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Endocrine disruptive activity and occurrence of pharmaceuticals and viral content in selected water sources in Melusi, Pretoria

MSc Environmental Health

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Date: October 2022

Summary

The quality of drinking water is of global concern with the increase in ineffective Wastewater Treatment Plants (WWTP) and human activity contributing to water pollution. These result in adverse health effects in various populations dependent on these water sources. Water sources are often contaminated with chemicals and pollutants, some of which have endocrine-disrupting chemicals (EDCs) properties. Additionally, poor water and sanitation services may lead to the presence of viruses in wastewater and potentially contaminate drinking water. Consequently, increased presence of compounds, such as EDCs and viruses in drinking water has the potential to threaten human health and place an additional burden on water treatment costs for a healthy drinking water supply and their risks require further investigation.

The breadth of such compounds is growing and therefore there is a constant necessity for reliable analytical methods for identification and their determination in order to detect the presence of these compounds at low levels. With limited research and development, monitoring of drinking water supply and management may be ineffective for regulating EDC exposure to humans via drinking water consumption. The study site in this study is Melusi, which is a non-sewage informal settlement in Pretoria North, home to over 3000 inhabitants dependent on external water sources for daily water use and consumption. Thus, it is imperative to screen for potential water contaminants, including EDCs and emerging contaminants, to identify sources of water contamination ensure drinking water supply security and resilience. This study aimed to determine the occurrence of endocrine-disrupting chemicals, pharmaceuticals, and viral content in drinking water sources in Melusi, Pretoria. This novel study is the one of the first to determine endocrine disruptive activity, emerging contaminant screening and viral content in an informal settlement in Pretoria.

Results show that Estrogenic activity was present in 13 out of 15 samples and the manner of how water was stored had an impact on the Estrogenic activity. There were three Pharmaceuticals quantified and Atrazine was the most prominent likely compound to be present across the samples. Lastly, there was no Norovirus detected however there was Adenovirus detected in the local dam water.

In conclusion, this research suggests that water is a potential source for human exposure to EDCs, pharmaceuticals and viruses which can result in that poverty-stricken rural communities such as Melusi are at a higher risk for exposure to these contaminants since the community lacks proper water systems. Exposure to these contaminants have the potential for health risks and warrants further investigation.

Keywords

Endocrine Disrupting Chemicals; Water; Pharmaceuticals; Viruses; Environmental Health; Public Health; Health; Hormone Disruption

ACKNOWLEDGEMENTS

I wish to express my appreciation to the following individuals and organizations that made this dissertation a reality:

I gratefully acknowledge the funding from the UP UNICEF ONEHEALTH FOR CHANGE (UP-OHC) and the University of Pretoria. This research was carried out at Environmental Chemical Pollution Health Research Unit.

First, I would like to thank my supervisor, Dr. Sean Patrick. This research would have not been possible without his constant advice and dedication over the last two years. Also, a big thanks to Dr Joyce Shirinde for supporting me throughout my research.
I extend my appreciation to the staff in the Environmental Chemical Pollution Health Research Unit, especially to Dr Catherina Van Zijl for her training, knowledge and endless assistance with the Bio-assays. I also want to say thank you to the Melusi Community for welcoming me into your homes and always being friendly and willing to assist.
Thank you to Dr Jan Hugo from the department of Architecture as well for all your assistance and support.

Finally, to all my family, and friends I would have never made it without their support and encouragement from the beginning. I can never thank them enough for everything they have done for me and I am here thanks to all of you.

A very special thank you to JC and Hannatjie, my parents, for always being there, encouraging and guiding me through everything in life. Finally, thank you to Danie for joining me through this journey, your enormous patience, support until the end. Even assisting me with join me to sampling visits and building maps.

Thank you! Kaleboga! Dankie!

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List of Abbreviations

AdV	Adenovirus
AP	Alkylphenols
AR	Androgen Receptor
ATP	Adenosine Triphosphate
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
E1	Estrone
E2	Estradiol
E3	Estriol
EDC	Endocrine-Disrupting Chemicals
EDTA	Ethylenediaminetetraacetic Acid
Eeq	Estradiol Equivalent Concentration
FBS	Fetal Bovine Serum
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid Receptors
MMTV	Mammary Tumour Virus Promoter
ng/g	Nanograms Per Gram
NoV	Norovirus
PAHs	Polycyclic Aromatic Hydrocarbons

PBA	Bisphenol A
PCP	Personal Care Products
p-NP	*Para*-nonylphenol
***p*-NPEC**	*Para*-nonylphenolethoxycarboxylic acids
***p*-NPEO**	*Para*-nonylphenolpolyethoxylates
***p*-OP**	*Para*-tert-Octylphenol
POP	Persistent Organic Pollutants
***p*-OPEO**	*Para*-tert-Octylphenolpolyethoxylates
PPCP	Pharmaceuticals and Personal Care Products
RLU	Relative Light Unit
RNA	Ribonucleic Acid
RT-PCR	Real-time Reverse Transcription PCR
RV	Rotavirus
SPE	Solid-phase Extraction
UPLC	Ultra-High-Performance Liquid Chromatography
WWTP	Wastewater Treatment Plants

CHAPTER 1: INTRODUCTION

1. Introduction

Globally, drinking water quality is a major concern and Africa is no exception.[1] With a major increase in the population density in third world countries which already, together with decreased clean water sources and sanitation services, result in water contamination with infectious agents and chemicals, which can have endocrine-disrupting properties.[2] An 'Endocrine Disrupting Chemical' or EDC is defined by the UNEP as "...an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations." [3]

Thus, compounds in water sources may have endocrine disrupting properties, that may mimic or block the action of naturally occurring hormones. As a consequence, compounds with EDCs properties may alter hormonal regulation and homeostasis. Numerous synthetic and natural compounds have been identified to have EDC properties. [4] Synthetic EDCs comprise a wide range of classes, including, polycyclic aromatic hydrocarbons (PAHs), Pharmaceuticals and Personal Care Products (PPCP), polychlorinated biphenyls (PCBs) and pesticides.[5]
Naturally occurring EDCs are found in phytoestrogens (such as genistein and coumestrol), which can mimic endogenous estradiol.[4] Additionally, natural, and synthetic hormones are frequently used in human oral contraceptives in agriculture and animal husbandry. Which form part of growth hormone regimens, to enhance reproductive success and meat production in livestock.[6] Interestingly, these compounds have been identified in runoff water and have been shown to have an endocrine disruptive effect, negatively affecting the male reproductive cycle.[7]

EDCs and toxic chemicals in water have been present for a long time, however, methods have only recently been developed in order to detect them due to their presence at low levels of concentration (pg/L to µg/L).[8-9]

While presence of EDCs are contributing to poor water quality, the presence of pathogens, including viruses are common in poor quality water sources in non-

sewered communities.[10-11] Viruses that are not commonly monitored can enter the aquatic environment and impair raw water quality.[12] The global increase of informal settlements in peri-urban areas, with a great need for water connections and proper sanitation, has significantly contributed to conditions under which viruses can thrive and replicate in sources of water.[13] Especially viruses like Norovirus and Adenovirus, which are known to cause diarrhoea and vomiting and ultimately have significant public health impact worldwide.[14]

1.2 Problem statement

Globally, drinking water quality is a major concern and Africa is no exception. Increases in the population in countries already experiencing a high burden of disease, coupled with decreased accessibility to clean water sources which leads to storage contamination, are likely to result in water contamination with infectious agents and chemicals, including those with endocrine-disrupting properties.

1.3 Aim and objectives of the study

This study aimed to determine the occurrence of endocrine-disrupting chemicals, pharmaceuticals and viral content in drinking water sources (JOJO tanks, household storage containers, dam and community tap) in Melusi, Pretoria.

The objectives were to:

1. Determine the estrogenic and anti-estrogenic activity (T47D-Kbluc assay), and androgenic and anti-androgenic activity (MDA-kb assay) in drinking water and wastewater samples using a battery of *in vitro* bioassays.

2. Screening for the occurrence of pharmaceuticals in drinking water and wastewater using the Agilent Forensic Toxicology Personal Compound Database and Library (PCDL) (library of 9200 compounds) and quantifying select pharmaceuticals using Ultra-high Pressure Liquid Chromatography

(UPLC) coupled to a Quadrupole Time of Flight Mass Spectrometer (Q-TOF/MS).

3. Determine the occurrence of viral contaminants in drinking water and wastewater samples using real-time reverse transcription-polymerase chain reaction (RT-PCR)

1.4 Structure of the dissertation

Chapter 1: Provides background, problem statement, aim and objectives and significance of the study.

Chapter 2: Provides a literature review of endocrine disrupting compounds, pharmaceuticals and viruses in water sources and the impact on a population's health.

Chapter 3: Describes the sampling methodology, and the statistical analysis tools used to achieve the set objectives

Chapter 4: Presents the results obtained from the sampling process and explains their significance.

Chapter 5: Discusses the results obtained in relation to current literature. The chapter also discusses the study's limitations and strengths. It provides the conclusion of the study, based on the results obtained, and recommendations for future studies and projects.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

Water is vital for all known forms of life, and water contamination can have detrimental health effects. Globally, the decreasing water quality and the sustainable supply of drinking water are becoming a source of concern.[15] Consequently, the effluent from sources such as wastewater treatment plants, municipal wastewater from households and hospitals, industrial wastewater from manufacturing processes, stormwater runoff and manure, have the potential to contaminate potable water sources.[2] Increasing contamination of water with viruses, pharmaceutical products, emerging contaminants and chemicals with endocrine-disrupting properties are seen worldwide.[15] Thus, human health and the environment can be heavily impacted by water contamination. Compounds that disrupt the body's natural endocrine system, resulting in adverse effects on hormone-dependent processes, such as fertility, feminisation, and manifest as lowered immunity and increased occurrences of cancers.[16] These compounds are referred to as endocrine-disrupting chemicals (EDCs).

2.2. Endocrine disrupting compounds (EDCs)

An endocrine disrupting chemical is released in the environment in two main sources, through human population and livestock, discharging 30,000 and 83,000 kg/year respectively.[17] Various compounds with EDC properties even at low ng/L concentration can alter hormonal dependent processes and disrupt homeostasis.[18] EDCs are capable of altering hormonal dependant processes such as reproduction and disrupting homeostasis.[19] Studies show that EDCs are not completely removed after water treatment, and therefore released into the environment and persistently observed,[20-22] Therefore, EDCs are the most investigated compounds in a compilation of studies between 1997 and 2007 all around the world.[23-24] Numerous synthetic and natural chemicals have been classified as EDCs. [25] Synthetic EDCs comprise a wide range of industrial chemicals and their metabolites. Classes of compounds that have EDC properties include polycyclic aromatic hydrocarbons (PAHs), Pharmaceuticals and Personal Care Products (PPCP), polychlorinated biphenyls (PCBs) and pesticides. Natural EDCs are found in phytoestrogens (which include genistein, coumestrol and zearalenone), which can mimic or inhibit the action

of endogenous estrogens.[25] The structure and uses of selected synthetic and natural EDCS are summarised in Table 1.[25-26]

2.3. Synthetic and Natural EDCs

Studies have shown that synthetic or naturally occurring compounds can be classified as EDCs.[18] Synthetic compounds with EDC properties are predominant in the manufacturing or pharmaceutical sectors.[27] Conversely, naturally occurring compounds with EDCs properties, such as phytoestrogens, can either mimic or block receptors and can illicit estrogenic, anti-estrogenic, androgenic or anti-androgenic responses.[18-19]

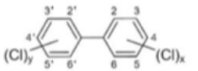
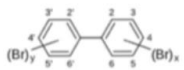
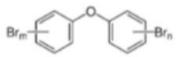
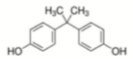
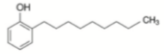
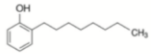
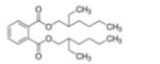
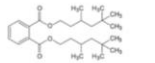
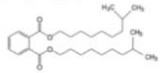
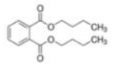
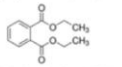
2.3.1 Synthetic EDCs

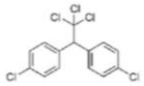
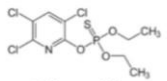
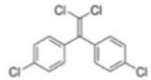
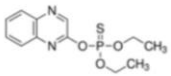
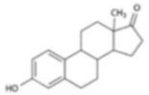
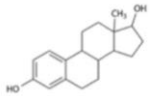
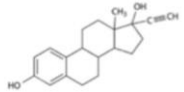
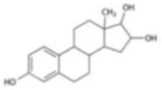
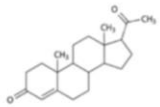
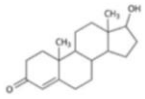
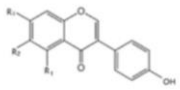
Polyhalogenated compounds are a class of synthetic compounds with EDC properties, which are commonly used in plastic manufacturing and flame retardants and ultimately end up in industrial wastewater.[28] Polyhalogenated compounds are listed as Persistent organic pollutants (POPs), which means these compounds are bio-accumulative, highly persistent and toxic.[29] Due to their persistent nature and physical, chemical and biological transformation these compounds can typically be fat-soluble and thus bioaccumulate in human and animal fat deposits, resulting in health risks and developmental impairments.[6]

Phenolic compounds including Bisphenol A (BPA), are common products used in manufacturing plastic bottles, containers, and water pipes.[30] Globally 2.9 billion kilograms of BPA are manufactured annually.[31] Studies have shown that chemicals in food packaging materials have the potential to leach into food in heated environments.[32] This is particularly concerning as only a select number of food packaging material has been certified BPA-free.[33] Due to the lipophilic nature of BPA, studies have shown bioaccumulation in adipose tissue accumulation during the development of a foetus, which is concerning during the hormone sensitive developmental programming windows.[27] Several governmental organizations have regulated the use of BPA in manufacturing, however, the presence of BPA in drinking water is still concerning. In a study by Xu *et al.*[34], BPA was detected with a maximum concentration of 317 nanogram per litre (ng/L) in Guangzhou in China, and the finding

signifying a lack of control and regulation, and calls for regular monitoring of water resources.[35]

Table 1*:* Selected compounds with Endocrine Disrupting Chemical properties. [25-4]

Chemical class	Structures	Known EDC activity	Sources
Industrial solvents/lubricants and combustion by-products - Polyhalogenated compounds	 Polychlorinated biphenyls (PCBs)  Polybrominated biphenyls (PBBs)  Polybrominated diphenyl ethers (PBDEs)  Perfluorooctanesulfonic acid (PFOS)  Perfluorooctanoic acid (PFOA)	• Estrogenic	• Flame retardants • Surfactants
Plastics and plasticizers - Phenolic compounds - Phthalates	 BisphenolA (BPA)  Nonylphenol (NP)  Octylphenol (OP)  Di-(2-ethylhexyl) phthalate (DEHP)  Di-isononyl phthalate (DINP)  Di-isodecyl phthalate (DIDP)  Dibutyl phthalate (DBP)  Dimethyl phthalate (DMP)  Diethyl phthalate (DEP)	• Thyrodogenic • Estrogenic	• Plasticisers • Surfactants • Lubricants • Fragrances • Antioxidants • Additives

Pesticides	 Dichlorodiphenyltrichloroethane (DDT)  Chlorpyrifos  Dichlorodiphenyldichloroethylene (DDE)  Quinalphos	• Estrogenic • Androgenic • Anti-androgenic	• Pest controls • Vector Control
Hormones	 Estrone (E1)  Estradiol (E2)  17α-ethynylestradiol (EE2)  Estriol (E3)  Progesterone  Testosterone  Phytoestrogen	• Estrogenic • Androgenic	• Growth stimulants • Hormonal therapies • Dietary intake

Alkylphenols (AP) are another phenolic compound typically seen in food because of migration through the packaging material and are found in detergents, pesticides and cosmetics.[36] Metabolites of AP are seen in high concentrations in our environment.[10] The high concentrations of AP can be attributed to high usage; as Nonylphenol derivates have been in use for more than 50 years, this means more than 500 000 tons of alkylphenol is produced yearly worldwide, and of that 500 000 tons 60% (300000 tons) of which end up in the environment.[37-38] In the manufacturing process, AP's are used due to their hydrophobic characteristics and low solubility, which means they are much more persistent in the environment and thus harm human and especially aquatic health.[39]

Phthalates are widely used in consumer products, including containers and toys as well as fragrances in cosmetics. These compounds are usually detected in food products and bottled water.[40] Phthalates have also been detected in urine, which suggests that one route of exposure may be dietary intake.[41] Additionally, non-dietary sources have also been suggested, where routes of exposure have been thought to be through evaporation of phthalates during the use of PCP and via inhalation and dermal absorption of dust and indoor air pollution.[14] Phthalates are chemically stable over a broad spectrum of temperatures as well as highly hydrophobic and lipophilic and thus persistent through water treatment.[42] The highest concentration of phthalates globally (6570,9 ng/L) has been observed in Northeast China with its high industrial sector.

