Systematic Review of the Occurrence and Health Risk Assessment of Antiretroviral Drugs (ARVDs) in Africa Water Resources

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

Antiretroviral drugs (ARVDs) are among the emerging environmental contaminants that has raised concern among researchers and public health experts in the recent times. Unabated release of pharmaceutical product residue into the water resources, especially in the developing countries, is a major threat to public health. The quality of water resources is steadily declining globally due to unabated release of contaminants into the river systems, despite a remarkable global awareness to improve water quality. This is due to discharge of toxic chemicals and emerging contaminants in the environment. Polluted wastewater and river systems pose serious public health risks, especially to resource constrained communities and those residing in informal settlements. Previous studies focusing on water quality in African settings have reported levels of various emerging contaminants including ARVDs residue in water resources such as surface water, wastewater, ground- and drinking water systems amongst others sources, however, a systematic review has not been conducted and there is limited studies focusing on health risk assessment (Ngumba et al., 2020; Nibamureke et al., 2019a; Swanepoel et al., 2015). It is therefore important to further investigate and systematically review the levels of ARVDs in water resources in Africa and understand their associated environmental and public health risks.

METHODS

A systematic review of the levels of ARVDs in African settings was conducted for the review period 2010-2021 using PRISMA guidelines. The ARVDs levels reported in the studies included in the systematic review was used to estimate the ecological risk in different African water systems including surface water and wastewater treatment plant (WWTP) effluents and influent by estimating the Risk Quotient (RQ). Human health risk assessment of ARVDs contaminated water resources was estimated with two risk-based exposure pathways, ingestion, and dermal contact. Levels of ARVDs obtained during the literature search were used to compute the non-carcinogenic and carcinogenic effects on human health (USEPA, 2005). Additionally, probabilistic hazard assessments to understand Africa environmental water quality concerns was conducted using the Weibull's' probabilistic approach. Probabilistic hazard assessment examined the likelihood that the four most common ARVDs reported in the systematic review as well as all the ARVDs in different water resources, exceeded the predicted no effect concentrations (PNEC) by The European Union European Medicines Agency (EU-EMA) for pharmaceuticals (proposed threshold value of 0.01 µg/L). The Australian Drinking water guidelines (DWG) (ranges between 0,0015 and 1000 (µg/L) for pharmaceuticals, the United States Food and Drugs Administration (USFDA) value of 1 µg/L for pharmaceuticals and the PNEC *R subcapi* for individual pharmaceuticals developed by the USEPA (Kuroda et al., 2021).

RESULTS AND DISCUSSION

Twenty-nine (29) studies were identified (Table 4.1). Most of the countries where the studies were collected in South Africa (n = 18, 62%), followed by Kenya (n = 8, 28%), Nigeria (n =2, 7%) and Zambia (n =1, 3%). Most of the studies identified were therefore conducted in Southern Africa (65%), followed by East Africa (28%) and West Africa (7%). Most of the water samples in the studies identified, were collected from surface water (23 studies, 79%) and wastewater treatment plants (WWTPs) (18 studies, 62%) with few (4 studies, 1.4%) collected from drinking and groundwater sources. The number of sites investigated in the studies varied from 2-50 sites and the number of samples varied from 2-812 samples. The duration of sampling in the studies varied from 3 days to 3 years with 9 studies (31%) sampling for at least a year during all four seasons.

There were 18 different ARVDs that were investigated in the identified studies. The ARVDs detected most frequently in the studies reviewed, include Nevirapine (NVP) (n = 21, 72%), Efavirenz (EFV) (n = 12, 41, 7%),

Lamivudine (3TC) (n =11, 37.9%), Zidovudine (ZVD) (10, 34.5%), Ritonavir (RIT) (n = 6, 21,0%) and Lopinavir (LPV) (3, 10.3%), with all the other ARVDs detected in less than 3 studies. Additionally, EFV, NVP, LPV, ZVD, Didanosine (DD) and RIT were not detected in some studies (number of studies = 3, 2, 2, 2 ,1 and 1 respectively). ARVDs were detected in ground and drinking water in 3 studies including NVP in 3 studies, EFV in 2 studies, LPV and (Oseltamivir) OSLT in one study. NVP was not detected in tap water in one study.

The highest concentrations of ARVs detected in surface and WWTP samples were 3TC and EFV with peak concentrations > 50 µg/L. The other ARVDs among the highest concentrations include LVP, FTC, ZDV, FTC, NVP, OSLT and Raltegravir (RTV). The highest concentrations of ARVDs were detected in Kenya and South Africa with most ARVDs detected in South Africa. South Africa has been found to have the highest consumption of ARVDs in Africa (Emeji, Ama, Khoele, Osifo & Ray, 2021).

Only one study conducted in SA, tested ARVDs in drinking water samples and detected up to 2.3 µg/L EFV, 0.904 µg/L LPV and 0.21 µg/L NVP. Although these concentrations are substantially lower than those detected in WWTP and surface water samples, their presence in drinking water is a concern. ARVDs were also detected in groundwater samples in a study conducted in Kenya in which NVP and EFV were detected at low concentrations (940 and 23.3 ng/L) and in Zambia in which NVP was detected (0-420 ng/L). Groundwater used as portable water resource especially for drinking could be a risk to exposed communities. Additionally, possible access to surface water sites could also present a risk to humans.

WWTP influent was the water systems that had the highest ecological risks with 3TC (5.68-3240), followed by Zidovudine (ZDV) (0.94-1224), Nevirapine NVP (up to 152) and then EFV (up to 2.33), the ARVD that had the highest risk. The highest RQ for WWTP effluent was for 3TC (6,22-6776), ZDV (0.18-68.27), while the RQ for surface water ranged between 1,78-62.50. The highest risk of ARVDs in water systems was found in Kenya, followed by South Africa while there was minimal risk to ARVDs in water resources in both Zambia and Nigeria. Cancer risks and non-cancer risks were very high for several ARVDs with 3TC being the highest, followed by Zidovudine (4.7 ng/L) and LPV (5.9 ng/L).

In probabilistic hazard assessments, the percentage exceedances of the PNEC EMEA and USFDA value for the four most common ARVDs were 100% for the vast majority of sites and water systems with a minimum 85%. The percentage exceedances for the 4 most common ARVDs of the Australian Drinking water guidelines for pharmaceutical residues in the different sites and water systems ranged between 55-100% with half having exceedances of 100%. The percentage exceedances of the PNEC *R subcapi* for the four most ARVDS ranged between 55-100% with 8% having exceedances of 100%. The percentage exceedances of the PNEC EMEA and USFDA guideline for all the ARVDs ranged between 87-100% and of the Australian Drinking Water Guideline ranged between 68-72% for all the ARVDs. This study therefore revealed that the ARVDs had a high percentage exceedance above permissible limits set for pharmaceuticals in water in most countries, hence ARVDs is a potential burden in both its surface water and other water resources.

The main limitation in the systematic review of the occurrence of ARVDs in African water systems is the representativeness of the countries in which studies was performed with studies only conducted in only 4 countries and predominantly in South Africa and Kenya. No studies were conducted in North Africa countries. Low turnout of scholarly output in Africa may be attributed to access to limit infrastructure in terms of research equipment and skilled manpower. The second important limitation is that that only one study investigated drinking water and another 2 investigated groundwater and in total only two ARVDs measured. Thirdly, the period of monitoring was over 1 year including all four seasons in only 9 (30%) of the studies. The limitation in the risk assessment was that there are not specific guidelines for ARVDs especially for African water systems.

CONCLUSIONS

This systematic review found the occurrence of 18 ARVDs in the water systems of 4 African countries with high concentrations of 9 ARVDs in wastewater and surface water samples. It is a concern, that ARVDs were also detected in drinking water and groundwater samples, although at low concentrations. The ecological risk of the ARVDs levels reported in African water WWTPs and surface water was high with the risk for several ARVDs exceeding one. The cancer and non-cancer risks were also high for several ARVDs in the 3 water systems. The probabilistic hazard assessments found a high proportion of exceedances of international water guideline for all sites in the 3 water systems. There is a need for more studies in African water systems, especially in drinking and groundwater samples, ideally over at least 12 months including all four seasons. Removal of ARVDs in accessible water systems in Africa, are recommended.

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ACRONYMS & ABBREVIATIONS

3TC	Lamivudine
ABC	Abacavir
ARVDs	antiretroviral drugs
ATV	Atazanavir
CE	Collision energy
CI	Confidence interval
DD/ddI	Didanosine
ddC	Zalcitabine
Di-EFV	8,4 dihydroxy Efavirenz
DRV	Darunavir
DS	Downstream
EC	Effect concentration
EFV	Efavirenz
ES	Ecosystem services
ESI	Electrospray ionization
ETR	Etravirine
FI	Fusion inhibitor
FRNA	F-specific RNA bacteriophages
FTC	Emtricitabine
GC	Gas Chromatograph
HI	Hazard index
HIV	Human immunodeficiency virus
HIV-ARV	HIV-antiretroviral
HPLC	High-pressure liquid chromatograph
HQ	Hazard quotient
INDV	Indinavir
LC	Liquid chromatography
LC-MS	Liquid chromatography-mass spectrometry
LOD	Limit of detection
LOP/LPV	Lopinavir
LOQ	Limit of quantification
LPV/r	Lopinavir/ritonavir
MEC	Measured environmental concentration
MRV	Maraviroc
NFV	Nelfinavir
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOEC	No-observed effect-concentration
NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
OSLT	Oseltamivir
PNEC	Predicted no-effect concentration
RQ	Risk Quotient
RTV	Raltegraviv
SQV	Saquinvir
STV	Stavudine
TEVD	Tenofovir Disoproxil

GLOSSARY

- **Antiretroviral:** Antiretroviral drugs (ARVDs) inhibit the reproduction of retroviruses (viruses composed of RNA rather than DNA) to aid in the treatment of HIV infections
- **HAART:** Refers to very potent ART regimen which almost invariably inhibits viral replication to undetectable levels in the blood. It comprises of a combination of ARVDs.
- **PRISMA-P:** Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

The quality of water resources is steadily declining globally due to unabated release of contaminants into the river systems (du Preez and van Huyssteen 2020; Tickner et al., 2020), despite a remarkable global awareness to improve water quality (Tickner et al., 2020). The decline in water quality is due to discharge of toxic chemicals and emerging contaminants into the environment. These toxic chemicals and emerging contaminants such as pharmaceutical residues including (ARVDs), endocrine disruption compounds (EDCs), nitrate and pesticides, perfluoroalkyl chemicals, personal care products among other contaminants (Elmonznino 2016). Polluted wastewater and river systems are serious public health risks, especially to resource constrained communities and those residing in informal settlements (Report 2015). Additionally, these resources are targeted as water sources for portable water, agricultural purposes, domestic and industrial water needs amongst others. Most of the previous research investigating water quality in African settings have reported levels of various emerging contaminants such as antiretroviral residue in surface water, wastewater, agricultural water systems amongst others sources, however there is limited studies focusing on health risk assessment (Ngumba et al., 2020; Nibamureke et al., 2019a; Swanepoel et al., 2015). It is therefore important to further investigate the occurrence of ARVDs into water resources and understand their associated environmental and public health risks.

Occurrence of elevated levels of ARVDs in various environmental matrices has been identified among transition pathways to the ecological cycle (Ngumba et al., 2020). ARVDs, like other emerging contaminants such as pharmaceuticals, personal care products (PPCPs), perfluoroalkyl chemicals, pesticides among others are ultimately discharged into wastewaters and surface waters. Potential health risk of ARVDs includes; health effects due to endocrine disruption, neurotoxicity, renal malfunctioning, cardiovascular effect, mitochondrial toxicity among others (Mosekiemang et al., 2019). Up to 90% of orally applied drugs are excreted (Ngumba et al., 2016) and end up in the sewage system where they occur unaltered or partially metabolized. Unused drugs or chemicals in the body systems are disposed from the body via excretion down to the sewage and ultimately reach wastewaters. To assess the accumulation profile of these ARVDs, environmental levels need to be established. Previous studies shows that ARVDs residues were detected in environmental matrices in South Africa (Abafe et al., 2018; Swanepoel et al., 2015) but it is unclear how much research is available in Africa. It is crucial to conduct a systematic review to establish the trend of occurrence and public health risk of ARVDs residue in the environmental matrix in Africa.

A large number of African countries still lack adequate accessibility to potable water resources (Oki and Quiocho 2020); this is due to insufficient availability of fresh water resources to meet the demands of water usage, which is exacerbated in this region by climate change, including altered weather patterns like droughts or floods, increased pollution, and increased human demand amongst other factors (Owusu-Sekyere et al., 2020). Most African countries still lack efficient wastewater management schemes with the exception of few countries. Due to inadequate fresh water supplies, water management scheme that incorporated treated wastewater reuse as an important source of its integrated water-management plans. Inefficient wastewater schemes could predispose humans to harmful contaminants such as ARVDs by way of the food chain (Adewumi et al., 2010). With South Africa's high prevalence of HIV/AIDS the use of ARVDs are likely to contribute to the burden of pharmaceutical residue in wastewater treatment plants and surface waters; this will be further amplified in the context of COVID19 where other chemicals and disinfectants are increasingly introduced to the environment. There are over 30 antiretroviral medications currently in use for the treatment of HIV/AIDS globally (Mlunguza et al., 2020). Previous studies reported elevated levels of various emerging contaminants such as pharmaceutical (ARVDs), EDCs, PPCP among other chemical have been detected in environmental matrix from different the geographical regions of Africa (Abafe et al., 2018). South Africa recorded the highest numbers of publications reporting the levels of ARVDs in aquatic environment relative other Africa regions in Africa (Nibamureke et al., 2019b).

Additionally, studies have shown levels of ARVDs in environmental matrices from some Southern Africa countries including Kenya, Zambia and Nigeria reported significant levels of ARVDs in water resources (such as WWTP, surface water, drinking and ground) (Corrales et al., 2015; Nibamureke et al., 2019b; Prasse et al., 2010). Disparity in the concentrations of ARVDs in Africa aquatic systems is attributed to prevailing environmental factors such as climate change, drought and flooding, poor approaches for waste disposal amongst others. Fewer studies focused on levels of ARVDs residues from Western and Central African countries despite of exponential increase in number of patients on ARVDs therapy in the region (Prasse et al., 2010). Studies focused on the probabilistic and health risk assessment of ARVDs in Africa regions are scanty. Researchers have been able to extrapolate the trend of contaminants in the environment matrix while predicting future scenarios the associated health and ecological risk using Weibull probabilistic risk assessment (USEPA 2006). However, there is limited works focusing on health risk assessment of ARVDs in water resources. We therefore propose to conduct systematic literature review of levels, distribution and health risk assessment of ARVDs in wastewater (influent and effluent), potable water, and surface water from river systems and community-based water pollution sources in Africa regions including Kenya, Zimbabwe, Botswana, Nigeria, Ghana, Congo and other Africa countries. In order to assess the health impact of ARVDs, Weibull probabilistic hazard assessment model will be applied to predict the likelihood of exceedance and predicted no effect concentrations (PNEC) values and other established standard regulatory limits for water in this study.

1.2 PROJECT AIM AND OBJECTIVES

The aim of the study is to review the concentrations of ARVDs in water bodies including wastewater, surface water, drinking water and ground water in Africa and to determine the risk to humans.

The objectives are:

1. To conduct a systematic literature review on reported levels and distribution of selected ARVDs in water bodies including wastewater, surface water, drinking water and ground water in Africa. Currently studies have been conducted in Africa countries including South Africa, Kenya, Zambia, Nigeria among others, but other countries for which data become available during the review period will be included.

2. To conduct health risks assessment on ARVDs in water resources using Weibull probabilistic risk assessment tools to estimates percentage exceedance in South Africa and in two other African countries (Kenya, Zambia with the highest and lowest ARVDs levels.

3. To forecast and model future scenarios of ARVDs including increased use and drought conditions in South Africa and the other two African countries (Kenya and Zambia).

1.3 SCOPE AND LIMITATIONS

The project is on track with the original timeline with Aim1 to be completed by the end of 2021 and Aim 2 and Aim 3 to be completed in 2022.So far, a background literature review on ARVDs was conducted, the search for studies and selection of studies for inclusion in the systematic review was completed. The death of data in Africa, especially with high quality data, is a limitation for the project. Although a large number of studies was identified before screening, only 23 studies measured the levels of ARVDs. The lack of exposure limits for ARVDs is also a limitation in evaluating the levels of ARVDs in the studies identified.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Antiretroviral drugs (ARVDs) are therapeutic class of pharmaceuticals chemicals that are mostly administered as remedy to reduce, eliminate and cure viral infections (Nannou et al., 2020). Due to astronomical increase in the incidence of viral infection across the globe has led to increase in therapeutic use and application of ARVDs. High prescription and consumption rates of ARVDs is due to global outbreak of viral infections such as Influenza, human immunodeficiency virus (HIV), COVID-19 caused by severe acute respiratory syndrome 2 (SARS-CoV-2) amongst other viruses are major contributor of these chemicals into the environmental (Boulware et al., 2014; Lee et al., 2021). For rapid efficacy of antiviral drugs in viral infected patients, several hundreds of milligrams of ARVDs are needed to be administered to keep the level of viruses present in the body at low level, in order to strengthen the patient immune system. Generally, ARVDs are mostly use for therapeutic purposes. They are popularly used for treatment of HIV, influenza, herpes, and hepatitis amongst other viral infections. Antiretroviral drugs are commonly prescribed for use in combinations as antiretroviral therapy (ART) such as taking two or more different ARVDs in one single dose for treatment of viral loads (Adeola et al. (2021). For example, ARV drugs such as lamivudine (3TC) and Emtricitabine are nucleoside analogue reverse transcriptase inhibitor frequently use for the treatment of HIV-positive and hepatitis B-positive patients (Fonseca et al., 2018). Efficacy and efficiency of ARVDs has been a major relief for public health, human existence, and world economy, as a whole. However, the extent of its ecological impacts on the environmental components has not been established. Different class of ARVDs are designed to helps the body system to recover immune that has been compromised due to viral infection (Cobb et al., 2020).

2.1.1 Class of ARVDs

Antiretroviral drugs are currently divided into six different classes, drugs and their physicochemical parameters are presented in (Table 2.1). This classification is based on the activities of ARVDs at different stages of activities targeting the viral application replication, inhibition of the key enzymes, reverse transcriptase, protease and integrase (Boulware et al., 2014).

2.1.1.1 Nucleoside reverse transcriptase inhibitors (NRTIs)

This is one of six classes of ARVDs. It is developed to interfere with the ability of a virus to multiply or reproduce. NRTIs functions by blocking the enzyme responsible to replicate itself. NRTIs require phosphorylation by cellular kinase in order to exert their activity. Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) are applied as single or combine therapy taking more than one active ingredient (Zhuang et al., 2020). Examples of NRTIS ARVDs are:

- Zidovudine (Retrovir)
- Lamivudine (Epivir)
- Abacavir sulfate (Ziagen)
- Didanosine (Videx)
- Delayed-release didanosine (Videx EC)
- Stavudine (ZeRTV)
- FTCricitabine (FTCriva)
- Tenofovir disoproxil fumarate (Viread)
- Lamivudine and zidovudine (Combivir)
- Abacavir and lamivudine (Epzicom)
- Abacavir, zidovudine, and lamivudine (Trizivir)
- Tenofovir disoproxil fumarate and Emtricitabine (Truvada)
- Tenofovir alafenamide and Emtricitabine (Descovy)

2.1.1.2 Non-nucleoside reverse transcriptase inhibitors (NNRTIs):

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are a class of ARV drug, also known as "non-nucleosides". NNRTI is similar to NRTIS, and also function by blocking the action of the enzyme. They attached themselves to reverse transcriptase which prevents the conversion RNA to DNA by the enzymes. Non-nucleosides are effective against the production of new virus as such in the case of blockage of replica of HIV virus (Engelman and Cherepanov 2021). Examples of NNRTIs include NVP, Delavirdine, Efavirenz (EFV) and Etravirine (ETR).

