any wastewater treatment equipment, must be maintained. Following the biological processes in both activated sludge and bio-filters, additional settling tanks (sometimes referred to as secondary sedimentation) are used to remove more of the suspended solids; these final clarifiers function similarly to the primary clarifiers, serving to clarify the effluent before it is discharged into a water resource (Boyd and Mbelu, 2009).

### 3.3.4 Ponds or constructed wetlands

A third typical method of secondary treatment is pond variations or created wetlands. The wastewater is filtered as it travels through a granular material bed, where reeds consume nutrients and convert ammonia to nitrogen gas. Ponds can efficiently polish and disinfect. Ponds and wetlands can act as a buffer in the event of a breakdown at the wastewater treatment plant, in addition to increasing the quality of the final effluent.

### 3.3.5 Tertiary treatment

Tertiary treatment is simply additional treatment performed after all previous procedures have been completed to eliminate contaminants or particular pollutants. In South Africa, tertiary treatment commonly includes phosphorus and nitrogen removal, as well as chemical disinfection. Tertiary treatment may remove practically all contaminants from sewage but is typically costly. Tertiary treatment is frequently employed to remove phosphorous or nitrogen, both of which contribute to eutrophication. To eliminate the burdens, conventional secondary treatment techniques are frequently changed. In certain WWTPs, chemicals are added to the effluent to cause the phosphates to coagulate and clear. Boyd and Mbelu (2009) include numerous phosphate removal agents, including ferric chloride, ferric sulfate, ferrous sulfates, and aluminum.

The quality of treated wastewater is monitored every week to ensure compliance with licensing conditions and national standards. Treated wastewater is re-used for irrigation or industrial use in the city. Cape Town's wastewater treatment works use different processes to clean the water (Table 4) (City of Cape Town, 2020)

*Table 4: Treatment methods at different wastewater treatment plants.*

| Name of wastewater treatment plant | Treatment method | Capacity (Ml/d) | Type of plant | Outflow |
|---|---|---|---|---|
| Bellville wastewater treatment plant | MBr, UV treatment, Chlorination with primary and secondary settling tanks. | 54.6 | Activated sludge | Kuilsriver |
| Mitchells plain wastewater treatment plant | Chlorination with primary settling tank. | 45.0 | Activated sludge | Sonwabe beach |
| Cape flats wastewater treatment plant | Chlorination with primary, secondary settling tanks and sludge removal | 200.0 | Activated sludge | Zeekoe Canal / Muizenburg beach |
| Athlone wastewater treatment plant | Chlorination treatment Primary and secondary settling tanks | 105.0 | Activated sludge | Blackriver / Saltriver |

| | | | | |
|---|---|---|---|---|
| Potsdam wastewater treatment plant | Phosphorous and chlorination treatment | 47.0 | Activated sludge | Diepriver/lagoon beach |
| Wesfleur wastewater treatment plant domestic waste | University of Cape Town process (anaerobic, anoxic and aerobic) Chlorination treatment | 8.0 | Activated sludge | Recycle basin 6<br>Atlantic<br>Recharge Ponds |
| Wesfleur wastewater treatment plant industrial waste | Modified Ludzack Ettinger process (anoxic and aerobic) | 6.0 | Activated sludge | Coastal recharge basin |

### 3.3.6 The Modified Ludzack Ettinger (MLE) process

This is one of the industrial waste treatment technologies utilized at the Wesfleur WWTP. The Modified Ludzack-Ettinger (MLE) procedure was designed to remove BOD, ammonia, and nitrate/nitrite simultaneously. The technique utilizes both an anoxic and aerobic zone. In the aerobic zone, nitrification (ammonia removal) happens. The nitrate-rich mixed liquor is recycled to the anoxic zone (through the internal recycle) for denitrification. IRQ rates typically vary from 200 to 400% of the process influent flow rate. Depending on the wastewater influent parameters, the MLE process can achieve a Total Nitrogen discharge of 6 to 8 mg/l. 2017 (Biochem Technology, 2017)

### 3.3.7 University of Cape Town (UCT) process

This is one of the treatment technologies used to handle residential waste at the Wesfleur WWTP. The authors offer a pilot-scale prototype activated sludge system that combines the University of Cape Town (UCT) concept with the step denitrification cascade for the removal of carbon, nitrogen, and phosphorus. An anaerobic selection and stepwise feeding in three

identical pairs of anoxic and anoxic tanks comprise the experimental setup. Raw wastewater with influent flow rates ranging from 48 to 168 l was supplied to the unit at hydraulic residence times (HRTs) ranging from 5 to 18 h and distributed to the anaerobic selection, second and third anoxic tanks in percentages of 60/25/15%, 40/30/30%, and 25/40/35%, respectively (influent flow distribution before the anaerobic selector). The findings for the entire study period indicated high removal efficiency of organic matter of 89% as total chemical oxygen demand removal and 95% removal for biochemical oxygen demand, 90% removal of total nitrogen removal by denitrification of 73%, mean phosphorus removal of 67%, and outstanding settling ability. 2017 (Biochem Technology)

## 3.4 Infiltration of storm water into the Zeekoe Catchment

The Zeekoe Catchment is now largely delineated by storm water drains, and the region served is a big shallow lake known as Zeekoevlei Lake, which is part of the catchment. The Great Lotus River, which was built primarily to drain the Cape Town International Airport, also drains the surrounding industrial region (Boquinar Industrial Area), as well as densely inhabited informal settlements, light industry, and low-middle class households. It also runs around the Philippi Horticulture Region (PHA), an urban agricultural area in Cape Town, along the route. Because of the places it drains, particularly the informal settlements, which are a source of grey and black water infiltration into storm water drains, the Great Lotus has the largest pollution load of all the streams in the area. The discharge from Zeekoevlei enters the Zeekoe Canal, which runs southwards beside the Cape Flats Waste Water Treatment Works before emptying into the sea (Muizenburg beach). All drains in the Zeekoe Catchment are maintained on a regular basis to remove excess plant growth, litter, and sediment deposits, with the goal of minimizing sediment and solid waste deposits and enhancing channel flow for flood control.

*Table 5: Witzand water treatment plant and Zeekoevlei information.*

| Name of plant | Treatment method | Capacity (Ml/d) | Water source |
|---|---|---|---|
| Witzand water treatment plant | pH level adjustment (sodium hydroxide), ion-exchange softening and chlorine disinfection | 14.0 | Witzand Wellfield (30 boreholes) Atlantis Aquifer |
| Zeekoevlei nature reserve lake (Zeekoe catchment) | Wetland filter | | Boquinar industrial area and populated informal settlements. |


*Figure 4**: Sampling point at Zeekoevlei.***

## 3.5 Wesfleur WWTP and Witzand water treatment plant recharge water system.

The primary aquifer at Atlantis (Western Cape, South Africa) is ideally suited for water supply and the indirect recycling of urban storm water runoff and treated domestic wastewater for potable purposes. The relatively thin, sloping aquifer requires careful management of the artificial recharge and abstraction for balancing water levels.

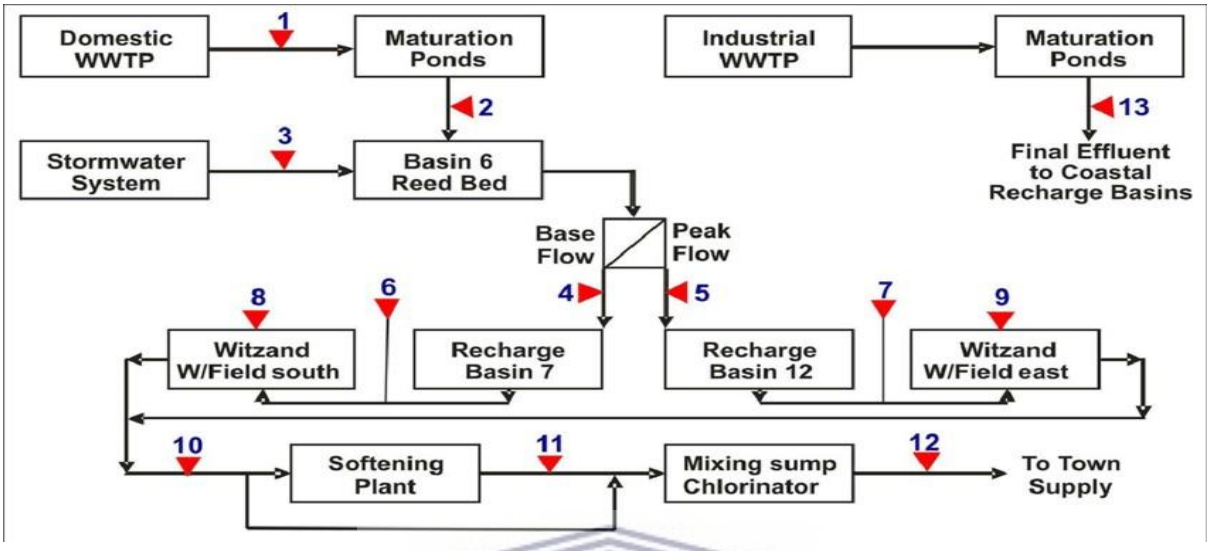


*Figure 5: **Schematic layout of the Atlantis water supply scheme showing monitoring points. (Tredoux et al., 1980)***

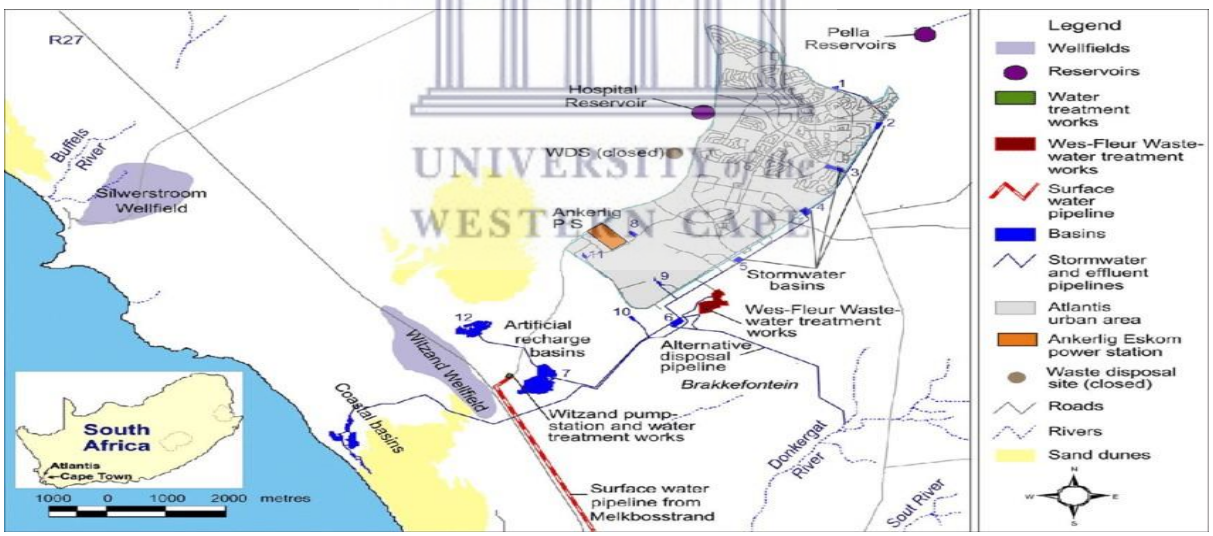


*Figure 6: **Location and layout of the Atlantis water supply system (Tredoux et al., 1980)***

Atlantis' indirect recycling of water began shortly after the town's construction began in the mid-1970s. This was prompted by pilot-scale experiments of artificial groundwater recharge as a technique of recycling treated residential wastewater on the Cape Flats (Tredoux et al., 1980).

- Initially, all wastewater was processed in a single facility, and all treated effluent was utilized for artificial recharge. Domestic wastewater is subjected to comprehensive secondary treatment, including nitrification and denitrification procedures (anaerobic-anoxic-aerobic). Secondary settling tank effluent is refined in a succession of maturation ponds.

- Storm water runoff created by urbanization is collected in large quantities via the storm water collection system, which consists of 12 detention and retention basins (numbered in Fig. 6) and accompanying interconnecting pipes. Detention basins 1–4, 8, and 11 were meant to be primarily dry and function as peak flow reduction basins, whereas basins 5, 6, 9, and 10 were designed to be wet basins with reed beds.

- In Basin 6, treated domestic effluent (from maturation ponds) is mixed with low-salinity urban storm water runoff before being dumped into the main recharge basins, namely retention Basins 7 and 12, for artificial replenishment up-gradient of the Witzand Well field (Fig. 6)

- More saline-treated industrial effluent is released into coastal recharge basins (CRBs), where it seeps into the ocean via the subsoil. This is a precaution against potential saltwater incursion. Groundwater is extracted from the well fields of Witzand (natural and recharged water) and
Silwerstroom (natural groundwater) (Fig. 6)



- The natural characteristics of the aquifer material influence groundwater quality; for example, calcrete and calcareous sands add high hardness to the water, vegetation contributes significantly to dissolved organic carbon, pyrite (and ferric rete in some areas) adds measurable dissolved iron, and high salinity occurs in isolated areas. Ion exchange softens a portion of the flow by reducing its hardness. Iron is also eliminated in the process, but the organic carbon remains. (Tredoux et al., 2011).

*Figure 7**: Atlantis water system basin 6 meeting with a combination of Basin 7 and 12.***

## 3.6 Landfill sites and groundwater pollution

Landfill leachates have been discovered as a substantial source of pharmaceutical and personal care products (PPCPs), which might endanger groundwater and surface water nearby. These medications wind up infiltrating the environment and, eventually, groundwater. Typically, expired or unused pharmaceuticals are dumped in landfills. Because wastewater treatment facilities are not prepared to remove medicinal pollutants from the liquid, this is considered a better method of disposal than flushing. Three landfill sites in the City of Cape Town will be evaluated, and samples from these boreholes will be obtained to determine the presence of antiretroviral medications. These landfills are the Bellville South dump, the Visserhok landfill, and the Coastal Park landfill.

*Table 6: landfill sites sampled around the City of Cape Town.*

| Landfill name | location | Borehole water level (m) | Type of disposal |
|---|---|---|---|
| Bellville south landfill | Bellville south | 6.0 | No longer operational was used for disposal of general waste in the past. Now it is only used for disposal of builders waste. |
| Visserhok landfill | Off N7, Frankdale Road, near Table View | 7.3 | Disposal of general and low to medium hazardous waste which cannot be reused or recycled. |
| Coastal park landfill | Baden Powell Drive, Muizenberg | 5.6 | Disposal of general waste which cannot be reused or recycled |

*Figure 8**: Visserhok landfill** site*

Large amounts of pharmaceuticals are being discharged continuously from wastewater treatment plants into African rivers due to the inefficiency of employed sewage treatment processes. Large portions of African communities do not even have proper sanitation systems which result in direct contamination of water resources with human waste that contains pharmaceutical constituents among other pollutants.