Pesticides are frequently used in agriculture (approximately two million tons per year) to secure crop production.[16] In Malaria endemic areas, pesticides and insecticides are used in integrated vector control [17]. In South Africa, some of the commonly used pesticides and insecticides are organochlorides, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), organophosphates and carbamates.[43] Organochlorides are listed as POPs and are banned in numerous countries except, for countries who are signatories to the Stockholm Convention.[44] These countries are permitted to use organochlorines for vector control, whilst seeking safe and sustainable alternatives. With the increase in pesticide and insecticide use, there is concomitant increase in pesticide residues and metabolites in the water cycle, including wastewater, aquatic systems, and water bodies.[45-46]

2.3.2 Natural EDCs

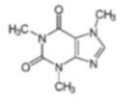
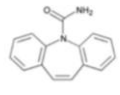
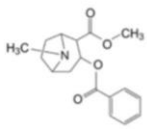
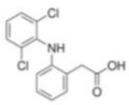
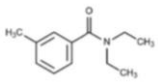
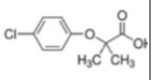
Natural hormones consist predominantly of steroidal hormones. Steroidal hormones regulate and control sexual development and reproduction.[4] Natural steroidal hormones include estrone (E1), estradiol (E2) and estriol (E3). Notably, hormones can be synthetic in origin and have EDC properties. Consequently, synthetic hormones include 17α-ethinylestradiol (EE2), a derivative of estradiol.[4,12]

These synthetic and natural hormones are common ingredients in human oral contraceptive medicines, and used in selected veterinary growth promotors used in farming for livestock's reproductive success. This means that these hormones frequently occur in the environment through human and animal excretion and agricultural runoff from manure and sewage used as fertilisers.[47-48] Most synthetic hormones such as EE2 can bioaccumulate and biomagnify in the aquatic environment.[49-50] Additionally, phytoestrogens are naturally occurring plant-derived compounds that are non-steroidal and present in cultivated and wild plants such as beans and legumes.[19] Thus, human dietary exposure to high levels of phytoestrogens have been suggested to have a negative impact on hormone dependant processes, such as reproduction.[51]

2.3.3 Emerging contaminants

Emerging contaminants are defined as chemicals of a synthetic origin or derived from natural sources that have recently been discovered and for which the public health and environmental risks are yet unestablished.[52] Emerging contaminants include pharmaceuticals, which are manufactured chemical compounds or drug that is designed to prevent or cure several diseases also adding value to the organism’s life.[53], and natural chemicals, such as caffeine, which is a stimulant that can be used as a somnolytic. High levels of caffeine are found in freshwater sources, and this poses a risk to the aquatic environment, and advocates for improved eco-pharmacovigilance practices.[54] Selected emerging contaminants, structure and sources are summarised in table 2 below.[2]

Table 2: Selected emerging contaminants, structure and use[3-4]

	Examples and structures	Known or suspected EDC Activity	Used as
Emerging contaminants	 Caffeine  Carbamazepine  Cocaine  Diclofenac  Diethyltoluamide (DEET)  Clofibric acid	• Estrogenic • Androgenic	• Hormonal therapies • Illicit drugs • Veterinary drugs • Agriculture • Antibiotics, • Anti-epileptic • Anti-anxiety medications

Pharmaceuticals are a significant class of emerging environmental micropollutants: their presence in water bodies is an increasing environmental concern.[55] One of the most important factors that governs the occurrence pattern of individual pharmaceuticals in the environment is their consumption trend. [56-57] A study by Kolpin *et al.*[20] showed that at least one pharmaceutical compound was found in 80% of 139 streams surveyed in the United States of America from 1999 to 2000. Currently, the abuse and misuse of pharmaceutical drugs and illegal drugs contribute to environmental contamination. This is due to pharmaceutical and illicit drugs which are continuously discarded or excreted into sewer and wastewater systems and left untreated by drinking water or wastewater treatment plants.[20]

Additionally, the manufacturing of pharmaceuticals has the potential to increase the discharge of compounds with suspected EDC properties in wastewater, which can lead to contaminants adsorbed into the soil.[58] According to a study by Phillips *et al.*[26] wastewater plants near pharmaceutical manufacturing plants contain 1000 fold more pharmaceutical products than WWTP near other manufacturing facilities. This partial elimination poses a threat to environmental health as numerous contaminants may not be removed from surface water and adsorbed into soil, leading to a higher bioavailability of contaminants.[59] Numerous emerging contaminants, including

pharmaceuticals, have been identified in seawater, because of the flow of wastewater effluents into rivers and other large bodies of water such as the ocean.[60] Furthermore, Richardson *et al.*[61] observed an increased concentration of pharmaceutical residues in the aquatic environment, and suggested the source may be drugs discarded by hospitals. Little progress has been made to remove pharmaceuticals from water sources and scientific evidence suggests that some pharmaceutical residues may disrupt an organisms' endocrine systems.[62] Table 3 below shows pharmaceuticals commonly found in the environment.[62-64]

Table 3: Common pharmaceutical compounds

Pharmaceutical compound	Description
Ampicillin	Penicillin antibiotics are used to treat or prevent many different types of infections such as bladder infections, pneumonia gonorrhea, meningitis, or infections of the stomach or intestines.[63]
Chloramphenicol	Broad-spectrum antibiotics are used in the management and treatment of superficial eye infections such as bacterial conjunctivitis, and otitis externa. It has also been used for the treatment of typhoid and cholera.[63]
Ciprofloxacin	Fluoroquinolone antibiotics are used to treat several bacterial infections. This includes bone and joint infections, intra-abdominal infections, certain types of infectious diarrhea, respiratory tract infections, skin infections, typhoid fever, and urinary tract infections, among others.[63]
Efavirenz	Antiretroviral medications are used to treat and prevent human immunodeficiency virus (HIV). [25]
Erythromycin	Antibiotics are used for the treatment of several bacterial infections. This includes respiratory tract infections, skin infections, chlamydia infections, pelvic inflammatory disease, and syphilis. It may also be used during pregnancy to prevent Group B streptococcal infection in the newborn, as well as to improve delayed stomach emptying.[25]
Fluconazole	Antifungal drugs are used to treat infections caused by different kinds of fungus species. The most common cause of fungal infections is a yeast, *Candida albicans*. Additionally, antifungal drugs are used to treat called cryptococcal meningitis, which is inflammation of the membranes covering the spinal cord and brain. [25]
Lopinavir	Antiretroviral drug which functions as a protease inhibitor and used

	against HIV infections as a fixed-dose combination with Ritonavir. [25]
Nevirapine	Non-nucleoside reverse transcriptase inhibitors (NNRTIs) which works by decreasing the amount of HIV in the blood. [25]
Ritonavir	Antiviral medication which functions as a protease inhibitor, used in preventing HIV replication. [25]
Sulfamethoxazole	Antibiotic are used for bacterial infections such as urinary tract infections, bronchitis, and prostatitis and is effective against both gram-negative and positive bacteria such as Listeria monocytogenes and *Escherichia coli*. [25]
Tetracycline	Antibiotics are used to treat many different bacterial infections of the skin, intestines, respiratory tract, urinary tract, genitals, lymph nodes, and other body systems. It is often used in treating severe acne, or sexually transmitted diseases such as syphilis, gonorrhea, or chlamydia. [25]
Trimethoprim	Antibiotics are used to treat and prevent urinary tract infections (UTIs), such as cystitis. Occasionally, used to treat other types of infections, such as chest infections and acne. [25]
Vancomycin	Antibiotics are used to treat complicated skin infections, bloodstream infections, endocarditis, bone and joint infections, and meningitis caused by methicillin-resistant *Staphylococcus aureus*. [25]

2.4. Mechanisms of action of EDCs

Our drinking water sources have been contaminated with a wide array of natural and synthetic compounds with EDCs properties. Environmental concentrations of EDCs in water systems are of great interest related to human and population health. Studies have shown that chronic exposure to EDCs can disrupt the endocrine system, which in turn causes immune and metabolic effects, reproductive abnormalities, reproductive abnormalities, diabetes, behavioural changes, obesity, cardiovascular diseases, neurological disorder, foetal development and growth disruptions and several different cancers.[1,3,4,12] Effects of exposure to EDCs are described in Figure 1.

Effects of EDCs can be seen at varying doses and are seen in the “something from nothing” phenomenon.[25] This phenomenon illustrates how weak EDCs can act together in a mixture at low and singly ineffective concentrations to cause an effect, thus this means from nothing (low concentrations of weak EDC) EDCs can cause

something (estrogenic activity).[19,25] Furthermore, the effect of low doses of EDCs is typically illustrated through a non-monotonic dose-response curve.[19] This curve shows the highest effect at a low dose or medium dose and is usually referred to as U-shaped or inverted U-shaped curves.[25] However, it is important to note that the exposure to EDCs during critical windows/developmental stages is often more important than the magnitude of the dose.[19] It has been shown that EDC exposures in far or low levels affect adults while the same EDC and dose may be hazardous to a growing fetus.[19]

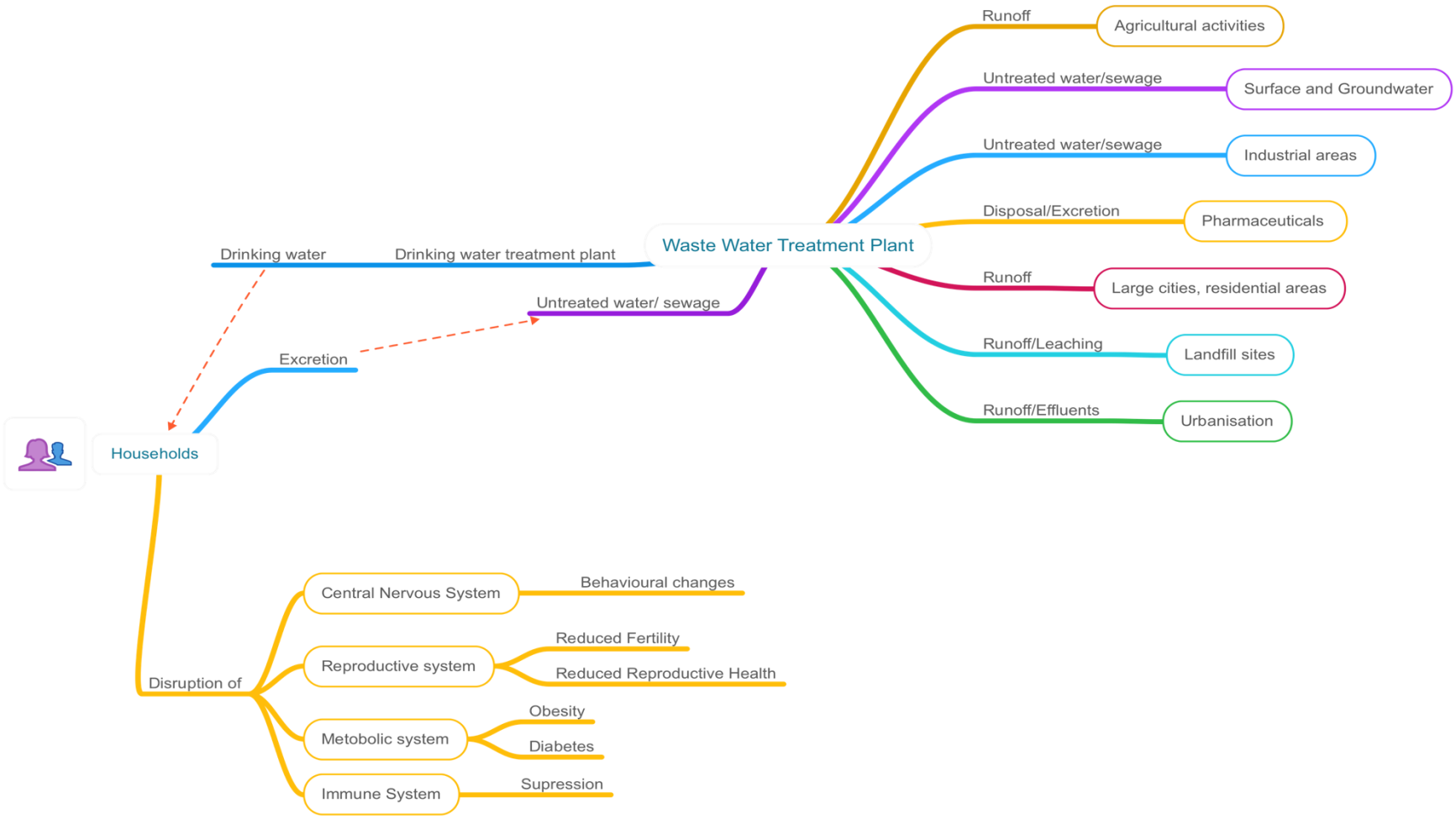


Figure 1: Routes of EDC exposure and potential disruption effects. [2-3,35-36]

Therefore, the adverse health effects of EDCs are dependent on factors such as the age, duration and dosage of exposure and the involvement of other pollutants.[65] It is known that EDCs interfere with the endocrine system's hormone signalling through antagonistic modes of action of the endogenous hormones. Thus, EDCs can impact the body in various ways. Firstly, to mimic the effects of endogenous hormones, which cause an insignificant response to a stimulus. Furthermore, by disruption of the endogenous hormone's synthesis and metabolism. Lastly, EDCs are also able to disturb the synthesis of specific receptors of hormones which causes the hormone to act additively, synergistically, or antagonistically.[31] Which could impact the natural hormone concentration and the downstream action of that hormone. This can impact the delivery of the hormone to the normal target cells or tissue. This ultimately means that EDCs can prevent the normal function of the hormone.

EDCs have the potential to target the HPG (Hypothalamus-Pituitary-Gonadal) axis and this affects several systems and consequently influences reproduction, growth, development, and results in metabolic syndromes.[66] Some EDCs are also known to disrupt metabolic processes. BPA and phytoestrogens, for example, can disrupt reproductive health by utero exposure and cause an increase in the duration of the estrous cycle.[25] In contrast, perinatal exposure causes several disruptions such as early suspension of cyclic activity and decreased ovarian weight. These exposures can lead to damage to reproductive health, including disorders of lactation and ovulation, endometriosis, and breast cancer.[3]

In a study by Forte *et al.*[67] on the association between EDC exposure, impaired fertility and pregnancy outcome, the human endometrium physiology was altered by triclosan and BPA, whereby human endometrial stromal cells stopped at the G2/M phase of the cell cycle, enhancing cell migration. Estrogenic compounds such as EE2 can influence populations at levels as low as 1ng/L. EE2 toxicity can reduce growth and development at early life stages as well as reproductive health. In turn, this can affect the population as a whole.[3,4] These effects were observed as behavioural changes, complete feminisation in male fish, reduced fertility and fecundity, increased plasma vitellogenin in male and female fish, and induction of intersex. A study done by Säfholm *et al*.[68] showed that synthetic and natural progesterone could cause

impairment in infertility and reproductive health by interruption of müllerian duct differentiation and oocyte formation.

Some pesticides, namely endosulfan and glyphosphate, are aromatase inhibitors known to cause sexual and reproductive dysfunction through inhibition of aromatisation.[69] Aromatisation is an enzymatic process for converting androgens to estrogens which is essential for reproductive success.[40] In addition, pesticides are known to induce feminisation and demasculinisation in male development across the vertebrate classes.[70] Furthermore, estrogenic effects, either directly by binding to estrogen receptors, thus increasing aromatase activity and estrogen sensitivity, or indirectly by their effect on gonadotropin-releasing hormone (GnRH), can result in early puberty disorders.[71] Exposure to EDCs can also disrupt the endocrine system with neurobiological and neurotoxic effects. Capolupo *et al*.[72] investigated the physiological stress response by the brain when induced by biological active pharmaceuticals. Their study showed an increase in DNA damage and an indication of neurological illnesses in the population, which can cause behavioural changes in individuals.[43]

Exposure to EDCs has been associated with an increased incidence of metabolic syndromes, including insulin resistance, type 2 diabetes mellitus, obesity, and cardiovascular diseases; thus, EDCs are referred to as metabolic disruptors.[3] In the framework of EDC exposure and metabolic disruption, the accumulation of EDCs in adipocytes, which reduce adiponectin levels, adjusts the modulation of glucose and lipid metabolism in insulin-sensitive tissues, associating obesity to type 2 diabetes mellitus.[73]

EDCs also have a notable effect on the immune system. Epidemiological research shows that there exists a relationship between EDCs and allergic diseases. Furthermore, EDCs can bind to the estrogen receptor and activate transcription factors such as proteins-1 (AP-1) and nuclear factor-kappa B (NF-κB) which are both involved in inflammation. An array of studies shows that EDCs have the ability to suppress immune function. For example, a study done in the United States showed that BPA and triclosan suppressed human immune function whereas there is a positive correlation between urinary concentration of triclosan showed with allergy

and hay fever diagnoses and BPA concentrations were correlated with elevated cytomegalovirus antibody concentrations.[74]

Pharmaceuticals in particular these antibiotic compounds are prone to have a high potential to bioaccumulate. The increase in bioaccumulation of these compounds can worsen abnormal hormonal control which can cause reproductive problems and persistent antibiotic resistance.[62] Hileman *et al.*[75] observed that antibiotic contamination at trace amounts in water can to the increased antibacterial resistance seen in the studied population. This increase in resistance has a widespread impact by increasing the medical costs of a population by the treatment costs and re-admission for hospitalisation.[62]

2.5 Environmental fate of known and suspected EDCs

Routes of exposure to EDCs can also occur through inhalation, dust ingestion and dermal absorption through dietary intake from EDC migration from packaging to food.[25] The level of contamination, exposure routes, and EDC's fate in the environment is of grave concern and is depicted in conceptual diagram 1[19,25,66,76] and shows the effects on human health.