2.1.1.3 Protease inhibitors (PI):

Protease inhibitors is another class of ARVDs that been used for combat virus infections. They are biological or chemical components that function by binding to the protease. Protease enzymes enhance the maturation of the viral particles budding from infected cells. The protease inhibitors function by blocking the enzymatic activity and this result into formation of non-effective virus. Protease inhibitors are pharmaceutically active without any form of catalyst (Suwannarach et al., 2020). Example of protease inhibitors (PI) include the following: Saquinavir mesylate, fortovase, RTVonavir, indinavir (INDV), Nelfinavir (NFV), Amprenavir, Fosamprenavir, Atazanavir (ATV), tipranavir and darunavir (DRV).

2.1.1.4 Integrase nuclear strand transfer inhibitors (INSTIs):

This is another class of ARVDs (INSTIs). They block the actions of integrase to function, for instance in the case of HIV virus. Integrase functions by assisting the viral DNA to integrate into the DNA of the host cells. INSTIs prevent replications of virus in the host. (INSTIs) are important components of drug formulations that are effective to treat people living with HIV. Examples of firs generation INSTIS are Raltegravir, (Isentress), Dolutegravir, (Tivicay), Elvitegravir, (Vitekta), and second-generation INSTIs was developed to overcome barriers of resistance in the in the first generation and the examples of second generation INSTIs include Dolutegravir, Bictegravir amongst others (Engelman and Cherepanov 2021).

2.1.1.5 Entry inhibitors (EI):

Entry inhibitors also known as post-attachment inhibitors or is another class of ARVDs. They function by bind to the viral receptor of the host which blocks the virus from attaching or fusion into co-receptor whereby entering into the cell of the host. The viral entry within the cell requires the attachment between HIV glycoprotein and the CD4 receptor of the host cell. Entry inhibitor enzymes specifically targets and blocks the chemokine co-receptor CCR5 which is used by HIV for fusion and cell entry (Bhardwaj et al., 2021). Entry inhibitors have also been used to treat other viral infectious conditions such as hepatitis D. Examples of EI are Enfuvirtide, (Fuzeon), Maraviroc (MRV), (Selzentry) amongst others.

2.1.1.6 Cytochrome P450-3A (CYP3A) inhibitor

Cytochrome P450-3A (CYP3A) inhibitor is new class of ARVDs that is currently in use. Cytochrome P450 3A4 (CYP3A4) is an enzyme found in the liver and intestine. It functions by oxidizing and remove any form of toxins or xenobiotic in the body. Examples of cobicistat, (Tybost), ketoconazole as an index inhibitor of cytochrome P450-3A (CYP3A) activity, but the mechanism of ketoconazole inhibition of CYP3A still is not clearly established (Engelman and Cherepanov 2021).

2.2 PHYSICOCHEMICAL PROPERTIES OF ARVDs

Summaries of the physiochemical characteristics of ARVDs

Table 2. 1: Classification and physicochemical parameters of ARVDs

	Class of Antiretroviral Drugs	Active ingredient (product)	Water solubility (mg mL ^{1})	pKa (base, acid)	Log Kow
1	**Nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs)**	Abacavir (Ziagen)	77	5.77, 15.41	1.18
		FTCricitabine (FTCriva)	112	2.65	0.43
		Lamivudine (Epivir)	70	4.3, 14.29	1.44
		Zidovudine (Retrovir)	20.1	9.72	0.05
		Didanosine	20.1, 9.13	9.72, .24	0.05
		Tenofovir(Viread)	13.14, 4.13,		18.59
		Stavudine(ZeRTV)	1003, 9.95	0.72	
2	**Non-nucleoside reverse transcriptase inhibitors (NNRTIs**)	Rilpivirine(Edurant)	<0.15.6, 12.93	3.93	
		Etravirine(Intelence)	0.0169, 4.13,		12.49
		Delavirdine (Rescriptor)	0.086, 6.82, .39		
		Efavirenz (Sustiva)	0.00855, 1.5,	4.7	12.52
		NVPirapine (Viramune)	0.7046, 2.8	2.5	
3	Protease inhibitors	Tipranavir (Aptivus)	7.17E-06	3.3, 5.96	6.71
		Indinavir, (Crixivan)	0.015, 7.37,		13.19
		Saquinavir, (Invirase)	0.0022, 8.31, 5.11	2.5	
		Fosamprenavir, (Lexiva)	0.0028, 2.45, 1.22	2.2	
		RTVonavir, (Norvir)	1.1E-07, 2.84,	6.27	13.68
		Darunavir, (Prezista)	0.0087, 2.38,	1.88	13.59
		Atazanavir, (Reyataz)	4e5, 4.42,	2.88	11.92
		Nelfinavir, (Viracept)	4.5, 8.18, 9.32	8.98	
		Amprenavir, (agenerase)	0.04, 2.39,	2.2	13.61
		Lopinavir, (Aluvan)	7.7E-06, 1.5,	5.94	13.39
4	Integrase nuclear strand transfer inhibitors (INSTIs),	Raltegravir, (Isentress)	5.39E-07, 1.5, 5.62	0.4	
		Dolutegravir, (Tivicay)	0.095, 0.51,	1.62	10.1
		Elvitegravir, (Vitekta)	0.0003, 0.53,	E	6.16
5	Entry & fusion inhibitors OR Post-attachment inhibitors	Enfuvirtide, (Fuzeon)	E	E	E
		Maraviroc, (Selzentry)	6.57E-06	10.13, 7.02	5.80
6	P450-3A inhibitors	Cobicistat, (Tybost)	0.1, 6.69	E	14.18

2.3 SOURCES OF ARVDs

Antiretroviral drugs are introduced into the environment from diverse sources such as point and non-pint sources. The disposal of unused or expired ARVDs most out of out-of-date medicine is another notable source of ARVDs into the environment (Abafe et al., 2018). Most of these unused medicines are generally disposed of by the patient into the toilet/sink and eventually end up in the sewage drains and WWTPs. High concentration of ARVDs residue regularly measure in wastewater treatment plants is a major indication as a possible source into the environment. Various sources such as domestic sewage, hospitals waste, industry effluent, agriculture runoff, aquaculture waste, and animal production amongst others sources empty their content into the WWTP reservoir (Adewumi et al., 2010). Design of most WWTP in Africa is obsolete and they are unfit to efficiently remove emerging organic contaminants during water treatment. Inefficient wastewater treatment (WWTP) is major source of ARVDs into the environment. Poor hygiene and sanitation programmes in most communities in Africa also contributed to the occurrence of ARVDs ground water and eventually in accessible water which mostly serves as drinking water (K'Oreje et al., 2016; Lapworth et al., 2017). Studies revealed that ARVDs are frequently detected both in influent and effluent of most WWTP in Africa, this is observation was attributed to large number of population living on ART in southern Africa region where most of this studies were conducted (Abafe et al., 2018; Almuktar et al., 2018). Kairigo et al. (2020) reveal that ARVDs in water systems through various sources is continuously increasing, as evidenced by their detection from several water resources in Africa water (Table 2.1). Different classes of ARVDs of have been reported in the literature such as EFV, NVD, ZDV among other. Concentration of ARVDs in the majority of investigated water resources across Africa indicated that domestic water is an important pointer to ARVDs sources into the African environment.

CHAPTER 3: METHODOLOGY

3.1 INTRODUCTION

3.1.1 Study design

A systematic literature review of published peer-reviewed studies was conducted to determine the reported levels of ARVDs in water resources in Africa. This information on levels of ARVDs in environmental matrices across Africa from the literature review was used to conduct probabilistic hazard assessments to determine the risk to humans.

3.2 SYSTEMATIC LITERATURE SEARCH

A systematic literature search was conducted on reported levels and distribution of selected ARVDs in water bodies including wastewater, surface water, drinking water and ground water in Africa was conducted for the period (2010-2021). The articles selected for these studies were extracted from the four databases including Scopus, Web of Science, PubMed, and Google scholar. South African university repositories were also screened for post-graduate theses on the occurrence of ARVDs although this did not yield any results. A verbal survey of stakeholders in hospitals, ARVDs clinical research and information from department of health for additional ARVDs terms was conducted. One stakeholder suggested searching the app SA clinical Guidelines and Essential Medicine List, that lists all the ARVs on offer in public sector, while other stakeholders directly suggested additional terms. All the additional terms were included in the search. The articles were screened to remove duplicates and furthermore screened by reading the abstracts and the full articles. Original studies that did not report levels of ARVDs in water resources were not selected.

Data identification, screening and checking the eligibility and the inclusion of the relevant studies were conducted using The Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P) guidelines (Moher et.al, 2009). The articles were reviewed by the project leader and his supervisor. In cases of uncertainty for inclusion, another reviewer was consulted, and a consensus was reached following detailed discussion.

3.2.1 Inclusion criteria of the selected papers

A study was considered eligible if it complied with the inclusion criteria listed below:

1. Studies published from Jan 2010 until 2021
2. Studies published in English
3. Studies reporting levels of ARVDs in wastewater, surface water, underground water, drains
4. Observational studies
5. Studies performed in Africa and any subgroup in Africa
6. Participants of all ages and genders

3.2.2 Exclusion criteria

Studies were not considered if they met the listed exclusion criteria listed below:

1. Studies published in other languages than English.
2. Studies reporting levels of ARVDs in the environment outside Africa.
3. Non-observational studies

3.2.3 Search Item:

((Antiretroviral drugs) OR (ARV drugs)) and ((Occurrence) OR (Concentrations) OR (Levels) OR (analysis)) AND ((Water resources) OR (wastewater) OR (Surface water)) AND ((Africa)) AND ((Health risk))
(((((((Antiretroviral drugs) OR (ARV drugs)) OR ((Lamivudine OR Abacavir OR Atazanavir OR Didanosine OR Darunavir OR 8,4 dihydroxy Efavirenz OR Efavirenz OR Lopinavir OR Ritonavir OR Nevirapine OR Stavudine OR Darunavir OR Indinavir OR Emitricitabine OR Oseltamivir OR Tenofovir Disoproxil OR Saquinvir OR Zidovudine OR Dolutegravir OR Raltegravir OR Tenofovir disoproxyl fumarate))) OR ((Antiretroviral OR anti-retroviral OR ARV))) AND ((Occurrence OR Concentrations OR Levels OR analysis))) AND (("Water resources" OR wastewater OR waste-water OR Surface water)))) AND ((Africa OR African OR Algeria OR Angola OR Benin OR Botswana OR "Burkina Faso" OR Burundi OR "Cabo Verde" OR Cameroon OR Cameroun OR "Canary Islands" OR "Cape Verde" OR "Central African Republic" OR Chad OR Comoros OR Congo OR "Cote d'Ivoire" OR "Democratic Republic of Congo" OR Djibouti OR Egypt OR Eritrea OR eSwatini OR Ethiopia OR Gabon OR Gambia OR Ghana OR Guinea OR Guinea-Bissau OR "Ivory Coast" OR Jamahiriya OR Kenya OR Lesotho OR Liberia OR Libya OR Madagascar OR Malawi OR Mali OR Mauritania OR Mauritius OR Mayotte OR Morocco OR Mozambique OR Namibia OR Niger OR Nigeria OR Principe OR Reunion OR Rwanda OR "Saint Helena" OR "Sao Tome" OR Senegal OR Seychelles OR "Sierra Leone" OR Somalia OR "St Helena" OR Sudan OR Swaziland OR Tanzania OR Togo OR Tunisia OR Uganda OR "Western Sahara" OR Zaire OR Zambia OR Zimbabwe))) AND (((((((health risk) OR (health effects)) OR (health factors)) OR (health impacts)) OR (health risk assessment)) OR (risk)) OR (risk assessment))

3.2.4 Filters applied:

Abstract, Free full text, Full text, Journal Article, in the last 16 years, English Language, Water, Environment

3.3 HEALTH RISK ASSESSMENT

The impact of ARVDs residue in wastewater, surface water and other water sources was evaluated to establish the extent of impact on ecological systems and direct links with human health problems. The health risk assessment equations that are mostly used for estimating exposure to contaminants was based on a previously developed method by United States Environmental Protection Agency (USEPA) and those reported in the literature (Prasse et al., 2010). The level of health risk posed by ARVDs in human and/or environmental matrices such as surface water, WWTP effluents and influent was characterized by the Risk Quotient (RQ), which was calculated, using equation 1.

$$RQ = \frac{MEC}{PNEC} \qquad (1)$$

$$PNEC = \frac{LC_{50}\ or\ EC_{50}}{UF} \qquad (2)$$

where MEC was the concentration of target compounds in the medium. The risk was classified into three levels: RQ 0.01-0.1, low risk; RQ 0.1-1, medium risk; and RQ >1, high risk (Hernando, Mezcua, Fernández-Alba, & Barceló, 2006). EC_{50} (effective concentration, reducing a biological process by 50%) or LC_{50} (lethal concentration, killing 50% of the organisms) was obtained from the literature or by using the US EPA Ecological Structure Activity Relationship (ECOSAR v1.10) model (Kuroda, Li, Dhangar, & Kumar, 2021)): The PNEC was estimated as the chronic toxicity value divided by a standard uncertainty factor (UF), UF value of 1000 was conventionally adopted to consider the intra- and interspecies variability in the sensitivity. Chronic toxicity of ARVDs as established in the literature using experimentally derived ecotoxicity predicted ecotoxicity by ECOSAR, a computerized structure activity relationship for aquatic toxicity (EPA, 2020) (https://cfpub.epa.gov/ecotox/).

Human health exposure and risk assessment model

Health risk assessment of ARVDs contaminated water resources was estimated with two risk-based exposure pathways, ingestion and dermal contact. Levels of ARVDs obtained during the literature search were used to compute the non-carcinogenic and carcinogenic effects on human health (USEPA, 2005).

Ingestion pathway for ARVDs

$$ADD_{ingestion} = \frac{CW \times IR \times EF \times ED}{BW \times AT} \quad (3)$$

where $ADD_{ingestion}$ is the average daily dose from ingestion pathway (mg/(kg day)), which mainly means the drinking water pathway; CW is the average ARVDs concentration in water samples (ng/L); IR is the average water intake rate of exposed population (2.000 L/day); EF is the exposure frequency (365 days/year); ED is the exposure duration viral infection patient (70 years); BW is the body weight of patients (60.45 kg); and AT is the averaging time for carcinogenic exposure (25 550 days)

Dermal contact pathway for ARVDs

$$ADD_{dermal} = CW \times K_i \times S_A \times \frac{EF \times ED \times EV \times CF}{BW \times AT} \quad (4)$$

where ADD_{dermal} is the average daily dose from dermal contact pathway (mg/(kg day)); K_i is dermal adsorption parameters (0.001 cm/h); S_A is body surface areas (16 600 cm^2); EV is exposure frequency of viral infected patient in Africa (1 times/day); and CF is a unit conversion factor (0.002 L/cm^3). Average weight of viral infected patient in Africa (57.45 kg); and AT is the averaging time for carcinogenic exposure (25 550 days). Carcinogenic and non-carcinogenic effects (*HQs*) could be quantified based on available data. The hazard index (HI) was further estimated from the *HQs*; when HI values are below 1, it indicates no significant carcinogenic effects, whilst HI values which are above 1 shows the tendency of carcinogenic effects (USEPA, 2006).

Non-carcinogenic risk estimation

$$HQ = \frac{ADD}{RfD} \quad (5)$$

where HQ is the hazard quotient (unitless); R*f*D is the reference doses [3E-04 or 0.0003 mg/(kg day) and 1.23E-04 or 0.000mg/(kg day)] for ingestion and dermal contact pathways, respectively (WHO, 2016). To assess the overall potential non-carcinogenic effects posed by more than one exposure pathway, the HQ is expressed as the summation of the HQ ingestion and HQ_{dermal}.

$$HQ = HQ_{ingestion} + HQ_{dermal} \quad (6)$$

where the $HQ_{ingestion}$ and HQ_{dermal} are calculated through ingestion and dermal contact pathways, respectively. An adverse non-carcinogenic risk is regarded as possible if the HQ value exceeds 1. On the contrary, no non-carcinogenic risks were expected.

Carcinogenic risk estimation

The carcinogenic risk associated with ARVDs is expressed as the excess probability of an individual contracting the cancer over a lifetime. The model for estimating target cancer risk is provided by the USEPA (2005), which can be used for each exposure pathway.

$$TR = ADD \times SF_o \quad (7)$$

where TR is the target cancer risk (unitless) and *SFo* is the cancer slope factor obtained from the integrated risk information system (IRIS) database (1.5 and 3.66 mg/(kg day) for ingestion and dermal contact pathways, respectively). For TR, the lowest safe standard for carcinogenic risk is 10E-06, as well as the highest safe standard for carcinogenic risk is 10E-04. The cumulative cancer risks are calculated as follows:

$$TR = TR_{ingestion} + TR_{dermal} \quad (8)$$

where the $TR_{ingestion}$ and TR_{dermal} are calculated through ingestion and dermal contact pathways, respectively (Martínez-Alcalá, Guillén-Navarro, & Lahora, 2021).

3.3.1 Probabilistic hazard assessment method

This study explored past and current trends of ARVDs occurrence in different environmental matrices to establish the pollutant distribution and partitioning. Information on levels of ARVDs in environmental matrices across Africa from the literature review will be used to conduct probabilistic hazard assessments to understand Africa environmental water quality concerns. A probabilistic hazard assessment is an ideal model for large distributions of data from multiple points. Reported concentrations of ARVDs will be selected as inputs to the exposure equation over the course of multiple simulations. As a result, the output of a probabilistic assessment is a distribution of potential exposure values. Probabilistic approaches are efficient for higher-tier assessments of environmental contaminants such as ARVDs. For example, probabilistic hazard assessments have been effectively applied to predict the future trend and risk related to contaminants in water resources in various countries globally.

The cumulative probability distributions of selected ARVDs in wastewater, surface water, and drinking water in African countries with the highest and lowest ARV levels was conducted. Levels of selected ARVDs from this study was subjected to probabilistic hazard assessment to predict the possibility of exceeding regulatory values in the sample matrices. The range of assumptions being made during this study, include a clear identification of the lack of data regarding risk – including the unavailability of any limits for ARVDs in water of any type so any discussions related to their limits will not be possible. To quantify the uncertainty of the risk assessment and to assess probabilistically regulation risks, probabilistic hazard assessment was applied to further predict the possibility of exceeding regulatory values in the sample matrices. Weibull's' probabilistic approach and percentile ranking were previously reported in literature (Berninger & Brooks, 2010; Connors et al., 2014; Corrales et al., 2015).