# Chapter 4 Research design and methodology

## 4.1 Introduction

## 4.2 Research design

This chapter describes the research design of the investigation and the methodology that was applied to achieve the aim. The research design is influenced by the objectives of this study which provide an answer to the research question. The research design utilized the relevant literature and attempts to explain the effectiveness of liquid chromatography coupled with mass spectrometry. The purpose of this chapter is to convey the research set up which is an attempt to determine antiretroviral drugs in water samples. This chapter concludes with establishing quality assurance, limitations, and ethical considerations undertaken during this study. This is done to encourage the validity and reliability of this report. The methodology includes all procedures and techniques for acquiring and analyzing data.

### 4.2.1 Research design approach

The study used a quantitative experimental design technique and a case study approach, which were influenced by a thorough peer review of the publication on the use of liquid chromatography (LC) combined with mass spectrometry (MS) to quantify the concentration of ARVs. This sort of study provides for a better knowledge of solid phase extraction (SPE) and LC/MS as methodologies for analyzing antiretroviral medicines in natural waterways, and has previously been applied in the detection of Oseltamivir Carboxylate in surface and wastewaters (Soderstrom et al., 2009 and Ghosh et al., 2009). The investigation includes hydro chemical monitoring of wastewater, surface water, and groundwater in order to assess quantities of Nevirapine, Lopinavir, Ritonavir, Tefonavir, and Efavirenz from various samples employing and analytical techniques. The LC/MS will be used to provide concentrations of antiretroviral drugs which will be used to determine the distribution of these drugs in the environment.



### 4.2.2 Analysis of sampling design and unit of analysis

Samples were collected using the grab sampling techniques at the wastewater treatment plants, landfills, storm water, vlei and boreholes around the Western Cape region, they were collected on a seasonally basis from the 19$^{th}$ of February 2021 to the 22$^{nd}$ of September 2021.These samples were then taken to the lab at the University of Stellenbosch clinical pharmacology

department to be evaluated using solid phase extraction for the extraction and isolation. Liquid chromatography coupled with tandem mass spectrometry was used to determine the analyte and therefore determine the concentration of the ARVs in the different samples provided. The study sites chosen for the present study were chosen based on the infection rate of HIV/AIDS and the amount of people that are on the ART program in each area. The study used samples from wastewater before and after treatment had occurred, rivers, canals, lagoon, beaches and boreholes of landfill sites used around the City of Cape Town. The Witzand water treatment plant in Atlantis was included in this study because of the interaction between the areas recharge system, groundwater and wastewater in that area.

## 4.3 Sampling design

Water samples were collected at various sites across the City of Cape Town for different seasons, sampling took place 4 times summer, autumn, winter, and spring. Water samples collected were natural waters (surface water), drinking water (from Water treatment plant), (raw wastewater) pretreatment after the screening, grit removal, final effluent from WWTPs after disinfection, and groundwater (landfill sites boreholes). It became clear that water samples had to be collected as close as possible in time for analyses, and had to be analyzed within a matter of days, to avoid any contamination or reactions within the samples. Water samples were collected in 500 mL plastic bottles that had been rinsed with distilled water. The pattern of sampling was determined firstly by the quantity of the ART program around the areas around the City of Cape Town such as Mitchell's Plain, Bellville, and the Cape flats, these areas have a high rate of HIV/AIDS within the city and also a high antiretroviral treatment program there is a higher possibility of the presence of ARVs in their water bodies. Other areas that were assessed were areas that had wastewater plants and landfill near them this was advantageous because the groundwater from the landfills and the flow pattern within each WWTP could be assessed from the treatment process to the discharge area that would lead the water back into the environment.

### 4.3.1 Sampling collection pattern

In each of the wastewater plants water samples were collected within 3 sections of the plant, first one was the raw wastewater after screening and grit removal this was wastewater that was

directly from the surrounding areas and facilities around the plant, and the industrial sector no treatment had been done at this point only sediments had been filtered out and screening had taken place so this is the pretreatment (Primary treatment). The second sample was after treatment (disinfection and filtration) had occurred the treatment used for each wastewater treatment plant was different some used UV filtration and chlorination while others also included MBR and phosphate treatment. The third water sample was taken at the discharge point of the wastewater plant this is the section where the treated water is discharged back into different water bodies. The fourth sample was collected in the different water bodies containing the final effluent and these water bodies included canals, vlei and rivers which eventually flowed into the ocean. Water from boreholes within or near the wastewater plant were taken, most of these boreholes are situated in landfill sites nearby such as Bellville south landfill, Visserhok landfill site and Coastal Park landfill site in Muizenburg and the Wavecrest primary school. The Witzand water treatment plant in Atlantis was also sampled to determine if there is any ARVs in water that is used for human consumption, this area is special because of the recharge system that the WWTP and WTP uses.

*Figure 9**: Collecting wastewater samples at the Bellville WWTP.***

## 4.4 Water sample collection method

A total of 33 samples were collected in each season these samples included groundwater samples, wastewater samples, and surface water samples, the first and second sets of samples were taken during summer and autumn (January and May 2021), and the third and fourth during winter and spring (July and September 2021) for analysis. Groundwater samples were sampled at different landfill sites boreholes, school boreholes, and a water treatment plant around the city (n=7). Wastewater samples were taken in different wastewater treatment plants, samples before and after treatment has occurred (n=18). Surface water samples were taken from a vlei in the Zeekoevlei nature reserve that gets storm water from the nearby community and industrial structures which also included, rivers, and ponds that got outflows from the wastewater treatment plant (n=8). The electrical conductivity, pH, and temperature were

monitored as the water was discharged at each sampling site using a Martini Mi 806 multimeter probe which was calibrated daily. A 500ml clear round plastic bottle with a plastic cap was used to collect all the water samples.

Samples from wastewater plants were collected by grab sampling from approximately 30 cm below the water surface using a bucket and a bailer which was washed with distilled water at every sampling point., samples were sometimes collected by hand in shallow areas that didn't require the use of a bailer or bucket mostly in the rivers or by the taps at the water treatment plant. Drinking water from taps was collected at the water treatment plant by hand and borehole water from groundwater sources was also collected by using a submersible pump in some areas and using a bailer in other areas when the pump malfunctioned. The water was allowed to flow for about 30 seconds from taps and about 10 minutes from boreholes when using a pump or discard 10 collections of the bailer before a sample was collected. After sampling all samples were labeled and stored in a cooler box with ice to limit the fractionation and or biological impacts. The water samples were then transported to the University of the Western Cape, Department of Earth Sciences refrigerator which has a temperature of 3 degrees Celsius before analysis, then later on samples were dispatched to the University of Stellenbosch for extraction and analysis.

*Table 7: Materials used for sampling*

| | |
|---|---|
| 1 | Bailor |
| 2 | 25l Bucket |
| 3 | Measuring tape |
| 4 | Martini Mi 806 multi meter probe |
| 5 | 500ml plastic bottles |
| 6 | Plastic bags |
| 7 | 20l cooler box |
| 8 | Ice |
| 9 | Submersible pump |



### 4.4.1 Wastewater

Wastewater samples were collected at different wastewater treatment plants around the city of Cape Town. A raw sample was collected this is wastewater that enters the plant only grit removal has occurred at this point, then the second set is taken after treatment has occurred and the wastewater is deemed safe to be released into the environment. The third set of samples is taken at the discharge point at the plant this is the point where the plant releases treated water into different water bodies such as canals, rivers, and lagoons. A bailor was used to collect some of the samples where the wastewater was at an unreachable level, the bailor would reach water level and then pull out quickly the wastewater was then poured into 500ml plastic bottles. Some of the samples were collected directly from the plant's infrastructure 30cm below the water surface using a 500ml plastic bottle.


*Figure 10**: Industrial wastewater samples before and after treatment at the Wesfleur WWTP.***

### 4.4.2 Surface water

Surface water samples were collected from the Kuilsriver, Zeekoevlei nature reserve, Zeekoe canal, Lagoon beach, Sonwabe beach, Blackriver, Saltriver, and Atlantis recharge pond 6. Samples were collected using the grab sample method on a seasonal basis from January to September 2021. A bailor was used and lowered into the Zeekoevlei, Zeekoe canal, Blackriver, Pond 6, and Saltriver into the deepest flowing part of the waters. Once the bailor was filled beneath the water surface, the bailor was lifted quickly, and the sample was poured into 500ml plastic bottles. The lagoon, Sonwabe beach, and Kuilsriver samples were collected directly 30cm below the water surface using 500ml plastic bottles.

*Figure 11: **Sample collection at the Kuilsriver***

### 4.4.3 Groundwater

Groundwater samples were collected from the Bellville South landfill site borehole, Visserhok landfill site borehole, Coastal park landfill site borehole, Wavecrest Primary School borehole, Milnerton residential borehole, Ronderbosch golf course borehole, and Witzand water treatment plant before/after treatment. The samples were collected using the grab sample method on a seasonal basis from January to September 2021. A submersible pump was sometimes used for the borehole samples; the borehole was purged for 10 minutes to remove any stagnant water. Then samples were decanted into the 500ml plastic bottles using the pump, during pump failure the bailor was used to collect water from the borehole. To remove stagnant water from the borehole the bailor is filled 10 times and the water is discarded before being decanted into the plastic bottles. At sites where the water was discharged by taps, water is left to run for 10 minutes to remove any stagnant before the sample is sampled.

## 4.5 Sample Preparation and Solid Phase Extraction

All 33 samples were firstly filtered by taking some water from the sample using a 10ml syringe attached to a nylon syringe filter with a 0.22 µm pore size. The filtered water was then transferred into a 5ml glass test tube with a cap. Before transferring the filtered sample containing the analyte into the Sep-Pak Vac 1cc (100mg) tc18 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 1 ml of deionized water, this was repeated twice using a pipette, and this process allows the sorbent to retain the analyte during the SPE procedure. After doing the above two steps the samples were then loaded into the cartridges using a 1000 µl pipette with a new pipette tip for every sample. The loading of the cartridge was repeated three times to ensure enough analyte was trapped by the sorbent. After the loading procedure, the tc18 cartridges were washed with deionized water, to remove any unwanted interference still present in the sorbent. The elution process was the last step of the SPE procedure to remove the analyte from the sorbent, this was done using 1ml methanol whereby the analyte were then transferred into glass tubes. The eluates were then evaporated to dryness using a rotary evaporator in a MiVAC Duo concentrator for 40 minutes at 45 degrees Celsius. The analyte was then reconstituted with 200 microliters of mobile phase solution (1:1 methanol and deionized water) and injected into vials for the LC/MS analysis.

Table 8: Materials used for the Solid Phase Extraction

| | |
|---|---|
| 1 | 10 ml syringes |
| 2 | Nylon syringe filter |
| 3 | 5 ml glass test tube |
| 4 | Sep-Pak Vac (100mg) 1cc tc18 cartridges (waters, microsep) |
| 5 | Methanol |
| 6 | Distilled water (Synergy, Millipore) |

| | |
|---|---|
| 7 | 1000 µl pipette |
| 8 | Pipette tips |
| 9 | Nevirapine reference standard |
| 10 | Efavirenz reference standard |
| 11 | Tenofovir reference standard |
| 12 | Lopinavir reference standard |
| 13 | Ritonavir reference standard |

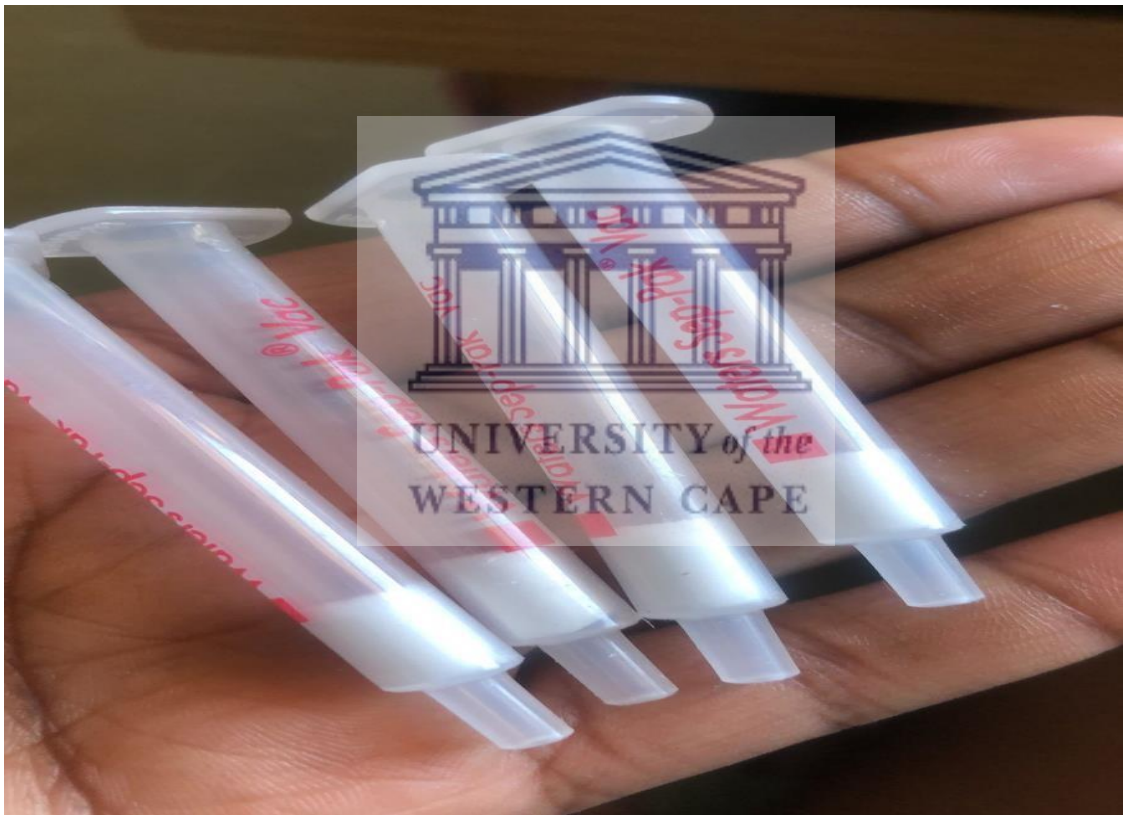


*Figure 12**: Sep-Pak tc18 cartridges (Company-Water, Microsep)***

### 4.5.1 Liquid chromatography coupled with mass spectrometry analysis

The procedure before analysis using LC/MS was first to create 5 standard reference materials for each pharmaceutical of interest to set up a calibration line with known analyte concentration. This was done by weighing 1mg of each standard reference material and placing it into vials that were mixed with methanol. The vials were then sonicated for 1 minute and then vortexed for 30 seconds to ensure adequate mixing of the solution.