With rapid urbanisation and the concomitant increase in population density, there is increased pressure on the drinking water supply.[62] This increased pressure, coupled with ineffective removal of emerging contaminants with hydrophobic and lipophilic properties, has negative impacts on water quality.[21 23] Several environmental factors such as rainfall and temperature, may have an effect on the bioaccumulation and biomagnification of EDCs.[77-78]

Barber *et al.*[10] reported that the concentration of a mixture of EDCs including, *para*-nonylphenol (*p*-NP), *para*-nonylphenolpolyethoxylates (*p*-NPEO), *para*-nonylphenolethoxycarboxylic acids (*p*-NPEC), *para*-tert-octylphenol (*p*-OP), *para*-tert-octylphenolpolyethoxylates (*p*-OPEO), bisphenol A, triclosan, ethylenediaminetetraacetic acid (EDTA), is significantly affected by water temperature, where the concentration of a particular EDC in autumn is 990 ng/g and as much as 5400 ng/g of EDC during springtime.

A study done by Shimazaki *et al.*[79] showed that in the exposure route from the river water into the groundwater, the rivers near informal settlements with landfills, waste disposal sites and agricultural land are the highest in EDCs. Exposures to EDCs can affect critical windows of exposure such as intrauterine, perinatal, juvenile, or puberty.[80] These are periods are when organisms are more sensitive to hormonal disruption compared to other periods.[81] The placenta and later the breast milk can expose the young foetus and child to EDCs.[29,82] Furthermore, there has been evidence of impaired reproductive development in children of pesticide-exposed workers[83], non-occupational exposure in young men[84] and adverse spermatogenesis seen in animal reproductive toxicology studies using cattle feedlot runoff water.[85]

A limited number of studies have been done on the impact contaminants have on drinking water supply systems.[25] Water treatment is a security step to ensure humans are protected from exposures to contaminants, including EDCs. However, emerging contaminants are not well understood, and water treatment facilities are not geared to effectively remove these contaminants from raw water (ref). Interestingly, higher concentrations of some EDCs were found in treated water compared to the corresponding raw water. [79] Apart from water treatment concerns, the epoxy-coated piping systems involved in transporting treated water can also cause BPA to leach into the drinking water supply.[86] Also, plastic packaging material that ends up in drinking water also leaches into the water, causing EDC exposure to humans and the environment.[76]

When compounds with known EDCs properties enter the aquatic system, they don't only leach into the water but can also be adsorbed into the surrounding soil. This results in hazardous compounds which can become bioavailable in the groundwater and soil.[27]

Mismanagement of groundwater along with its deteriorating quality and increasing manufacturing are major concerns in developing countries may lead to groundwater to be potentially contaminated with EDCs. [87] This contaminated groundwater can in turn lead to contaminated soil and sediments. Furthermore, research suggest that EDCs can be a potential threat to soil ecosystems, especially EDCs such as Bisphenol A.[88] This is because Bisphenol A is hypothesised to be easily absorbed in the soil and to bioaccumulate in organisms and ultimately affect the worms, snails

and insects eaten by birds and fish.[89] All while the demand for bisphenol A is increasing for it is widely used in the manufacturing of plastics, thermal papers for receipts and flame-retardant chemicals that are used in everyday life worldwide.[89] As the demand of Bisphenol A products rises, so does the amount of Bisphenol rise in the environment and has a dire impact on the environment.[89]

2.6 Mechanisms of action and Environmental fate of viruses in water

As mentioned above EDCs have the potential to suppress an organism's immune system. This means that if a population experiences exposure to EDCs the population will be more susceptible to other pathogens that may be present in the water such as viruses.

Viruses that are uncommonly monitored can enter the aquatic environment and impair the quality of raw water.[90] With the global increase of informal settlements in rural and peri-urban areas, there is a great need for water connections and proper sanitation. This has significantly contributed to conditions under which viruses can thrive and replicate in sources of water.[90] Several infectious diseases are associated with faecal contaminated water and are worldwide a significant cause of increased morbidity and mortality.[90]

The infection of waterborne pathogenic viruses is depended on several factors.

1. The survival of viruses in the water environment.
2. The infectious dose of the virus is required to cause disease in susceptible individuals.
3. The physio-chemical microbiological quality of the water.
4. The presence or absence of water treatment at the WWTP.
5. The season of the year.
6. and other factors[90]

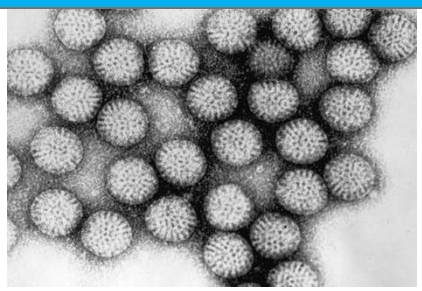
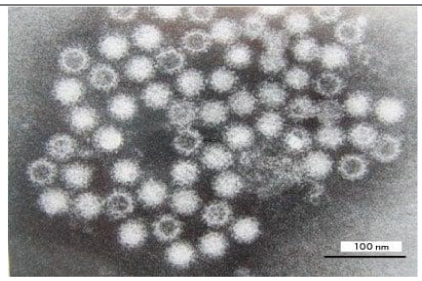
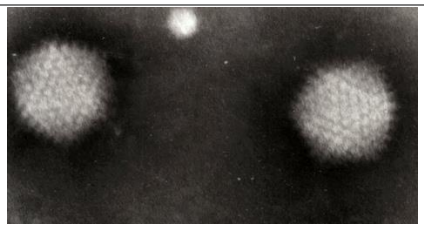
The survival of viruses in aquatic environments depend on the nutrients present and also the temperature of the environment.[64] The dose of infection of viruses has been established to be as little as 1 to 10 infectious particles.[48] Viruses cannot replicate outside living cells; however, viruses can survive for long periods in water

environments. This is why waterborne viruses are one of the leading causes of severe diarrhoea.[91] Diarrheal diseases are known to be a significant public health concern worldwide.[49] About 0.8 Million deaths per year related to diarrheal disease caused by enteric viruses are reported.[92] These viruses include rotavirus (RV), norovirus (NoV) and adenovirus (AdV).[92] These viruses' structures are shown in Table 4.[93-94] The 2009 annual South African surveillance review reported diarrhoeal disease data from four sentinel sites in three provinces (Gauteng, North-West and Free State). The diarrhoeal surveillance detected RV in 12%, NoV in 13% and AdV in 22% of children hospitalised with diarrhoea at the sentinel sites.[94]

The RV belongs to the Reoviridae family and Rotavirus A is most commonly found in water.[91,93] The RV is considered to be one of the most common causes of diarrheal disease, especially in children younger than 5 years old. It is estimated that RV infections caused 453000 deaths among children younger than 5 years old per year in developing counties.[95] In 2005 the RV was the responsible agent in a large outbreak of watery diarrhoea in the Northern Cape.[94] Another provincial hospital-based study in 2010 showed that in the Northern Cape and Gauteng 1 in every 43 children in the chosen areas are likely to be hospitalised because of RV diarrhoeal infections before the age of 2 years old.[96] The presence of the RV in hospitalised children has remained stable in recent years and decreased from 2017.[97]

The NoV is also a common cause of Diarrheal disease and falls within the Caliciviridae family.[91] The NoV is classified into 6 groups, where groups 1 and 2 are associated with diarrheal disease.[49] The NoV is predominantly seen in South African river samples. In a study done between 2008 to 2011, NoV was found in 63% of the sewage-polluted river water samples.[98] It is estimated that annually 218000 children younger than 5 years old die from the NoV annually in developing countries.[98] It seems that the presence of NoV group 2 is decreasing in comparison to the percentage observed in 2018 and 2019.[97]

Table 4: Waterborne diarrheal viral disease. [91,93]

Virus	The structure shown by transmission electron microscope images	Disease
Rotavirus		Diarrhoea
Norovirus		Vomiting Diarrhoea
Adenovirus		Diarrhoea

The AdV is a part of the Adenoviridae family and has also contributed to diarrheal disease.[91] In recent years, between 2015 to 2017, the prevalence of AdV worldwide has increased by 12,8 %.[49] The group of AdV which are significantly associated with paediatric diarrheal disease is Adenovirus 31,40 and 41.[99]

Rationale

Research on exposure to known EDCs, emerging contaminants such as pharmaceutical products and residues, and viral content of potable water sources has recently been highlighted, as water quality and water stress have been of great concern. Consequently, screening, and regular monitoring of potable water sources is becoming vital as water insecurity is threatening human health and ecosystems. The effective removal of contaminants such as EDCs and emerging contaminants from water sources are of concern. Due to the persistent nature of environmental contaminants, such as EDCs, emerging contaminants, (pharmaceutical products and residues) and viruses (Nora and G-1 and 2), the potential adverse health effects in non-sewered communities are concerning. Some of the contributing factors of these aquatic pollutants' ion are the increasing number of informal settlements with poor waste removal and poor water quality. The study's conceptual framework in Figure 2 illustrates the layout of the study. The study is novel in that although these contaminants have been reported in the literature, not much has been reported in South Africa. Thus, the results are critical in giving an idea of the extent of the problem from these contaminants.

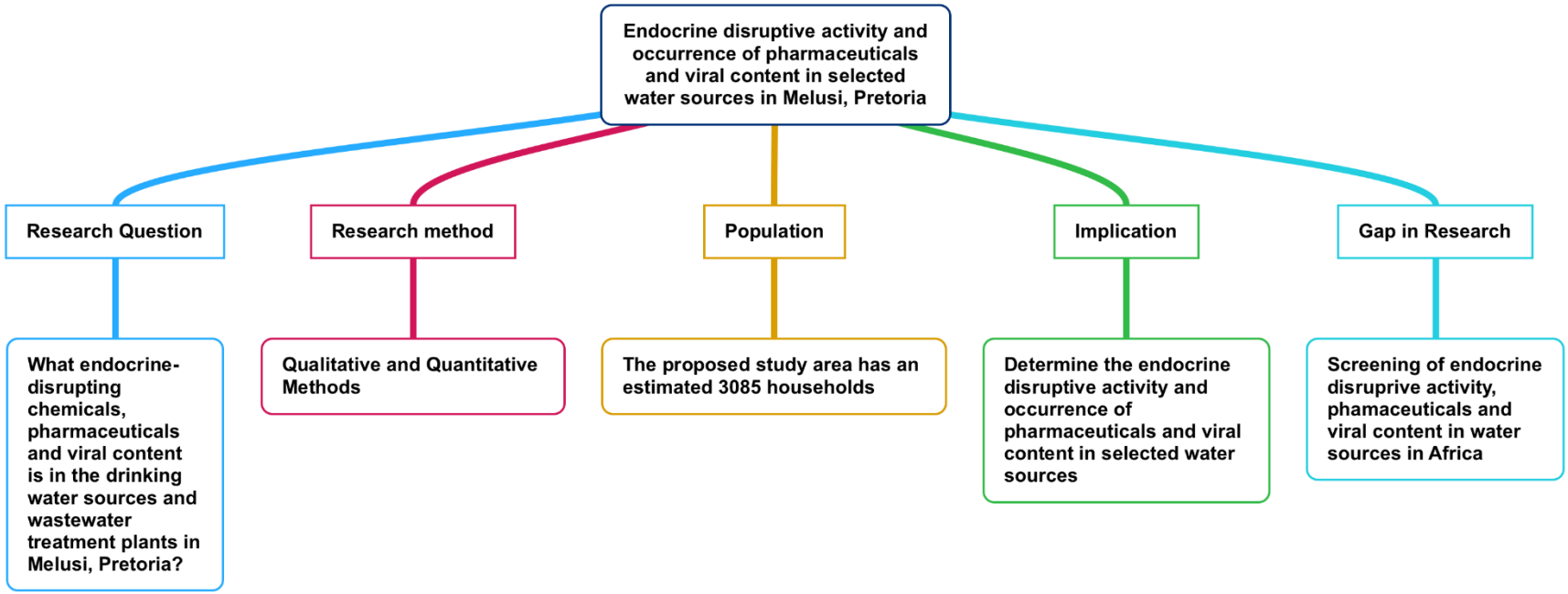


Figure 2: Conceptual framework for study

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Setting

The study was conducted in the West of Pretoria, in the Daspoort area. This area includes the informal settlements of Melusi (See Figure 3 for a Photograph of the Community and Figure 4 for map of the study area). Samples were collected at the houses summarised in Table 5.

Figure 3: Photograph of the Melusi Community

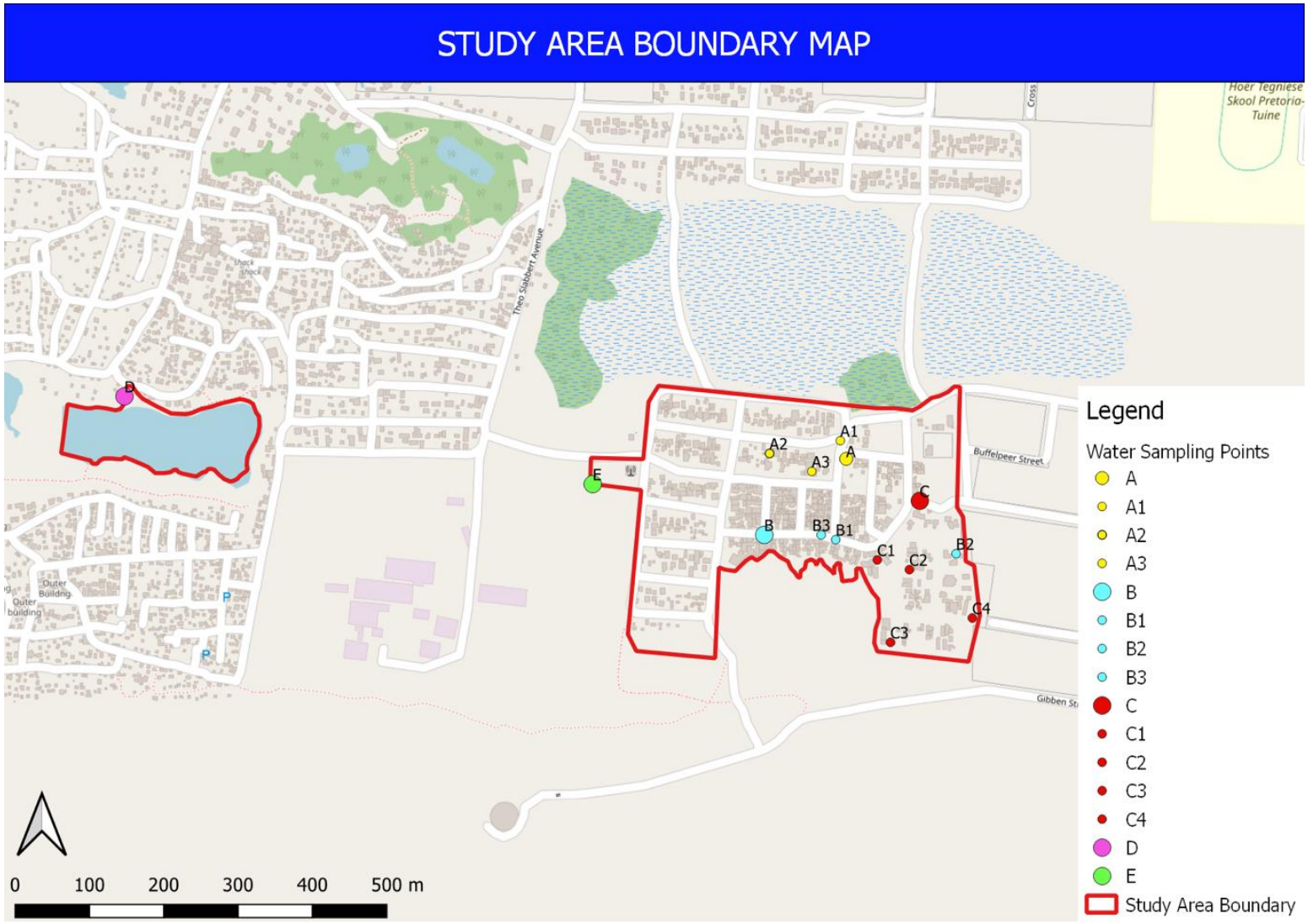


Figure 4: Map of the study area, with the study site indicated with red outline. (Courtesy of Mr Danie Labuschagne, Kuhle Environmental Consult)

Table 5: Sampling codes, description, and appearance and storage

Sample code	Location Description	Appearance and storage
A	JOJO A	Clear, JOJO tank
A1	House 1 from JOJO A	Clear, paint container, shade
A2	House 2 from JOJO A	Clear, paint container, sun
A3	House 3 from JOJO A	Green with algae, white container, shade
B	JOJO B	Clear, JOJO tank
B1	House 1 from JOJO B	Clear, white container, shade
B2	House 2 from JOJO B	Clear, paint container, shade
B3	House 3 from JOJO B	Clear, blue container, sun
C	JOJO C	Clear, JOJO tank
C1	House 1 from JOJO C	Clear, paint container, sun
C2	House 2 from JOJO C	Clear, paint container, sun
C3	House 3 from JOJO C	Clear, white container, shade
C4	House 4 from JOJO C	Clear, white container, shade
D	Dam	Pungent smell and cloudy
E	The community tap from borehole	Little cloudy but clear

3.2 Study population and sampling

3.2.1 Study population

The study area has an estimated 1906 households. There is no running water in the houses but those living nearby use the main line at communal JOJO tanks, using any available containers as shown in figure 5, and carry it back home. Some of the JOJO tanks are sometimes not filled for month and only a few is then filled. Some people from the community reported that if they do not have water in the JOJO tanks, they use the community dam or community tap. There are no municipal waste-removal containers for refuse collection and household refuse is dumped alongside the road, burned, or buried.