In this study, uncertainty analysis was used to quantify the expected risk estimation of the parameters, which was then used to rank the importance of the parameters by sensitivity analysis. After simulations, the correlation coefficients between each input and output were calculated by Spearman rank correlation method to assess the sensitivity of each parameter related to the output, and then the rank correlation coefficients were squared and normalized to 100% to estimate each input contribution to the output parameter. A probabilistic hazard assessment will be employed to examine the likelihood of exceeding predicted no EC for reported levels of ARVDs above standard regulatory limits such as those proposed by USEPA, Environmental Canada and UKEA, amongst other standard regulatory limits (Zinabu, Kelderman, van der Kwast, & Irvine, 2018). Future scenarios and trend of ARVDs in aquatic systems in Africa will be predicted from the data. Future scenarios of ARVDs levels will be forecasted including increased use and drought conditions in Africa countries.

3.3.2 Weibull's' probabilistic approach

Data was evaluated and analysed using percentile ranking and Weibull health risk assessment tools to establish their health risk and percentage exceedance during the second objectives. Public health concern of elevated levels of ARVDs in aquatic systems was identified from the outcome of systemic review study. Health risks assessment on ARVDs in water resources using Weibull probabilistic risk assessment tools to estimates

percentage exceedance in African countries with the highest and lowest ARVDs levels reported. Weibull's' probabilistic approach and percentile ranking were previously reported in literature (Connors et al., 2014).

Weibull Distribution Model

The Weibull Distribution Model is a statistical distribution function of wide applicability. The objection to this theory has been stated that this distribution function has no theoretical basis. Weibull's theory claims that when the data distribution and fit the parameters seemed too good to be true. It could be applied to improve the variables. Weibull analysis is among the leading method for fitting data (Datsiou & Overend, 2018). Some of advantages of Weibull model include.

- Its flexible shape and ability to model a wide range of failure rates, the Weibull has been used successfully in many applications as a purely empirical model.
- The Weibull model can be derived theoretically as a form of Extreme Value Distribution, governing the time to occurrence of the "weakest link" of many competing failure processes. This may explain why it has been so successful in applications in engineering and research.
- Weibull is applicable when the shape parameter is 2. The distribution is called the Rayleigh Distribution and it turns out to be the theoretical probability model for the magnitude of radial error when the x and y coordinate errors are independent normal with 0 mean and the same standard deviation (Datsiou & Overend, 2018).

3.3.3 Weibull Modulus and predictive failure analysis

$$F = 1 - \ exp^{\left(\frac{\delta_f}{\delta_o}\right)^m} \qquad (9)$$

F represent the probability of failure or measured concentrations (0 < F < 1). F is derived from 0.5 means of average concentration and 50% failure. δ_f strength of failure, δ_o scaling parameters and *m* represent the Weibull Modulus. Higher Weibull modulus is a narrower distribution. Equation 9 can be express as;

$$\frac{1}{1-F} = exp^{\left(\frac{\delta_f}{\delta_o}\right)^m} \qquad (10)$$

$$\mathrm{Ln}\left(\frac{1}{1-F}\right) = \left(\frac{\delta_f}{\delta_o}\right)^m \qquad (11)$$

Linea representation of equation 11 is illustrated in equation 12.

$$\ln\left(\ln\left(\frac{1}{1-F}\right)\right) = m\ ln\ \delta_f - m\ ln\delta_o \qquad (12)$$

Where m represents Weibull modulus values is assigned to observed probabilities. The $\boldsymbol{\delta_f}$ and $\boldsymbol{\delta_o}$ values were derived from the graph and data obtained will be fitted into a straight-line graph (Datsiou & Overend, 2018).

Statistical analysis

Descriptive statistics such as sum, mean, medium, to maximum parameters will be calculated using Origin Pro and Microsoft Excel tools. The data was subjected to one-way and two-way ANOVA for repeated measures and Spearman and Pearson correlation using SPSS 24 statistical software. Level significant differences among the sample types was determined.

3.4 ETHICS

This study offers no risk of harm to human participants and there would be no risk from COVID-19 as research was conducted online. The protocol was approved by the Departmental Research Committee of the School of Public Health and Family of the University of Cape Town (UCT) and the UCT's Faculty of Health Sciences Research Ethics Committee confirmed that no Ethics Approval is required.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 SCREENING RESULTS

Figure 4.1 summarises the studies screened.

Total number of 2089 studies were identified using the search terms from the data bases: Web of Science (n = 65, PubMed (n = 179), Scopus (n = 735) and Google Scholar (n = 1110). Sixty-five (65) duplicated studies were excluded. Two thousand and nine (2009) articles were excluded based on title and abstract. The full text of one hundred and eighty (180) articles were screened and assessed for eligibility for the study and ninety-seven (97) articles not measuring Antiretroviral drugs (ARVDs) levels were excluded, and therefore leaving twenty-nine (29) studies included in systematic review.

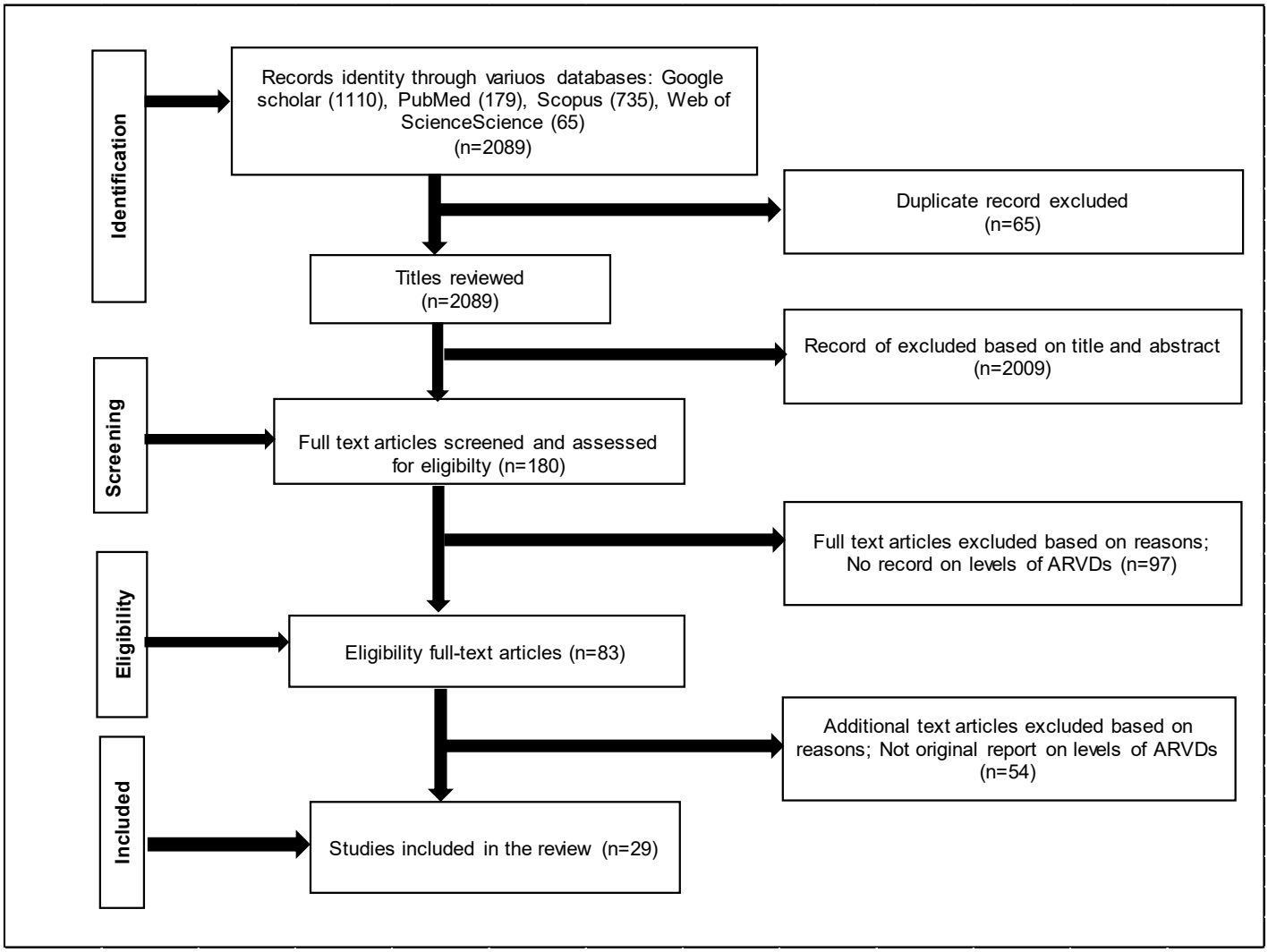

Figure 4. 1: PRISMA flow chart of process of how papers were screened.

4.2 OVERVIEW OF STUDIES INVESTIGATING ARVDs IN AFRICAN COUNTRIES

Twenty-nine studies (29) were identified (Table 4.1). Most of the studies countries were conducted in South Africa (n = 18, 62%), followed by Kenya (n = 8, 28%), Nigeria (n =2, 7%) and Zambia (n =1, 3%). Most of the studies identified were therefore conducted in Southern Africa (65%), followed by East Africa (28%) and West Africa (7%). Most of the water samples in the studies identified, were collected from surface water (23 studies, 79%) and wastewater treatment plants (WWTP) (18 studies, 62%) with few (4 studies, 1.4%) collected from drinking and groundwater sources. The number of sites investigated in the studies varied from 2-50 sites and the number of samples varied from 2-812 samples. The duration of sampling in the studies varied from 3 days-3 years with 9 studies (31%) sampling for at least a year during all four seasons.

Table 4. 1: Summary of studies reporting concentrations of ARVDs in African water resources.

N/S	Year	References	Country & location	No of Sampling sites & size	Sampling period and duration	Analytical method and MDL	Media	Summary of ARVDs detected
1	2012	(K'oreje et al., 2012)	Kenya	8 sites, 24 samples	2010 (3 days in July (27,28 & 30th)	HPLC-MS/MS MDL; <50 ng/L	Surface water	NVP 2000, ZVD 9000, 3TC 1200 ng/L
2	2015		South Africa:	24 sites	2014	GC-TOF/MS	WWTP effluent	NVP 350, EFV 7100 ng/L
			WTW, Gauteng	24 samples	(3 days)	LOQ: Nev 6.0, EFV 25.9 ng/L	WWTP influent	NVP 2100, EFV 174000 ng/L
3	2015	(Wood et al., 2015)	South Africa (Gauteng)	29 sites 29 samples	2013-2014 (Between: 25-Feb 2011-22 Aug 12014)	UPLC-ESI-MS/MS MDL: Nev 92.7, EFVfz 519.0 ng/L	Surface water	NVP (0-1480)360, ZVD (0-973)319, Dida (0-54) 54,3TC (0-242)160. STVD (0-778043),TVN ng/L
4	2016	(K'oreje et al.. 2016),	Kenya	19 sites	2012-2013	HPLC-ESI/MS/MS	Surface water	NVP 2246.3, EFV 176.42, ZVD 4000.7, 3TC 58254.3 ng/L
			Nairobi and Kisumu city (WWTP), Kenya	24 Samples	(Sept 2012-July 2013)		WWTP effluent	NVP 1723.3.3, EFV 106.6, ZVD 96.9, 3TC 26946.6.3 ng/L
							WWTP Influent	NVP 2076.6, EFV 753.3, ZVD 15166.6, 3TC 40683.3 ng/L
							Ground water	NVP 940, EFV 23.3 ng/L
5	2016	(Ngumba et al., 2016)	Kenya	40 sites	2014	LC-ESI/MS/MS	Surface water	NVP 4859, ZVD 7684, 3TC 5428 ng/L
			Nairobi River Basin	120 samples	(October dry season)	LOQ: Ranged btw 8-122 ng/L	WWTP effluent	NVP 2110, ZVD 100 ng/L
6	2017	(Schoeman et al., 2017)	South Africa	7 points	2016	GC-HT/TOF/MS LOQ: NVP 6.0, EFV 25.9 ng/L	WWTP effluent	NVP 258, EFV 3959 ng/L
			WWTP, Gauteng, SA	28 samples	(25/05 & 14/06 June) 4wks		WWTP influent	NVP 9653.5 ng/L
7	2017	(Wood et al., 2017)	South Africa (from; Selected rivers across: RDS, RD, OWS,CR)	31 sites 72 samples	2013-2016	UPLC-QTOF/MS LOQ: Nev 1.7, ZDV 0.4, EFV 10.93 3TC 1.2 ng/L	surface water	NVP (0-3740123, LPV (1-359)204, RTV (59-1130)481, EFV 93-696)174, 3TC (0-21) EMT (0-361 ng/L
8	2017	(Wooding et al., 2017)	South Africa Pretoria (Rietvlei Nature Reserve)	6 sites 18 samples	2015 & 2016 (2/6/2015, repeated 15 /3/ 2016)	GC/GC-TOF/MS LOQ: NVP 148, EFV N/A ng/L	Surface water	NVP (0-227)65.7
9	2018	(Abafe et al., 2018)	South Africa	6 Sites	2016	LC-ESI/MS/MS)	WWTP effluent	NVP 1900, LPV (1900-3800), RTV (460-1500), EFV 34000, ZVD (87-500), 3TC (0-130), MRV 29, RALT (0-35000, DRV (130-17000), ATV (78-740), Ind (24-42) ng/L
			WWTPs & DEWATS KwaZulu-Natal	18 Samples	(Btw 15-19 Aug 2016)	LOQ: Ranges btw 12-65 ng/L	WWTP influent	NVP (24000-34000), LOP (1200-2500), RIT (0-1400), EFV (8049-22000, ZVD (6900-53000), 8,4 Di EFV (61-11700), TVN(0-180), MAV (82-320) 29, RALT (61-11700,

N/S	Year	References	Country & location	No of Sampling sites & size	Sampling period and duration	Analytical method and MDL	Media	Summary of ARVDs detected
								DRV (69-4300), SQV(0-180) ATV (64-1400), IND (260-590), ABC (0-1400) ng/L
10	2018	(K'Oreje et al., 2018)	Kenya	28 sites	2015-2016	UPLC-QTOF/MS	Surface water	NVP 6000, ZVD 1300, 3TC 19670 ng/L
			WWTP & Nzoia Basin, Kenya	198 samples	(Aug-Sept 2015, May 2016)	LOQ: Nev 0.2-49, ZDV 73-132 ng/L	WWTP effluent	NVP 9500, ZVD 1500, 3TC 100000 ng/L
							WWTP influent	TVN (6000-50000), EFV 58000-405000 ng/L
11	2018	(Rimayi et al., 2018)	South Africa Hartbeespoort Dam catchment & uMngeni River	14 sites 36 samples	2014-2016 (Btw Nov 2014-Sept 2015 & 19 May 2016)	LC-ESI/MS/MS) LOQ: Nev 0.67, EFV 0.3 ng/L	Surface water	NVP (23-68), EFV (12-225) 138, 3TC (0-2), EMT (2-8) ng/L
12	2019	(Gerber 2019)	South Africa	7 sites	2017	UPLC-QTOF/MS	Surface water	NVP (0-300), LOP (800-12750), RTV (640-1960)148, EFV 8830, ZVD 3630 ng/L
			(Gauteng, Ekurhuleni and Tshwane catchment area)	26 samples	monthly (Btw 10/2017-2018,	LOQ: Nev 932, EFV 3375, ZDV 3346 ng/L	Drinking water	NVP (0-210), Lop (0-904), EFV (0-2330) ng/L
13	2019	(Mtolo et al., 2019)	South Africa (WWTPs Durban	9 sites	2018	LC-PDA (photodiode array)	WWTP effluent	EFV 66560 ng/L
			and Msunduzi River Pietermaritzburg, Durban)	12 samples	(May & October)	LOQ: Nev1.39 ug/L	Surface water	EFV 975-2450 ng/L
14	2019	(Mosekiemang et al., 2019)	South Africa	3 sites	2016-2018 (April-July 2016 April 2018)	UPLC-QTOF-MS/MS	Surface water	NVP (658-1430), RTV (780-20000), 3TC (3670-48700), EMT (721-3520), 8,4 Di EFV (1480-15200) ng/L
			WWTP Western Cape province	20 samples		MLD: Nev 932, EFV 0.004, NVP 0,003, RTV 0,005, 3TC 0.004 ng/mL	WWTP effluent	NVP (0 681), EFV (1420-15400), 3TC (3670-20900), EMT (31300-17200) ng/L
15	2020	(Kairigo et al., 2020)	Kenya	6 points	2019	LC-ESI/MS/MS)	Surface water	NVP (900-2300), ZVD (1100-2100), 3TC (219000-228000) ng/L
			(WWTP Machakos &	(14 samples)	(Jan & Sept)	MQL: 3TC 15, ZDV 53, NVP 19 ng/L	WWTP effluent	NVP (9500), ZVD (1400), 3TC (847000) ng/L
							Sediment	NVP (101-955), ZVD (118-510), 3TC (107-491) µg/kg
16	2020	(Kandie et al., 2020)	Kenya Lake Victoria South Basin	50 sites 50 samples	2017 (Sept & Oct)	HPLC-LC-HRMS /MS)	Surface water	NVP (0-12660)770, 3TC (0-50300)27200 ng/L
17	2020	(Mlunguza et al., 2020)	South Africa	10 sites	2019	UHPLC-QTOF-HRMS	Dam/ surface water	TEV (0-110)
			(WWTP Around Durban city &	30 Samples		LOQ: EFV 0.3-0.5, EMT 0.033 ug/L	WWTP effluent	NVP 1720, EFV (0-3270), EMT 359 ng/L
			Johannesburg, Hartbeespoort dam, Pretoria)				WWTP influent	NVP 3270-37300, OSLT (200-250), EMT(1-3100) ng/L

N/S	Year	References	Country & location	No of Sampling sites & size	Sampling period and duration	Analytical method and MDL	Media	Summary of ARVDs detected
18	2020	(Muriuki et al., 2020a)	Kenya	3 sites	2019	LC-MS/MS	WWTP effluent	NVP (2006-3214), ZVD (2415-19464), 3TC (1131-69681) ng/L
			Machakos and Nyeri counties	24 samples	(Sept 2019)	LOQ: Ranged between 0.1-1.9 ng/L	WWTP Influent (SPM)	NVP (563-3795), ZVD (3596-4447), 3TC (1248-30761) ng/L
19	2020	(Muriuki et al., 2020b)	Kenya	6 sites	2019	LC-ESI/MS/MS)	Drains surface water	NVP (1400-145000)3600, ZVD (6200-34000)15000, 3TC (124000-913000) 532000 ng/L
			Juja town	14 samples	(August 2019)	LOQ: 3TC 3.1, NVP 0.5, ZDV 2.9 ng/L	Drains sediments)	NVP (527-1480)1000, ZVD (106-8590)3370, 3TC (125-10200)447 ng/L
20	2020	(Ngumba et al., 2020)	Zambia	33 sites	2016	LC-ESI/MS/MS)	Surface water	NVP (210-220), ZVD (1280-9670), 3TC (42630-49700) ng/L
			(Chunga,	33 samples	(June 2016 period for a week)		WWTP effluent	NVP 1720, ZVD 37140, 3TC 55760 ng/L
							Ground water	NVP (0-410)150 ng/L
21	2020	(Ogunbanwo et al., 2020)	Nigeria	22 sites	2017-2018	HPLC-MS/MS	Surface water	OSLT (910-3380) ng/L
			Lagos	22 sites	(Apr & Jul 2017, Oct 2017 & Jan-March 2018)	LOQ: OSLT 13.3 ng/L	Sewage effluent	OSLT (690) ng/L
22	2020	(Omotola and Olatunji 2020)	South Africa KwaZulu-Natal	20 sites	2018	LC-QTOF/MS	Surface water	3TC 2354-3176 ng/L
				60 samples		LOQ: 3TC 0.1462 ug/L	Dam inlet	3TC 3870 ng/L
							Dam outlet	3TC 12670 ng/L
23	2020	(Vogt et al., 2020)	South Africa	33 sites	2013-2014 &	UPLC-QTOF/MS	Surface water	NVP (0-1300) ng/L
			(Gauteng and North West)	72 samples	Sept-Oct 2017	LOQ: EFV 3375, LOP 248, NVP 1074, ZDV 1111 ng/L	WWTP effluent	NVP (0-1300), Lop (0-20110), EFV (0-20400), ZVD (0-3680), 3TC (0-29) ng/L
24	2021	(Madikizela & Ncube, 2021)	South Africa	6 sites,	2020	UHPLC-QTOF-MS	Surface water	EFV ND- <LOD ng/L
				48 samples	(4hr between	LOD 0.01-0.106, <LOQ 0.01-0.352 ng/L		
					10am-2.00pm)			
25	2021	(Yao et al., 2021)	South Africa	2 Location	2020	UHPLC-QTOF-MS	Surface water	EFV ND- <LOD ng/L
				10 sites		MDL; <50 ng/L	WWTP effluent	
26	2021	(Holton et al., 2021)	South Africa	10 sites	10 months	LOQ: NVP 0.05, LOP 1.94, RIT 0.8, DID 0.05 EFV 1.69, ZVD 1.67ug/L	Surface water	NVP <LOQ, LOP <LOQ, <LOQ, DID <LOQ EFV <LOQ, AZT <LOQ ug/L
				812 samples	July 2018-May 2019		WWTP influent	
27	2021	(Horn et al., 2022)	South Africa	22 sites	3 months	UHPLC-QTOF-MS	WWTP	AZT <LOQ, EFV <LOQ-25.0, RIT <LOQ-1.9, LOP <LOQ-39, NVP <LOQ-1.4.