All calibration curves for the analyte of interest obtained a regression square of 0.99, suggesting satisfactory calibration curves. The Shimadzu Triple Quadrupole LCMS-8040 was used to construct the LC-MS/MS technique, which included the use of LC-20ADXR binary pumps, a SIL-20ACXR auto sampler, a CTO-20A column oven, and Lab Solutions software (Shimadzu Corporation, Kyoto, Japan). An Agilent Poroshell 120EC-C18 column (3.0 x 100 mm, 2.7m; Agilent Technologies Inc., California, United States) was used for LC-MS/MS separation. The solvents, which contain the mobile phases, were pushed through the liquid chromatography apparatus. The instrument's process entailed injecting 3 ml of sample concentration into 0.2 ml (15-fold concentration) of mobile solution. To establish carry over to the highest standard, blank samples were injected following the highest standard. Using two procedures, a volume of 10 l of each of the 33 samples was injected into LC-MS (the one in the negative ion mode and the other in the positive ion mode). So the molecular weight and structure of the compound influence whether it can attract or lose a proton, and it has a preference for how the compound wants to ionize, which is regulated to stimulate the ionization in the proper way using various combinations of mobile phases. Because chemicals that ionize in the negative ion mode require different mobile phases than compounds that ionize in the positive ion mode, two independent methodologies were developed for this investigation.

The first approach was the negative ion mode, which consists of two mobile phases: mobile phase A is 10 mm ammonium acetate in water, and mobile phase B is acetonitrile. The column was an infinite Lab Poroshell 120 EC-C18 (3.0 X 100 mm 2.7 m/z) at 30 degrees Celsius. The gradient is 60 to 95% B over 3 minutes, 95% up to 3.5 minutes, 95 to 60% up to 4 minutes, and 60% till 7 minutes. 0.5 ml/min was the flow rate.

The second approach was the positive ion mode, which consists of two mobile phases: mobile phase A, which contains 5mm of ammonium formate with 0.1% formic acid in water, ACN; 95;5, and mobile phase B, which contains 2mm of ammonium formate with 0.1 formic acid in water, ACN;95;5. The column utilized is the inifinityLab Poroshell 120 EC-C18 (3.0X100mm, 2.7um) at 30 degrees Celsius. The gradient is 10 to 95% B over 5 minutes, 95% up to 5.5 minutes, 95 to 10% up to 6 minutes, and 10% till 9 minutes. The gradient of flow was 0.45 ml/min.

The samples were then injected into a mass spectrometer and separated using reversed-phase chromatography. The flow was passed through three quadrupole mass analyzers: the first quad looked for the precursor mass of the compounds and ignored any other compounds present; the second quad had the presence of argon gas molecules, so the masses collided with the argon mass molecules and were broken up into fragment ions; and the third quad collected those fragment ions and used them to improve the specificity of the method, so that each compound had a unique figure print. The detector then sent a signal to the program, which generated peak graphs based on the mass to charge ratio. This is the molecular ion ratio, which indicated which drug was present in each sample.

*Table 9: Mass transitions*

| Antiretroviral Drugs | Mass to charge ratio |
|---|---|
| Tenofovir | 287.9□176.0, 159.0 |
| Nevirapine | 267.0□226.1, 80.1 |
| Lopinavir | 629.3□156.1, 429.2 |
| Ritonavir | 721.4□296.1, 268.2 |
| Efavirenz | 314.0□244.0, 242.1 |

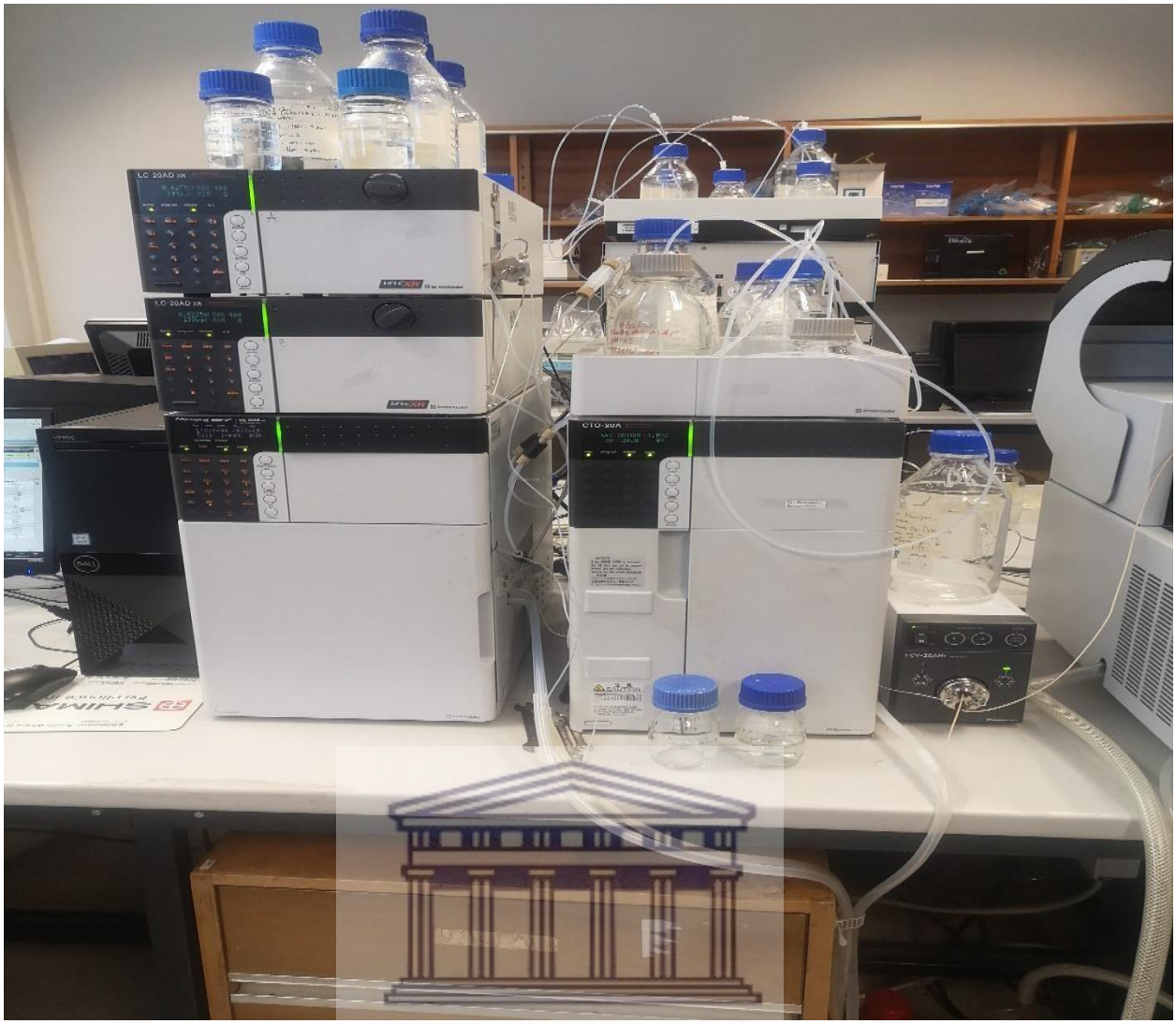

*Figure 13**: Liquid Chromatography instrument***


*Figure 14**: LCMS-840 Shimadzu Triple Quadrupole***

# Chapter 5 Results and Discussion

## Introduction

Chapter 5 discusses the results which were obtained during the quantitative assessment of antiretroviral drugs in water bodies around the Western Cape. This chapter addresses objective 3 the analysis of samples. This focuses on applying liquid chromatography and mass spectrometry. The central argument is the presence and concentration of antiretroviral drugs in different water bodies around the City of Cape Town and the effectiveness of wastewater and water treatment plants in removing these drugs before the water is discharged into the environment. This section will highlight the spatial distribution and the concentration of these drugs around the study areas assessed.

## 5.1 Identifying and prioritizing ARV in South Africa.

South Africa has the biggest HIV epidemic in the world with an estimated prevalence of 12.7% of the overall population (Statistics South Africa, Mid-year population estimates 2019, Statistical Release PO302, www.statssa.gov.za). In South Africa, the most often used drugs include NRTIs such as Zidovudine, Stavudine, Didanosine, Tenofovir, Lamivudine, and Abacavir, NNRTIs such as Efavirenz, Nevirapine, and PIs such as Lopinavir, Ritonavir, Saquinavir, and Nelfinavir. This study only examined five of these prescription drugs: nevirapine, Lopinavir, ritonavir, Tenofovir, and Efavirenz. The country has made great strides in reducing the HIV/AIDS pandemic by implementing the largest antiretroviral treatment (ART) program globally. The majority of patients are on a regimen that includes zidovudine, Tenofovir, lamivudine and Abacavir, but in order to make the regimen simpler, more effective and cheaper, in 2015 a fixed dose combination (FDC) of antiretroviral drugs (ARVDs) was introduced as the first-line treatment of HIV positive patients in addition to other commonly used ARVDs. FDC involves a single pill that contains a combination of two or more drugs. The single pill contains a combination of Tenofovir, emtricitabine and Efavirenz (NDoH & Sahivsoc, 2015). In South Africa over three million people are on ART (UNAIDS, Global AIDS Update 2016). If one assumes a daily dose of HIV-ARVDs of 991 mg/day/person (mean, range 590–1996) (Swanepoel et al., 2015) that would equate to a total of over 1085 metric tons

of HIV-ARV compounds ingested per year in South Africa. The excretion of HIV-ARVDs varies depending on the compound.

The excretion of HIV-ARVDs varies depending on the compound. Irrespectively, South Africa still has one of the highest HIV incidence rates in the world, the largest treatment program, and therefore the greatest consumption of antiretroviral drugs per capita (Wood et al., 2014), with prescribed amounts of up to several tons per year (Swanepoel et al., 2015). The national rollout of antiretroviral began in 2005, with the objective of one service point in each of the 53 districts of South Africa. Since then, it has improved the quality of life and the historical pattern of mortality in South Africa. However, there may be subtle, yet unquantified effects and processes that need to be better understood. These include environmental concentrations of the drugs, secondary human exposures, effects on aquatic life, and social considerations.

## 5.2 Source emission of antiretroviral drugs into different water bodies

ARVDs, like other pharmaceutical and personal care products (PPCPs), are emerging contaminants that are ultimately discharged into wastewater streams, fresh water and groundwater. In communities with inadequate sanitation and/or no centralized WWTPs, infiltration from pit latrines and direct discharge of untreated wastewater and excreta to aquatic and terrestrial habitats constitute a substantial entry route for pharmaceuticals into the aquatic environment (Ngumba et al., 2016b; K'oreje et al., 2016; Rehman et al., 2013; Madikizela et al., 2017). Pharmaceuticals may be discharged into the environment through various routes including domestic (solid) and sewage (liquid) wastes. Therefore, it is incumbent to investigate water quality up- and downstream from wastewater treatment plants (WWTPs), as well as other factors that may play a role in their release and changes (Refer to figure 1).



Alternative decentralized ecological sanitation methods must be investigated in order to reduce environmental pollution and improve nutrient recycling (Simha and Ganesapillai, 2016; Udert et al., 2016; Richert et al., 2010). The high concentrations seen in surface waterways can be attributable to the direct release of untreated residential trash and effluent from wastewater treatment plants. Previous research by C Swanepoel, H Bouwman, R Pieters, and C Bezuidenhout discovered that the primary pathway for pharmaceutical chemicals to enter the

aquatic environment is through human urine. The high concentration of medicines in urine indicates that source separation offers the benefit of pooling the bulk of pharmaceutical residues into tiny amounts that may be easily disposed of.

The high concentrations seen in surface waterways can be attributable to the direct release of untreated residential trash and effluent from wastewater treatment plants. The high concentration of medicines in urine indicates that source separation offers the benefit of pooling the bulk of pharmaceutical residues into tiny amounts that may be easily disposed of. To safeguard both human health and the environment, initiatives to enhance centralized and decentralized wastewater management should be prioritized.

## 5.3 Spatial distribution of antiretroviral drug around the City of Cape Town

The total bioavailable ARV compounds in surface water and effluents must be determined since this reflects the quantity of compounds that might possibly be taken up by biota in water. HIV-ARV medications were discovered in varying amounts in several water sources in Germany. 33 samples were taken from different areas around the City of Cape Town which included wastewater treatment plants, water treatment plants, rivers, landfills, and boreholes. These samples included surface water, groundwater, and storm water, four sets of samples were taken the first set was taken in summer on 3 consecutive days beginning from the 13th of January 2021 to the 15th of January 2021. The second set of samples was taken in autumn on 3 consecutive days beginning from the 12th of May 2021 to the 14th of May 2021. The third sample was taken in winter on 3 consecutive days beginning from the 14th of July 2021 to the 16th of July 2021. The last set of samples was taken in spring for 3 consecutive days beginning from the 8th of September 2021 to the 10th of September 2021. The analysis of this set of samples was done by the clinical pharmacology department at the University of Stellenbosch.



The drugs that were assessed were present in a majority of the sampling sites with varying concentrations. Several factors contribute to these concentrations such as the number of people that are taking ARVs in the area, treatment methods used at each wastewater treatment plant, and weather conditions that can affect how the drugs behave in different water sources. Other factors that influence the concentration of the drugs in different water bodies include the pH,

electrical conductivity, and total dissolved solids of the samples taken. The presence of ARVDs has been reported in surface waters (rivers and man-made lakes [dams]), as well as wastewater influents and effluents in Africa (Wood et al. 2015; Ngumba et al. 2016b, 2020; Abafe et al. 2018; Nibamureke et al. 2019). Surface waters around the globe have scarcely been studied for the presence of ARVDs; however, ARVDs have been detected in rivers and lakes in South Africa, Kenya, Zambia, and other parts of the world (Aminot et al. 2015; Funke et al. 2016; K'Oreje et al. 2016; Wooding et al. 2017, Mosekiemang et al. 2019; Madikizela et al. 2020; Ngumba et al. 2020).