3.2.2 Sampling method

In August 2021, water samples were collected at the study site, community tap, household water, and the water from the community dam. Samples were collected in 1000 ml glass triplicate glass Schott bottles which were prepared by rinsing the bottles with HPLC grade methanol. A possible source of EDC contamination was prevented by lining the lids with tin foil in order for the plastic lids not to come into contact with the samples. The samples were stored and transported at 4°C. Each sample received a code as described in Table 5.

Samples were collected from each site during the sampling visit as detailed in Table 6 and shown in figure 6. Water samples for the detection of viruses were collected in one 10L container and no duplicate samples are collected. Water collected for EDC activity and Pharmaceutical Screening were collected in 1L glass bottles. Two samples from each sampling point were used for determining each of the following, endocrine disruptive screening, and pharmaceutical screening and quantification.

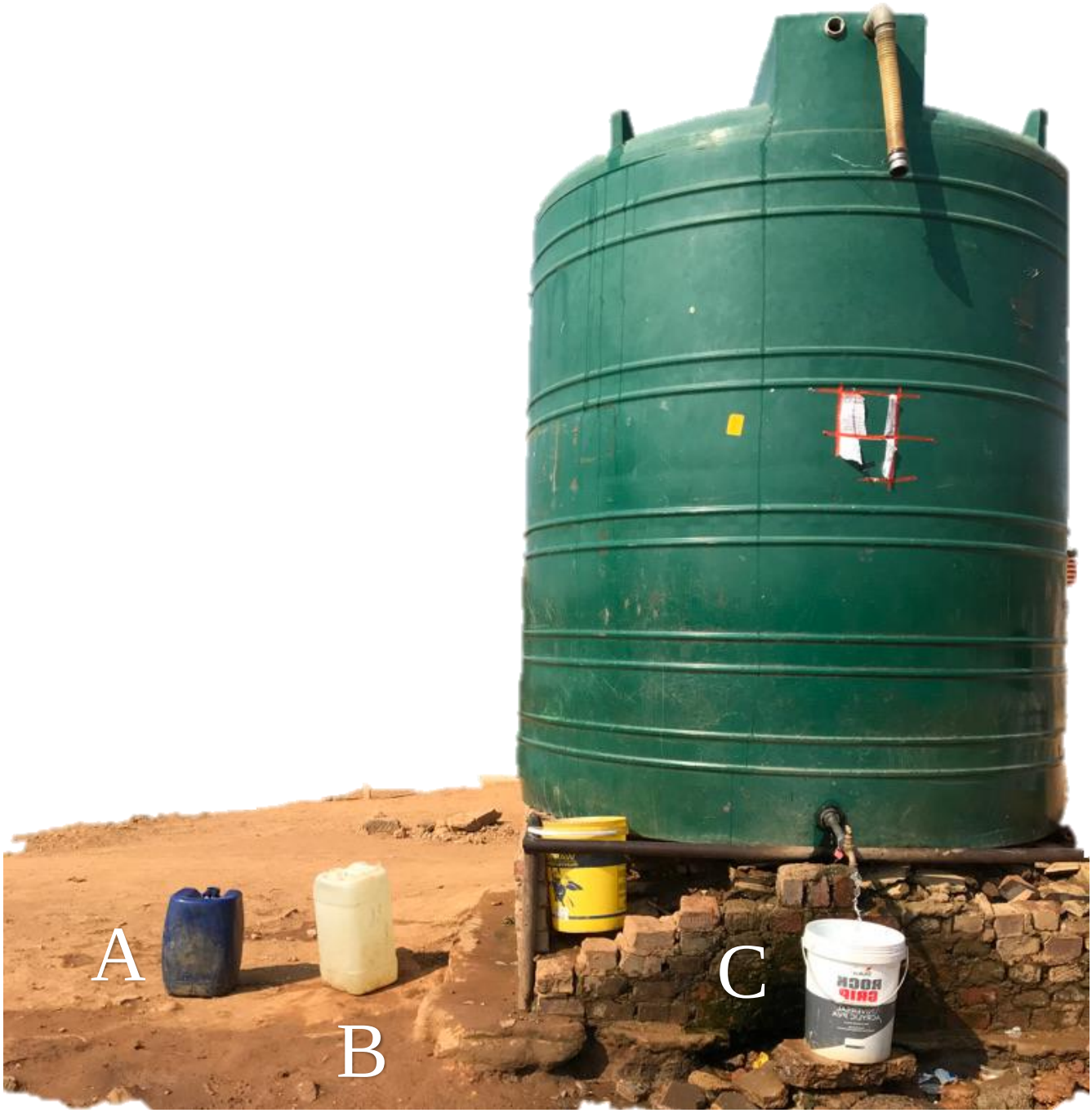


Figure 5: Containers used to collect water

A- 20 L Blue container

B- 10 L White container

C- 20 L Paint container

Figure 6*:* Different sources of water of Melusi Community.

1. JOJO tanks across the community where water is collected in buckets
2. Sampling from containers at homes
3. Sampling from JOJO tank
4. Sampling from the local dam

Table 6: Water sample collection distributed per site, per sampling period

Analysis	Sampling Sites		
	Community Tap	Household water	Dam
Viral content	0	0	1
Endocrine Disruptors	1	13	1
Pharmaceuticals	1	13	1

Total	**2**	**26**	**3**

3.3 Measurements

3.3.1 Endocrine Disrupting Screening

Water extraction

Extracting water samples before analysis in bioassays aid in the successful discovery of the active compounds to achieve reliable results in bioassays. This is due to water extraction concentrating the water samples. Water extraction cleans and purifies the water samples so that only the analytes of importance are analysed and that the water does not affect the cell system being used. The process followed was described by the Oasis glass cartridges care and use manual and also according to the South African Water Research Commission (WRC) Toolbox project: K5-1816.[100-101]

Materials

Oasis hydrophilic-lipophilic balance (HLB) Solid phase extraction (SPE), glass pipettes and disposable serological pipettes were the apparatus used. The compounds used were Methanol (MeOH), Ethanol (EtOH) and methyl tertiary-butyl ether (MtBE). The solid-phase extraction (SPE) apparatus components are depicted in figure 7.

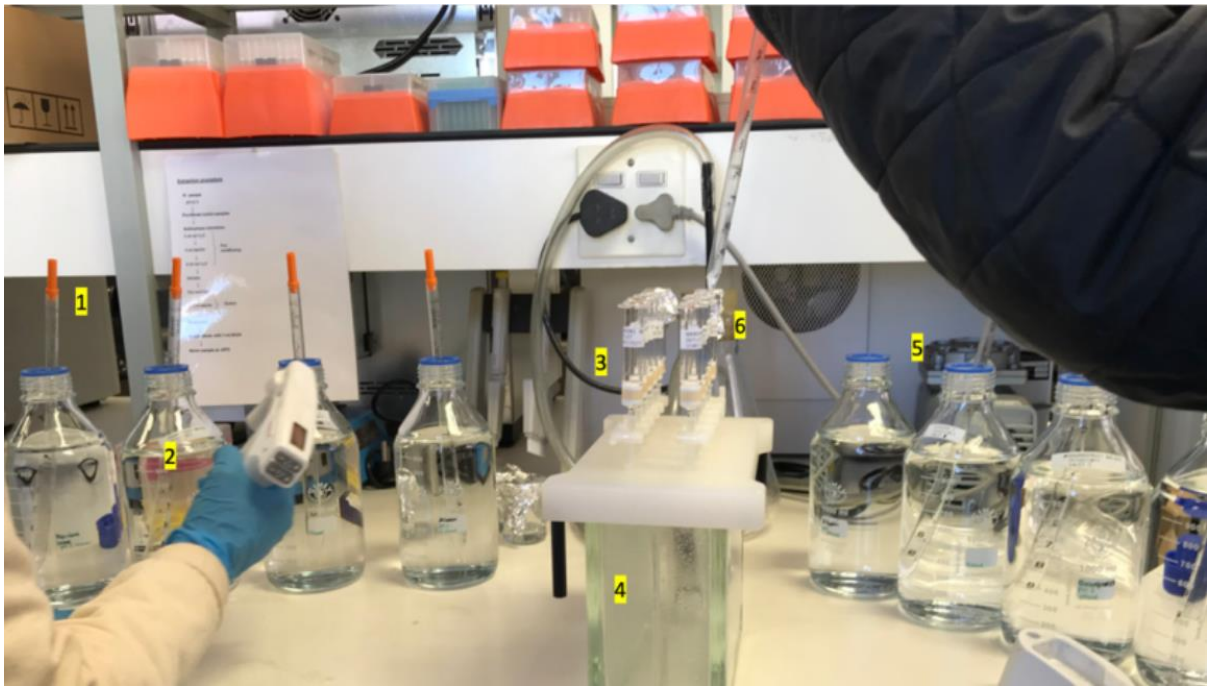


Figure 7: Solid Phase Extraction Setup and apparatus

1. Glass pipette- To collect the sample and transport to cartridges.
2. Glass Schott bottles containing the sampled water- holds the sample to be extracted.
3. Glass Cartridges- Glass hinders leaching and contains Silica-based packing material where though the sample is extracted.
4. Vacuum manifold- Collects the remaining sample that filtered through the cartridge
5. Vacuum Pump- Provides vacuum source for vacuum manifold.
6. Vacuum Flask- Where waste can collect from the Vacuum Manifold.[101-102]

Methods

The water's pH was adjusted to 3 using concentrated HCl (32%) and pH indicator strips. The samples were stored at 4°C in the dark until extraction to minimize sample degradation. After the samples were collected all the samples were filtered through a glass fibre filter connected to a vacuum pump to remove any solid matter. As shown below in Figure 8.

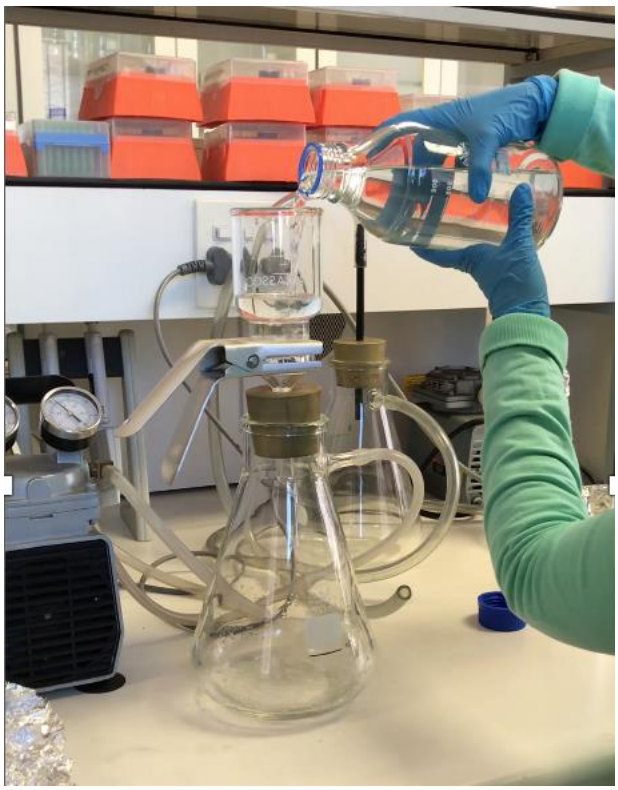


Figure 8: Glass Fibre Filter with Vacuum Pump to remove solid matter

Seven days following the sample collection, one litre of sampled water was subjected to a SPE (Figure 9) using the SPE-DEX system to protect against biodegradation.[57] Pre-conditioning of the SPE cartridges was done by 5mL 10% MeOH in MtBE and thereafter 3mL of MeOH. Furthermore, the SPE cartridges were equilibrated with 3 mL ddH_2O before andy samples passed through. The cartridges were also washed with 3mL of 5% MeOH in ddH_2O and dried. Elution of the samples was achieved through 6mL of 10% MeOH in MtBE. The sample then passed through a sorbent bed and analytes were retained on the sorbent while the sample matrix liquid passes through, and the purified analytes were subsequently eluted from the column using solvents. While throughout the process 10 mL/minute flow rate was never exceeded. The cartridge was not left to run dry until the entire sample passed through the cartridge. Furthermore, the eluent was then concentrated to near dryness using nitrogen gas and heat in the Dry-Vap system. The samples were then reconstituted in 1 mL EtOH and filtered with polytetrafluoroethylene filters before they were subjected to biological and chemical screening.[100-101]

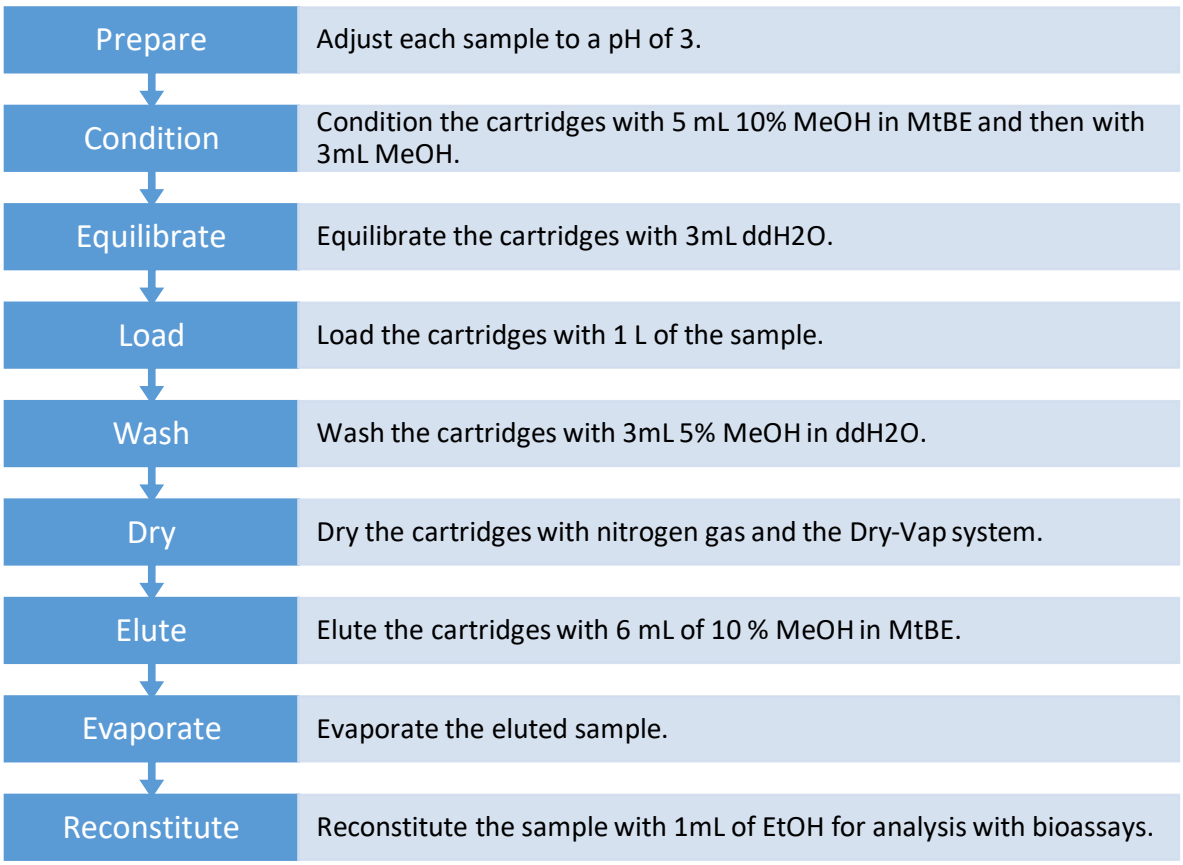


Figure 9: Summary of steps for SPE[100-101]

Bioassays to screen for estrogenic and anti-estrogenic activity

EDC activity in the samples were determined by bioassays, T4TD-Kbluc assay which was described by Wilson *et al*.[103-104] and the MDA-kb assays described also by Wilson *et al*.[105] in another study. The protocol followed was according to the South African Water Research Commission (WRC) Toolbox project: K5-1816.[101] Also, Nitrile (latex free) gloves was worn when preparing the assay components and while doing the assay. Working with latex gloves could have affected the outcome of the assays.[101]

Preparation of all glassware used

All glassware was prepared by washing in chromic acid a then rinsing in tap water, double distilled EDC free water and HPLC grade ethanol consecutively. The glassware was dried in the oven and covered with foil. The glassware was then sterilized by autoclaving at 121°C for 20 minutes.[101]

The T47D-KBluc assay

The T47D-Kbluc reporter gene assay was established to be a sensitive and specific assay for samples screening for estrogenic and anti-estrogenic activities. Human breast cancer cells,T47D cells, can naturally express estrogen receptor alpha and beta. These cells were transfected with an estrogen-responsive element luciferase reporter gene construct. Active ligands that bind to the estrogen receptor result in the luciferase reporter gene's activation and dose-dependent production of the luciferase enzyme.