N/S	Year	References	Country & location	No of Sampling sites & size	Sampling period and duration	Analytical method and MDL	Media	Summary of ARVDs detected
			Gauteng	22 samples	Sep-17	LOD: 0.01-0,5 µg/L		
28	2021	(Späth et al., 2021)	South Africa	12 sites	3 consecutive days (29-31 March 2016)	UHPLC-QTOF-MS	WWTP Influent	ABC 100, ATV 3100, DAR 14000, 3TC 74000, NVP 350, RALT 4100
			Gauteng	82 samples		LOD: 0.01-0,5 µg/L	WWTP Effluent	ABC 540, ATV 3000, DAR 10000, 3TC 130000, NVP 350, RALT 3500
29	2021	(Hu et al., 2021)	Nigeria,	14 sites	December 2nd and December 17th, 2017	UHPLC-QTOF-MS	Sewage TP	
			Lagos, Sango Ota	14 samples		LOQ: 0.01-0.03 µg/L	Surface water	NVP <LOQ
							Tap water	

3TC: Lamivudine, ABC: Abacavir, ATV: Atazanavir, ddl: Didanosine, DRV: Darunavir, Di-EFV: 8,4 dihydroxy Efavirence, EFV: Efavirenz, LPV: Lopinavir, RIT: Ritonavir, NVP: Nevirapine, STV: Stavudine, DRV: Darunavir, INDV: Indinavir. FTC: Emitricitabine, OSLT: Oseltamivir, TEVD: Tenofovir Disoproxil, SQV: Saquinvir, ZVD: Zidovudine, DTG: Dolutegravir, RAL: Raltegravir, TDF: Tenofovir disoproxil fumarate)

4.3 ANTIRETROVIRAL DRUGS DETECTED IN AFRICAN WATER SYSTEMS

There were 18 ARVDs that were investigated in the identified studies. The ARVDs detected most frequently in the studies reviewed, include NVP (n = 21, 72%), EFV (n = 12, 41, 7%), 3 TC (n =11, 37.9%), ZVD (10, 34.5%), RIT (n = 6, 21,0%) and LOP (3, 10.3%), with all the other ARVDs detected in less than 3 studies. Additionally, EFV, NVP, LOP, AZT, DD and RIT were not detected in any studies (number of studies = 3, 2, 2, 2 ,1 and 1 respectively). ARVDs were detected in ground and drinking water in 3 studies including NVP in 3 studies, EFV in 2 studies, LOP and Oseltamivir (OSLT) in one study. NVP was not detected in tap water in one study.

Figures 4.2-4.4 presents the ARVDs detected in surface and wastewater samples, with the highest concentrations in the studies included in the review. The highest concentrations of ARVs detected in surface and WWTP samples were 3TC and EFV with peak concentrations > 50 0000 ng/L. The other ARVDs among the highest concentrations include LVP, FTC, ZDV, FTC, NVP, OSLT and Raltegravir (RTV). The highest concentrations of ARVDs were detected in Kenya and South Africa with most ARVDs detected in South Africa. South Africa has been found to have the highest consumption of ARVDs in Africa (Emeji, Ama, Khoele, Osifo, & Ray, 2021).

Lamivudine (3TC) is a one of the most prominent and frequently used ARVDs for the treatment of HIV. It is highly soluble in water (solubility of 70,000 mg/L and polarity: log Kow = -2.62) (Funke, Prasse, & Ternes, 2016). Levels of 3TC detected in other settings were much lower. In German wastewaters, 3TC was detected at concentrations up to 720 ng/L (Funke et al., 2016). In another study, concentrations of 3TC up to 55 ng L in influents and 22 ng L in effluents (Ngumba et al., 2016). 3TC is the most prescribed ARVDs, since it constitutes the first line daily dose antiretroviral regimen for people living with HIV.

Efavirenz (EFV) belongs to the class NNRT, it is often used as constituent of a cocktail ART doses prescribed to HIV patients (Mbuagbaw et al., 2016). Efavirenz is excreted from the body as both unaltered and metabolites. Efavirenz transformed into several hydroxylated metabolites in human body before excretion. Efavirenz is persistence and stable in the water with low log K_{ow} value which indicates its availability and potential to bind to the solid phase in the environment. some authors suggest that elevated levels of ARVDs in most WWTP could also be attributed to purification process such as short hydraulic residence time of wastewater in the activated sludge system during water treatment, and the formation of recalcitrant ARVDs during metabolism (Abafe et al., 2018; Madikizela et al., 2020). Efavirenz is one the frequently used ARVDs that is detected in water resources at higher concentrations relative to other commonly used ARVDs (Mosekiemang et al., 2019).

Nevirapine (NVP) is another ARVD that have been widely used for treatment antiretroviral infections HIV in humans. Only one study conducted in SA by Gerber (2019), tested ARVDs in drinking water samples and detected up to 2330 ng/L EFV, 904 ng/L LPV and 210 ng/L NVP. Although these concentrations are substantially lower than those detected in WWTP and surface water samples, their presence in drinking water is a concern. Reproductive effects such as testicular morphological changes and decreased sperm quality has been associated with exposure to elevated concentrations of ARVDs in humans (Ahmad et al., 2011, Azu et al.). ARVDs were also detected in groundwater samples in a study conducted in Kenya (Koreje et al., 2021) in which NVP and EFV were detected at low concentrations (940 and 23.3 ng/L) and in Zambia (Ngumba et al., 2020b) in which NVP was detected (0-420 ng/L). Groundwater used as portable water resource especially for drinking could be a risk to exposed communities. Additionally, possible access to surface water sites could also present a risk to humans.

Concentrations of abacavir (ABC), zidovudine (ZVD), nevirapine (NVP), ritonavir, 3TC, lopinavir (LPV), and EFV have been reported in other parts of the world, in countries such as France, Germany, Finland (Aminot et al., 2015; Funke et al., 2016; Ngumba et al., 2016).

With limited or no experimental data available for evaluation, ecological risk assessment (ERA) could be estimated using recommended EC50 or LC50 values from the USEPA. Ecological Structure Activity Relationships Class Program (ECOSAR database) (Horn et al., 2022). Derived information of the level of toxicity of the compounds could be used to define the predicted-no-effect-concentration (PNEC) for the lowest effective concentration. Also, risk quotient (RQ) can be calculated based on the PEC/PNEC ratio and used to characterize the risk in relation to aquatic ecosystems (Kumari & Kumar, 2022).

Recent studies have reported on the possible ecotoxicity of ARVDs on the environment and living components (Kudu, Pillay, & Moodley, 2022). Lamivudine was found to pose an ecological health risk at different trophic levels, to both flora and fauna, at concentrations previously found in the environment (Omotola, Oluwole, Oladoye, & Olatunji, 2022). NRTIs has been found to cause ecotoxicological effects in exposed organisms by integrating the nucleoside structure of an organism into DNA or RNA-strains (Azu, 2012; Sigonya, 2021). Ritonavir has also been reported to display a high ecotoxicity potential (Escher et al., 2011). A mixture of ARVD residues with other compounds in aquatic environment have the potential to produce an increased ecological impact compared to single antiretroviral chemical in the environment (Peng et al., 2014).

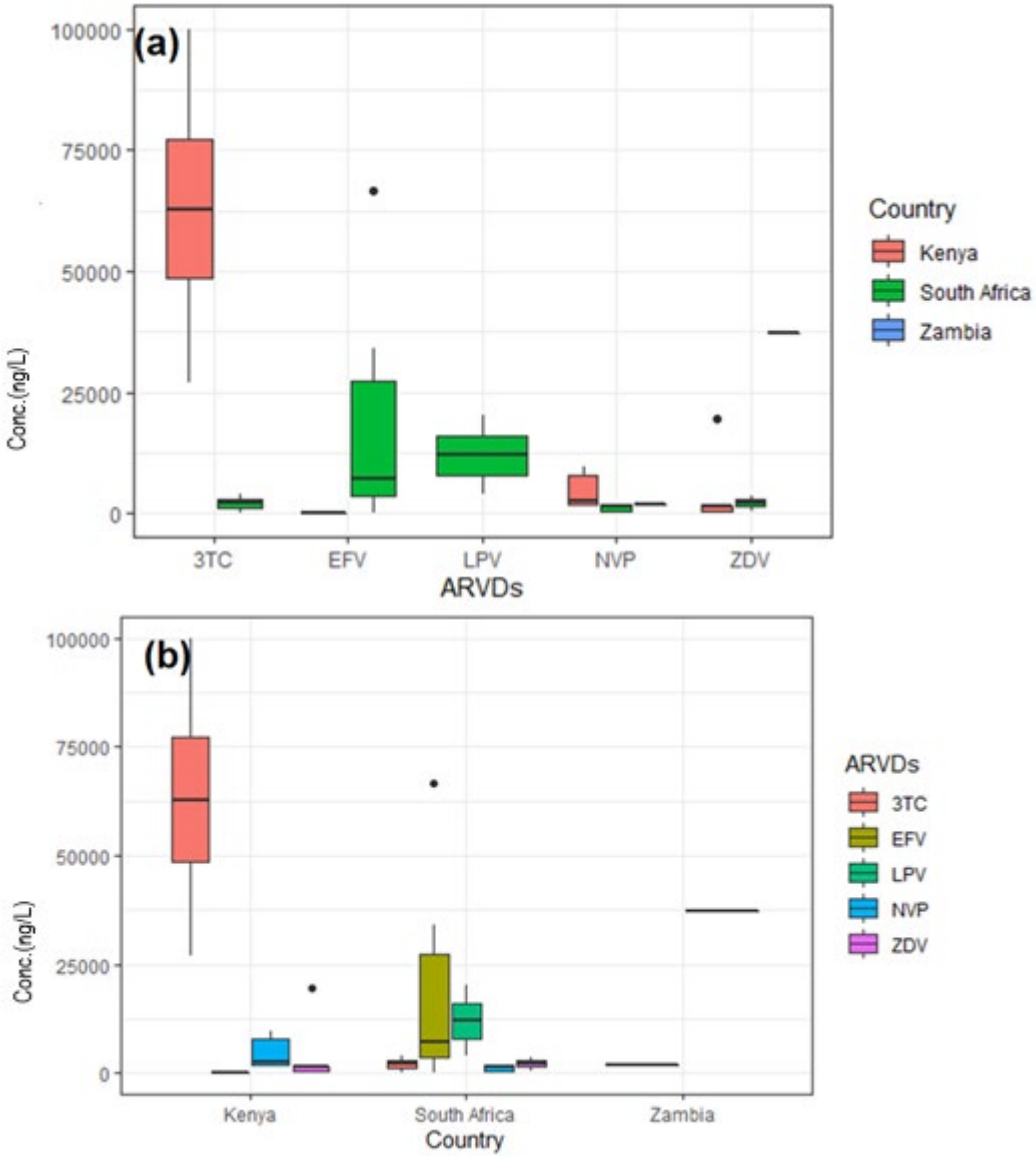

Figure 4. 2: Levels of ARVDs in (influent) WWTP (a) ARVDs compounds (b) different countries

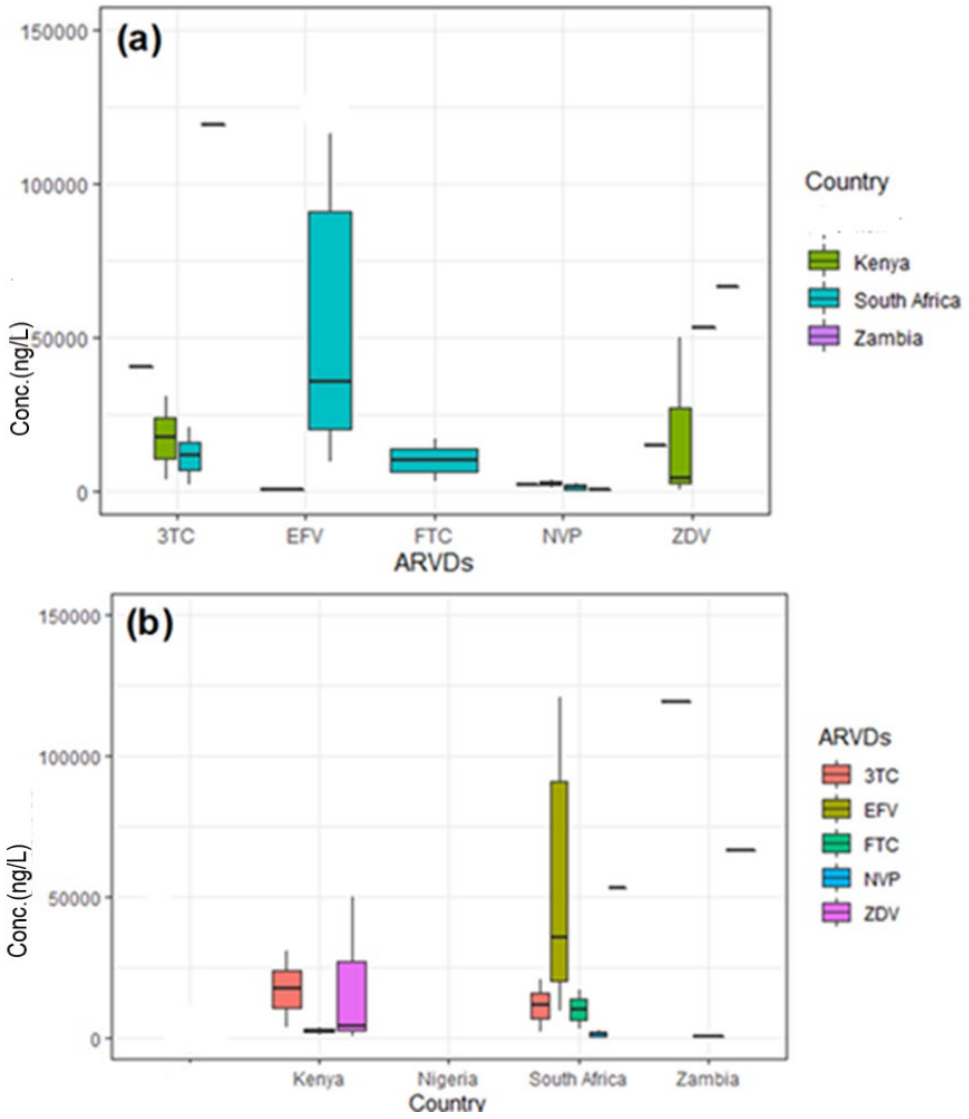

Figure 4. 3: Levels of ARVDs in (effluent) WWTP (a) concentrations of ARVDs compound (b) concentration in different countries

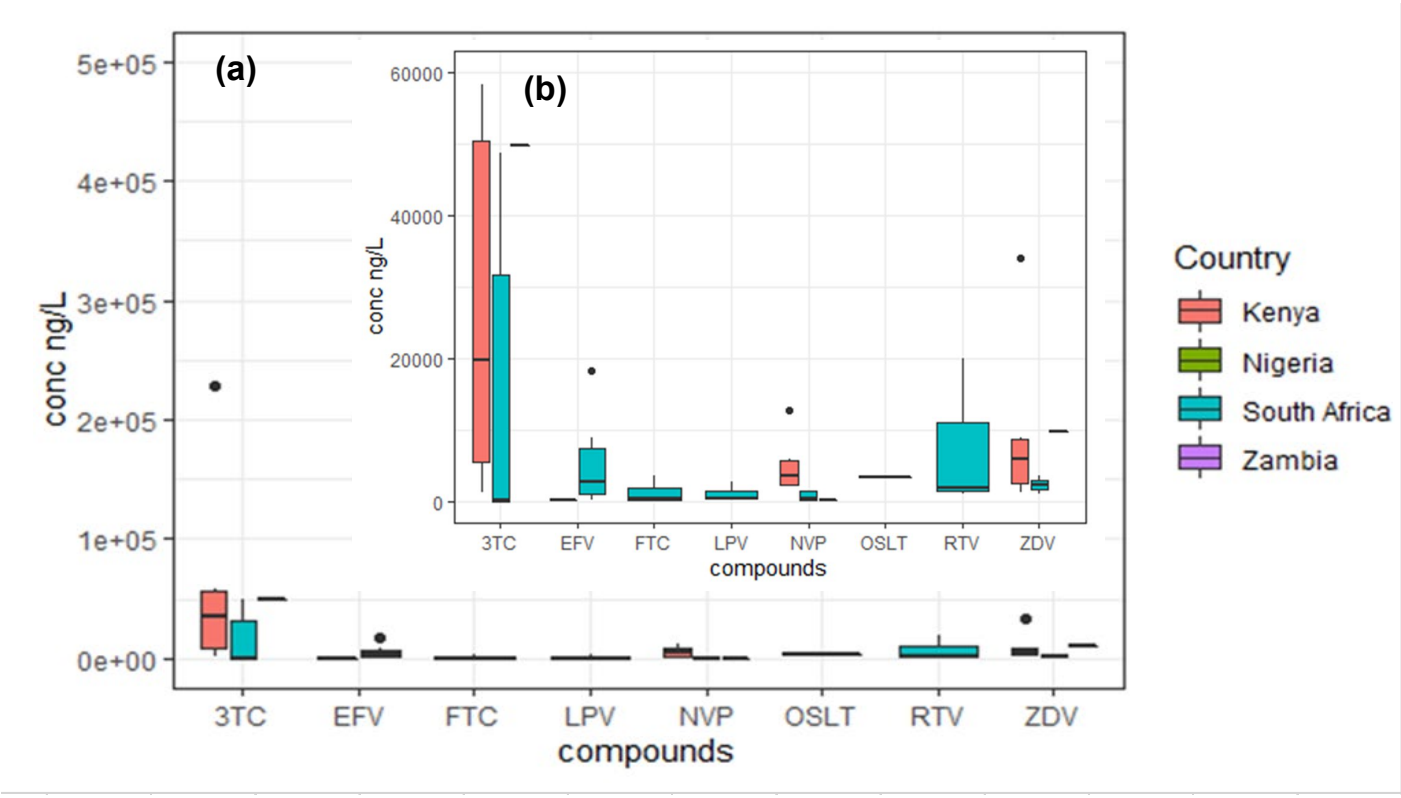

Figure 4. 4: Levels of ARVDs in surface water (a) concentration range (b) concentration range in different countries

4.4 ANALYTICAL METHODS FOR MEASURING ARVDs IN THE ENVIRONMENT

Current advances in instrumentation and analytical capabilities have enabled determination of low concentration of micro contaminants such has ARVDs in the environment matrices including surface waters, wastewater, groundwater, drinking water, soil, and aquatic organisms (Madikizela et al., 2020; Ngqwala & Muchesa, 2020). Due to the complexity and dynamics surrounding most of these environmental matrices, there is need for robust, sensitive, and efficient protocol that can accurately measure their levels in the water resources. Like other pharmaceuticals and other contaminants in the environment, ARVDs residues co-exists with other chemicals and components in the environment. Analysis of ARVDs can be discussed based on the method mode of extraction, instrumental analysis for identification, recovery method for the analysis.