### 5.3.1 Nevirapine spatial distribution

The amounts of nevirapine in surface water varied from 0.0855 ng/l to 6.28 ng/l, with the peak values occurring during the wet season in July and September 2021. The greatest nevirapine concentrations were recorded during the rainy seasons, whereas concentrations were largely low during the dry seasons. Because it is resistant to biodegradation (Vankova M, 2011) and the chlorination process used in wastewater treatment plants in the country results in the formation of various NVP degradation products, some of which have similar antiviral activity to the parent compound, nevirapine (NVP), a first-line ARV prescribed in South Africa, has been repeatedly detected in surface water throughout the country (Wood TP, 2015). (Wood TP, 2016).

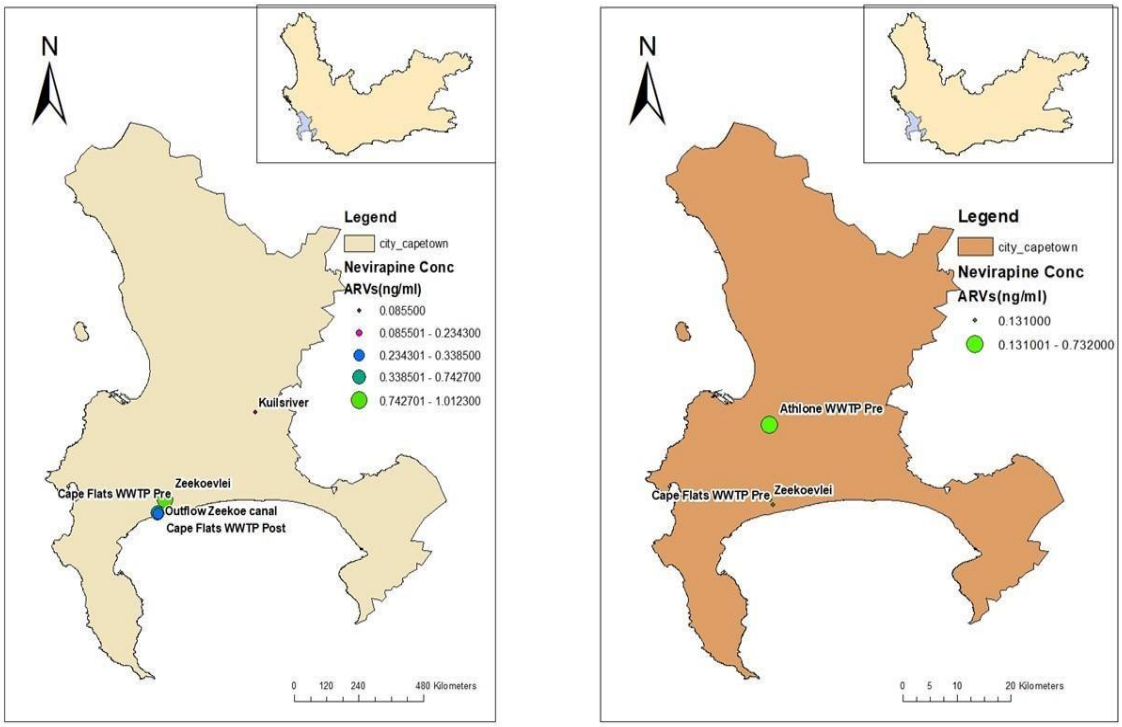


*Figure 15: Spatial distribution maps of nevirapine during summer and autumn.*

Nevirapine during summer was present mostly in the Cape flats WWTP from pre-treatment to post-treatment. There is a decrease in concentration as wastewater moves through the different treatment within the Cape flats WWTP plant. It is also present at Kuilsriver which gets final effluent from the Bellville WWTP, concentrations of the drug range between 0.7ng/ml at Cape flats to 0.005 ng/ml at Kuilsriver. During this season the drug is mostly concentrated around the Cape flats WWTP (Appendix A). Nevirapine is only present in 3 sampling areas during autumn these areas include Athlone and Cape flats WWTP pre-treatment and Zeekoevlei.

Concentrations of the drug range from 0.73 ng/l to 0.13 ng/l between the 3 areas (Appendix B), the presence of the drug in Zeekoevlei could be caused by the neighbouring area's storm water and possible leakages from the Cape flats WWTP that goes into the vlei. A study done in South Africa found that the concentrations of nevirapine in wastewater influent and surface water were found to be relatively high, within a range of 2100 to 17 400 ng L–1and<LOD to 1480 ng L–1, respectively, with a treatment efficiency of approximately 50%, which accounts for

effluent concentrations of nevirapine within the range of 350 to 7 100 ng L–1, respectively (Wood et al. 2015; Schoeman et al. 2017).

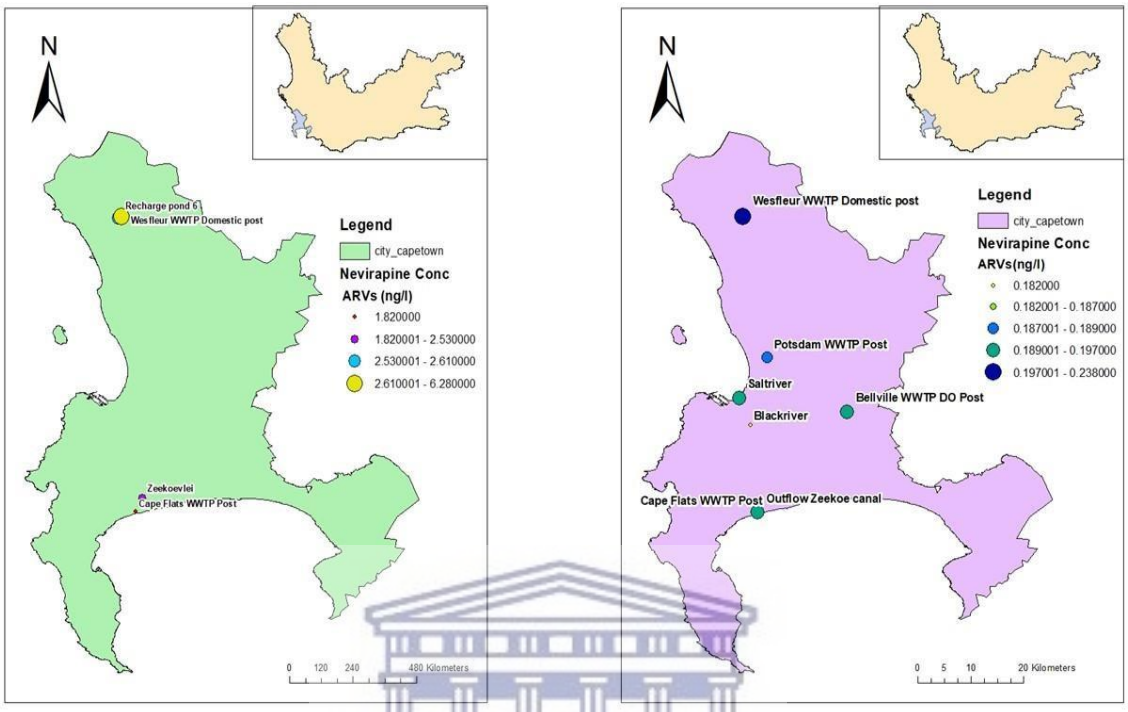


*Figure 16: Spatial distribution maps of nevirapine during winter and spring.*

Nevirapine is present in 4 sampling areas during winter these areas include Cape flats and Wesfleur WWTP post-treatment, recharge pond 6, and Zeekoevlei. Concentrations of the drug range from 2.25 ng/l to 1.62 ng/l between the 4 areas (Appendix C). During there was sampling period there was a leakage at the Cape flats WWTP into the Zeekoevlei this could have affected the concentration and presence of the drug in the vlei. The drug was distributed in the cape flats area and the Wesfleur area in varying concentrations. Nevirapine is only present in 6 sampling areas during spring these areas include Cape flats, Potsdam, Bellville and Wesfleur WWTP post-treatment, black river, Salt River, and Zeekoe canal. Concentrations of the drug range from 0.23 ng/l to 0.18 ng/l between the 4 areas (Appendix D). The drug was distributed and present in more areas compared to winter. It was discovered that shallow wells with proximity

to latrines contained the recalcitrant antiretroviral, nevirapine at concentrations as high as 1000 to 2000 ng L−1, which may likely be due to underground seepage and groundwater movement; unfortunately, this untreated well serves as a drinking water source in Machakos Town, Kenya. A high concentration ranges of nevirapine (1.1–228μgL–1) was also detected in grab samples from a river in Machakos Town, Kenya, and ARVDs were more prevalent than antibiotics (Kairigo et al. 2020)



*Figure 17: Wastewater leakage from Cape Flats WWTW into Zeekoevlei.*

This substance is commonly detected in high amounts during the wastewater and water treatment processes in sampling regions such as Cape Flats. It is found in the WWTP before treatment, after treatment, and in the final effluent dumped at the Zeekoe canal (Appendix A). This can be related to differing treatment methods utilized in different WWTP investigations in different countries, which have demonstrated that existing wastewater treatment plant technologies do not remove, or only partially remove, several medications, including ARVs. (C. Prasse and M. Schlusener (2010) (Madikizela LM, 2017). Furthermore, certain medications

create compounds that are more active than their parent substance during the disinfection process. NVP concentrations were discovered in a German WWTP. NVP concentrations in a German WWTP were found to be in the low ng/L range, rising by around 50% in the final effluent. A researcher in Finland found similar values, but with up to a 50% drop in ultimate effluent concentration, Kenyan concentrations were nearly 100 times higher. This is due to the high prevalence of AIDS in Kenya (6.3% in 2008), when ARVDs accounted for 15% of all pharmaceutical medications taken in Nairobi (T.A. Ternes, 2003). Concentrations of nevirapine of around 1 mg/L were found in WWTP influents and effluents, indicating that no nevirapine removal occurred in the WWTP (T.A. Ternes, 2003). As a result of everyday use, NVP is continually added to surface water.

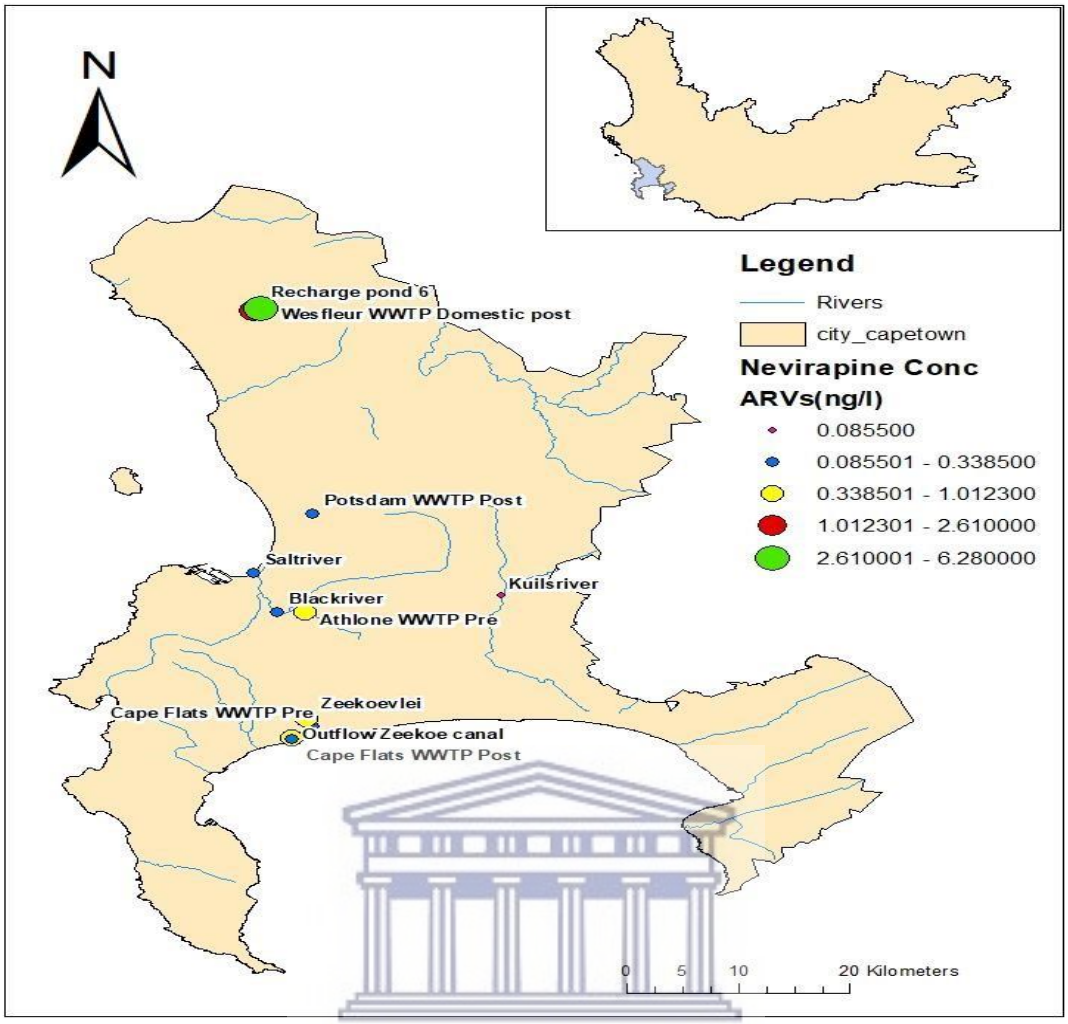




*Figure 18: Spatial distribution of nevirapine during all sampling periods.*

### 5.3.2 Efavirenz spatial distribution

Efavirenz concentrations in surface water ranged between 0.118 and 1.59 ng/l, with the highest concentrations occurring during the wet season in September 2021 (Appendix C). A high concentration of 2.77 ng/l was also found in September 2021, and most of the sample sites had no presence of the drug. It was observed that the highest Efavirenz concentrations were measured in the wet season, whereas during the dry season concentrations were mostly low or below detection levels. Concentrations of Efavirenz were found in wastewater before treatment

at Mitchells plain WWTP and the final effluent from Cape Flats WWTP and Athlone WWTP (Appendix A). Data for the occurrence of Efavirenz in WWTP effluents is not widely available. A study by the U.S. Geological Survey (S.D. Zaugg, 2013) investigated 2 regions, 1 in New York City in which Efavirenz was not detected and 3 in Oregon where Efavirenz was detected at one site at a concentration of 78 ng/L. The analysis of 2 WWTP effluents done in South Africa showed a concentration of 74.1 ng/L at one of the WWTPs and it was not detected at the other WWTP (T.P. Wood, 2015). The most prevalent ARVD detected in South African aquatic environments has been Efavirenz with a concentration as high as 140μgL–1(Durban WWTP influent sample), whereas lower concentrations ranging from 0.002 to 2.45μgL–1have been detected in surface water samples (Rimayi et al. 2018; Mtolo et al. 2019; Ngqwala and Muchesa 2020). The highest concentration of Efavirenz in WWTP influents reported in Kenya was 12.4μgL–1, concentrations as high as 119 and 140μgL–1have been reported in Zambia and South Africa, respectively (Madikizela et al. 2020). The concentration of efavirenz detected in surface water is highest in Kenya (228μgL–1) and lower in South Africa (2.45μgL–1; Mtolo et al. 2019, Kairigo et al. 2020), possibly as a result of differences in waste management and WWTP efficiencies.