Materials

The materials used included T47D-Kbluc cells, RPMI 1640 powder, sodium bicarbonate ($NaHCO_3$), glycylglycine, adenosine 5'-triphosphate (ATP), bovine serum albumin (BSA), magnesium chloride ($MgCl_2$) solution, E2, D(+)-glucose, Ethanol (EtOH), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer solution, sodium pyruvate, antibiotic/antimycotic solution, Hank's buffered salt solution (HBSS), trypsin, phosphate buffered saline (PBS), recovery cell culture freezing media, Fetal bovine serum (FBS), charcoal/dextran treated FBS (c/d FBS),

reporter lysis buffer, beetle luciferin and ICI 182,780. The apparatus used was 25cm^2 and 75 cm^2 tissue culture flasks 96-well luminometer plates, cryovials and 50 mL centrifuge tubes and CoolCell freezing containers.

Methods

When testing chemicals using the T47D-Kbluc cells, estrogen is defined as "a chemical that induced a dose-dependent luciferase activity, which could be specifically inhibited by the anti-estrogen ICI 182,780."[104] T47D-Kbluc cells were maintained in RPMI growth media supplemented with 2.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, 1.5 g/L $NaHCO_3$, 10% fetal bovine serum (FBS), 100 µg/mL penicillin, 100 U/mL streptomycin and 0.25 µg/mL amphotericin B. One week before the assay, cells were placed in growth media modified by replacing 10% FBS with 10% dextran-charcoal treated FBS, excluding antibiotic supplements.[104]

As per figure 10, cells were then seeded at 5 x 104 cells per well in 96-well luminometer plates which was then allowed to attach overnight. Dosing dilutions was prepared in growth media containing 5% dextran charcoal treated FBS, and vehicle (ethanol) did not exceed 0.2%. Each plate contained a positive agonist control (E_2), negative control (solvent only), antagonist control (E_2 plus ICI) and background control (solvent plus ICI). Each sample was tested separately and also for the presence of 0.1 nM E_2 or ICI. Cells were incubated 24h with 100 µL/well dosing solution at 37°C, with 5% CO2.

Following the incubation period, cells were washed with phosphate-buffered saline at room temperature and lysed with 25 µL lysis buffer. The microtiter plate luminometer determined Luciferase activity and was quantified as relative light units (RLU). Each well received 2 5µℓ reaction buffer (25 mM glycylglycine, 15 mM $MgCl_2$, 5 mM ATP, 0.1 mg/mL BSA, pH 7.8), followed by 2 5µL 1mM D-luciferin 5 s later. RLU were then converted to a fold induction above the vehicle control value.

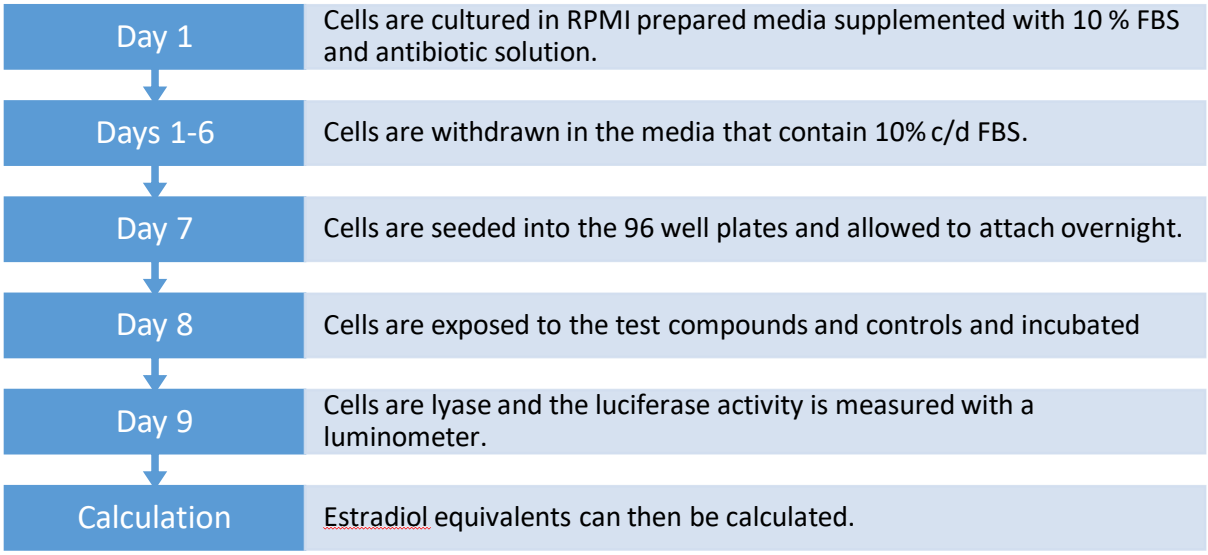


Figure 10: Summary of steps of a The T47D-Kbluc assay.[100-101]

Bioassay for androgenic and anti-androgenic activity

MDA-kb assay

The MDA-kb2 is a cell line developed to screen androgen agonists and antagonists. This was done to characterize its specificity and sensitivity to endocrine-disrupting chemicals. The breast cancer cell line, MDA-MB-453, was stably transformed with the mouse mammary tumour virus promoter (MMTV). luciferase.neo reporter gene construct. Since both glucocorticoid receptors (GR) and androgen receptors (AR) are present in the MDA-MB-453 cells. These two receptors can act through the MMTV promoter, compounds that act through either AR or GR and thus activate the MMTV luciferase reporter.[105] A Novel Cell Line, MDA-kb2, that Stably Expresses an Androgen- and Glucocorticoid-Responsive Reporter for the detection of hormone receptor agonists and antagonists.[105]

Materials

The materials used were MDA-kb2 cells, Lebovitz's L-15 growth media, 10% FBS, Penicillin, streptomycin, amphotericin B. The apparatus used was 25 cm^2 and 75 cm^2 tissue culture flasks, 96-well luminometer plates, centrifuge tubes, cryovials and CoolCell freezing containers

Methods

As per figure 11, MDA-kb2 cells were maintained in Lebovitz's L-15 growth media (Cat. No. 41300-021, Gibco, Scientific Group, SA) supplemented with 10% FBS (characterised, Cat. No. SH30071.03, Hyclone, Separations, SA), 100 ug/m; penicillin, 100 U/m; streptomycin and 0.25 ug/m; amphotericin B (Cat. No. 15240-062, Gibco, Scientific Group, SA) as described in Wilson *et al*. [105]

Cells were seeded at 5 x 104 cells per well in 96-well luminometer plates and allowed to attach overnight. Dosing solutions was prepared in growth media and vehicle (ethanol), not exceeding 0.2%. Each plate contained a positive agonist control (dihydrotestosterone, DHT), negative control (vehicle only), antagonist control (DHT plus flutamide) and background control (vehicle plus flutamide). Each sample was tested alone as well as in the presence of 0.1 nM DHT or flutamide. Cells were incubated for 24hrs with 100; dosing solution per well at 37°C, without supplemental CO_2. After the incubation period, cells were lysed, and luciferase activity determined as described for the Kbluc assay.

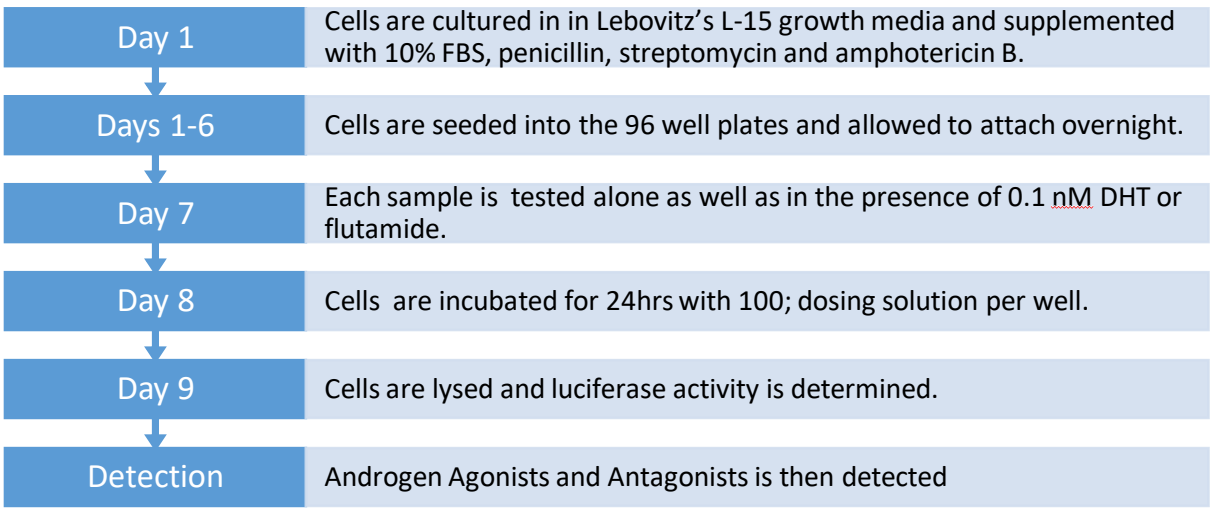


Figure 11: Summary of steps of the MDA-kb2 assay. [100-101]

3.3.2 Pharmaceutical Screening

Chemical screening of the water extract was conducted using a UPLC (ultra-high-performance liquid chromatography) system consisting of an Agilent 1290 Infinity Binary pump (G4220A);1290 Infinity Autosampler (G4226A); and 1290 Infinity

Thermostatted Column Compartment (G1316C) coupled to an Agilent 6540 Accurate mass Q-TOF/MS (G6540A) (Agilent Technologies, Santa Clara, CA, USA). Agilent's Personal Compound Database and Library software, 7 500 compound forensic/toxicology database, and 2 500- compound MS/MS Broecker, Herre & Pragts PCDL library enabled identification of a long list of compounds. This list includes human doping drugs, designer drugs, veterinary drugs, pesticides, mycotoxins, cannabinoids, hallucinogens, stimulants, benzodiazepines, hypnotics, neuroleptics, barbiturates, antidepressants, cardiovascular medicine, anti-epileptics, opioids, anabolic agents, pharmaceuticals and personal care products and hormones. The software used was MassHunter's data acquisition (version 10.1), qualitative analysis (version 10.0) and quantitative analysis (version 10.1). The mass axis calibration of the QTOF was performed daily for positive and negative ionisation with a tuning mix (G1969-85000, Agilent). A reference solution with masses of 121.050873 [M+H] and 322.048121 [M+H] was constantly infused to serve as an accurate mass reference.

Liquid chromatography (LC) and mass spectrometry (MS) parameters

The injection volume for analysis was 1 µL. A Poroshell 120 Bonus-RP column (Agilent, 2.1 x 100 mm, 2.7 µm) kept at 25°C was used for separation. The mobile phase for positive ionisation consisted of water (solvent A) and acetonitrile (solvent B) both containing 0.1% formic acid.[106] The mobile phases for negative ionisation was water (solvent A) and methanol (solvent B) without modifiers.

Qualitative screening of unknown compounds

The data obtained after the chromatographic analysis were utilised to screen for and identify some of the compounds present in the extracts. Compound possibilities were generated based on molecular features and subjected to the Agilent Forensic Toxicology Personal Compound Database and Library (PCDL). The library includes 9 200 compounds including human doping drugs, designer drugs, veterinary drugs, pesticides, mycotoxins, cannabinoids, hallucinogens, stimulants, benzodiazepines, hypnotics, neuroleptics, barbiturates, antidepressants, cardiovascular medicine, anti-epileptics, opioids, anabolic agents, pharmaceuticals and personal care products and

hormones. This PCDL combined with the accurate mass capabilities of the Q-TOF instrument confirm the presence of compounds based on accurate monoisotopic mass, isotope patterns, fragment confirmations and retention time.

Quantitative analysis of specific target compounds

Matrix-matched-calibration.

The matrix to be analysed was drinking and surface water. To account for matrix effects during quantification an external matrix-matched calibration curve was used. To mimic natural water in the matrix-matched calibrations, deionised water (18.2 mΩ·cm) was supplemented with 0.7 mM $NaHCO_3$, 2 mM $CaCl_2 \cdot 2H_2O$, 0.5 mM $MgSO_4 \cdot 7H_2O$, and 75 µM KCl to create artificial freshwater (ISO, 2012). This water was inoculated with 50 mL of the same water after 25 mature specimens of the freshwater snail *Bulinus tropicus* had been living in one litre of the first type for at least 24 hours. This was done to assist to simulate at least some form of organic content.

The concentrations used in the calibration curve were determined based on the expected levels of pharmaceuticals present in the sample (after enrichment) and the performance of the instrument. Samples were concentrated 2 000 times during extraction. This should be accounted for when choosing a calibration range (Table 7). Matrix-matched calibration curves were prepared by extracting 1L of the prepared water, evaporating the extract to dryness and resuspending it in 500 µL. This reconstitute was spiked with a mixture of pharmaceuticals and internal standards at the calibration range concentrations. Serially dilutions were not prepared but originated from different stocks. These standards were analysed in triplicate to assess the reportable range (Westgard, 2008). They were injected in order of increasing concentration, with blank injections between batches to prevent carry-over.

Table 7: Concentration range of calibration curves used for pharmaceuticals quantification

Pharmaceutical compound	Calibration curve range (ug/mL)
Ampicillin	0–10 (1.2x serial dilution)
Chloramphenicol	0–10 (1.2x serial dilution)
Ciprofloxacin	0–2 (1.2x serial dilution)

Efavirenz	0–2 (5x serial dilution)
Erythromycin	0–8 (1.2x serial dilution)
Fluconazole	0–8 (5x serial dilution)
Nevirapine	0–10 (5x serial dilution)
Lopinavir	0–10 (5x serial dilution)
Sulfamethoxazole	0–5 (1.2x serial dilution)
Trimethoprim	0–0.5 (1.2x serial dilution)
Tetracycline	0–4 (1.2x serial dilution)
Ritonavir	0–10 (5x serial dilution)
Vancomycin	0–20 (1.2x serial dilution)

Precision and accuracy

Precision (repeatability, in terms of % RSD) and accuracy (percentage recoveries) were estimated by recovery experiments at two spiked levels, each one analysed in triplicate.

Accuracy was determined as the recovery of spiked samples (Table 8).

Precision was calculated using % RSD = (SDEV of QCs/mean of QCs) x 100 (Table 7).

Linearity

The linearity of the calibration curve was assessed by determining the R^2 value. Good linearity is indicated with R^2 as close to 1 as possible (at least 0.9).[107] The linearity of the calibration curve for this analysis can be found in table 8.

Limit of detection (LOD)/Limit of quantification (LOQ)

Sensitivity of an analytical method is defined as “the increased response of the analyte linear to the analyte concentration.”[108] A calibration curve and the slope of the calibration curve is used as a display. By using linear regression statistics, the uncertainties of the calibration curve can be used to calculate limit of detection(LOD) and limit of quantification(LOQ) for the method from the external matrix-matched

calibration curve. By use of the y = m x +c model, LOD is calculated by 3*Sa/b and LOQ by 10*Sa/b; where Sa is the SD of the intercept (abundance) and b is the slope of the calibration curve.[109]

Table 8: Method validation parameters

Pharmaceutical compound	Precursor ion (m/z)	RT (min)	R^2	LOD (ug/mL)	LOQ (ug/mL)	Accuracy (%)	Precision (%)
Ampicillin	350.1500	3.3	0.993	0.96	3.19	83	8
Chloramphenicol	320.9358	0.9	0.995	0.73	2.44	73	7
Ciprofloxacin	332.1446	3.464	0.999	0.09	0.30	93	5
Efavirenz	314.0193	2.0	0.988	3.06	10.23	78	12
Erythromycin	734.7401	8.914	0.996	0.60	2.01	48	10
Fluconazole	307.1111	5.007	0.998	0.44	1.48	71	4
Nevirapine	267.1224	7.58	0.993	1.48	4.93	87	9
Sulfamethoxazole	254.0588	9.2	0.994	0.48	1.61	75	16
Trimethoprim	291.1473	3.181	0.997	0.03	0.11	111	5
Tetracycline	445.1649	3.4	0.988	1.9	6.5	72	15
Vancomycin	1447.1446	3.2	0.998	0.91	3.04	61.8	7

3.3.3 Viral Content

Virus Concentration and Nucleic Acid Extraction

The glass wool adsorption-elution technique was used for viral recovery from water samples.[110] The recovered viruses were eluted from glass wool using 100 millilitres (mL) of glycine-beef extract buffer (pH 9.5) (Glycine; Merck KgaA) (BBL™ Beef Extract; Becton, Dickinson and Company, Sparks, MD) and the pH of the eluate was adjusted to pH 7 using 1 M HCl (Merck KgaA). The viruses in 100 mL eluate were further concentrated into a final volume of 10 mL in phosphate-buffered saline (PBS) (pH 7.4) (Sigma-Aldrich Co., St. Louis, MO) by polyethylene glycol 8000/sodium chloride (PEG8000/NaCl) precipitation (PEG8000; Amresco LLC, Solon, OH) (NaCl;

Merck KgaA).[111-112] The extracted nucleic acids will be eluted in a final volume of 100 µL and stored in 10 µL aliquots at −70 °C.