Extraction methods

Solid phase extraction (SPE) method is the most reported method frequently used for routine extraction of organic contaminants like ARVDs in environmental matrices. Most articles included in this study reported the use of offline-mode SPE for extraction of ARVDs in water resources (Abafe et al., 2018; Madikizela et al., 2020; Mosekiemang et al., 2019; Prasse et al., 2010) while there are no studies reporting the application of the on-line mode SPE. Also, it was noted that among the available SPE sorbent for sample clean-up, hydrophilic lipophilic balance (HLB)(Oasis) were frequently used, this is due to its versatility and efficiency for a wide spectrum of organic chemicals with different physicochemical attributes (Abafe et al., 2018; Nannou et al., 2020). Other SPE cartridges also in use include mixed-mode sorbent (MCX), Bond Elut SCX, Isolute ENV+, Strata SDB-L, Cleanert PEP, Bond Elut Plexa amongst other sorbents available in the market (Kapoor,

Khandavilli, & Panchagnula, 2006; Madikizela & Chimuka, 2017). Studies also showed that combination of two or more sorbents shown some improvement in recovery of analytes in the literature (Horn et al., 2022; Yao, Chen et al., 2021). Examples of such combinations was described by Li, Undeman, Papa, and McLachlan (2018) where HLB, Strata X-CW, Strata X-AW and Iso lute ENV+ Strata WCX, ZT, WAX (Azuma et al., 2017) and Isolute ENV+ (Thompson, van den Heever, & Limanowka, 2019), HLB and Bond Elut SCX (Azuma et al., 2017). Alternatively, other extraction methods reported include the application of molecularly imprinted polymer (MIP) SPE (Mtolo, Mahlambi, & Madikizela, 2019) and extraction on an in-house disposable loop sampler (Wooding, Rohwer, & Naudé, 2017) which have been successfully applied in laboratory scale. Also, direct injection of sample into the LC-MS/MS system have also been considered with outright elimination of extraction step during analysis, this method excludes chances of determining ultra-trace concentration of ARVDs (Battal, Süküroğlu, Alkaş, & ÜNLÜSAYIN, 2021). However, these optional extraction procedures come with their peculiar limitations which may affect the overall purpose of extraction using the sorbents.

Identification and quantification of ARVDs

For reliable identification and quantification of ARVDs residue in water resources, several analytical protocols have been developed and applied in the literature. Liquid chromatography (LC) coupled to (tandem) mass spectrometry (LCMS/MS) is the most prominent among list due to its versatility and efficiency (Battal et al., 2021; Mosekiemang et al., 2019; Rimayi, Odusanya, Weiss, de Boer, & Chimuka, 2018). Development of high-resolution mass spectrometers such as Orbitrap and Q-Tof technology which allows wide spectrum of analytes to be identified both target and non-target compounds is a great advantage for determination of ARVDs in complex matrices (Kapoor et al., 2006). On the other hand, application of gas chromatography mass spectrometry (GC-MS) is less popular, hence, is not frequently use for analysis of ARVDs. Other workers reported the application of a two-dimensional gas chromatography with time-of-flight mass spectrometry (GC-TOFMS) (Teehan, Schall, Blazer, & Dorman, 2022). The major advantage of LC-MS over the GC-MS include the ability to identify and measure a broader range of compounds with minimal sample preparation. For efficient and stability of the column for both LC and GC compartment, attachment of a guard column is advised to prevent damaging the separation column in use (Andries, Rozenski, Vermeersch, Mekahli, & Van Schepdael, 2021).

4.5 RISK ASSESSMENT OF ARVDs DETECTED IN AFRICAN WATER SYSTEMS

Levels of ARVDs reported in studies in the literature review was used to estimate ecological risk by determining the risk quotients, non-cancer health risk in different water resources including wastewater Influent, effluent and surface water using. The permissible levels and published PNEC values from literature and previously derived by EMA, WHO and USFDA for pharmaceutical in water were used in this study.

4.5.1 Assessment of ecological risk of detected ARVDs

Figure 4.5 summarises the ecological risk of the ARVDs detected in African water systems identified in the systematic literature review. WWTP influent was the water systems that had the highest ecological risks with 3TC (5,68 – 3240), followed by ZDV (0,94 – 1224), NVP (up to 152) and then EFV (up to 2.33), the ARVD that had the highest risk. The highest RQ for WWTP effluent was for 3TC (6,22 – 6776), ZDV (0,18 – 68,27), while the RQ for surface water ranged between 1,78 – 62,50. Recent studies have reported possible ecotoxicity of ARVDs on the environment and living components (Kudu, Pillay, & Moodley, 2022). Lamivudine was found to pose an ecological health risk at different trophic levels, to both flora and fauna, at concentrations previously found in the environment (Omotola, Oluwole, Oladoye, & Olatunji, 2022). NRTIs has been found to cause ecotoxicological effects in exposed organisms by integrating the nucleoside structure of an organism into DNA or RNA-strains (Azu, 2012; Sigonya, 2021). Ritonavir has also been reported to display a high ecotoxicity potential (Escher et al., 2011). A mixture of ARVDs residues with other compounds in aquatic environment have the potential to produce an increased ecological impact compared to single antiretroviral chemical in the environment (Peng et al., 2014).

3TC is the most prescribed ARVDs in South Africa (Funke et al., 2016) and this pose environmental, and health concerned due to its high ecotoxicological risk because of its exceptionally high hydrophobicity (Escher et al., 2011). The highest risk of ARVDs in water systems was found in n Kenya, followed by South Africa while there was minimal risk to ARVDs in water resources in both Zambia and Nigeria (Figure 4.6).

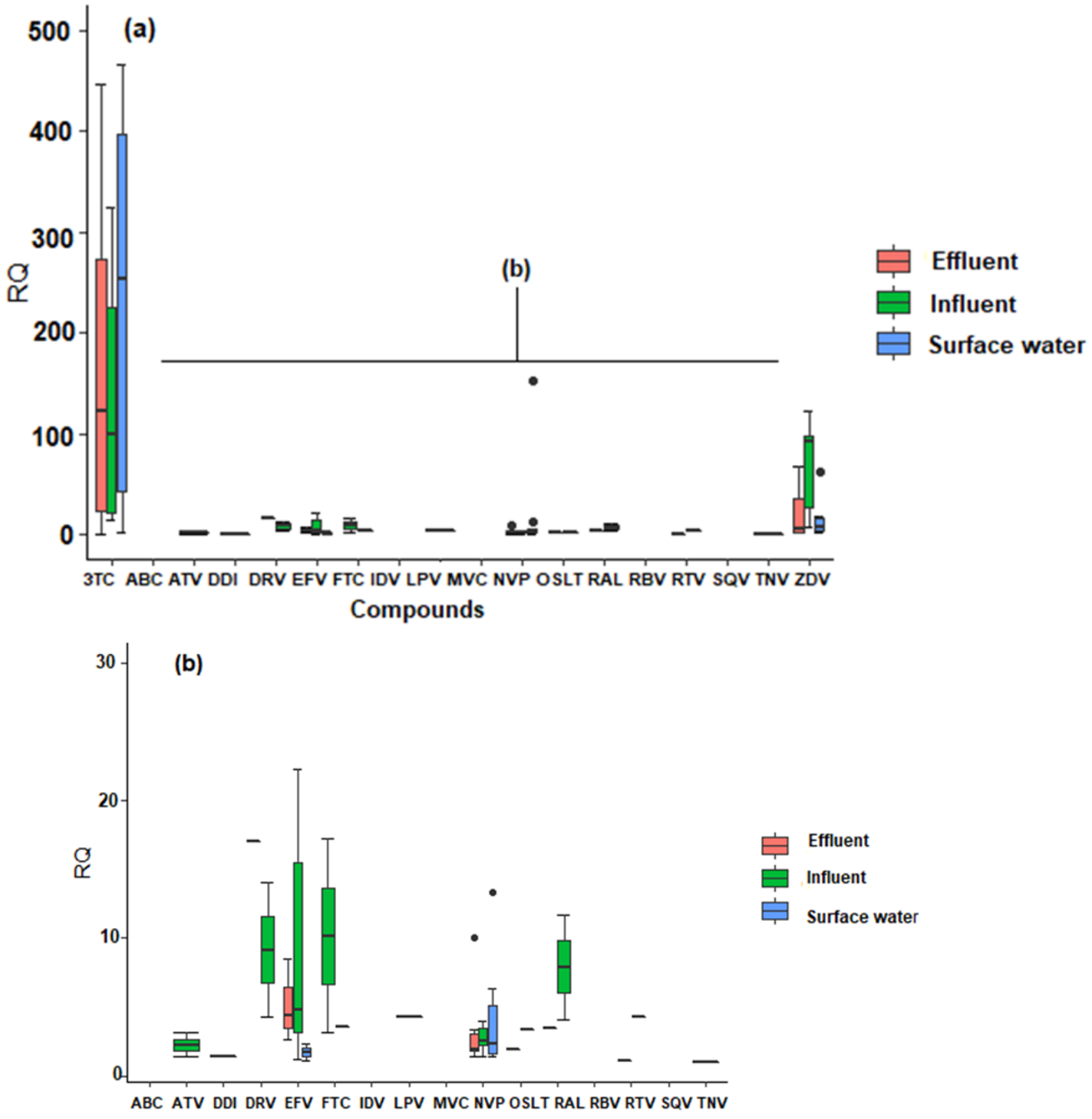

Figure 4. 5: (a) Risk quotient (RQ) for ARVDs in Effluent, influent and surface water (up to 500 ng/L) (b) RQ for ARVDs below 30 ng/L

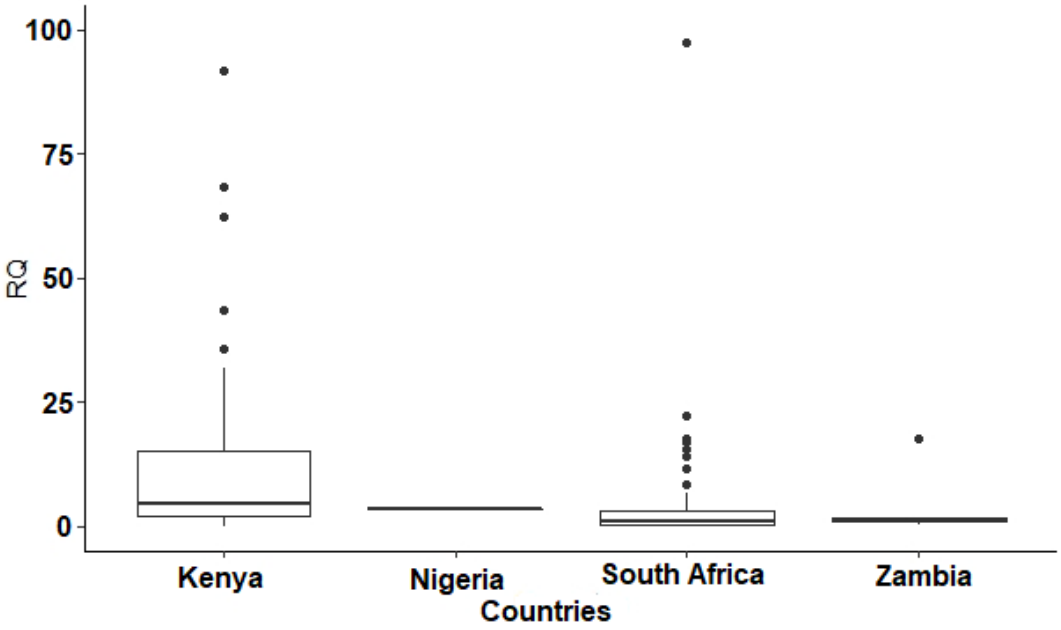

Figure 4. 6: Box-Whisker plot summarizing the risk quotient (RQ) of antiretrovirals in water resources in Africa countries.

4.5.2 Assessment of human health risk of detected ARVDs

Cancer risks and non-cancer risks were very high for several ARVDs with 3TC, followed by ZVD (4.7 ng/L) and LPV (5.9 ng/L). It should be pointed out that the risks for humans were calculated based on the assumptions of for drinking water which is not applicable for most of the water resources monitored in the studies. But the modelling demonstrates the high levels of ARVD in these waters.

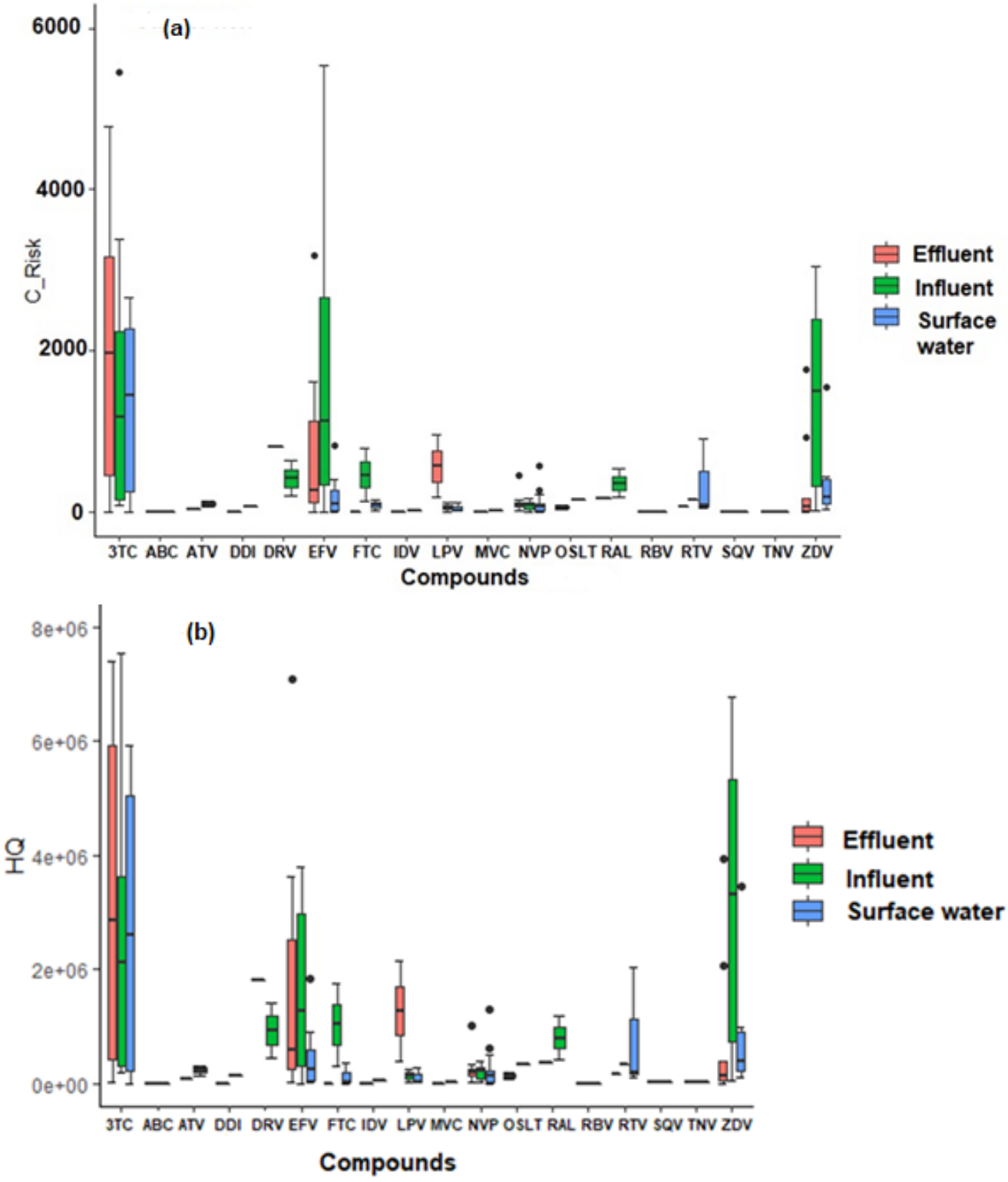

Figure 4. 7: Estimated (a) cancer risk and (b) Hazard quotient (HQ) for ARVDs in Effluent, influent and surface water /L) (b) RQ for ARVDs below 30 ng/L

4.5.3 Weibull probabilistic risk assessment of ARVDs

Probabilistic hazard assessment was employed to examine the likelihood that the four most common ARVDs reported in the systematic review (Table 4.2) as well as for all the ARVDs (Table 4.3) in different water

resources, exceeded the PNEC by The European Union European Medicines Agency (EU-EMA) for pharmaceuticals (proposed threshold value of 0.01 µg/L or 10 ng/L), The Australian Drinking water guidelines (DWG) (ranges between 0,0015 and 1000 (µg/L) for pharmaceuticals, the United States Food, and drugs Protection Agency (USFDA) value of 1000 ng/L for pharmaceuticals and the PNEC R subacid for individual pharmaceuticals developed by the USEPA (Kuroda et al., 2021). As indicated before, the risks for humans were calculated based on the assumptions for drinking water which is not applicable for most of the water resources monitored in the studies. But the modelling demonstrates the high levels of ARVD in these waters if they were they were to be consumed.