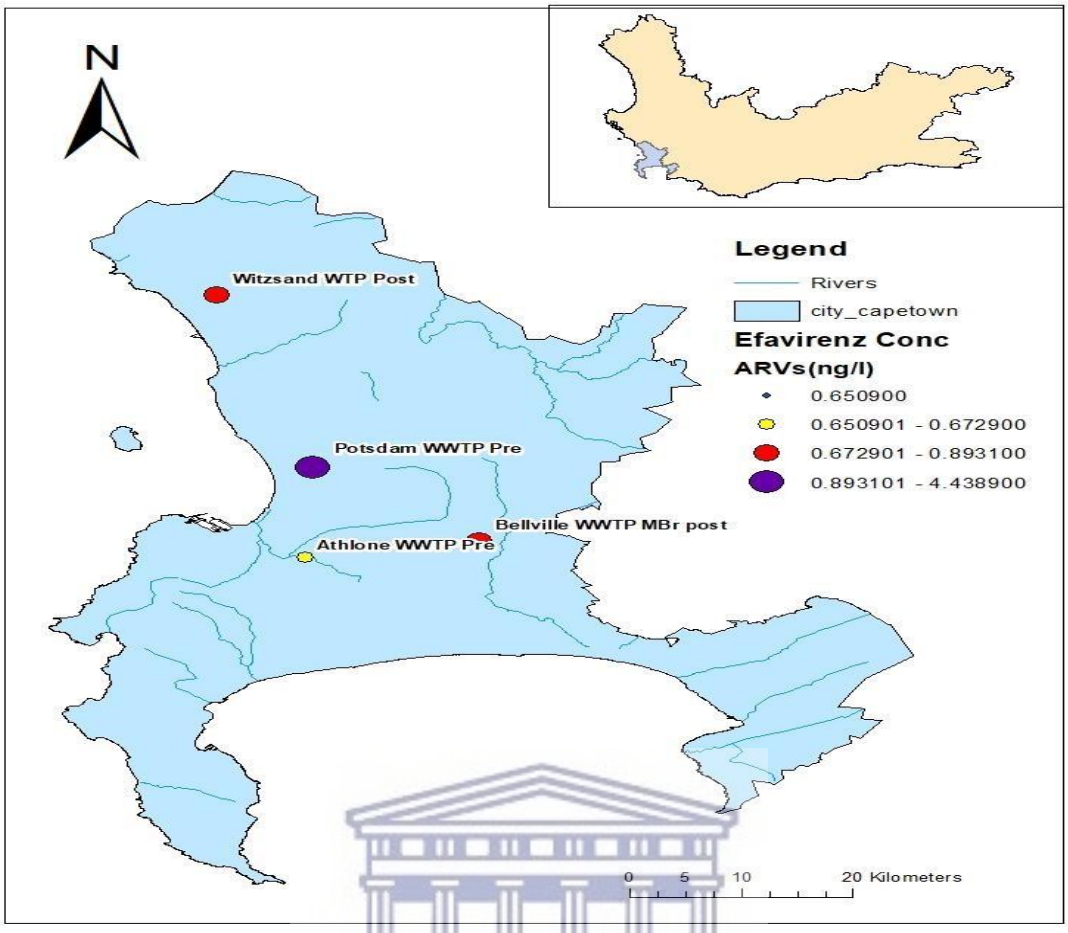


*Figure 19: Spatial distribution of Efavirenz during all the sampling periods.*



### 5.3.3 Lopinavir spatial distribution

Lopinavir concentrations in surface and groundwater ranged between 0.401 and 2.7573 ng/l, with the highest concentrations occurring during the wet season in September 2021. A high concentration of 4.43 ng/l was also found in September 2021, and most of the sample sites have the presence of the drug. It was observed that the highest Lopinavir concentrations were measured in both the summer and winter seasons, whereas during the autumn and spring season concentrations were mostly low or below detection levels. Sample sites included WWTP pre and post-treatment, final effluents, discharge water bodies, and landfill boreholes (Appendix A-D).

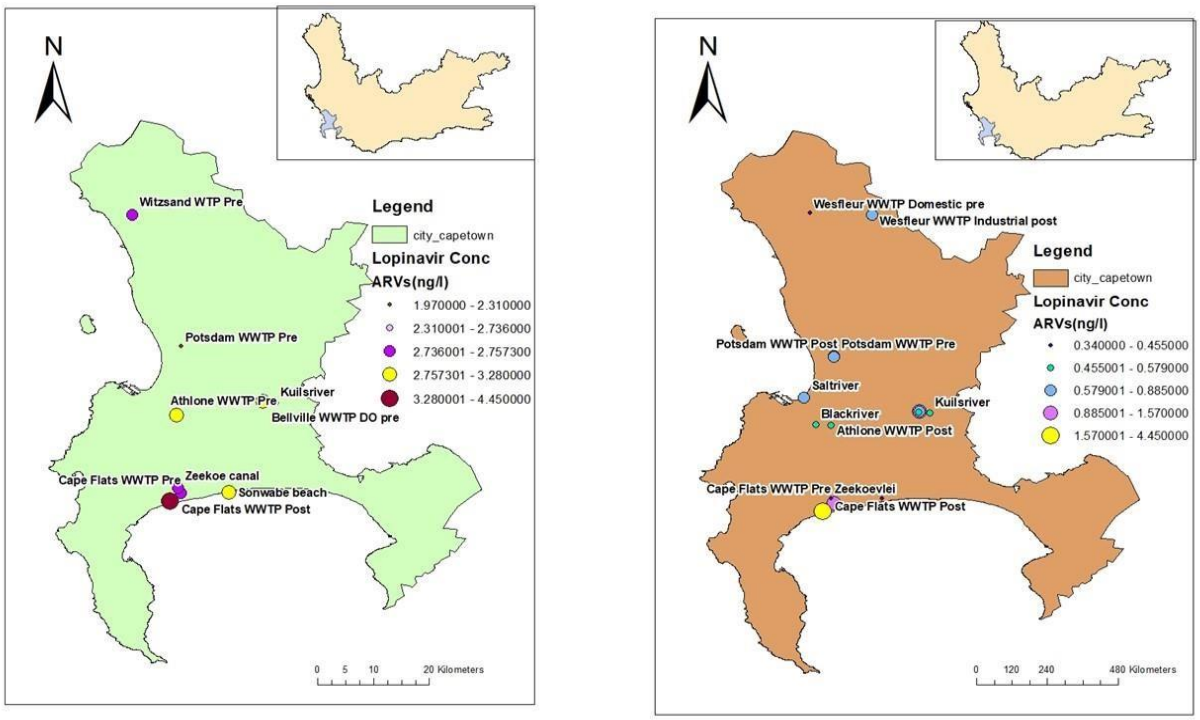


*Figure 20: Spatial distribution of Lopinavir during summer and autumn.*

The distribution of Lopinavir is throughout the study area during summer with concentrations being found in different water samples at varying concentrations. Lopinavir shows presence at Cape flats, Bellville, Potsdam and Athlone WWTPs, before and after treatment concentration which further proves the lack of removal of the drug in wastewater. Water bodies that have the presence of the drug include Kuilsriver, Sonwabe beaches, Zeekoe canal, and the Witzand water treatment plant. Concentrations range between 4.4 ng/ml to 1.0 ng/ml during summer (Appendix A). Lopinavir is present during autumn in most of the study sites from WWTP pre-treatment, after treatment to the final effluent discharge. The drug is distributed throughout the study area at varying concentrations that range from 4.45ng/l to 0.34ng/l (Appendix B). Cape flats area has higher concentrations and Wesfleur WWTP pre-treatment has a lower concentration. There is a significant decrease in concentration before and after treatment in all the WWTP, concentrations are lower at the final effluent point compared to before treatment. A study done in South Africa shows that in the Vlakplaats and Waterval system, the highest

mean concentrations were for Lopinavir 1.53 µg/L. The Welgedacht sites had measurable concentrations of Lopinavir with the highest mean concentrations of 1.78 µg/L during summer.

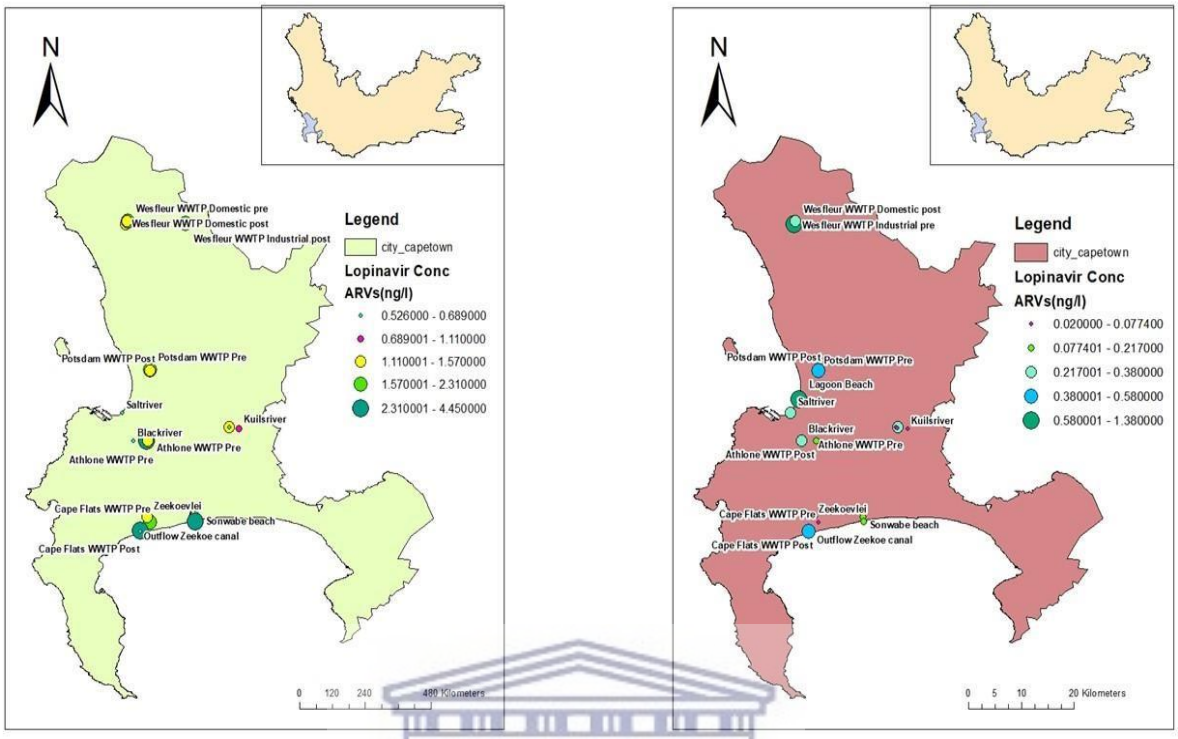


*Figure 21: Spatial distribution of Lopinavir during winter and spring.*

Lopinavir is present in most of the study sites during winter from WWTP pre-treatment, after treatment to the final effluent discharge. The drug is distributed throughout the study area at varying concentrations that range from 4.46 ng/l to 0.52 ng/l (Appendix C). Mitchell's plain area has higher concentrations and the Athlone area has a lower concentration. There is a significant decrease in concentration before and after treatment in all the WWTP, concentrations are lower at the final effluent point compared to before treatment. Lopinavir during spring is present in most of the study sites from WWTP pre-treatment, after treatment to the final effluent discharge. The drug is distributed throughout the study area at varying concentrations that range from 1.38 ng/l to 0.02 ng/l (Appendix D). Atlantis area has higher concentrations and Athlone, Mitchell's plain area has a lower concentration. There is a significant decrease in concentration before and after treatment in all the WWTP,

concentrations are lower at the final effluent point compared to before treatment. Lopinavir had the highest concentrations of any of the targeted compounds in the Klip River ranging from 6.25 □g/l to 1.23 □g/l during a South African study during winter.