Detection and Quantification of Norovirus (NoV) and Adenovirus (AdV)

Commercial real-time reverse transcription-PCR (RT-PCR) kits were used for the detection and quantification of NoV and AdV GI and GII using CeeramTools™(Ceeram S.A.S, La Chapelle Sur Erdre Cedex, France) and (norovirusGII@ceeramTools™) (Ceeram S.A.S). All kits used contained internal, positive, and negative PCR inhibition controls to monitor the efficiency of target amplification. According to the manufacturer's instructions, Nov and AdV GI and GII were detected and quantified using one-step real-time qRT-PCR. Norovirus and Adenovirus standard curves were generated using plasmid DNA standards (Norovirus and Adenovirus GI Q Standard) (Norovirus and Adenovirus GII Q Standard) (Ceeram S.A.S). Norovirus and Adenovirus concentrations were adjusted to compensate for extraction efficiencies below 100% and expressed as genome copies/litre (gc/L). All NoV and AdV-negative samples were re-tested using a 1:10 dilution of RNA in nuclease-free water to exclude possible false-negative results due to inhibition.

3.4 Data Management and Analysis

3.4.1 Assays to assess endocrine disruption

T47-Kbluc-Assay

The E2 standard curve was fitted (sigmoidal function, variable slope) using GraphPad Prism (version 4), which was then used to calculated the minimum, maximum, slope, EC50 value and 95% confidence limits. The EEq values of extracts with greater than a two-fold induction above the vehicle control was interpolated from the estradiol standard curve and corrected with the appropriate dilution factor for each sample.

MDA-kb assay

Each well was receive 25; reaction buffer (25 mM glycylglycine, 15 mM MgCl2, 5 mM ATP, 0.1 mg/m; BSA, pH 7.8), followed by 25mM; 1 mM D-luciferin 5s later. The RLU

was converted to a fold induction above the vehicle control value. The curves for DHT and selected EC samples was fitted (sigmoidal function with variable slope) using GraphPad Prism (version 4), then the minimum, maximum, slope, EC50 value and 95% confidence limits was calculated.

3.4.2 Pharmaceutical screening

Quantitative screening of known compounds

The concentration of target compounds was calculated by using the below formula:

Xcompound = ((native/stable isotope)-c) / m) x ISO conc

where:

Xcompound = calculated analyte concentration native = native abundance

stable isotope = stable isotope abundance
c = calibration curve is the y-intercept
m = slope of the calibration curve
ISO conc = stable isotope concentration

Qualitative screening of unknown compounds

The data obtained after the chromatographic analysis were utilised to screen for and identify some of the compounds present in the extracts. Compound possibilities were generated based on molecular features and subjected to the Agilent Forensic Toxicology Personal Compound Database and Library (PCDL). This PCDL combined with the accurate mass capabilities of the Q-TOF instrument confirm the presence of compounds based on accurate monoisotopic mass, isotope patterns, fragment confirmations and retention time

3.5 Ethical and legal considerations

Ethical approval was attained from the University of Pretoria's Faculty of Health Sciences Research Ethics Committee with the ethical approval numbers 151/2021 and letter attached at Annexure A.

The risk of the study was negligible since water sampling was used. Principles of Helsinki and Good Clinical Practice was adhered to, and there were no conflicts of interest. No samples or information were obtained from the community or other people and no experimental animals were used. Permission to conduct the study was obtained from the City of Tshwane, please see Annexure B.

CHAPTER 4: RESULTS

4.1 Estrogenic activity: T46D-Kbluc-assay

T46D-Kbluc-assay results indicated that water samples analysed from selected sample points contained compounds with estrogenic activity. Estrogenic activity was detected in thirteen samples with only 2 samples (C3 and E) below the detection limit (dl). The estradiol equivalency (EEq) values ranged from below the dl to 0.216. The samples collected from the houses in samples A1, A2 and A4 were collected from JOJO A (sample A). Samples collected from JOJO tank A's EEq values (average of 3 houses= 0.028 ng/L) were higher than their source (JOJO A). Higher activity was seen in the samples collected from the houses compared to source JOJO was also seen in samples B1, B2 and B3 (average= 0.03 ng/L) from the source, JOJO B. Higher or same EEq values were also seen in the houses' samples (C1, C2 and C4 except sample C3) compared to source JOJO C. The average across all these houses was 0.018 ng/L. As seen in the graph in Figure 10 sample B3's EEq value was 193.65% more than the source JOJO B and the house with the highest EEq value. The average EEq value across all the houses was 0.025 ng/L and the EEq value average of the 3 JOJO tanks (samples A, B and C) were 0.012 ng/L which is almost double that of the houses. The JOJO tank with the highest EEq value was JOJO C.

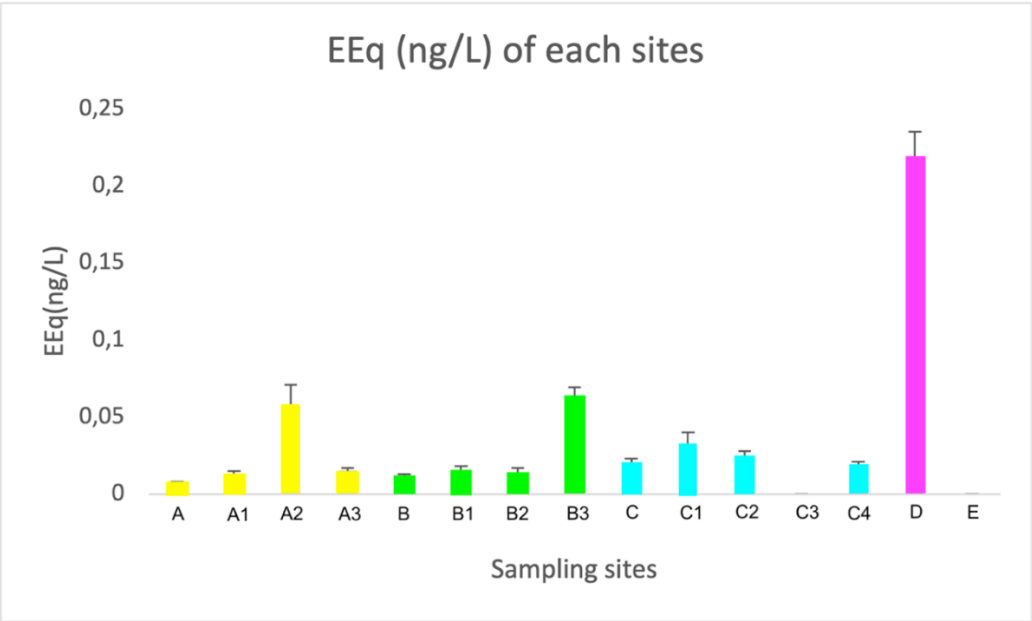


Figure 12: Estradiol equivalency (EEq) across all samples.

The only house with an EEq below the level of quantification of the bioassay was house sample C3. The dam (sample D) had the highest EEq value of 0.216 also seen how high the EEq value is compared to other samples. The community tap (Sample E) which is sourced from a borehole had an EEq value below the level of quantification of the bioassay. No cytotoxicity or anti-estrogenic activity was detected in any of the samples at the concentrations tested (Table 9).

Table 9: Estrogenic and Cytotoxic activity across all samples

Sample code	EEq (ng/L)	Cytotoxicity
A	**0.007 ± 0.001**	**-**
A1	0.013 ± 0.002	-
A2	0.057 ± 0.014	-
A3	0.015 ± 0.002	-
B	**0.010 ± 0.003**	**-**
B1	0.015 ± 0.003	-
B2	0.013 ± 0.004	-
B3	0.062 ± 0.007	-
C	**0.019 ± 0.004**	**-**
C1	0.030 ± 0.010	-
C2	0.024 ± 0.004	-
C3	<loq	-
C4	0.019 ± 0.002	-
D	0.216 ± 0.019	-
E	<loq	-

4.2 Androgenic activity: MDA-kb2 reporter gene assay

No androgenic activity was observed in any of the water samples, as none of the samples had a RLU (Relative light unit) values above the level of quantification (loq) for each plate. Therefore, none of the samples were able to suppress the activity of DHT, which shows none of the samples have anti-androgenic activity at the tested concentrations.

4.3 Emerging contaminant screening and quantification

4.3.1 Emerging contaminant screening

The most predominant compound seen across all samples is Atrazine-desisopropyl a common herbicide. The second most possibly detected compound is also an herbicide, Trietazine. These two herbicides are seen in almost all samples. The most common pharmaceuticals possibly detected was Enprofylline, a Bronchodilator and Valdetamide, a sedative (Table 10).

Table 10: Frequently screened compounds across all the samples

Ranking	Samples containing compounds in top 5	Name	Used as a
1	A, A1, A3, B1, B2, B3, C, C1, C3, C4	Atrazine-desisopropyl	Herbicide
2	A1, A2, B2, B3, C, C3, E	Trietazine	Herbicide
3	A, A3, B3, C1. E	Enprofylline	Bronchodilator
3	A1, B1, C, C2, D	Valdetamide	Hypnotic; Sedative
3	A, B3, C, C3, E	Propisochlor	Herbicide
4	A, A1, A3, C1	Dimazole	Antimycotic
5	C3, C4, E	Atrazine	Herbicide
5	A3, B2, D	Nonoxinol 9	Spermicide
6	A, C3	Atraton	Herbicide

There is one pharmaceutical which may be prevalent in JOJO A and all the corresponding houses, A1, A2 and A3, namely Dimazole which is known to be an Antimycotic (An antifungal medication). In addition, Atrazine-desisopropyl (herbicide

metabolite) is also seen in JOJO A as well as houses A1, A3. Another pharmaceutical, Enprofylline (Brochodilator) which is seen in JOJO A as well as house A3. All the top 5 compounds seen in JOJO A is also seen in all the other samples' top 5 compounds. For example, Enprofylline is seen in 4 other samples, Atraton seen in 1 other sample, Atrazine-desisopropyl is seen in 9 other samples, Propisochlor is seen in 4 other samples and Dimazole is seen in 3 other samples. In other words, there is no compound unique only in JOJO A. (Table 11)

Table 11: Compounds with highest screening scores from samples A,A1,A2 and A3

Sample A CAS		Notes	Mass (DB)	Score (DB)	Formula	Height
41078-02-8	Enprofylline 5	Bronchodilator	194.0804	97.48	C8 H10 N4 O2	135982
1610-17-9	Atraton 2	Herbicide	211.1433	96.39	C9 H17 N5 O	61908
1007-28-9	Atrazine-desisopropyl 10	Herbicide metabolite	173.0468	95.72	C5 H8 Cl N5	80764
86763-47-5	Propisochlor 5	Herbicide	283.1339	94.56	C15 H22 Cl N O2	73747
95-27-2	Dimazole 4	Antimycotic	293.1562	93.88	C15 H23 N3 O S	176583
Sample A1						
1007-28-9	Atrazine-desisopropyl 10	Herbicide metabolite	173.0468	94.95	C5 H8 Cl N5	50459
95-27-2	Dimazole 4	Antimycotic (skin infections)	293.1562	93.12	C15 H23 N3 O S	89286
1912-26-1	Trietazine 7	Herbicide	229.1094	88.53	C9 H16 Cl N5	89902
15534-92-6	Terbuficin	Antilipidemic (cholesterol medication)	468.324	87.81	C30 H44 O4	10821
512-48-1	Valdetamide 5	Hypnotic; Sedative	155.131	87.28	C9 H17 N O	10000
Sample A2						
15793-40-5	Terodiline 2	Coronary Dilator	281.2143	98.02	C20 H27 N	28521
511-96-6	Gitogenin	Biomolecule	432.324	93.49	C27 H44 O4	75369
	Nonoxinol 15	Evidence of developmental/endocrine/reproductive	880.5759	93.08	C45 H84 O16	5000

		effects; chronic aquatic toxicity; acute aquatic toxicity				
1912-26-1	Trietazine	Herbicide	229.1094	90.34	C9 H16 Cl N5	22404
56695-65-9	Rosaprostol	Anticulcerative	298.2508	88.8	C18 H34 O3	11259
Sample A3						
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	96.52	C5 H8 Cl N5	26845
95-27-2	Dimazole	Antimycotic	293.1562	96.39	C15 H23 N3 O S	52505
9016-45-9	Nonoxinol 9- 3	Spermicide	616.4186	95.15	C33 H60 O10	37821
41078-02-8	Enprofylline	Bronchodilator	194.0804	93.24	C8 H10 N4 O2	53701
23790-08-1	Moxipraquine	Antiamebic (parasites)	414.2995	86.6	C24 H38 N4 O2	14097

House A1 also may contain Atrazine-desisopropyl and Dimazole which may also be in JOJO A. House A1 may also have Trietazine (an herbicide) which is seen in 6 other samples as well especially sample A2. Furthermore, House A1 may also contain Valdetamide (Hypnotic; a sedative) which is seen is 4 other samples. House A1 may contain a unique compound, Terbuficin, which is only seen in house A1's top 5 compounds and not in another sample's top 5. Terbuficin is known to be an Antilipidemic which is used as a cholesterol medication.

House A2 may have 3 unique compounds namely, Gitogenin, Nonoxinol 15, Rosaprostol. Gitogenin is known to be a biomolecule and Rosaprostol is known to be an anticulcerative. Interestingly Nonoxinol 15, is known to show evidence of developmental/endocrine/reproductive effects, chronic aquatic toxicity, and acute aquatic toxicity.

House A3 may contain Nonoxinol 9, a spermicide, which is also seen in 2 other sample's top 5 possible compounds. In Sample A4 there is also one unique possible compound, namely Moxipraquine which is an antiamebic.

JOJO B shares no possible compounds in its top 5 compounds with any other sample. Propentofylline (Vasodilator), Cholesta-3,5-dien-7-one (biomolecule found in high concentrations in fatty/cirrhotic alcoholic liver), delta8-Tetrahydrocannabinol (Psychedelic), Hetramine (Antihistamine), Phenamidine (Chemotherapeutic) are all unique to sample B (Table 12).

Sample B1, B2 and B3 all share a possible compound, Atrazine-desisopropyl. Sample B1 has 3 unique compounds, namely Tacrolimus (immunosuppressant), Norephedrine (Phenylpropanolamine) and Tolylethanol.

Table 12: Compounds with highest screening scores from samples B, B1, B2 and B3

Sample B CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
55242-55-2	Propentofylline	Vasodilator	306.1692	92.61	C15 H22 N4 O3	11094
567-72-6	Cholesta-3,5-dien-7-one	High concentrations in fatty/cirrhotic alcoholic liver	382.3236	89.03	C27 H42 O	191958
28646-40-4	delta8-Tetrahydrocannabinol	Psychedelic	330.2195	85.56	C21 H30 O3	16367
531-08-8	Hetramine	Antihistamine	256.1688	84.76	C15 H20 N4	5337
101-62-2	Phenamidine	Chemotherapeutic	254.1168	82.58	C14 H14 N4 O	3528
Sample B1						
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	92.09	C5 H8 Cl N5	11816
104987-11-3	Tacrolimus	Immunosuppressant	803.482	91.02	C44 H69 N O12	37646
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	87.35	C9 H17 N O	45758
14838-15-4	Norephedrine (Phenylpropanolamine)	Anorectic, sympathomimetic; synonym = Phenylpropanolamin with Pragst ID = P158	151.0997	87.33	C9 H13 N O	38868
536-50-5	Tolylethanol		136.0888	87.13	C9 H12 O	15066
Sample B2						
142-91-6	Isopropyl palmitate	Dermatic	298.2872	94.49	C19 H38 O2	16805
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	93.73	C5 H8 Cl N5	13342
1912-26-1	Trietazine	Herbicide	229.1094	91.21	C9 H16 Cl N5	8938

9016-45-9	Nonoxinol 9	Spermicide	616.4186	89.29	C33 H60 O10	235240
120-80-9	Pyrocatechol	Insecticide	110.0368	87.78	C6 H6 O2	10405
Sample B3						
86763-47-5	Propisochlor	Herbicide	283.1339	92.92	C15 H22 Cl N O2	76134
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	92.27	C5 H8 Cl N5	53431
1912-26-1	Trietazine	Herbicide	229.1094	90.61	C9 H16 Cl N5	96920
60762-57-4	Pirlindole	Antidepressant	226.147	85.7	C15 H18 N2	5320
835-31-4	Naphazoline	Vasoconstrictor	210.1157	85.18	C14 H14 N2	8703

Sample B2 and B3 share 2 possible herbicide compounds, Atrazine-desisopropyl and Trietazine. Sample B2 has 2 unique possible compounds, Isopropyl palmitate and Pyrocatechol which are a dermatic and an insecticide, respectively. Sample B3 and 2 other samples share the possibility of containing Pirlindole, an anti-depressant. Sample B# has 1 unique possible compound Naphazoline, which is a vasoconstrictor.