The percentage exceedances of the PNEC EMEA and USFDA value for the 4 most common ARVDs were 100% for vast majority of sites and water systems with a minimum 85%. The percentage exceedances for the 4 most common ARVDs of the Australian Drinking water guidelines for pharmaceutical residues in the different sites and water systems ranged between 55-100% with half having exceedances of 100%. The percentage exceedances of the PNEC R subcapi for the four most ARVDs ranged between 55-100% with 8% having exceedances of 100%. The percentage exceedances of the PNEC EMEA and USFDA guideline for all the ARVDs ranged between 87-100% and of the Australian Drinking Water Guideline ranged between 68-72% for all the ARVDs (Table 4.5). This study therefore revealed that the ARVDs had a high percentage exceedance above permissible limits set for pharmaceuticals in water in most countries, hence ARVDs are a potential burden in both its surface water and other water resources.

Table 4. 2: Equation for regression lines and values corresponding to percentile for environmental concentration distribution of ARVDs in surface water, WWTP influent and effluent (ng/L)

	Compounds	Weibull plots				(%) Centile value			(%) Exceedance values			
Sample matrices	**ARVDs**	**n**	**R^2**	***m***	**δ_f**	**25th**	**50th**	**75th**	**PNEC (EMEA)**	**(DWG) Australia**	**USFDA values**	**PNEC (R. subcapi)**
Surface water	EFV	7	0,9208	0,65	3,38	225	2450	8830	100%	100%	93,33%	57,14%
	NPV	15	0,8631	1,42	1,38	300	1480	4859	100%	66,67%	100%	66,67%
	ZDV	9	0,9114	0,87	1,72	2100	4000	9000	100%	100%	100%	88,89%
	3TC	13	0,9575	2,94	0,14	1200	31760	50300	84,60%	84,61%	84,60%	76,92%
WWTP Influent	EFV	9	0,9384	0,41	3,79	9653,5	34000	120700	88,89%	88,89%	88,89%	77,78%
	NPV	10	0,8369	0,46	3,61	680	2076	2100	90%	60%	90%	60%
	ZDV	6	0,9421	0,61	3,49	3985	30761	74000	100%	100,00%	100%	83,30%
	3TC	9	0,9115	0,63	3,49	4447	15166	50000	100%	100%	100%	100%
WWTP Effluent	EFV	8	0,9284	0,48	3,63	1300	1720	2110	100%	100%	100%	75%
	NPV	12	0,8891	1,08	3,03	138	3959	20400	100%	80%	100%	80%
	ZDV	9	0,8819	0,54	3,50	500	1400	3680	100%	55,56%	100%	77,77%
	3TC	7	0,9625	0,42	3,78	3985	55760	100000	100%	100%	100%	85,71%

Percentage Exceedance is based on the Predicted No Effect Values (PNEC) values *R. subcapitata* (EFV= 0,0078; 3TC= 0,125; ZDV= 0,544 $\mu g/L$) Ref: a= (Almeida, Mattos, Dinamarco, Figueiredo, & Bila, 2021), b = (Cid et al., 2021). Drinking water guidelines (DWG) ranges between 0,0015 and 1000 (µg/L). USFDA set 1 µg/L or 1000 ng/L maximum for pharmaceutical in water. The European Union ha European Medicines Agency (EMEA) guidelines proposed a threshold value of 0.01 µg/L or 10 ng/L for based on the extrapolation of predicted environmental concentrations in the environment (PEC) or on the measure of pharmaceutical concentrations in the environment (MEC).

Table 4. 3: Equation for regression lines and values corresponding to percentile for environmental concentration distribution of all ARVDs distribution in surface water, WWTP influent and effluent (ng/L)

Compounds	Weibull plots				(%) Centile value			(%) Exceedence values		
ARVDs	**n**	**R2**	***m***	**δ_f**	**25**	**50**	**75**	**PNEC (EMEA)**	**DWG (Australia)**	**FDA (USA)**
Surface water	54	0,92	1,89	0,96	374	2459	9670	96,29	92,59	70,37
WWTP Influent	54	0,97	2,00	0,81	690	3200	20900	96,29%	92,59%	72,22%
WWTP Efluent	47	0,87	1,97	1,88	350	1900	1700	100%	87,03%	68,08%

Percentage Exceedance is based on the Predicted No Effect Values (PNEC (EMEA) 1000 ug/L or 100000 ng/l, FDA (USA) limits 1000 ng/L and DWC (Australia) 100 ng/l for pharmaceutical in water.

4.6 ECOLOGICAL IMPLICATIONS OF ARVDS IN THE ENVIRONMENT

Eco-toxicological implications of emerging contaminants such as ARVDs in the environmental matrices is crucial to establish the potential harmful effect on exposed organisms (Z. Li et al., 2018). When there is limited or no experimental data available for evaluation, ecological risk assessment (ERA) could be estimated using recommended EC50 or LC50 values from the U.S. Environmental Protection Agency (EPA) Ecological Structure Activity Relationships Class Program (ECOSAR database (Horn et al., 2022). Derived information of the level of toxicity of all the compounds could be used to define the predicted-no-effect-concentration (PNEC) for the lowest effective concentration. Also, risk quotient (RQ) can be calculated based on the PEC/PNEC ratio and be used to characterize the risk in relation to aquatic ecosystems (Kumari & Kumar, 2022). Generally, environmental levels (µg/L) of composite concentration of ARVDs does not pose significant risk to the ecological system. Workers report that exposure to multiple pharmaceutical drugs would be different based on individual responses (Kumari & Kumar, 2021). Currently there are limited information on characterization and ecological implication of elevated levels of ARVDs.

Recent studies reported some of the possible impact and ecotoxicity posed by ARVDs on the environment and living components (Kudu et al., 2022). For example, OSLT and OSLT carboxylate were frequently detected in water sample (Ashauer & Escher, 2010; Zeeshan et al., 2022). (Omotola et al., 2022) explore environmental threat 3TC poses to aquatic fauna and flora in ecosystems using *Daphnia magna* (filter feeders), the Ames bacterial mutagenicity test, *Lactuca sativa* (lettuce), the study showed that 3TC poses an ecological health risk at different trophic levels, to both flora and fauna, at concentrations previously found in the environment. Studies showed concentrations of EFV, and 3TC are often detected in the environment do not appear to cause adverse effects in aquatic animals as they are usually below the therapeutic concentrations (Brumovský et al., 2016; Ngqwala & Muchesa, 2020). Additionally, ecological studies suggest that class of ARVDs such as NRTIs can cause ecotoxicological effects in exposed organism such that the nucleoside structure of the organism can be integrated into DNA or RNA-strains (Azu, 2012; Sigonya, 2021). Another class of ARVDs (Protease Inhibitors), for example Ritonavir has also been reported to display a high ecotoxicity potential (Escher et al., 2011).

The overall implication of ARVDs on the ecosystem may not be noticeable without scientific investigation, however, a growing number of studies from sub-Sahara Africa confirms the elevated levels in water resources (Mosekiemang et al., 2019; Ngqwala & Muchesa, 2020; Omotola et al., 2022). Additionally, presence of ARVDs such as ZDV, RTV, LPV and telbivudine could pose in ecological risks to aquatic life (Z. Li et al., 2018). Mixture

of ARVDs residue with other compounds in aquatic environment have the tendency to produce a significant ecological impact when compared to singly existing antiretroviral chemical in the environment (Peng et al., 2014).

4.7 HUMAN HEALTH IMPLICATIONS OF ARVDs IN THE ENVIRONMENT

Drinking and consumption of untreated surface water and aquatic organisms such as fish and other biotas from ARVDs laden aquatic environment can lead to adverse effects on human health (Khan et al., 2021). A review conducted by Azu (2012) highlights the views of experts focusing therapy and prolongation of survival in HIV/AIDS patients, studies suggested that testicular morphologic changes are considered to be a potential outcome of ARVDs. In a similar study, findings indicate that higher doses of ARVDs (saquinavir) have adverse effects on sperm motility, mitochondrial potential and significantly decreases total sperm count and increases the proportion of abnormal sperm forms (Ahmad et al., 2011). The route of exposure to ARVDs include drinking contaminated water, consumption of contaminated food that led to bioaccumulation and biomagnification. Consumption of unprescribed ARVDs is another route of exposure that could lead to possible adverse health implications, hence, self-medication has been strongly discouraged among the population (Ebele et al., 2017) However, another study focusing on endocrine disrupting effects of HIV ARVDs established there are ARVDs is limited endocrine disrupting activities of ARVDs in the aquatic environment at environmental level (Gerber, 2019).

4.8 FORCASTING AND MODELLING FUTURE SCENARIOS OF INCREASED USE OF ARVDS AND IMPACT ON EXTREME WEATHER CONDITIONS IN AFRICA

In Africa, the increased use of antiretroviral drugs has resulted in a significant reduction in HIV-related deaths and has also contributed to the control of the HIV/AIDS epidemic. However, as the demand for antiretroviral drugs continues to grow in Africa, it presented several challenges that may impact the future of the ecological systems and public health. Presence of ARVDs in Africa can have potential impacts on water resources and in turn, on extreme weather conditions.

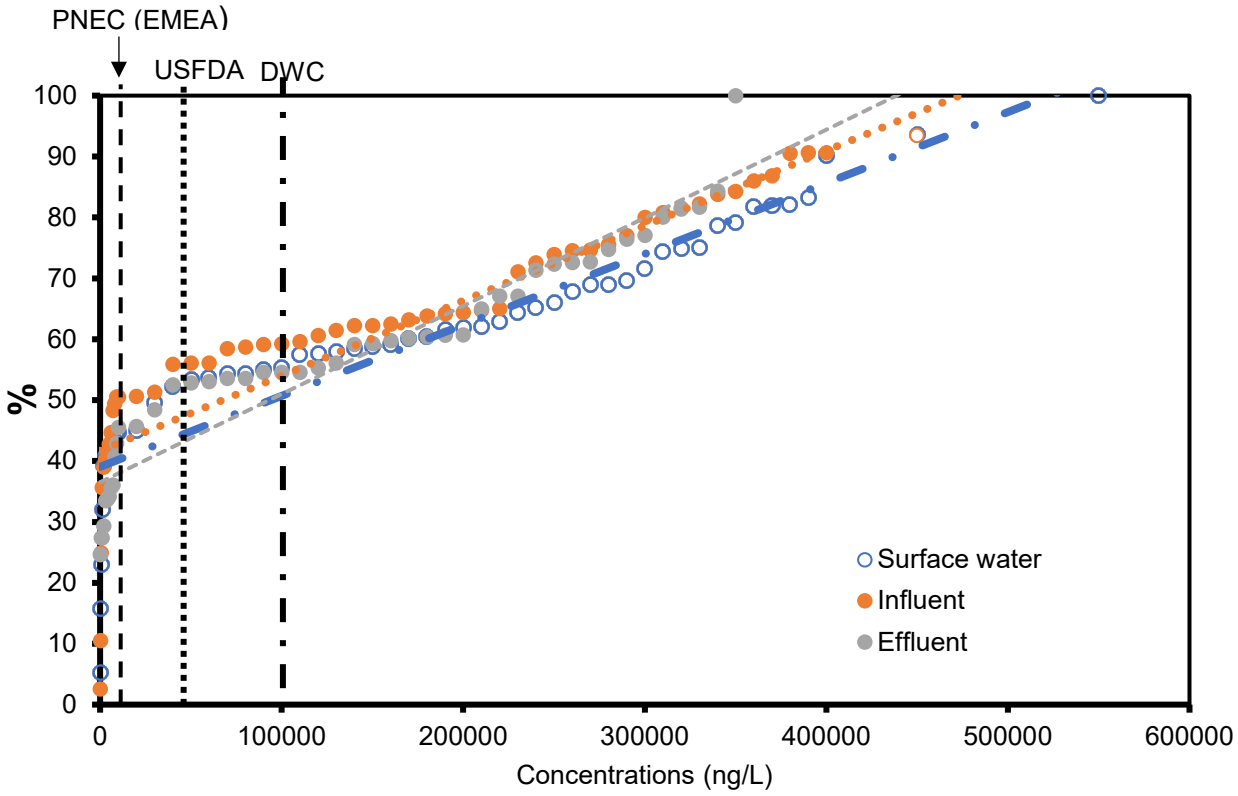

Figure 4. 8: Hazard probabilistic chart summarizing percentile exceedance of antiretrovirals in surface-water, WWTP influent and effluent.

Based on the results obtained from this study using Weibull model presented in Figure 4.8, some of the future scenarios that could emanate from the study include:

1. Increased use of ARVDs drug residues in wastewater and sewage can leads to contamination in water sources, leading to potential health and environmental hazards.
2. The presence of ARVD residues in water resources can cause a reduction in water quality, affecting both aquatic life and human health. This can result in decreased water availability for drinking, irrigation, and other purposes.
3. Impact of ARVDs in extreme weather conditions such as climate change: The contamination of water resources can impact on extreme weather conditions by altering the water cycle and disrupting the balance of the ecosystem. For instance, changes in the water cycle can lead to increased frequency and severity of droughts and floods, affecting agriculture and food security.

Changes in the climatic indicators such as temperature could affect environmental components such water resources. Rise in temperature will favours increase availability of ARVDs in water thereby increase contamination water sources. This will in turn can make it unfit for consumption during the extreme weather conditions. To mitigate the impact drought and other extreme weather conditions, it is important to develop a comprehensive strategy that addresses the future scenarios of further intrusion of ARVDs into our water ways. These strategies involve development of frequent monitoring campaign to measure levels of pharmaceutical chemicals including ARVDs in water resources, conservation and protection of available freshwater resource from contamination, planting of drought-resistant crops, development of governmental policies and guidelines that encourages wastewater treatment procedure in various chemical industries and discouragement of strategic stockpiling and illegal disposal of chemical such as antiretroviral drugs.

4.8.1 South Africa

South Africa recorded the largest number of ART regime in Sahara Africa with large volume of scholarly publications indicating presence of ARVDs in water resources. Weibull modulus and predictive analysis outcome may be applicable reducing levels of ARVDs residue presence in the water resources in the future. Meanwhile, precautionary measure such as innovative and efficient water treatment technologies, public health awareness again wide spread of HIV and other viral infections method among others. Future of antiretroviral drugs in South Africa is closely linked to the stable of the supply of ARVDs due to increase in prescription from medical centres, and the inability to remove residual ARVDs from water resources such as WWTP, surface water, drains among others. Proactively, Department of Health, South Africa can ensure disposal awareness among the population and provision of efficient water treatment process for complete removal of ARVDs from wastewater.

4.8.2 Kenya

Kenya is situated in the eastern part of Africa and has recorded significant level of ARVDs in the water resources. The increased use of antiretroviral drugs (ARVDs) in Kenya could pose potential future scenarios in terms of its impact on the environment, particularly with regards to extreme weather conditions based on reported studies. The rise in ARVDs use could lead to an increase in the amount of drug residues present in the environment, particularly in water sources. This could have a negative impact on the health of aquatic life and other wildlife, as well as potentially pose a risk to human health if the ARVDs contaminated water is used for drinking or irrigation purposes. Like other Africa countries, Kenya is agrarian country with massive economic dependant on agricultural produce. Increased release of ARVDs into the environment could lead to changes in the soil and water chemistry, which could in turn affect the growth of crops and other vegetation. This could have implications for food security and potentially exacerbate the impacts of extreme weather events such as droughts or floods. Continuous release of ARVDs in the environment could result in the development of drug-resistant strains of viruses and bacteria, which could further compound the effects of extreme weather events on public health. Unabated prescription of ARVDs will increase its availability in wastewater and drains which has the potential to have a significant impact on the environment and public health. Further research and monitoring campaign necessary to fully understand the potential impacts of ARVDs in Kenya water resources.

4.8.3 Zambia

Elevated concentration of ARVDs were reported in studies conducted in Zambia similar to levels recorded in South Africa and Kenya. The increased use of antiretroviral drugs (ARVDs) in Zambia could have various future scenarios in terms of its impact on the environment, particularly in relation to extreme weather conditions. The increase in the prescription of antiretroviral drugs to patients with viral infections may lead to higher detection levels of the drug residues in water resources. Elevated levels of organic contaminants such as ARVDs has the potential to harm aquatic life and wildlife and potentially pose a threat to human health. The increased in availability if ARVDs and other organic chemicals could alter soil and water chemistry, affecting crop growth and exacerbating the impact of droughts or floods on food security. Additionally, the development of drug-resistant viruses and bacteria may occur, exacerbating the effects of extreme weather events on public health in Zambia.

4.9 REMOVAL OF ARVDS FROM WASTEWATER TREATMENT PLANTS (WWTP)

Antiretroviral drugs are constantly released from sewage and drains into the WWTP and ultimately finds its way into surface waters with little or no impediments. Currently, there is scanty information on removal efficiency in most wastewater treatment plants in Africa for emerging organic contaminants. In KwaZulu-Natal in South Africa, negative results were obtained with using the CAS treatment process for removing LPV, ritonavir and 3TC in influents and effluents from WWTPs in KwaZulu-Natal, South Africa (Abafe et al., 2018). However, in a similar study, negative result was recorded during WWTP for removal of 3TC (25 ng/L) in the influents and increased to 220 ng/L in the effluents (Funke et al., 2016).

Another study, Mosekiemang et al. (2019) reported levels of ARVDs including atazanavir (ATV), EFV, LPV, 3TC and NVP in the effluent samples from WWTPs were significantly removed from tertiary stage of WWTP with biological processes compared to MBR treated waste with subsequent UV-irradiation. Yao et al. (2021) reported the removal and mass loads of ARVDs in seven wastewater treatment plants with various treatment processes. The latter study found that most of antiviral drugs cannot be efficiently removed in these WWTPs in Guangdong province of China. It is observed that some ARVDs such as NVP exhibits a desorptive preference as the raw effluent enters water bodies, so it is diluted by environmental water (Bagnis et al., 2018). It is important to note that there is no single wastewater treatment that has the capacity to resolve most pharmaceuticals such as ARVDs in water resources, hence they do reach surface water.

4.9.1 Technologies for removal ARVDs in water resources

Unarguably, complete removal of organic contaminants such ARVDs during the water treatment process could not be established. Released from domestic and municipal sources is the major reason why this class of organic micro-pollutants is detected in surface and drinking water. Water treatment conditions play important roles in the efficiency of conventional WWTPs and membrane bioreactor treatment plants. Some of the factors identified that could affect the optimum performance of water treatment plants include retention time in the sludge, available concentration of pollutant, system temperature, the pH, and pKa of micropollutants, as well as membrane fouling (Adeola and Forbes, 2021; Cirja et al., 2008).

4.9.1.1 Adsorption technology

Application of activated carbon using adsorption technology is among the early technics useful for removal of contaminants, improve taste and repulsive from water. Several activated carbons have been generated from various materials has been experimented for potential removal of organic compounds such as pharmaceuticals chemicals including ARVDs from drinking water (USEPA, 2018). Various activated carbon products show wide variability in adsorption performance towards different pollutants. Activated carbons were typically applied as powdered activated carbon (PAC) and granular activated carbon (GAC). PAC are dosed early into the treatment process and removed via sedimentation, while GAC on the other hand is used in fixed beds either in combination with filtration or as a post-filtration step (Chowdhury, 2013; USEPA, 2018). Some of the adsorption mechanisms identified in literature including multi-layer adsorption, micelle and hemi-micelle formation (Chen et al., 2017), acid base interactions, and the electrostatic and hydrophobic effects (Du et al., 2014). Adsorption mechanism is primarily controlled by surface chemistry. Efficiency of adsorption mechanisms for removal contaminants depend on the physicochemical properties of the adsorbents such as specific surface area, volume of pores, surface functional groups, hydrophobicity and chemical bonds for example the electrostatic forces, π-π bond, hydrogen bond (Zhang et al., 2011).