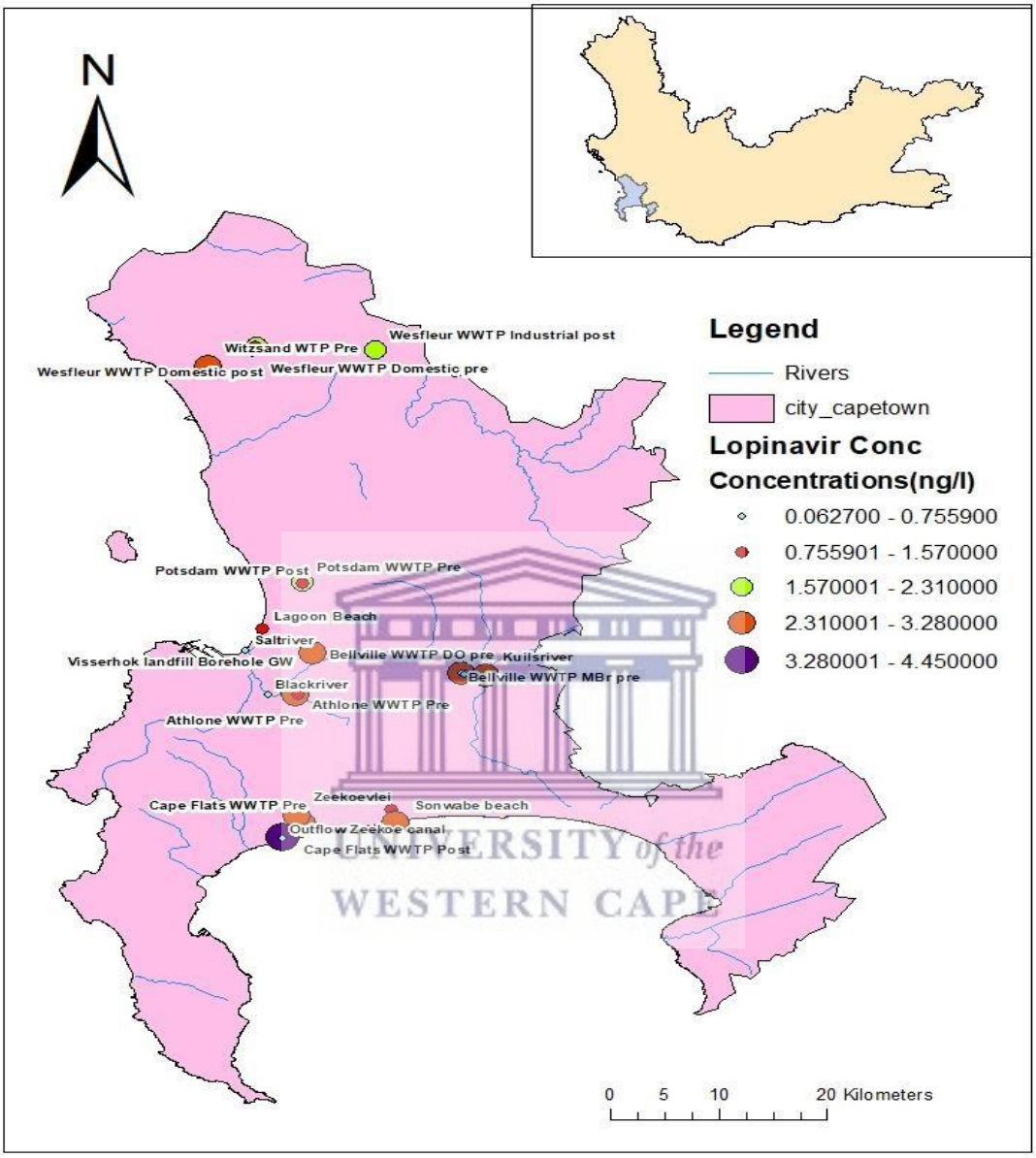


*Figure 22: Spatial distribution of Lopinavir during all the sampling periods.*

### 5.3.4 Ritonavir spatial distribution

Ritonavir concentrations in surface and groundwater ranged between 0.0695 and 2.6578 ng/l, with the highest concentrations occurring during the wet season in September 2021(Appendix D). A high concentration of 2.8558 ng/l was also found in February 2021, with autumn not having any presence of the drug in any of the sampling sites (Appendix A). It was observed that the highest ritonavir concentrations were measured during the summer season, whereas during the winter and spring season's concentrations were mostly low but frequently present in most of the sampling sites. Ritonavir is present in all of the WWTPs and WTP throughout the process from pre-treatment, post-treatment, and final effluent to the final discharge water bodies. This is most evident in spring (Appendix D) with concentrations increasing and decreasing at different parts of the treatment process. Ritonavir was reported as the most commonly consumed (1,026 g/year) and found in high concentrations of effluent from a hospital in France (Jean et al. 2012). Ritonavir has gained substantial attention for bioaccumulation potential in the environment.

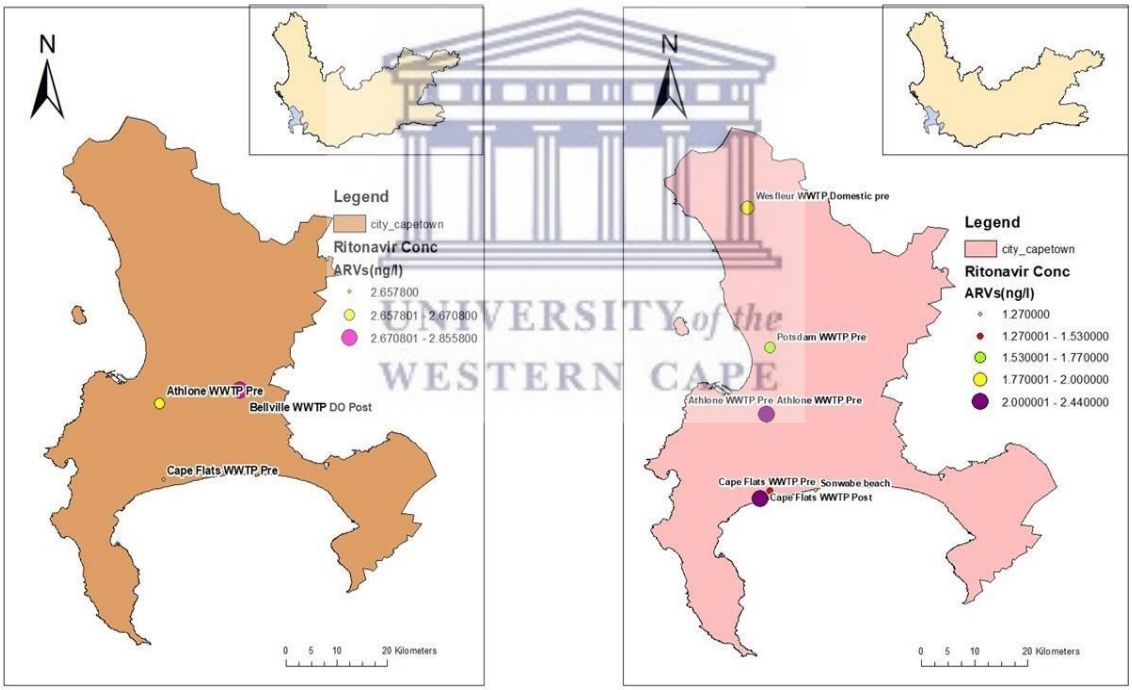


*Figure 23: Spatial distribution of ritonavir during summer and winter.*

Ritonavir is only present in 3 of the sampling area during summer this could be caused by the effectiveness of the treatment methods used at the WWTPs and WTP in removing the drug. It is present in the pre-treatment samples at Cape flats WWTP, Athlone WWTP, and Bellville WWTP after treatment. Concentrations range between 2.8 ng/ml to 2.0 ng/ml during this sampling season (Appendix A). Ritonavir is present throughout the study area of the sampling area during winter. It is present in the pre-treatment samples at Cape flats WWTP, Athlone WWTP, Potsdam, and Wesfleur WWTP after treatment, meaning it was removed during treatment in most areas and was not present at all in most of the sites. Concentrations range between 2.4 ng/ml to 1.2 ng/ml (Appendix C).

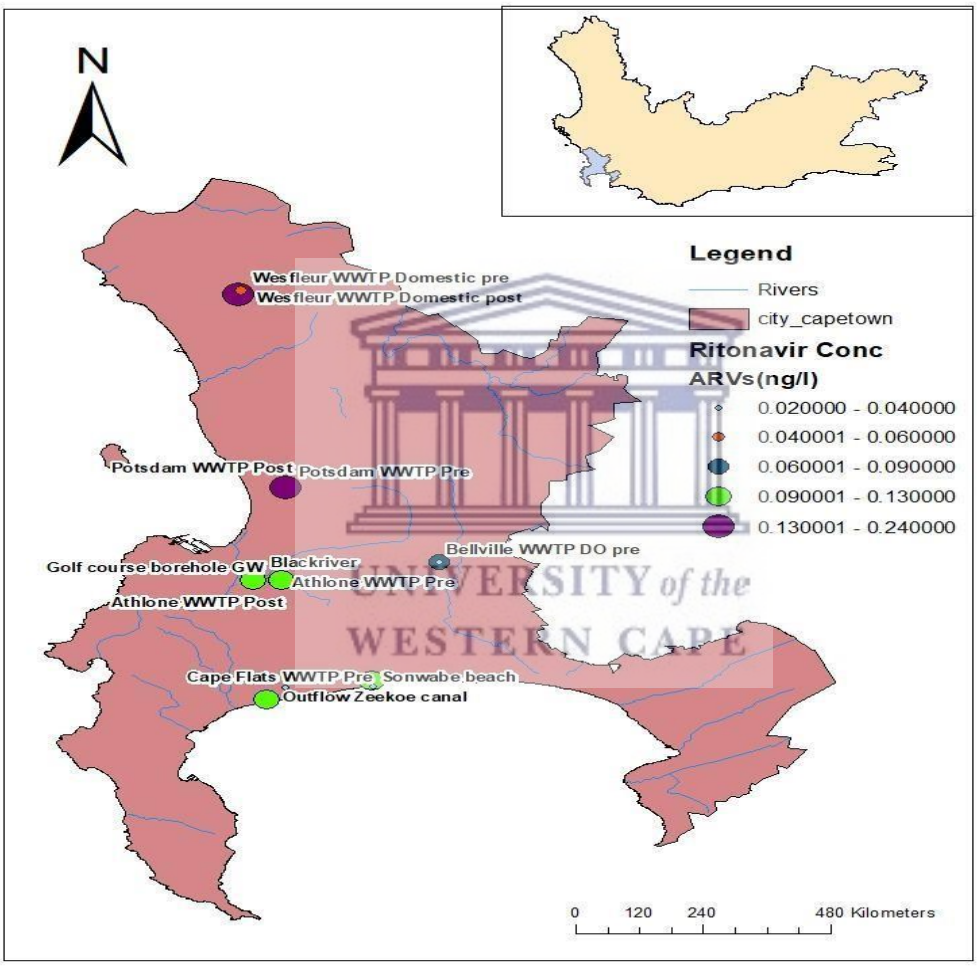


*Figure 24: Ritonavir spatial distribution in spring.*

Ritonavir is only present throughout the sampling areas during this season these areas include 5 WWTPs post-treatment, Black river, Sonwabe beach, Salt River, Zeekoe canal, and a groundwater sample at Rondebosch golf club. Concentrations of the drug range from 0.23 ng/l to 0.02 ng/l between the 4 areas. (Appendix C)

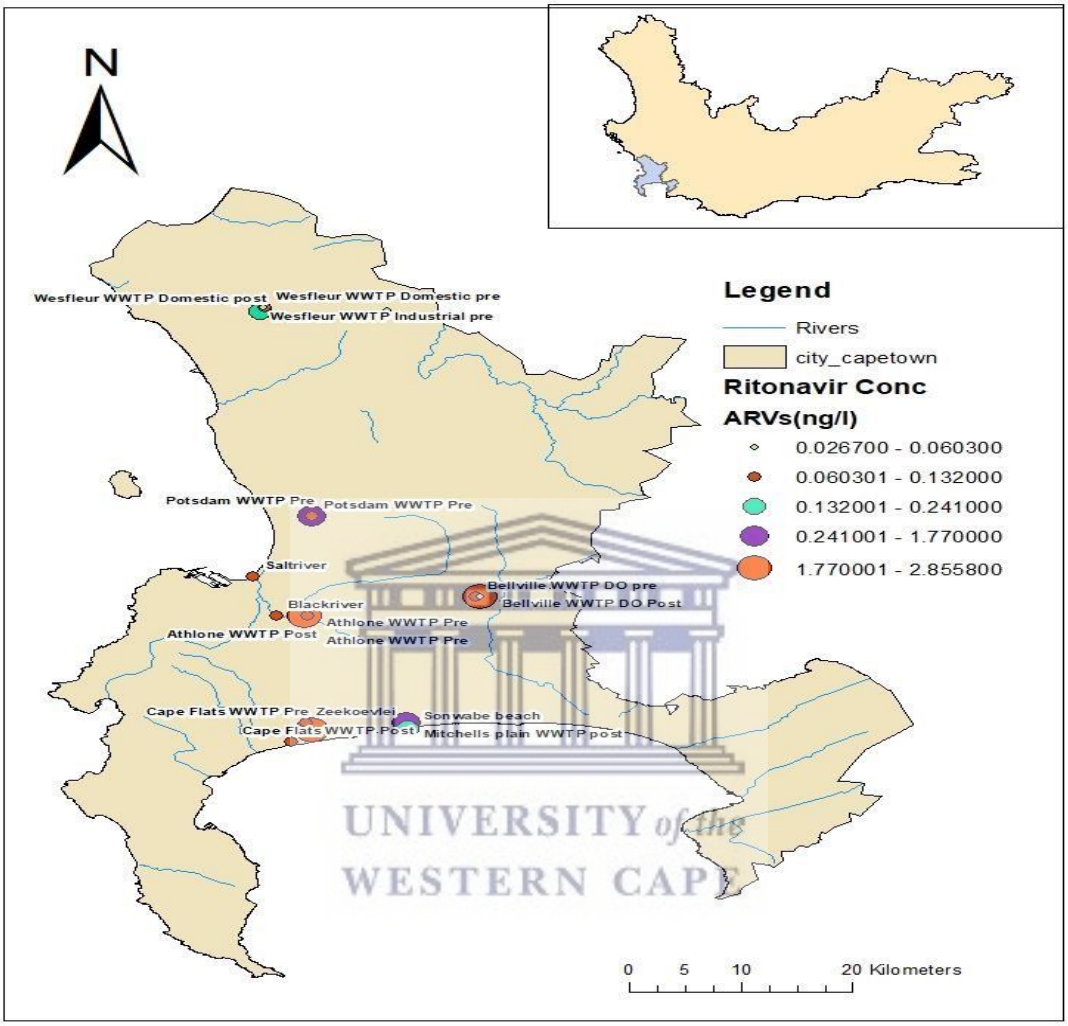


*Figure 25: Spatial distribution of ritonavir during all the sampling periods.*

### 5.3.5 Tenofovir spatial distribution

Tenofovir concentrations on the surface ranged between 0.6509 and 1.5097 ng/l, with the highest concentrations occurring during the dry season in February 2021. A high concentration

of 4.4389 ng/l was also found in February 2021, with the rest of the seasons having below detection levels of the drug in any of the sampling sites (Appendix A). It was observed that the highest ritonavir concentrations were measured during the summer season, whereas during the winter, autumn and spring season's concentrations were mostly below detection levels in all the sampling sites. Tenofovir is largely and rapidly excreted unchanged in the urine. Al-Rajab et al. (2010) reported that bio solids or recycled wastewater could contain trace concentrations of Tenofovir that are found to be persistent in soils and are expected to limit availability for biodegradation. Of the four WWTP treated effluent samples taken in South Africa North west 2018, Tenofovir (1.6 ng/l) were quantified in two of the WWTPs.