Sample C, which is one of the JOJO tanks may possibly have 5 compounds which is seen across all corresponding houses' samples. Atrazine-desisopropyl is seen in sample C1, C3 and C4. Propisochlor is also seen in sample C3 and Valdetamide is possibly seen in sample C2 (Table 13). Sample C1 shows to possibly have 2 unique compounds namely N-Desalkyl-pentazozin an opioid medication and Hydracarbazine, a diuretic. Sample C2 possibly has 2 unique compounds one an anabolic and another an antimycotic, which is Zearalenone and N-(2-hydroxyethyl)-10-Undecenamide, respectively. Sample C3's top 5 possible compounds are Herbiceds, namely trazine, Propisochlor, Atrazine-desisopropyl, Trietazine, Atraton. There are also no unique compounds only seen in sample C3's top 5 compounds. Atrazine is also seen in 2 other samples, sample C4 and the community tap sample E Sample C4 shows to possibly have 3 unique compounds. Th first unique compound in this sample's top 5 possible compounds is Leucomalachite green, which is a dye used as a dye for materials such as silk, leather, and paper and controversially as an antimicrobial in aquaculture. In addition, 2 another unique compound possibly in sample 3 is Octamylamine which is a Parasympatholytic and Cassaidine which is used as a Cardiotonic.

Table 13: Compounds with highest screening scores from samples C, C1, C2, C3 and C4

Sample C CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	97.44	C5 H8 Cl N5	17823
86763-47-5	Propisochlor	Herbicide	283.1339	89.1	C15 H22 Cl N O2	19968
1912-26-1	Trietazine	Herbicide	229.1094	88.67	C9 H16 Cl N5	30555
60762-57-4	Pirlindole	Antidepressant	226.147	86.69	C15 H18 N2	33572
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	85.91	C9 H17 N O	32861
Sample C1						
	N-Desalkyl-pentazozin	Opioid pain medication	217.1467	98.93	C14 H19 N O	50724
41078-02-8	Enprofylline	Bronchodilator	194.0804	96.19	C8 H10 N4 O2	78072
3614-47-9	Hydracarbazine	Diuretic	153.0651	92.84	C5 H7 N5 O	55862
95-27-2	Dimazole	Antimycotic	293.1562	88.72	C15 H23 N3 O S	92153
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	88.22	C5 H8 Cl N5	37083
Sample C2						
15793-40-5	Terodiline	Coronary Dilator	281.2143	95.32	C20 H27 N	54998
17924-92-4	Zearalenone	Anabolic	318.1467	89.27	C18 H22 O5	20912
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	86.62	C9 H17 N O	16097
60762-57-4	Pirlindole 3	Antidepressant	226.147	86.58	C15 H18 N2	23884
20545-92-0	N-(2-hydroxyethyl)-10-Undecenamide	Antimycotic	227.1885	84.34	C13 H25 N O2	7223
Sample C3						

1912-24-9	Atrazine	Herbicide	215.0938	96.45	C8 H14 Cl N5	23895
86763-47-5	Propisochlor	Herbicide	283.1339	96.28	C15 H22 Cl N O2	57211
1007-28-9	Atrazine-desisopropyl	Herbicide metabolite	173.0468	93.77	C5 H8 Cl N5	41379
1912-26-1	Trietazine	Herbicide	229.1094	89.62	C9 H16 Cl N5	78236
1610-17-9	Atraton	Herbicide	211.1433	86.15	C9 H17 N5 O	8082
Sample C4						
	Leucomalachite green	Dye unique	330.2096	99.62	C23 H26 N2	96731
502-59-0	Octamylamine	Parasympatholytic	199.23	98.99	C13 H29 N	76941
26296-41-3	Cassaidine	Cardiotonic	407.3036	97.54	C24 H41 N O4	17452
1007-28-9	Atrazine-desisopropyl	Bronchodilator	173.0468	96.72	C5 H8 Cl N5	29585
1912-24-9	Atrazine 3	Herbicide	215.0938	94.65	C8 H14 Cl N5	11836

Sample D shares the possibility to have Nonoxinol 9 present in the sample with samples A3 and B2 as well as Valdetamide with 4 other samples. Simvastatin a cholesterol synthesis inhibitor is a unique to the dam as well as Leptacline (Stimulant) and Enprazepine (antidepressant). Sample D contain no Herbicides (Table 14).

Table 14: Compounds with highest screening scores from sample D

Sample D CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
79902-63-9	Simvastatin	Cholesterol synthesis inhibitor	418.2719	96.26	C25 H38 O5	26105
9016-45-9	Nonoxinol 9	Spermicide	616.4186	89.49	C33 H60 O10	203820
512-48-1	Valdetamide	Hypnotic; Sedative	155.131	86.59	C9 H17 N O	29045
5005-72-1	Leptacline	Stimulant	181.183	86.16	C12 H23 N	7513
47206-15-5	Enprazepine	Antidepressant	292.1939	85.52	C20 H24 N2	8060

Sample E, which is the community, tap at the clinic, contains 3 herbicides, 1 insecticide and 1 pharmaceutical, with only 1 unique compound in its top 5 possible compounds, namely Fenazaquin, which is and insecticide (Table 15).

Table 15: Compounds with highest screening scores from sample E

Sample E CAS	Name	Notes	Mass (DB)	Score (DB)	Formula	Height
1912-24-9	Atrazine	Herbicide	215.0938	93.12	C8 H14 Cl N5	14287
86763-47-5	Propisochlor	Herbicide	283.1339	89.23	C15 H22 Cl N O2	42812
1912-26-1	Trietazine	Herbicide	229.1094	89.09	C9 H16 Cl N5	66791
41078-02-8	Enprofylline	Bronchodilator	194.0804	86.36	C8 H10 N4 O2	10117
N/A	Fenazaquin	Insecticide	306.1732	82.9	C20 H22 N2 O	14050

4.3.2 Quantification of Pharmaceuticals

The UPLC analysis showed the presence of the 3 antibiotics, Ciproflaxin, Sulfamethoxazole and Vancomycin (Table 16). Samples A1, A3, B, C1, C3, and E showed the presence of Ciproflaxin ranging between 0.2 ug/L - 0.3 ug/L. Sulfamethoxazole was quantified in 2 samples namely sample A3 (0.6 ug/L) and B (1ug/L). Only sample A3 was quantifiable for Vancomycin at a concentration of 8 ug/L. In addition, sample A3 was the only sample where all three antibiotics, Ciproflaxin (0.3 ug/L), Sulfamethoxazole (0.6 ug/L) and Vancomycin (8 ug/L) wat quantified. JOJO tank B was the only source JOJO to show the presence of a pharmaceutical, namely Sulfamethoxazole. Interestingly, the community tap sample showed the presence of Ciproflaxin at a concentration of 0.2 ug/L. It is important to note that sample B3 was lost through analysis.

Table 16: Pharmaceuticals quantification (ug/mL) using UPLC/Q-TOF/MS

Sample	Ciprofloxacin	Erythromycin	Fluconazole	Sulfamethoxazole	Trimethoprim	Vancomycin	Nevirapine	Tenofovir	Tetracycline	Efavirenz	Chloramphenicol
A	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
A1	**0.2±0.1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD
A2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
A3	**0.3±0.1**	<LOD	<LOQ	**0.6±0.5**	<LOD	**8±6**	<LOD	<LOD	<LOD	<LOD	<LOD
B	<LOD	<LOD	<LOD	**1±0.08**	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
B1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
B2	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
B3	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
C	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C1	**0.3±0.1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C3	**0.3±0.4**	<LOQ	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
C4	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
D	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
E	**0.2±0.1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

4.4 Screening and quantification of Viruses: RT-PCR for viruses

Two samples were taken from the dam for RT-PCR to detect the presence of Norovirus and Adenovirus. Norovirus GI and GII were undetected in both samples. Both samples exhibited inhibition of the internal control when testing the concentrated nucleic acids. Thus, both samples were also tested at 1 in 10 dilutions of the nucleic acids. The inhibition was resolved when the nucleic acids were diluted, but norovirus GI and GII were still not detected.

However, Adenoviruses were detected in the concentrated screen, 1 and 2. Sample 1 was detected at a concentration of 36.8 and 2 at a concentration of 40, which is the cut-off for positive samples. Both samples showed weak, but clear positives. (Table 17)

Table 17. Noro GI, GII and Adenovirus detection in dam water in Melusi informal settlement

Sample ID	Nucleic acid concentration	IC	Noro GI	IC	Noro GII	IC	Adenovirus
1	Concentrated	34,5	ND	ND	ND	31,5	**36,8**
2	Concentrated	34,5	ND	ND	ND	31,5	**40,0**

CHAPTER 5: DISCUSSION

This study seeked to determine the estrogenic, anti-estrogenic, androgenic and anti-androgenic activity in drinking water sources using a battery of in vitro bioassays. Additionally, the occurrence of selected pharmaceuticals and viruses will be determined to assess the water quality and propose possible strategies to minimise adverse health effects and health risk for the community of Melusi, Pretoria.

There is a paucity of evidence in South Africa regarding personal exposure to multi-pollutants. Thus, our aim with screening and quantifying multi-pollutants, has the potential to reveal multi-pollutant exposure patterns that could inform future toxicology and epidemiology research. Findings will contribute to a transdisciplinary strategy to reduce exposure to multi-pollutants (SDG 3 – indicator 3.9) in water sources (SDG6 – 6.3, 6b) by using novel technology through partnerships (SDG 17 – 17.6) and educational tools (SDG 4) to achieve resilient and sustainable environments (SDG 11 – 11a) to ensure health and wellbeing (SDG 3) for communities like the community of Melusi, Pretoria.

Table 18 below summarizes all the results obtained through our investigation across all samples tested.

Table 18: Comprehensive summary of all results across all samples

Sample	Appearance and storage	EEQ	Pharmaceuticals	Shared compounds	Unique compounds	Viruses
A	Clear, JOJO tank	0.007	-	• Enprofylline • Atraton • Atrazine-desisopropyl • Propisochlor • Dimazole		Not Applicable
A1	Clear, paint container, shade	0.013	Ciprofloxacin	• Atrazine-desisopropyl • Dimazole • Trietazine • Valdetamide	• Terbuficin	Not Applicable

A2	Clear, paint container, sun	0.057	-	• Terodiline • Trietazine	• Gitogenin • Nonoxinol 15 • Rosaprostol	Not Applicable
A3	Green with algae, white container, shade	0.015	Ciprofloxacin Sulfamethoxazole Vancomycin	• Atrazine-desisopropyl • Dimazole • Nonoxinol 9 • Enprofylline	• Moxipraquine	Not Applicable
B	Clear, JOJO tank	0.010	Sulfamethoxazole		• Moxipraquine • Propentofylline • Cholesta-3,5-dien-7-one delta8- • Tetrahydrocannabinol • Hetramine • Phenamidine	Not Applicable
B1	Clear, white container, shade	0.015	-	• Atrazine-desisopropyl • Valdetamide	• Tacrolimus • Norephedrine • Tolylethanol	Not Applicable
B2	Clear, paint container, shade	0.013	-	• Atrazine-desisopropyl • Trietazine • Nonoxinol 9	• Isopropyl palmitate • Pyrocatechol	Not Applicable
B3	Clear, blue container, sun	0.062	N/A	• Propisochlor • Atrazine-desisopropyl • Trietazine • Pirlindole	• Naphazoline	Not Applicable
C	Clear, JOJO tank	0.019	-	• Atrazine-desisopropyl • Propisochlor • Trietazine • Pirlindole • Valdetamide		Not Applicable
C1	Clear, paint container, sun	0.030	Ciprofloxacin	• Enprofylline • Dimazole • Atrazine-desisopropyl	• N-Desalkyl-pentazozin • Hydracarbazine	Not Applicable
C2	Clear, paint container, sun	0.024	-	• Terodiline • Valdetamide • Pirlindole	• Zearalenone • N-(2-hydroxyethyl)-10-Undecenamide	Not Applicable

C3	Clear, white container, shade	<loq*	Ciprofloxacin	• Atrazine • Propisochlor • Atrazine-desisopropyl • Trietazine • Atraton		Not Applicable
C4	Clear, white container, shade	0.019	-	• Atrazine-desisopropyl • Atrazine	• Leucomalachite green • Octamylamine • Cassaidine	Not Applicable
D	Pungent smell and cloudy	0.216	-	• Nonoxinol 9 • Valdetamide	• Simvastatin • Leptacline • Enprazepine	Adenovirus
E	Little cloudy but clear	<loq*	Ciprofloxacin	• Atrazine • Propisochlor • Trietazine • Enprofylline	• Fenazaquin	Not Applicable

*<loq is below limit of detection.

Table 18 shows that estrogenic activity was detected in most samples (13 out of 15 samples) in this study using the T47D-KBluc bioassay, with the EEq values ranging from below limit of detection (<loq) to 0.216 ng/L. However, none of the samples exceeded the trigger value of 0.7 ng/l for estrogenic activity in drinking water.[113] If the trigger value is exceeded, possible adverse health effects are implicated and warrants further investigation and continued testing of the water.[114] Estrogens (estradiol, estrone, estriol) are female hormones responsible for the maintenance and development of reproductive tissues and secondary sex characteristics in females.[115] In addition, estrone is also the main metabolite of 17 β estradiol ($C_{18}H_{24}O_2$, a natural estrogen) and reaches the environment via the sewer system or animal excretion.[116] It should also be kept in mind that estradiol equivalents are only rough estimates, as the intricacy of the sample, the pH of the water, extraction procedure used and the nature of the assay (i.e. a biological system) might all have an influence on the results.

As the trigger value, these ranges were determined by assuming long-term continuous use (lifelong exposure) and incorporating a margin of safety. In conjunction with the South African National Standards (SANS), the water-quality guidelines do not take into consideration the very sensitive receptors among humans which EDCs are known to effect, especially infants and children. Also the latest edition of the SANS has a limited

requirement in terms of known EDC which needs to be considered more thoroughly in the following editions which will ultimately contribute to SDG 6 which focuses on ensuring a clean and stable water supply and effective water sanitation for all people by the year 2030.

There was no cytotoxicity detected in the samples. Additionally, no anti-estrogenic activity was detected in any of the water samples in this study. It should also be kept in mind that a water sample consists of a complex mixture of chemicals with possible (anti)-androgenic and (anti)-estrogenic activity, as well as other chemicals not measured, that could affect the outcome of the assay. In a Dutch study, by Van der Linden et al.[117] no antagonist activity was reported in various water sources and this was ascribed it the complex mixture of agonist and antagonist interaction which could be masking the contribution of each individual compound. The androgenic activity measured in the MDA-kb2 assay was absent and may be attributed to the complexity of the samples. This could be that possible androgens present were also below the limit of detection of the MDA-kb2 assay. Furthermore, Blake *et al.*[118] concluded in their study that the steroidal estrogen estradiol possibly could bind to the androgen receptor in MDA-kb2 cells. Agonism and antagonism is showed by Estradiol in MDA-kb2 cell line, however only at high concentrations. Steroidal estrogens therefore potentially interfere with the response of the cells to androgens.[118] So, in future, methods such as effect-based monitoring should be explored to identify the activity of these complex environmental mixtures.[119]

It is interesting to note that the estrogenic activity is more in the samples taken from the homes than in the sourced JOJO tank. This can be attributed to the way the water is stored in the homes, either in blue or white containers or paint containers and predominantly stored outside in the sun. These containers are made from Base polyethylene with pigment antioxidants and UV stabilizers to ensure extended service life. Studies have shown that High-Density Poly Ethylene (HDPE), is a thermoplastic polymer made from petroleum[120] is currently considered an EDC, as contains nonylphenol, which has been found to be dangerous to aquatic life.[121-122] It is suggested that HDPE is UV resistant, however, according to a study done by Said et al.[123], polypropylene fibres can only withstand approximately 6 days of exposure to high-intensity UV light before losing 70% of their strength to resist UV rays. These

containers are used not only for six days but for several years. Ultimately this enclosed environment, coupled with potentially poor water quality may lead to a source for estrogenic contamination.

JOJO A had the lowest estrogenic activity of all the JOJO tanks and the third lowest of all the samples, this may be because it was noted from the community that this JOJO tank is used by the most people and is regularly filled by the municipality. Therefore, the water does stay for a long period of time in the JOJO tank and limited leaching from the JOJO tank can occur. When focusing on the homes, houses A2 and B3 had the highest EEq value compared to all the other houses. When screening water from House A2, the presence of Nonoxinol 15 was identified in the samples. Nonoxinol 15 is part of the Nonoxinols group which is produced by ethoxylation of alkylphenols and vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups. Nonoxynols have been used as detergents, emulsifiers, and wetting agents in cosmetics, including hair products, and defoaming agents.[124] Only nonoxynol-9 with 9 repeating ethoxy groups, has been used as a spermatocide, which is seen in samples A3, B2 and D.