Qwane et al. (2020) reported the synthesis, characterised a molecularly imprinted polymer (MIP) for selective adsorption of abacavir from contaminated water. MIP selectively adsorbed abacavir from water in the presence of other ARVDs such as tenofovir disoproxil and efavirenz. The maximum adsorption capacity obtained for abacavir in the study was 5.98 mg g^{-1}. The adsorption mechanism was best described by the Freundlich isotherm and pseudo-second-order kinetic model, which were translated to multilayer coverage and chemisorption influenced by electrostatic attractions, respectively. The removal efficiencies achieved for

abacavir in wastewater influent, effluent and estuarine water were 67,74 and 76%, respectively. The synthesized MIP sorbent shows reusability of at least 10 consecutive times for adsorption of abacavir from polluted water without losing its extraction efficiency. This study suggest that adsorption techniques could be explore for removal of ARVDs in water resources at minimal cost.

Yao et al. (2021) reported the removal mechanisms of selected ARVDs in WWTPs, the mean aqueous removal efficiency range of −6.2% (NVP) between 100% (3TC) and total removal efficiency were from −1.2% (NVP) up to 100% (3TC). It was observed that removal efficiency for some ARVDs including zidovudine, nevirapine, and lopinavir exhibited negative values for the water treatment processes identified in the study. The trend was attributed to the reconversion of metabolites to their corresponding parent compounds, or the unaccounted hydraulic retention time during sampling (Mosekiemang, 2021; Muriuki et al., 2020). Previous investigations reported substantial removal for abacavir and lamivudine from WWTPs using conventional activated sludge (CAS) treatment processes up to 75-100% (Abafe et al., 2018; Funke et al., 2016; Mosekiemang, 2021; Prasse et al., 2010) was observed in removal efficiency in lopinavir and ritonavir in WWTPs using modified advance oxidation treatment processes (i.e. 96-98% and 97-99%) relative to CAS treatment process (i.e. -200% to -50% and 25-65%) (Abafe et al., 2018; Chèvre et al., 2013).

Adeola and Forbes (2021) summarized removal of ARVD by conventional WWTP in influents and effluents in WWTPs in African countries where studies have been conducted in comparison to report from other parts of the world. There are higher efficiencies in removal of ARVDs in water in developed countries relative to those from Africa due to application sophisticated wastewater treatment methods adopted than with the conventional wastewater treatment procedures used in South Africa and Kenya. (Abafe et al., 2018) reported levels of efavirenz and nevirapine in WWTPs in eThekwini Municipality in KwaZulu-Natal, South Africa were reduced (influent to effluent) from 21 to 34 µg/L and 1.9 to 3.4 µg/L respectively. It was similarly observed that concentration of efavirenz and nevirapine were reduced in decentralized wastewater treatment facility; from 0.670 to 24µg/L and 0.540 to 3.3 µg/L in Northern WWTP; and from 2.8 to 3.4 µg/L and 1.4 to 2.0 µg/L in Phoenix WWTP respectively (Abafe et al., 2018).

4.9.1.2 Biological method

Antiretroviral drugs like other emerging organic contaminants find its way in water resources via several sources. Water resources laden with high concentration of organic pollutants including pharmaceuticals such as ARVDs and other chemical components nitrogen, phosphorus among others can leads to environmental problem for aquatic biota which could hinder grow and potability if released into natural water bodies untreated (Mahari et al., 2022). Application of biological method such as microalgae and phytoremediation agent are termed biological method and they are often used to remove contaminants from water bodies has continue to gain interest of the researchers globally(Abdelfattah et al., 2022). Some of the potential microalgae is the fact that they have a high growth rate which makes them suitable for remediation purpose. Studies have identified some strains of micro-organisms that are productive like plants which makes them suitable for CO_2 sequestration and phytoremediation of wastewater (Shackira et al., 2021). Unique characteristic of microalgae is the ability utilized excess carbon dioxide produced during photosynthesis process and assimilate organic compounds in wastewater and biodegrade the pollutants into non-toxic compounds, thereby improving the water quality indices (Al-Jabri et al., 2020). Additionally, previous workers affirms that biological method using plant-based component have been recognized as an environmentally friendly alternate and sustainable option to remove pollutants during wastewater at conventional wastewater treatment facilities (Al-Jabri et al., 2020; Reddy et al., 2021; Renuka et al., 2021). Additionally, Reddy et al. (2021) reported the treatment of antiretroviral drugs in wastewater using Algae-mediated processes, the study showed that removal of pharmaceutical chemicals such as ARVDs from wastewater is possible through different processes such as biosorption, bioaccumulation, and intra-/inter-cellular degradation. This study reveals that algal species shown could tolerate high concentrations of ARVDs than the reported concentrations in the environmental matrices (Reddy et al., 2021). Biological approach also have added advantage regardless of it removal capabilities and wastes derived from microalgal biomass during the waste water treatment process can be explore for various applications such as biofuels, biofertilizers, pigments and feed among others(Abdelfattah et al., 2022; Ferreira et al., 2019).

4.9.1.3 Advance oxidation process (AOP)

Advance oxidation method (AOP) is another method that have been developed to remove or degrade recalcitrant organic compounds such as active pharmaceutical chemicals including ARVDs in water resources to barest minimum (Pelaez et al., 2012). The advance oxidation process is a removal technology that relies on the generation of highly reactive non-selective radicals. Hydroxyl radical (•OH) and fluorine are the most utilized radical with high oxidation potential (2.8 V) and (3.03 V) respectively (Shikuku and Nyairo, 2020). Moreso, application of photocatalysis with titanium dioxide (TiO_2) is among the frequently applied techniques of AOP. This is due to it cost effectiveness, stability, and safety. The process can be initiated by UV light which comes at additional cost for AOPs photocatalytic treatment (Preetha and Lekshmi, 2022).

Ngumba et al. (2020b) reported the post-treatment degradation of selected antibiotics and antiretroviral drugs by direct UV photolysis and advanced oxidation processes (UV/H_2O_2 and UV/Cl_2) using low-pressure mercury lamp. ARVDs zidovudine were among the pharmaceutical chemicals readily degraded above 90% using direct UV photolysis. It was also observed that addition of Cl_2 and H_2O_2 to the UV process enhance the rate of degradation of pharmaceutical chemicals (Miklos et al., 2019). Other application of UV-based AOPs have been extensively studied for the degradation of recalcitrant organic micropollutants in water (Wei et al., 2020). Additionally, advanced oxidation processes (AOPs) have been Hybridized with another water treatment processes such as biological treatment for enhance its efficiency to treat organic contaminant persistent pollutants such as PPCPs, EDCs and pharmaceutical chemical present in wastewater (Wardenier et al., 2019). However, oxidation process has some setbacks such as to achieve complete mineralization of target chemical is expensive due to resistance of oxidation intermediates formed during treatment tend to complete chemical degradation. Additionally, cost of energy consumption such as (radiation, ozone, etc.) and chemical reagents (catalysts and oxidizers) increases with prolong treatment time (Miklos et al., 2019).

4.9.1.4 Nano technology

Advance research using nano technology has also been explored for removal of organic contaminants such as pharmaceuticals chemical like ARVDs. Studies have shown the potential of nano material such as carbon nanotubes (CNTs) for water treatment, this is due to their specific chrematistics including developed pore structure, specific surface area among others which are suitable for adsorbing of micro contaminants during WWTP. Nano materials such CNTs have been widely accepted for treatment of contaminated water resources due to their unique characteristics such as large specific surface area, high pore volume, hydrophobic walls, electrical conductivity, stable chemical properties that gave them excellent environmental applications for removal of organic compounds. Studies revealed that combined carbon nano tubes with other removal techniques such as adsorption and membrane filtration demonstrated excellent removal of organic pollutants up to~95% from water in an optimum experimental condition (Kurwadkar et al., 2019; Preetha and Lekshmi, 2022). Similarly reports from literature established that nanocomposite membranes have showed promising outcome for the removal of pharmaceutical chemicals such as triclosan, acetaminophen, ibuprofen among others (El-Fattah et al., 2023). Nano material also possess excellent photocatalytic activity such that it generates reactive oxygen species that can oxidize contaminants to CO_2 and water. Recent research conducted by Bhunia et al. (2023) has shown the effectiveness of nanocomposite in photocatalytic degradation of organic compounds Summary of studies affirms that the hybrid TiO2/CNT photocatalyst outperforms the conventional WWTP process in terms of removal and degradation of some organic contaminants in water (Lashuk and Yargeau, 2021). Recent advance in research into application of nano technology such as carbon nanotubes (CNTs) have been applied in conjunction with other water treatment method including membrane filtration showed potentially high removal efficient rate for organic contaminants.

4.10 REQUIREMENTS FOR IMPROVEMENT IN HEALTH RISK ASSESSMENT OF ARVDS IN AFRICA

The health risk assessment conducted in this study was conducted using the standard regulation set in other countries and that are based on non-ARVD chemicals. In order to conduct effective health risk assessment data on specific ARVDS used in Africa is needed including laboratory experiments and modelling in order to develop standard regulation in African settings.

Additionally, there are several other research gaps that need to be addressed including:

- A need for better monitoring and detection methods to accurately measure ARVDs levels in African waters. There is a need for the development of cost-effective and sensitive analytical methods that can detect ARVDs even at low concentration in nanograms (ng) in water. This is crucial for understanding the extent of contamination and its potential impact on aquatic life and human health.
- The identification of the sources of ARVDs residues in African water resources including wastewater (WWTP), pharmaceutical manufacturing facilities, biological wastewater from hospitals and domestic wastewater from households among others. This will help to identify the most effective interventions to reduce ARVDs intrusion into the water resources.
- More research on the environmental fate and transport of ARVDs in African waters. Further studies are needed on their interaction and degradation with other chemicals in the environment.
- More data on the ecological effects of ARVDs in African waters. This includes the potential impact on aquatic life and the food chain, as well as the potential for ARVDs to be degraded in the environment.
- More research on the associated human health risks with exposure to ARVD residues in African waters. Implication of short-term and long-term health effects on ecological and human is yet to be established, as well as the potential interaction with other contaminants in the environment.

Recommendations to minimize the risk of ARVDs residue contamination of water resources include:

1. Improvement in the waste management programme through proper disposal of ARVD-containing waste, including unused and expired drugs, is essential to prevent contamination of water resources. Health facilities and households should be educated on the proper disposal of ARVD waste.
2. Improvement in wastewater through proper treatment of wastewater from manufacturing and health facilities, and households to remove ARVDs residues before being released into the environment.
3. Regular monitoring and testing of water resources for ARVD residues is crucial to assess the extent of contamination and to determine the effectiveness of waste management and sewage treatment practices.
4. Extensive research is needed to understand the fate and behaviour of ARV residues in the environment and to develop effective methods for their removal and remediation.
5. Effective management of ARVDs residues in water resources requires collaboration between various stakeholders, including government agencies, health facilities, communities, and the private sector.
6. The implementation of international guidelines, such as the World Health Organization's Guidelines for Drinking-Water Quality, can help ensure that water resources are free from ARV residues and are safe for human consumption.

4.11 LIMITATIONS IN THE STUDIES REVIEWED AND RISK ASSESSMENT

The main limitation in the systematic review of the occurrence of ARVDs in African water systems is the representativeness of the countries in which studies was performed with studies only conducted in only 4 countries and predominantly in South Africa and Kenya. No countries were conducted in North Africa. Low turnout of scholarly output in Africa may be attributed to access to limit infrastructure in terms of research equipment and skilled manpower. The second important limitation is that that only one study investigated drinking water and another 2 investigated groundwater and in total only two ARVDs measured. Thirdly, the period of monitoring was over 1 year including all four seasons in only 9 (30%) of the studies. The limitation in the risk assessment was that there are not specific guidelines for ARVDs especially for African water systems.

CHAPTER 5: CONCLUSIONS

This systematic review found the occurrence of 18 ARVDs in the water systems of 4 African countries with high concentrations of 9 ARVDs in wastewater and surface water samples. It is a concern, that ARVDs were also detected in drinking water and groundwater samples, although at low concentrations. The ecological risk of the ARVDs levels reported in African water WWTPs and surface water was high with the risk for several ARVDs exceeding one. The cancer and non-cancer risks was also high for several ARVDs in the 3 water systems based on assumptions for drinking water. The probabilistic hazard assessments found a high proportion of exceedances of international water guideline for all sites in the 3 water systems. There is a need for more studies in African water systems especially in drinking and groundwater samples ideally over at least 12 months including all four seasons. Removal of ARVDs in accessible water systems in Africa, are recommended. For instance, Abafe et al. (2018) reported the efficient removal of LPV and ritonavir during WWTPs processes ranging between 96-98% and 97-99%, respectively.

CHAPTER 6. DISSEMINATION OF INFORMATION

Outcomes from this study will be disseminated through various platforms.

1. *Publication in peer reviewed scientific journal:*

   The findings from this study will be published in international accredited peer-reviewed scientific environmental health journals. The following manuscripts are in preparation:

   - A systematic review of ARVDS in African water systems
   - Risk assessment of antiretroviral drugs (ARVDS) in Africa water resources

   This will serve as a vital means of disseminating our findings to a wider audience and made available to the public. This allows other researchers, academics, and practitioners to access and build upon the findings, helping to advance the field. With the increasing availability of online journals, the dissemination of research this the publication has become more accessible and efficient, making it easier for researchers to reach a global audience and have a greater impact on their field.

2. *Presentation at scientific conferences*

   Dr BO Fagbayigbo presented part of this study at the First African Chapter Conference of the International Society of Environmental Epidemiology (ISEE) in October 2022, that was held virtually (Title: Occurrence of antiretroviral drugs (ARVDs) in Africa water resources and probabilistic hazard assessment: A systematic review.) The final results are intended to be presented at international and local scientific conferences.

   This allows the research team to engage with experts and receive feedback from peers, industry experts, and potential collaborators in real-time. The conference presentations provide opportunity to showcase the findings and receive valuable feedback and network with others in their field and learn about the latest developments and trends in their area of study. This can lead to future collaborations, funding opportunities, and the development of new research ideas. Conference presentation is a valuable tool for disseminating research findings and advancing the researcher's career.

3. *WRC website*

   The final report of this study will be publish on WRC website. Disseminating research findings through WRC website is an effective way to reach a wider audience both nationally and internationally. WRC typically have a large network of researchers, academic institutions, and organizations, who are interested in the latest developments in their respective fields. Final outcomes of this research results will showcase the work relevant audience, increase visibility, and impact, and build a stronger online presence. Moreover, WRC website will also provide a platform for researchers to engage with experts in the field and discuss the implications of their findings. This can facilitate collaborations and help in other research areas and provides valuable opportunity to communicate further and reach a wider audience.

4. *Stakeholders*

   The final report of this study will be distributed to government such as The Department of Environmental Affairs and Development Planning, Department of Health, various NGO’s and community stake holders to raise awareness and highlight the need for intervention and

This will help to build strong relationships between researchers and stakeholders, this will lead to further collaboration and support for future research. By engaging with the stakeholders will leads to valuable feedback and insights which can help to inform future research efforts.

5. *Workshop*

An online workshop was successfully held on 23 March (10-11 am) to inform and gather input from stakeholders on the study finding and recommendations. The stakeholders invited to the meeting include WRC staff, the reference panel for this project, academic and research staff and relevant government, industry, and community representatives. (Appendix 6 list the invitation flyer).

Ten participants took part including representatives from academic, scientific councils and govern health departments. The input was incorporated into this final report.

6. *Policy reports and brief.*

A policy brief (Appendix 7) was developed based on the findings of the study.

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APPENDICES

APPENDIX 1: LEVELS OF ARVDS IN WWTP INFLUENT IN REVIEWED STUDIES

Country	Year	Nevirapine	Efavirenz	Zidovudine	Lamivudine	Emtricitabine
South Africa	2013					
South Africa	2015	2100	174000			
South Africa	2017	154,25	9653,5			
South Africa	2018	670-2800	24000-34000	6900-53000	8040-2200	
South Africa	2019		2790-120700			
South Africa	2019	0-681	1420-15400		3670-20900	31000-17200
South Africa	2019		(2790-120700)	109000		
South Africa	2020		3270-37300			0-3100
Zambia	2020	680		66590	118970	
Kenya	2016	1357		513	3985	
Kenya	2016	2076,6	753,3	15166,6	40683,3	
Kenya	2018			6000-50000	58000-405000	
Kenya	2020	563-3795		3596-4447	1248-30761	
Nigeria	2020					
South Africa	2021					

APPENDIX 2: LEVELS OF ARVDS MEASURED IN EFFLUENT IN STUDIES REVIEWED

	NVP	EFV	ZDV	3TC	**Lopinavir**
South Africa					
South Africa	350	7100			
South Africa	258	3959			
South Africa		138		130	
South Africa	1900	34000	500		
South Africa		66560			3800
South Africa	1300	20400	3680		
South Africa	1720	0-3270		3985	20110
Kenya	1357		513	55760	
Zambia	1720		37140		
Kenya	2110		100	26946,6	
Kenya	1723,3	106,6	96,9	100000	
Kenya	9500		1500	69681	
Kenya	3214		19464	847000	
Kenya	9500		1400		
South Africa					

APPENDIX 3: HEALTH RISK, CANCER RISK AND RISK QUOTIENT FOR ARVDS IN SURFACE WATER MEASURED IN REVIWED STUDIES

Country	Years	MEC (µg/L)	ARVD	MW	log Kw	PNEC values	HQ	Cancer risk	RQ
2015	South Africa	1,48	NVP	266,3	3,89	950[a]	150761,75	67,84	1,55
2017	South Africa	0,37	NVP	266,3	3,89	950[a]	38097,90	17,14	0,39
2017	South Africa	0,23	NVP	266,3	3,89	950[a]	23123,59	10,41	0,23
2019	South Africa	0,30	NVP	266,3	3,89	950[a]	30559,81	13,75	0,31
2019	South Africa	1,43	NVP	266,3	3,89	950[a]	145668,45	65,55	1,51
2018	South Africa	0,07	NVP	266,3	3,89	950[a]	6926,89	3,12	0,07
2020	South Africa	1,30	NVP	266,3	3,89	950[a]	132425,86	59,59	1,36
2020	Zambia	0,22	NVP	266,3	3,89	950[a]	22410,53	10,08	0,23
2012	Kenya	2,00	NVP	266,3	3,89	950[a]	203732,09	91,68	2,11
2016	Kenya	2,25	NVP	266,3	3,89	950[a]	228821,70	102,97	2,36
2016	Kenya	4,86	NVP	266,3	3,89	950[a]	494967,12	222,73	5,12
2018	Kenya	6,00	NVP	266,3	3,89	950[a]	611196,28	275,04	6,32
2020	Kenya	2,30	NVP	266,3	3,89	950[a]	234291,91	105,43	2,42
2020	Kenya	12,66	NVP	266,3	3,89	950[a]	1289624,10	580,33	13,32
2020	Kenya	145	NVP	266,3	3,89	950[a]	14770577,00	6646,75	152,63
2015	South Africa	0,30	LPV	628,8	5,94	4700[b]	31069,14	13,98	0,06
2017	South Africa	0,35	LPV	628,8	5,94	4700[b]	36569,91	16,45	0,07
2019	South Africa	2,75	LPV	628,8	5,94	4700[b]	280131,63	126,06	0,58
2017	South Africa	1,13	RTV	720,94	6,29	4700[b]	115108,63	51,79	0,24
2019	South Africa	1,95	RTV	720,94	6,29	4700[b]	198638,79	89,39	0,41
2019	South Africa	20,00	RTV	720,94	6,29	4700[b]	2037320,90	916,79	4,25
2017	South Africa	0,69	EFV	315,67	4,15	7800[c]	70898,76	31,90	0,09
2019	South Africa	8,83	EFV	315,67	4,15	7800[c]	899477,19	404,76	1,13
2019	South Africa	2,45	EFV	315,67	4,15	7800[c]	249571,81	112,31	0,31
2019	South Africa	18,2	EFV	315,67	4,15	7800[c]	1853962,00	834,28	2,33
2018	South Africa	0,225	EFV	315,67	4,15	7800[c]	22919,86	10,31	0,03
2019	South Africa	2,88	EFV	315,67	4,15	7800[c]	293374,21	132,02	0,37
2016	Kenya	0,17642	EFV	315,67	4,15	7800[c]	17971,208	8,09	0,02
2015	South Africa	0,973	ZDV	267,24	−7,05	544[c]	99115,663	44,60	1,78