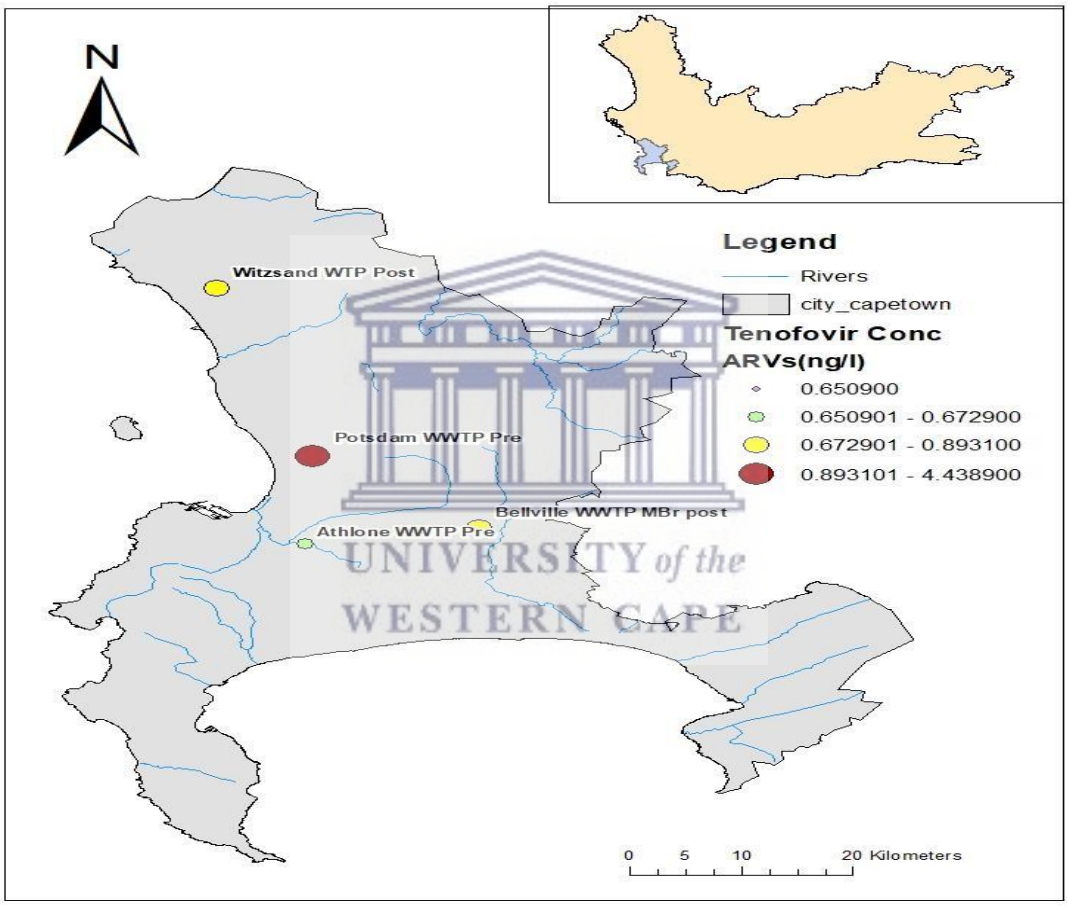


*Figure 26: Spatial distribution of Tenofovir during all sampling periods.*

# Chapter 6 Conclusions and recommendations

## 6.1 Introduction

The project aimed to establish the presence, concentrations, and distribution of HIV-ARVs in final treated wastewater effluent, natural receiving waters, tap water, and groundwater. By firstly identifying different antiretroviral drugs and their prioritization in South Africa. This was done by researching the drugs distributed to people living with HIV/AIDS in South Africa and South African laws prioritization in including these drugs in the national water act or national water treatment guidelines in wastewater and water treatment plants. Secondly to identify the sources of emission of these drug into different water types. This was done by assessing the pathway of these drugs from when they are given to the patients, to them being present in different water types. Lastly to sample and analyze the different emission sources to identify the distribution and concentrations of the drugs. Liquid chromatography coupled with mass spectrometry was used to identify the concentrations therefore providing an indication of the distribution and concentration of the drugs.

## 6.2 Antiretroviral drugs in South African waters and its inclusion in the national law

HIV-ARVs were discovered at detectable amounts in all water sources. In South Africa, the most often used drugs include NRTIs such as Zidovudine, Stavudine, Didanosine, Tenofovir, Lamivudine, and Abacavir, NNRTIs such as Efavirenz, Nevirapine, and PIs such as Lopinavir, Ritonavir, Saquinavir, and Nelfinavir. This study only examined five of these prescription drugs: nevirapine, Lopinavir, ritonavir, Tenofovir, and Efavirenz. Each of these drugs were present in some of the selected study areas at different concentrations. Antiretroviral drugs are not listed in the national water treatment guideline or the national waters legislation in South Africa. This makes monitoring of these medications in various water types challenging because it is not prioritized. This leads to drugs being found in different water bodies with no monitoring or removal being done therefore causing an accumulation of the drugs in the environment.



## 6.3 Identifying the sources of emission of antiretroviral drugs

Many pharmacological drugs are eliminated in biologically active form through the urine because they are not digested in the body. Wastewater treatment facilities (WWTPs) are not

intended to manage pharmaceutical products and remains deposited in water systems and the environment as a result of human intake and excretion, as well as incorrect disposal of these goods. ARVD pollution can occur through leaking septic tanks, subterranean sewage pipelines, and runoff from rainfall on landfills. Improper household and sewage waste disposal, drug manufacturing, hospital waste disposal, and agro-products containing metabolized and untransformed parent chemicals are also significant causes of ARVD contamination. This concludes that there are different sources of emission that contribute to the presence of ARVs in different water bodies, these drugs are transported in different pathways and end up in water bodies and the environment. As sources of antiretroviral drugs emissions, this study found landfills, wastewater treatment facilities, human excrement, hospital, domestic garbage, and leaking pipes. ARVs released into the aquatic environment via wastewater may cause environmental contamination and risks due to bioaccumulation and non-target exposures if they are not biodegraded or eliminated in WWTPs.

## 6.4 identifying the concentration and distribution of the selected antiretroviral drugs

The findings of this study revealed the existence and quantities of HIV-ARVs in water sources, as well as effective analysis and quantification using the methodologies employed. Many of the water samples collected during the water treatment process had minimal measurable residues. All five HIV-ARVs were measured at least once, however several samples included multiple compounds. HIV-ARVs were found in several wastewater samples and biota. The concentrations of nevirapine in surface water varied from 0.0855 ng/l to 6.28 ng/l, with the highest quantity being 6.28 ng/l. The quantities of Efavirenz in surface water varied from 0.118 to 1.59 ng/l, with the highest concentrations occurring during the rainy season. The amounts of ritonavir in surface and groundwater varied from 0.0695 to 2.6578 ng/l, with the highest concentrations occurring during the rainy season. Tenofovir concentrations on the surface varied from 0.6509 to 1.5097 ng/l, with the highest concentrations seen during the dry season. The amounts of Lopinavir in surface and groundwater varied from 0.401 to 2.7573 ng/l, with the highest concentrations occurring during the rainy season. This indicates that indeed the selected antiretroviral drugs are present in different water types at varying concentrations. These drugs are distributed throughout the selected study areas and throughout the different

water types identified. Future study should focus on assessing the danger of ARV hotspots, the fate, environmental behavior, and ecotoxicology of ARVs, and cost effective measures to reduce the related health concerns. However, the availability of analytical standards may limit the scope of this inquiry.

## 6.5 Recommendations

The following recommendations are made in light of these findings:

It is recommended that possibly higher concentrations of stable breakdown products be studied by looking at the clinical literature, where the pharmacodynamics and pharmacokinetics of certain substances are likely to be better understood.

- The amount of HIV-ARVs utilized in South Africa should be continually monitored and, if feasible, measured.

- To predict changes in the quantities utilized and released, keep track of HIV-ARVs that are taken out of circulation and novel compounds introduced.

- It may one day be feasible to predict increases and hazards for individual substances based on their physical and chemical properties, use patterns, and environmental behaviour. This monitoring will also enable for the evaluation of the sufficiency of existing systems.

- Existing extraction and analytical processes may be enhanced, which will aid in eventual relative risk judgments as well as provide information on better wastewater treatments, drinking water preparation, groundwater protection, and conservation.

- The danger of external contamination is quite minimal due to the sampling protocols utilized, but any potential concerns should be addressed.

## 6.6 Wastewater recommendations

HIV-ARV concentrations were measurable in all of the final treated wastewater effluents. This appears to be related to the perceived efficiency of the WWTPs. It also suggests that WWTPs do not remove all HIV-ARVs below the lowest quantitative limit (LOQ). The presence of HIVARVs in final treated wastewater implies that it will also be present in greater amounts in

the inflow. It is uncertain what effects these substances may have on the viral-bacterial ecology of the different therapeutic procedures, but the possible repercussions are concerning. Based on this, the following are suggested:

- Attempts should be made to link inflow concentrations with watershed parameters (relating to ART) of the WWTPs, including hospitals and clinics.

- Conduct studies on the effects of HIV-ARVs on the viral ecology of WWTPs.

## 6.7 Surface waters recommendations

The concentrations of measurable chemicals in the receiving waters varied. The current sampling technique made it impossible to establish whether HIV-ARVs were transferred downstream, but there are hints that they were. As a result, it is likely that poorly performing WWTPs might pollute downstream populations' drinking water supplies. People who consume surface water directly may also be exposed. The implications of HIV-ARVs on the natural aquatic viral component of stream ecology remain unclear. The following recommendations can be made based on these findings:

- Research the half-lives of HIV-ARVs in natural waters.

- Analyze the waters downstream of WWTPs to see if HIV-ARVs are transported far enough to possibly infect downstream towns' drinking water treatment facilities.

- What could happen in the future, when more patients are anticipated to receive ART, should be considered.



## 6.8 Groundwater recommendations

Groundwater appears to have lower amounts than surface water, as assessed by the measurable presence of HIV-ARVs. There are also some signs that WWTPs pollute groundwater around them. The presence of HIV-ARVs in groundwater suggests that persons who drink groundwater may consume water carrying HIV-ARVs. The following recommendations can be made for further studies:

- Conduct an investigation into the origins of HIV-ARVs in groundwater, plume investigations of the substances may point to possible origins.
- Conduct investigations on possible human absorption and exposure in bore-hole waterdependent populations.
- Human exposure should be explored, and risk assessments should be carried out if proven.

## 6.9 Water from water treatment plant recommendations

The presence of detectable and quantifiable HIV-ARVs in tap water was varied. This shows that the treatment techniques used to produce drinking water may be incapable of lowering the amounts in raw intake water below the LOQ. It also implies that those who drink tap water may be vulnerable. It is suggested for further research that:

- Extensive time-based investigations are being undertaken on the concentrations of HIVARVs in raw water utilized to derive drinking water through treatment, as well as the product.
- Extensive time-based research is undertaken on the eventual water reaching consumers.
- If such exposures are verified, risk assessments must be carried out.

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# Appendices

## Appendix A: Antiretroviral drugs actual concentrations for summer sampling

| **Site parameters** | **Nevirapine (ng/ml)** | **Lopinavir (ng/ml)** | **Ritonavir (ng/ml)** | **Tenofovir (ng/ml)** | **Efavirenz (ng/ml)** | **EC (us)** | **TDS (mg/l)** | **pH** |
|---|---|---|---|---|---|---|---|---|
| **Mitchells plain** | | | | | | | | |
| Pre treatment | - | - | - | - | 0.118 | 1430 | 710 | 7.72 |
| Post treatment | - | - | - | - | - | 645 | 315 | 7.69 |
| Borehole Wavecrest school | - | - | - | - | - | 625 | 315 | 6.96 |
| Outflow Sonwabe beach | - | 2.7757 | - | - | - | 895 | 450 | 6.67 |
| **Cape flats** | | | | | | | | |
| Pre treatment | 0.3376 | 2.7536 | - | - | - | 1170 | 565 | 7.36 |
| Post treatment | 0.2212 | 2.8035 | 2.6578 | - | - | 1160 | 585 | 6.55 |
| Borehole coastal park landfill site | - | - | - | - | - | 490 | 2.6g/l | 6.53 |
| Zeekoevlei | 0.2027 | - | - | - | - | 645 | 320 | 9.21 |
| Outflow Zeekoe canal | 0.3760 | 2.7573 | - | - | - | 1570 | 820 | 6.62 |
| **Athlone** | | | | | | | | |
| Pre treatment | - | - | 2.6708 | 0.6729 | - | 1675 | 840 | 6.81 |
| Post treatment | - | - | - | - | - | 2510 | 465 | 7.88 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Borehole Ronderbosch golf course | - | - | - | - | - | 1305 | 640 | 6.42 |
| Black river | - | - | - | - | - | 585 | 285 | 7.87 |
| Salt river | - | - | - | - | - | | | 7.51 |
| **Bellville** | | | | | | | | |
| Pre-DO | - | 3.0179 | - | - | - | 725 | 375 | 7.51 |
| Post-DO | - | - | 2.8558 | - | - | 985 | 480 | 7.36 |
| Pre-MBR | - | - | - | 0.6509 | - | 1640 | 815 | 6.95 |
| Post-MBR | - | - | - | 0.8931 | - | 830 | 420 | 7.04 |
| Final Effluent | - | - | - | - | - | 960 | 505 | 7.49 |
| Kuilsriverrietvlei | 0.3385 | 2.7536 | - | - | - | 905 | 450 | 7.65 |
| Bellville South Landfill borehole | - | - | - | - | - | 3.85 | 1.85 | 6.83 |
| **Potsdam** | | | | | | | | |
| Pre-DO | - | 2.7573 | - | 4.4389 | - | 1945 | 965 | 7.33 |
| Post-DO | - | - | - | - | - | 1190 | 600 | 6.75 |
| Lagoon beach | - | - | - | - | - | 1855 | 6.20g/l | 6.88 |
| Milnerton residential borehole | - | - | - | - | - | 1495 | 765 | 6.32 |
| Visserhok landfill | - | 2.7794 | - | - | - | 950 | 465 | 7.11 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Atlantis** | | | | | | | | |
| Pre-domestic | - | 2.7573 | - | - | - | 980 | 490 | 7.30 |
| Post-domestic | 0.0855 | - | - | - | - | 755 | 380 | 7.7 |
| Pre-industrial | - | - | - | - | - | 3.80 ms | 1.90g/l | 7.66 |
| Post-industrial | - | - | - | - | - | 1295 | 645 | 6.98 |
| Pond 6- | - | - | - | - | - | 540 | 275 | 6.59 |
| Witzand Pre Water treatment plant | - | 2.755 | - | - | - | 685 | 345 | 6.62 |
| Witzand Post water treatment | - | - | - | 0.8931 | - | 375 | 190 | 6.78 |



Appendix B: Antiretroviral drugs actual concentrations for Autumn sampling

| Site location | Nevirapine (ng/ml) | Lopinavir (ng/ml) | Ritonavir (ng/ml) | Tenofovir (ng/ml) | Efavirenz (ng/ml) | EC (us) | Temp (Celsius) | pH |
|---|---|---|---|---|---|---|---|---|
| Mitchells plain | | | | | | | | |
| Pre treatment | 0 | 0 | 0 | 0 | 0 | 1731 | 22.2 | 8.20 |
| post treatment | BLQ | O.348 | 0 | 0 | 0 | 916 | 18.9 | 7.51 |
| Borehole Wavecrest school | 0 | BLQ | 0 | 0 | 0 | 726 | 20.3 | 6.48 |
| Outflow Sonwabe beach | BLQ | BLQ | 0 | 0 | 0 | 924 | 18.9 | 7.40 |
| Atlantis | | | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Witzand Pre water treatment | BLQ | 0 | 0 | 0 | 0 | 816 | 18.7 | 7.83 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Witzand Postwater treatment | 0 | 0 | BLQ | 0 | 0 | 273 | 18.5 | 8.14 |
| Pre domestic | BLQ | 0.401 | 0 | BLQ | 0 | 1247 | 21.2 | 8.06 |
| Post domestic | BLQ | BLQ | 0 | 0 | BLQ | 823 | 16.5 | 8.62 |
| Pre industrial | BLQ | 0 | 0 | 0 | 0 | 2598 | 23.0 | 7.63 |
| Post industrial | BLQ | 0.827 | 0 | 0 | 0 | 2423 | 17.7 | 8.19 |
| Recharge Pond 6 | BLQ | BLQ | BLQ | 0 | 0 | 706 | 16.4 | 7.70 |
| Potsdam | | | | | | | | |
| Pre pots | 0 | 0.885 | 0 | 0 | 0 | 1636 | 22.2 | 7.55 |
| Post pots | BLQ | 0.348 | 0 | 0 | 0 | 1404 | 19.5 | 7.65 |
| Milnerton residential borehole (3.4m) | BLQ | 0 | 0 | 0 | 0 | 1652 | 21.4 | 5.71 |
| Lagoon beach | BLQ | BLQ | 0 | 0 | 0 | 3459 | 18.9 | 7.63 |
| Visserhok landfill borehole (5.3m) | BLQ | 0 | 0 | 0 | 0 | 887 | 21.6 | 7.53 |
| Bellville | | | | | | | | |
| MBR pre | 0 | BLQ | 0 | 0 | 0 | 1143 | 23.0 | 7.69 |
| MBR post | BLQ | BLQ | 0 | 0 | 0 | 887 | 25.0 | 7.30 |
| DO pre | BLQ | 0.455 | BLQ | 0 | 0 | 1387 | 22.6 | 7.71 |
| DO post | BLQ | 0.575 | 0 | BLQ | 0 | 1081 | 21.1 | 7.69 |
| Final effluent | BLQ | 0.692 | 0 | 0 | 0 | 1078 | 20.8 | 7.98 |
| Kuilsriver | BLQ | 0.494 | 0 | 0 | BLQ | 1963 | 20.7 | 7.95 |

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|---|---|---|---|---|---|---|---|---|
| Bellville south Landfill borehole (3.2m) | BLQ | 0 | 0 | 0 | 0 | 5344 | 20.8 | 7.46 |
| Athlone | | | | | | | | |
| Pre treatment | 0.732 | BLQ | BLQ | 0 | 0 | 1442 | 22.4 | 7.81 |
| Post treatment | 0 | 0.570 | 0 | 0 | 0 | 1267 | 23.1 | 7.60 |
| Blackriver | BLQ | 0.579 | 0 | 0 | 0 | 1199 | 19.3 | 7.77 |
| Borehole Ronderbosch golf course (3m) | BLQ | 0 | 0 | 0 | 0 | 1429 | 19.6 | 6.67 |
| Saltriver | 0 | 0.759 | 0 | 0 | 0 | 1320 | 17.6 | 7.24 |
| Cape flats | | | | | | | | |
| Pre treatment | 0.131 | 1.41 | BLQ | 0 | 0 | 1160 | 21.0 | 7.68 |
| Post treatment | BLQ | 0.735 | BLQ | 0 | 0 | 1242 | 19.6 | 7.47 |
| Zeekoevlei | 0.131 | 0.340 | 0 | 0 | 0 | 1307 | 17.3 | 8.10 |
| Coastal park Landfill borehole | BLQ | 0 | 0 | 0 | 0 | 4387 | 20.4 | 7.68 |
| Zeekoe canal | BLQ | BLQ | 0 | 0 | 0 | 1276 | 17.3 | 8.13 |



Appendix C: Antiretroviral drugs actual concentrations for winter sampling.

| **Site location** | **Nevirapine (ng/ml)** | **Lopinavir (ng/ml)** | **Ritonavir (ng/ml)** | **Tenofovir (ng/ml)** | **Efavirenz (ng/ml)** | **EC (us)** | **Temp (Celsius)** | **pH** |
|---|---|---|---|---|---|---|---|---|
| **Mitchells plain** | | | | | | | | |
| Pre treatment | 0 | 0.962 | BLQ | 0 | 0 | 1331 | 17.2 | 7.86 |
| post treatment | BLQ | 1.27 | BLQ | 0 | 0 | 925 | 12.8 | 7.41 |

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|---|---|---|---|---|---|---|---|---|
| Wavecreast school Borehole | BLQ | 0 | 0 | 0 | 0 | 798 | 18.1 | 7.25 |

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|---|---|---|---|---|---|---|---|---|
| Outflow Sonwabe beach | BLQ | 2.99 | 1.27 | 0 | 1.83 | 932 | 12.1 | 7.61 |
| **Atlantis** | | | | | | | | |
| Witzand Pre water treatment | BLQ | 0 | 0 | 0 | 0 | 840 | 18.7 | 7.83 |
| Witzand Postwater treatment | BLQ | 0 | BLQ | 0 | 0 | 269 | 18.5 | 8.14 |
| Pre domestic | BLQ | 2.31 | 2.00 | 0 | 0 | 1293 | 21.2 | 8.06 |
| Post domestic | 6.28 | 1.23 | 0 | 0 | BLQ | 745 | 16.5 | 8.62 |
| Pre industrial | BLQ | 1.22 | BLQ | 0 | 0 | 2189 | 23.0 | 7.63 |
| Post industrial | BLQ | 2.06 | 0 | 0 | BLQ | 1676 | 17.7 | 8.19 |
| Recharge Pond 6 | 2.61 | BLQ | 0 | 0 | BLQ | 762 | 16.4 | 7.70 |
| **Potsdam** | | | | | | | | |
| Pre pots | 0 | 1.97 | 1.77 | 0 | 0 | 1663 | 16.9 | 7.99 |
| Post pots | BLQ | 1.24 | BLQ | 0 | 0 | 980 | 14.3 | 7.53 |

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|---|---|---|---|---|---|---|---|---|
| Milnerton residential borehole (3.4m) | 0 | 0 | 0 | 0 | 0 | 1557 | 19.8 | 5.58 |
| Lagoon beach | BLQ | 1.24 | BLQ | 0 | 0 | 1611 | 10.4 | 7.56 |
| Visserhok landfill | 0 | 0 | 0 | 0 | 0 | 1330 | 19.4 | 7.04 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| borehole (5.3m) | | | | | | | | |
| **Bellville** | | | | | | | | |
| MBR pre | BLQ | BLQ | 0 | 0 | 0 | 990 | 18.0 | 7.55 |
| MBR post | BLQ | BLQ | 0 | 0 | 0 | 911 | 18.8 | 7.35 |
| DO pre | BLQ | BLQ | 0 | 0 | BLQ | 1316 | 18.1 | 5.21 |
| DO post | BLQ | 1.57 | BLQ | 0 | 0 | 966 | 16.4 | 7.35 |
| Final effluent | BLQ | 0.532 | 0 | 0 | BLQ | 966 | 15.6 | 7.79 |
| Kuilsriver | 0 | 1.11 | 0 | 0 | BLQ | 860 | 13.8 | 7.88 |
| Bellville south Landfill borehole (3.2m) | BLQ | 0 | 0 | 0 | 0 | 2117 | 17.8 | 7.69 |
| **Athlone** | | | | | | | | |
| Pre treatment | 0 | 3.28 | 2.44 | 0 | 0 | 1237 | 18.5 | 7.49 |
| Post treatment | BLQ | 1.21 | 0 | 0 | BLQ | 969 | 18.4 | 7.03 |
| Blackriver | BLQ | 0.689 | 0 | 0 | BLQ | 1015 | 14.4 | 7.59 |

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|---|---|---|---|---|---|---|---|---|
| Borehole Ronderbosch golf course (3m) | 0 | 0 | 0 | 0 | 0 | 1386 | 18.9 | 6.55 |
| Saltriver | BLQ | 0.526 | 0 | 0 | 1.65 | 942 | 13.0 | 7.35 |
| **Cape flats** | | | | | | | | |
| Pre treatment | BLQ | 1.95 | 1.53 | 0 | 0 | 1024 | 19.2 | 7.56 |
| Post treatment | 1.82 | 4.43 | 2.28 | 0 | 2.77 | 1075 | 13.5 | 7.44 |
| Zeekoevlei | 2.53 | 1.18 | BLQ | 0 | 0 | 1089 | 13.4 | 7.50 |
| Coastal park Landfill borehole | BLQ | 0 | 0 | 0 | 0 | 492 | 19.2 | 7.71 |
| Zeekoe canal | BLQ | 0.651 | BLQ | 0 | 0 | 880 | 12.5 | 7.92 |

Appendix D: Antiretroviral drugs actual concentrations for spring sampling.

| **Site location** | **Nevirapine (ng/ml)** | **Lopinavir (ng/ml)** | **Ritonavir (ng/ml)** | **Tenofovir (ng/ml)** | **Efavirenz (ng/ml)** | **EC (us)** | **Temp (Celsius)** | **pH** |
|---|---|---|---|---|---|---|---|---|
| **Mitchells plain** | | | | | | | | |
| Pre treatment | BLQ | BLQ | 0.0267 | BLQ | BLQ | 1472 | 18.3 | 8.32 |
| post treatment | BLQ | O.174 | 0.103 | BLQ | BLQ | 1042 | 16.4 | 7.90 |
| Wavecreast school Borehole | BLQ | BLQ | BLQ | BLQ | BLQ | 775 | 19.8 | 7.79 |
| Outflow Sonwabe beach | BLQ | 0.191 | 0.0459 | BLQ | BLQ | 1052 | 16.5 | 7.93 |

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|---|---|---|---|---|---|---|---|---|
| **Atlantis** | | | | | | | | |
| Witzand Pre water treatment | BLQ | BLQ | BLQ | BLQ | BLQ | 877 | 18.8 | 8.0 |
| Witzand Post-water treatment | BLQ | BLQ | BLQ | BLQ | BLQ | 238 | 15.7 | 8.36 |
| Pre domestic | BLQ | BLQ | 0.0695 | BLQ | BLQ | 1184 | 17.3 | 8.87 |
| Post domestic | 0.238 | 1.38 | 0.241 | BLQ | BLQ | 742 | 16.5 | 8.19 |
| Pre industrial | BLQ | 0.3801 | 0.0603 | BLQ | BLQ | 2530 | 19.7 | 8.11 |
| Post industrial | BLQ | 0.134 | 0.0267 | BLQ | BLQ | 1984 | 17.1 | 8.14 |
| Recharge Pond 6 | BLQ | BLQ | BLQ | BLQ | BLQ | 774 | 17.4 | 7.90 |
| **Potsdam** | | | | | | | | |
| Pre pots | BLQ | 0.140 | 0.0841 | BLQ | BLQ | 1941 | 18.6 | 8.47 |
| Post pots | 0.189 | 0.580 | 0.208 | BLQ | BLQ | 1569 | 17.8 | 7.88 |
| Milnerton residential borehole (3.4m) | BLQ | BLQ | BLQ | BLQ | BLQ | 1854 | 19.1 | 5.67 |
| Lagoon beach | BLQ | 0.0511 | BLQ | BLQ | BLQ | 12830 | 14.4 | 8.12 |
| Visserhok landfill borehole (3.2m) | BLQ | BLQ | BLQ | BLQ | BLQ | 1670 | 19.7 | 7.19 |
| **Bellville** | | | | | | | | |
| MBR pre | BLQ | 0.0637 | BLQ | BLQ | BLQ | 1023 | 18.5 | 7.77 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| MBR post | BLQ | 0.0624 | BLQ | BLQ | BLQ | 1017 | 18.9 | 7.81 |
| DO pre | BLQ | BLQ | 0.0697 | BLQ | BLQ | 1457 | 18.5 | 8.58 |
| DO post | 0.197 | 0.282 | 0.0934 | BLQ | BLQ | 1147 | 17.3 | 7.88 |
| Final effluent | BLQ | 0.253 | 0.0267 | BLQ | BLQ | 1140 | 16.7 | 8.02 |
| Kuilsriver | BLQ | 0.0774 | BLQ | BLQ | BLQ | 978 | 14.4 | 8.25 |
| Bellville south Landfill borehole (1.83m) | BLQ | BLQ | BLQ | BLQ | BLQ | 5820 | 17.2 | 7.61 |
| **Athlone** | | | | | | | | |
| Pre treatment | BLQ | 0.0737 | 0.0937 | BLQ | BLQ | 1336 | 18.8 | 7.33 |
| Post treatment | BLQ | 0.217 | 0.103 | BLQ | BLQ | 1224 | 19.7 | 7.39 |
| Blackriver | 0.182 | 0.328 | 0.132 | BLQ | BLQ | 1304 | 18.8 | 8.11 |
| Borehole Ronderbosch golf course (3m) | BLQ | BLQ | 0.0697 | BLQ | BLQ | 1414 | 18.9 | 6.76 |
| Saltriver | 0.197 | 0.359 | 0 | BLQ | BLQ | 1185 | 17.2 | 7.90 |
| **Cape flats** | | | | | | | | |
| Pre treatment | BLQ | 0.0337 | 0.0364 | BLQ | BLQ | 1195 | 17.9 | 7.79 |
| Post treatment | 0.187 | 0.280 | 0.117 | BLQ | BLQ | 1150 | 16.9 | 7.46 |
| Zeekoevlei | BLQ | 0.0461 | BLQ | BLQ | BLQ | 878 | 16.2 | 9.94 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Coastal park Landfill borehole (3.49m) | BLQ | BLQ | BLQ | BLQ | BLQ | 4850 | 19.2 | 7.97 |
| Zeekoe canal | 0.197 | 0.554 | 0.103 | BLQ | BLQ | 1286 | 16.6 | 7.87 |