2019	South Africa	3,63	ZDV	268,24	−7,06	544[c]	369773,75	166,39	6,67
2020	Zambia	9,67	ZDV	269,24	−7,07	544[c]	985044,66	443,27	17,78
2012	Kenya	9,00	ZDV	270,24	−7,08	544[c]	916794,41	412,56	16,54
2016	Kenya	4,00	ZDV	271,24	−7,09	544[c]	407535,49	183,40	7,35
2016	Kenya	7,68	ZDV	272,24	−7,10	544[c]	782738,70	352,23	14,13
2018	Kenya	1,30	ZDV	273,24	−7,11	544[c]	132425,86	59,59	2,38
2020	Kenya	2,10	ZDV	274,24	−7,12	544[c]	213918,70	96,26	3,86
2020	Kenya	34,00	ZDV	275,24	−7,13	544[c]	3463445,60	1558,54	62,50
2015	South Africa	0,242	3TC	230,24	−2.62	125[c]	24651,58	11,09	1,93
2017	South Africa	0,021	3TC	231,24	−2.63	125[c]	2139,18	0,96	0,16
2019	South Africa	48,70	3TC	232,24	−2.64	125[c]	4960876,40	2232,39	389,60
2018	South Africa	0,002	3TC	233,24	−2.65	125[c]	203,73	0,09	0,016
2020	South Africa	31,76	3TC	234,24	−2.66	125[c]	3235265,60	1455,86	254,08
2020	Zambia	49,70	3TC	235,24	−2.67	125[c]	5062742,50	2278,23	397,6
2012	Kenya	1,20	3TC	236,24	−2.68	125[c]	122239,26	55,01	9,6
2016	Kenya	58,25	3TC	237,24	−2.69	125[c]	5934135,20	2670,36	466,03
2016	Kenya	5,428	3TC	238,24	−2.70	125[c]	552928,90	248,82	43,42
2018	Kenya	19,67	3TC	239,24	−2.71	125[c]	2003705,1	901,67	157,36
2020	Kenya	228,00	3TC	240,24	−2.72	125[c]	23225458	10451,44	1824
2020	Kenya	50,30	3TC	241,24	−2.73	125[c]	5123862,1	2305,74	402,40
2020	Kenya	532,00	3TC	242,24	−2.74	125[c]	54192736	24386,71	4256,00
2020	Nigeria	3,38	OSLT	321,33	0,64	1000[d]	344307,24	154,94	3,38
2017	South Africa	0,36	FTC	247,24	-0,43	1000[d]	36773,643	16,55	0,36
2019	South Africa	3,52	FTC	247,24	-0,43	1000[d]	358568,48	161,35	3,52
2018	South Africa	0,01	FTC	247,24	-0,43	1000[d]	814,93	0,36	0,01

Predicted No Effect Values (PNEC) values R. subcapitata (EFV= 0,0078; 3TC= 0,125; ZDV= 0,544 µg/L) Ref: a= (Almeida, Mattos, Dinamarco, Figueiredo, & Bila, 2021), b = (Cid et al., 2021). Drinking water guidelines (DWG) ranges between 0,0015 and 1000 (µg/L). USFDA set 1 µg/L or 1000 ng/L maximum for pharmaceutical in water. The European Union ha European Medicines Agency (EMEA) guidelines proposed a threshold value of 0.01 µg/L or 10 ng/L for based on the extrapolation of predicted environmental concentrations in the environment (PEC) or on the measure of pharmaceutical concentrations in the environment (MEC).

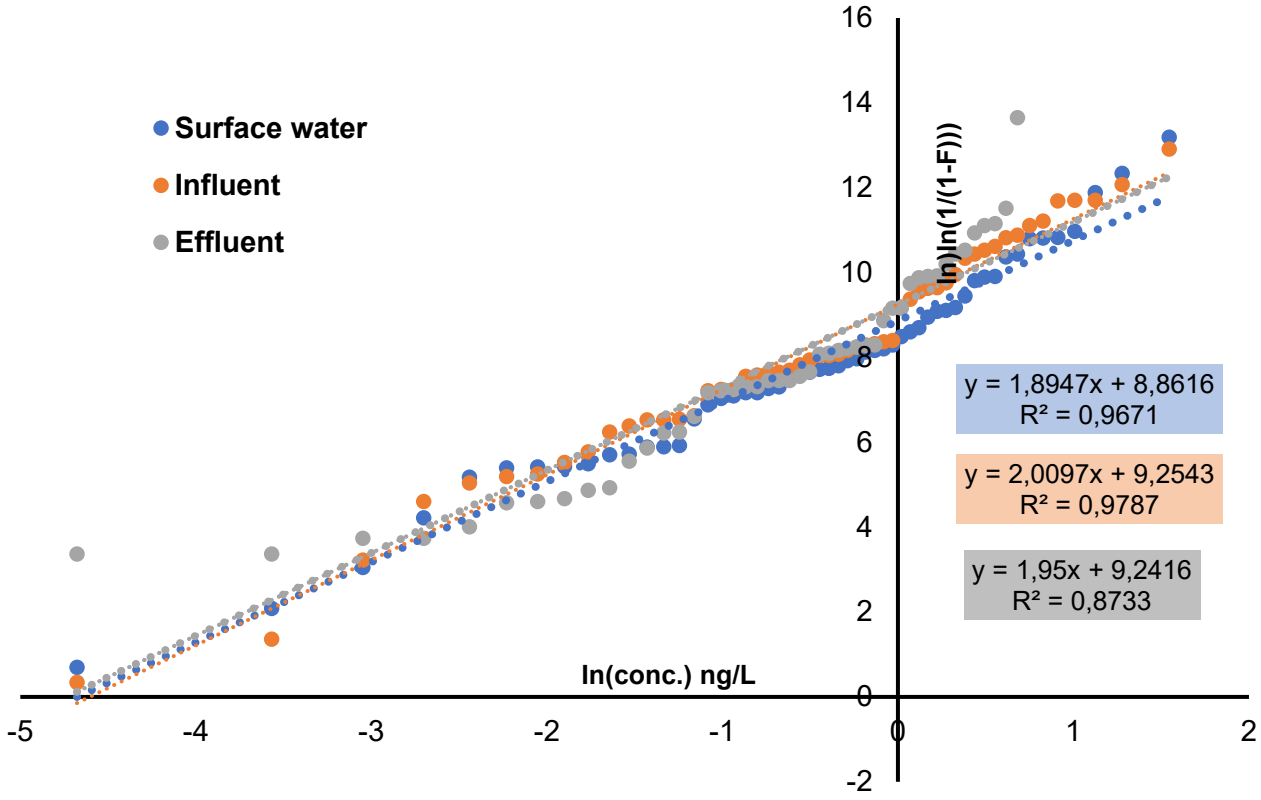
APPENDIX 4: HEALTH RISK, CANCER RISK AND RISK QUOTIENT OF ARDVS MEASURED IN INFLUENT IN STUDIES REVIEWED

Country	Years	MEC (µg/L)	ARVD	MW	log Kw	PNEC values	HQ	Cancer risk	RQ
South Africa	2013	2100,00	NVP	266,3	3,89	950[a]	213912,3	96,26	2,21
South Africa	2015	154,25	NVP	266,3	3,89	950[a]	15712,37	7,07	0,16
South Africa	2017	2800,00	NVP	266,3	3,89	950[a]	285216,40	128,34	2,95
South Africa	2019	681,00	NVP	266,3	3,89	950[a]	69368,7	31,22	0,72
South Africa	2020	680,00	NVP	266,3	3,89	950[a]	69266,84	31,17	0,72
Zambia	2020	1357,00	NVP	266,3	3,89	950[a]	138228,1	62,20	1,43
Kenya	2016	2076,60	NVP	266,3	3,89	950[a]	211528,7	95,18	2,19
Kenya	2018	3795,00	NVP	266,3	3,89	950[a]	386570,1	173,95	3,99
South Africa	2021	1,40	NVP	266,3	3,89	950[a]	142,6082	0,06	0,00
South Africa	2021	3500,00	NVP	266,3	3,89	950[a]	356520,5	160,43	3,68
South Africa	2018	2500,00	LPV	628,8	5,94	4700[b]	254657,5	114,59	0,53
South Africa	2021	3,90	LPV	628,8	5,94	4700[b]	397,2657	0,17	0,00
South Africa	2021	190,00	LPV	628,8	5,94	4700[b]	19353,97	8,70	0,04
South Africa	2018	3200,00	RTV	720,94	6,29	2900[c]	325961,6	146,68	1,10
South Africa	2015	174000,00	EFV	315,67	4,15	7800[c]	17724162	7975,86	22,31
South Africa	2017	9653,5	EFV	315,67	4,15	7800[c]	983334,5	442,50	1,24
South Africa	2018	34000	EFV	315,67	4,15	7800[c]	3463342	1558,50	4,36
South Africa	2019	120700	EFV	315,67	4,15	7800[c]	12294864	5532,68	15,47
South Africa	2019	15400	EFV	315,67	4,15	7800[c]	1568690	705,91	1,97
South Africa	2019	120700	EFV	315,67	4,15	7800[c]	12294864	5532,68	15,47
South Africa	2020	37300	EFV	315,67	4,15	7800[c]	3799490	1709,76	4,78
Kenya	2016	753,3	EFV	315,67	4,15	7800[c]	76733,4	34,53	0,10
South Africa	2021	25	EFV	315,67	4,15	7800[c]	2546,575	1,14	0,00
South Africa	2018	53000	ZDV	267,24	−7,05	544[c]	5398739	2429,43	97,43
Zambia	2020	66590	ZDV	267,24	−7,06	544[c]	6783057	3052,37	122,41
Kenya	2016	513	ZDV	267,24	−7,07	544[c]	52255,72	23,52	0,94
Kenya	2016	15166,6	ZDV	267,24	−7,08	544[c]	1544915	695,21	27,88
Kenya	2018	50000	ZDV	267,24	−7,09	544[c]	5093150	2291,92	91,91
Kenya	2020	4447	ZDV	267,24	−7,10	544[c]	452984,8	203,84	8,17
South Africa	2017	1400	DDI	236,2	−7,11	1000[d]	142608,2	64,17	1,40
South Africa	2021	74000	3TC	230,24	−2.62	125[c]	7537862	3392,04	592,00
South Africa	2018	2200	3TC	230,24	−2.63	125[c]	224098,6	100,84	17,60
South Africa	2019	20900	3TC	230,24	−2.64	125[c]	2128937	958,02	167,20
Zambia	2020	118970	3TC	230,24	−2.65	125[c]	12118641	5453,38	951,76
Kenya	2016	3985	3TC	230,24	−2.66	125[c]	405924,1	182,66	31,88
Kenya	2016	40683,3	3TC	230,24	−2.67	125[c]	4144123	1864,85	325,47
Kenya	2018	405000	3TC	230,24	−2.68	125[c]	41254515	18564,51	3240,00
Kenya	2020	30761	3TC	230,24	−2.69	125[c]	3133408	1410,032	246,09
Kenya	2020	1960	3TC	230,24	−2.70	125[c]	199651,5	89,84	15,68
South Africa	2013	1900	OSLT	321,33	0,64	1000[d]	193539,7	87,09	1,90
Kenya	2020	690	OSLT	312,4	1,20	4700[b]	70285,47	31,63	0,15

South Africa	2019	17200	FTC	247,24	-0,43	1000[d]	1752044	788,42	17,20
South Africa	2020	3100	FTC	247,24	-0,43	1000[d]	315775,3	142,09	3,10
South Africa	2020	250	TNV	561,48	-1,57	248[c]	25465,75	11,46	1,01
South Africa	2018	320	MVC	513,67	5,8	1000[d]	32596,16	14,67	0,32
South Africa	2018	11700	RAL	485,53	0,4	1000[d]	1191797	536,31	11,70
South Africa	2018	4300	DRV	547,67	1,88	1000[d]	438010,9	197,10	4,30
South Africa	2018	180	SQV	670,841	2,5	1000[d]	18335,34	8,25	0,18
South Africa	2018	1400	ATV	704,856	2,88	1000[d]	142608,2	64,17	1,40
South Africa	2018	590	IDV	613.8	2,66	1000[d]	60099,17	27,04	0,59
South Africa	2021	100	ABC	384,41	1,54	1000[d]	10186,3	4,58	0,10
South Africa	2021	3100	ATV	704,856	2,88	1000[d]	315775,3	142,09	3,10
South Africa	2021	14000	DRV	547,67	1,88	1000[d]	1426082	641,73	14,00
South Africa	2021	4100	RAL	485,53	0,4	1000[d]	417638,3	187,93	4,10

Percentage Exceedance is based on the Predicted No Effect Values (PNEC) values *R. subcapitata* (EFV= 0,0078; 3TC= 0,125; ZDV= 0,544 µg/L) Ref: a= (Almeida, Mattos, Dinamarco, Figueiredo, & Bila, 2021), b = (Cid et al., 2021), c= (Kuroda, Li, Dhangar, & Kumar, 2021), d = USFDA set 1 µg/L or 1000 ng/L maximum for pharmaceutical in water. Drinking water guidelines (DWG) ranges between 0,0015 and 1000 (µg/L). The European Union ha European Medicines Agency (EMEA) guidelines proposed a threshold value of 0.01 µg/L or 10 ng/L for based on the extrapolation of predicted environmental concentrations in the environment (PEC) or on the measure of pharmaceutical concentrations in the environment (MEC).

APENDIX 5: WEIBULL MMODULUS ANALYSIS CHART FOR ARVDS IN WATER RESOURCES



APENDIX 6: WORKSHOP INVITATION FLYER

APENDIX 7: POLICY BRIEF

Policy brief for "Systematic review of the occurrence and health risk assessment of antiretroviral drugs (ARVDs) in African water resources**".**

This policy brief is targeted at national and provincial policy makers, health managers, non-governmental organisations, and experts in the sphere of environmental science with an interest in public health, guideline development and implementation.

Background

Antiretroviral drugs (ARVDs) are among emerging environmental contaminants of concern. Unabated release of pharmaceutical product residue into the water resources, especially in developing countries, can pose a major threat to public health. Polluted wastewater and river systems are serious public health risks, especially to resource constrained communities and those residing in informal settlements where drainage systems and water access are limited. Previous studies focusing on water quality in African settings have reported levels of various emerging contaminants including ARVDs residue in water resources, such as, surface water, wastewater, ground and drinking water systems, however, until now a systematic review has not been conducted and there are limited studies focusing on health risk assessment around this topic. Here we present some findings related to a study aimed at further investigating and systematically reviewing the levels of ARVDs in water resources in Africa, with efforts to understand associated environmental and public health risks.

Current policy on ARVDs contamination of water resources in Africa

- Current environmental policy does not address the risk of ARVDs in South Africa and other African countries. For instance, South Africa water quality guidelines does not include ARVDs and other pharmaceutical chemicals in the list of prioritized chemicals.
- The WHO's Guidelines for pharmaceuticals in drinking water and guidelines in regions are used in African settings.

Study objectives

- To conduct a systemic literature review (period: 2010-2021) on reported levels and distribution of selected ARVDs in water bodies including wastewater, surface water, drinking water and ground water in Africa.
- To conduct ecological and health risks assessment based on the levels of ARVDs in water resources reported in the systematic literature review using the Risk Quotients and Weibull probabilistic risk assessment tools to estimate exceedance of international health standards for pharmaceuticals.
- To forecast and model future scenarios of ARVDs including increased use and drought conditions in South Africa and 2 other African countries.

Study findings and conclusions

1. This systematic review found the occurrence of 18 ARVDs in the water systems of 4 African countries (South Africa, Kenya, Zambia and Nigeria) with high concentrations of 9 ARVDs in wastewater and surface water samples.
2. ARVDs were also detected in drinking water and groundwater samples at low concentrations.
3. The ecological risk selected ARVDs (3TC, EFV, NVP, ZDV, FTC, ATV, DRV) levels reported in African wastewater and surface water was high with the risk quotient exceeding one (>1).
4. The cancer and non-cancer risks was also high for several ARVDs in the 3 water systems.
5. The probabilistic hazard assessments found a high proportion of sites/countries such as Kenya and Zambia exceeding international water guidelines in the 3 water systems.
6. There are no wastewater treatment plants in Africa with the capacity to completely resolve most pharmaceuticals such as ARVDs in water resources, as such, these are evident in water resources.
7. There is a need for more studies on African water systems, especially on drinking and groundwater samples, ideally over at least 12 months to ensure analysis includes all four seasons.

Recommendations

- Health risk assessment data on specific ARVDS used in Africa is needed; this includes laboratory experiments and modelling to develop standard regulation in African settings.
- Better monitoring and detection methods are needed to accurately measure ARVDs levels in African waters.
- Sources of ARVDs residues in African water resources such as wastewater (WW) treatment plants, pharmaceutical manufacturing facilities, biological wastewater from hospitals and domestic wastewater from households among others is needed to be monitored.
- Further studies are needed on the environmental fate and transport of ARVDs, interaction and degradation with other chemicals in the environment.
- More data on the ecological effects of ARVDs in African waters in needed. This includes the potential impact on aquatic life and the food chain, as well as the potential for ARVDs to be degraded in the environment.
- More research on the associated human health risks with exposure to ARVD residues in African waters is needed.

Policy recommendations:

1. International standard policy and regulation on ARVDS in water resources should become a priority. This involves collaboration with international stakeholders.
2. Monitoring of ARVDs in African water resources to understand changes over time and between seasons.
3. Investment in clean water infrastructure to improve water quality. Application of modern water treatment techniques or the upgrade of existing ones to remove organic contaminants such as ARVDS from the water resources.
4. Increased awareness among the public about the risks associated with poor disposal of ARVDs which subsequently leads to high concentrations in the water system.
5. Adherence to international environmental health treaties.
6. Funding should be directed to research on ARVDs.