House B3 had an EEq value almost twice that of its source JOJO B. It is interesting to note that House B3 with its high EEq value was also the only house to store water in a blue container. This high EEq value may be from phthalates used to produce this plastic container, or from the blue dye, or another unidentifiable source. Phthalates are known EDCs and have an estrogenic effect.[125] Phthalates may induce alterations in puberty, the development of testicular dysgenesis syndrome, cancer, and fertility disorders in both males and females.[125-126] However, there was no possible Phthalate found in the Qualitative screening of unknown compounds, only herbicides and pharmaceuticals which can also contribute to estrogenic activity.

JOJO C had the highest estrogenic activity compared to JOJO A and B. It is important to note that according to the community, this JOJO tank was filled for the first time in 4 months. This may contribute to the EEq value observed for the JOJO tank heated up to a higher degree for there was no water inside to cool it down and this could cause leaching from the JOJO tank to the water. In addition temperature shows to affects the rate of chemical reactions in a stored water body and it plays an important role in the survival of microorganisms.[127-128] The highest noted temperature in water storage tanks was reported to be 23.1°C which exceeds the WHO permissible limit of

drinking water guidelines of 15°C. Furthermore, high temperature favoures the re-growth of bacteria.[128] The storage tank material and colour can affect the temperature of the water in the JOJO tanks. Additionally placing the JOJO tank in a shaded location can reduce temperature meanwhile microbial contamination.[129]

House C3 had the lowest estrogenic activity seen across all houses. This is quite interesting for the owners of this house, who pertinently stated that they wash their white container every time before refilling it and never leave it in the sun.

The Melusi community lies adjacent to a dam, which is a decommissioned quarry. The dam had a very pungent smell and was cloudy in appearance with a very high EEq value compared to the other samples. It is evident that this water is polluted and not safe to drink. Water pollution causes 1.8 million deaths worldwide, according to a study published in 2022.[130] Together with that Contaminated water sickens about 1 billion people worldwide.[130] And low-income communities like Melusi are disproportionately at risk because their homes are often closest to the most polluting industries, like mines and are exposed to the results such as this decommission quarry hole. [130]

While residents reported that they do not usually use the dam water for drinking, they do regularly use the community tap. The community tap had an EEq value below the detection limit, however almost all the top 5 possible compounds were Herbicides. This may be attributed to the community tap being near a nursery where a high concentration of Herbicides is potentially used. These herbicides may leach into the underground water and ultimately end up in the borehole from which the water is extracted.[131] Additionally, In this study, herbicides, were 5 out of the 10 most frequently possibly detected compounds across all the samples. This can be because herbicides are one of the most major contaminants in the environment as they are widely use in agriculture and are transported into the environment after their application[132] contributing to water quality degradation.[133]

When analysing the data from all the sample sites, the most predominant compound across all samples was Atrazine, and its environmental transformation product atrazine-desisopropyl. Atrazine has been shown to increase the conversion of testosterone and other androgens into estrogens, especially estradiol, by increasing the activity of the enzyme aromatase, which is responsible for the conversion.[134] Resent research, which compared women in Illinois to women in Vermont in the United

States showed that women who drink water contaminated with low levels of atrazine may be more likely to have irregular menstruation and low estrogen levels.[135]

Ciprofloxacin is an antibiotic drug that targets Gram-negative and Gram-positive bacteria and is prescribed to millions of individuals annually.[136] Low-income countries like South Africa has a higher rate of infectious diseases and generally higher rate of over-the-counter self-medication.[57] Ciprofloxacin which is widely used in South Africa, it is used to treat microbial infections, and belongs to the fluoroquinolones group. It is widely prescribed and frequently found in sewage due to its incomplete uptake and metabolism in patients.[137] Studies have shown the presence of ciprofloxacin in sewage treatment effluents, wastewaters, and domestic waters, which ranged from 0.03 µg/L to 5.6 µg/L.[138-139] In this study, ciprofloxacin was detected in five samples with concentrations ranging from 0.2-0.3 ug/L. In other studies, ciprofloxacin has been detected in South African river systems at 0.71 µg/L observed concentration[140], and in other countries, the presence of ciprofloxacin has been confirmed in their municipal wastewaters and drinking water.[141-143] The estimate of at which concentration Studies negative health effects or estrogenic effects are associated with ciprofloxacin require investigation. This is concerning as ciprofloxacin has been shown to have endocrine-disrupting effects.[138,144] This may possibly contribute to the estrogenic activity seen in samples A1,A3 and C1 which contain ciprofloxacin.

Another antibiotic drug screened for and detected was Sulfamethoxazole. This regularly prescribed antibiotic is known to upregulate CYP17 and CYP19 gene expression in the human adenocarcinoma cell line (H295R) and also increase the estradiol hormone levels and aromatase enzyme activity in male fish.[144-145] Sulfamethoxazole was also found in the Surface water and WWTW influent7 in KwaZulu-Natal[140], WWTW influent and WWTW effluent in Gauteng[146] and the WWTW influent and sewage treatment works effluent in the Western Cape.[147]

Vancomycin is seen in only one sample, sample A3 at a concentration of 8ug/L, which is the highest concentration of any pharmaceutical screened for across all samples. This is significant because sample A3 is also the only sample with no clear appearance but rather a green appearance with algae. In a study done by Cheng et al.[148] that even at trace concentrations of (0.01–2 mg L^{-1}) antibiotics may enhance the growth of microalgae, which may be the reason for the algae seen in the sample taken from

house A3. Vancomycin is also prone to antimicrobial resistance [149]and is shown to cause a reduction in spermatozoa integrity, hormonal levels and sperm morphology that contribute to male infertility.[150]

Antibiotics such as the ones detected, Ciprofloxacin, Sulfamethoxazole and Vancomycin are biologically active and can cause non-target toxicity to aquatic organisms. They are increasing concern due to their continuous exposure threatening human health through diet and environmental ecosystems. Even at low concentrations they can produce antibiotic resistant bacteria, which has been detected in sludge, ultimately used as a fertilizer on agricultural fields.[151] Antibiotic resistant bacteria is generated resistance which occurs through mutations in their genes or by acquisition of foreign DNA coding through horizontal gene transfer, and the bacteria can survive, and even grow, in the presence of antibiotic drugs, leading to a condition under which the drugs become noneffective on the patient, which aggravates with time and, at the end, may lead to death Moreover, it has been reported that microplastics can increase the accumulation of these analytes in fish and algae.[152] However it would be advised to perform an Environmental and Human Health risk assessment to understand and evaluate the risk of these antibiotics in the waters of the Melusi community.

All of the above-mentioned possible contaminants have an impact on the homeostatic systems of the body, especially the immune system.[153] Homeostatic control is affected by the fact that most EDCs tend to bind to steroid hormone receptors including the estrogen receptor (ER), progesterone receptor (PR) and androgen receptor (AR). As EDCs disrupt the actions of endogenous hormones, they may induce abnormal reproduction, stimulation of cancer growth, dysfunction of neuronal and immune system.[154] In general, sex hormones such as testosterone help stimulate the immune system. [154]This logically implies that immune system is sensitive to EDCs in a manner similar to that of endogenous hormones. However, synthetic non-steroidal compounds such as DES are potent suppressors of thymus-dependent cellular immune responses *via* gene expression alterations in animal.[154] Susceptibility of the immune system to toxic chemicals is increased during the perinatal period as shown by *in vivo* studies of various compounds such as dioxin.[154]

Given the wide range of emerging contaminants present in the JoJo tanks and the household water, the presence of Nonoxinol 9, Valdetamide, Simvastatin, Leptacline and Enprazepine in the dam water, is of concern as the presence of a DNA virus was also present. Human adenovirus was the only virus to present in the viral screening and is ubiquitous in the environment and humans are the only reservoir for them.[155] The virus is excreted in large numbers in human faeces and although adenoviruses have been reported to infect a variety of animals, they are more reported in humans to be highly specific to them.[156] The viruses persist wherever the environment has been polluted by human faeces or sewage[157-159]

Therefore, in natural aquatic environments, the incidence of human adenovirus is probably attributable to contamination with untreated or inefficiently treated sewage.[159] Various variants of adenovirus have been identified, and over 50 serotypes are known throughout the world.[160-161] The burden of disease is caused by adenoviruses manifests as pneumonia, bronchiolitis, otitis media, conjunctivitis, and tonsillitis. The public health implications of these viruses depend upon the physiological status of the wastewater microbial community. The presence of Adenovirus signifies the imminent danger posed to public health by the discharge of poorly treated effluent into the environment because the adenoviral species have been implicated in clinical illnesses. The complex mixture of emerging contaminants and adenovirus in the water from the dam is concerning and an effect-based monitoring approach should be explored to assess the extent of the endocrine disrupting activity the water poses. These are in line with SDG 3 and 6 about health and wellbeing and access to clean and safe drinking water.

CHAPTER 6: CONCLUSION

The exposure to EDCs in our environment through; water, air, soil, food, personal care products and medical devices, are unavoidable. Due to the ubiquity of EDCs in the environment and endocrine disruptive activity, the potential impact of EDCs on public health is a great reason for concern.

There is limited information available on estrogenic activity and pharmaceutical and viral content in drinking and dam water in South Africa. This study has shown the presence of herbicides, pharmaceuticals, and adenovirus in various water sources in the Melusi Community.

The objectives of this study were to:

1. **Determine the estrogenic and anti-estrogenic activity (T47D-Kblassay), and androgenic and anti-androgenic activity (MDA-kb assay) in drinking water and wastewater samples using a battery of *in vitro* bioassays.**

   Findings: Androgenic or anti-androgenic activity together with no anti-estrogenic activity was observed. However estrogenic activity was seen in the tested samples ranging from <loq to 0.216 ng/L. Additionally, estrogenic activity was higher in the samples taken from the homes compared to samples taken from the JOJO tanks. This can be attributed to the manner in which the water is collected and stored in the homes. Understanding the water collection and storage practices would aid in interventions to improve potable water. An interesting observation was that JOJO A (which is frequently re-filled) had the lowest estrogenic activity compared to JOJO C (which was left empty for 4 months) which had the highest estrogenic activity. This high EEq difference observed between the samples from these two tanks requires further investigation. In addition, cleaning containers and removing them from UV exposure may also impact estrogenic activity and require investigation. House C3 has the lowest estrogenic activity seen across all houses and the water storage containers are washed regularly and left in the shade. Investigating community water collection and storage practices may aid in improving access to safe and clean water in the community. Exposure to a large

range of environmental contaminants may n also impact estrogenic activity as seen in the estrogenic activity seen in the sample from the dam and warrants further investigation.

2. **Determine the occurrence of selected pharmaceuticals in water samples using ultra-high-performance liquid chromatography (UPLC)**

   Findings: Quantitative screening showed that three different antibiotics could be detected, Ciprofloxacin, Sulfamethoxazole and Vancomycin in six out of the 15 samples. The qualitative screening showed that the most predominant possible compound across all samples was Atrazine and its metabolite atrazine-desisopropyl. The community tap, near a nursery, is interesting to note as it contains herbicides and requires further investigation.

3. **Determine the occurrence of viral contaminants in drinking water and wastewater samples using real-time reverse transcription-polymerase chain reaction (RT-PCR).**

   Findings: None of the 2 samples taken from the showed to contain any Norovirus GI or GII, however Adenovirus was detected in varying concentrations in both samples and further investigation is needed.

It is evident that water is a potential source of human exposure to EDCs and pharmaceuticals and viruses, for the drinking water was found to be oestrogenic and can contain pharmaceuticals and adenovirus. Also, poverty-stricken rural communities such as Melusi will be at higher risk since they lack proper water services in the area. The findings suggest that community knowledge, attitudes and practices to water pollution and water storage practices would be essential. This information, coupled with the findings from this study, would enable community information sessions and workshops to be held to improve the safe handling of waste, and practices that improve the safe storage of water.

CHAPTER 7: RECOMMENDATIONS

Low levels of estrogenic activity were frequently detected in most samples a monitoring strategy is therefore recommended for the municipalities filling the JOJO tanks. Continued monitoring and constant refilling of JOJO tanks is vital to reduce any potential estrogenic activity of the water in the JOJO tanks (for example the high EEq concentration seen at JOJO C after being left empty) to identify the source and take remedial action as soon as possible. Also, more focus on EDC in water quality guidelines will contribute to better monitoring of water quality regarding EDCs.

This study used a grab sample approach, and a follow up study should be conducted to monitor the changes in concentrations of the emerging contaminants over time with an increased sample size. Effect-based monitoring of water sources would need to be explored to fully elucidate the effects of the complex mixtures seen in the water sources in Melusi.

Based on the estrogenic activity and presence of a wide range of emerging contaminants in the water, future studies should explore a health risk assessment, to understand the risks exposure to these aquatic pollutants may have on the health of the Melusi community.

More awareness can be created of the harm that endocrine disruptors and pharmaceuticals can cause. This research project was presented at a United Nations Children's Fund One Health for Change (UNICEF-OHC) Symposium and more people need to be made aware of the impact that these contaminants have on human health.

The community must be more empowered to better equip them to the harm that these contaminants can cause. This research will contribute to a Community Resilience Planning Guide and the Community Resilience Development Framework that will be developed specifically for the South African context and will address the community's short term and long-term needs. Further research needs to develop strategies to improve community health and well-being, especially through an integrative approach.

This study shows that water is a potential source of human exposure to EDCs and pharmaceuticals, for the drinking water was found to be oestrogenic and contain pharmaceuticals and viruses. Also, poverty-stricken rural communities such as Melusi will be at higher risk since they lack proper water services in the area. Together with other sources of exposure, the potential for health risks needs further investigation. With regards to sustainability going forward our country aims to move toward establishing sustainable cities (SDG 11), access to water and sanitation (SDG 6), and an environment free from chemicals and other pollutants (SDG 3), this study contributed to attaining health and well-being for all.

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APPENDIX

Ethics Approval Letter

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 18 March 2022 and Expires 18 March 2027.
- IORG #: IORG0001762 OMB No. 0990-0278 Approved for use through August 31, 2023.

Faculty of Health Sciences **Research Ethics Committee**

15 June 2022

Approval Certificate
Annual Renewal

Dear Miss HJ Swanepoel,

Ethics Reference No.: 151/2021 – Line 1
Title: Endocrine disruptive activity and occurrence of pharmaceuticals and viral content in selected water sources in Melusi, Pretoria

The **Annual Renewal** as supported by documents received between 2022-05-18 and 2022-06-15 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2022-06-15 as resolved by its quorate meeting.

Please note the following about your ethics approval:

- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2023-06-15.
- Please remember to use your protocol number (151/2021) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
Deputy Chairperson *of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health)

Tshwane Approval Letter

CITY OF TSHWANE
IGNITING EXCELLENCE

City Strategy and Organisational Performance

Room D2EO01 | 2nd Floor, Block D | Tshwane House | 320 Madiba Street | Pretoria | 0002
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My Ref:	**Research Permission Letter\UP**	Tel:	(012) 358 4559
Contact Person:	**Pearl Maponya**	Email:	PearMap3@tshwane.gov.za
Section/Unit:	**Knowledge Management**	Date:	28 April 2021

The Principal Investigator
School of Health Systems and Public Health
University of Pretoria
Private Bag x20
Hatfield, Pretoria
0028

Dear Dr Patrick,

RE: ARCHITECTURE AND PUBLIC HEALTH NEXUS: AN INTERSECTORAL APPROACH TO HEALTH AND WELL-BEING

Permission is hereby granted Dr Sean Mark Patrick, the principal investigator at the University of Pretoria (UP) Architecture; Public Health and Chemical Engineering Departments, to conduct research in the City of Tshwane Metropolitan Municipality.

It is noted that the study aims to investigate the interaction between living spaces, environmental pollution, and diseases in communities to developing a strategy to improve health and well-being. The City of Tshwane further notes that all ethical aspects of the research will be covered within the provisions of the UP Research Ethics Policy. You will be required to sign a confidentiality agreement form with the City of Tshwane prior to conducting research.

Relevant information required for the purpose of the research project will be made available as per applicable laws and regulations. The City of Tshwane is not liable to cover the costs of the research. Upon completion of the research study, it would be appreciated that the findings in the form of a report and or presentation be shared with the City of Tshwane.

Yours faithfully,

Pearl Maponya (Ms.)
DIRECTOR: KNOWLEDGE MANAGEMENT

Biostatistics Letter

Date: 26/04/2021

LETTER OF CLEARANCE FROM THE BIOSTATISTICIAN

This letter is to confirm that the student, with the Name(s)

HJ SWANEPOEL (student number 17091315)

Studying at the University of PRETORIA discussed the Project with the title:

"Endocrine disruptive activity and occurrence of pharmaceuticals and viral content in selected water sources in Melusi, Pretoria"

with me.
I hereby confirm that I am aware of the project and also undertake to advise on the Statistical analysis of the data generated from the project. The analytical tool that will be used will be:

T47-KBluc-Assay
The E2 standard curve will be fitted (sigmoidal function, variable slope) using Graphpad Prism (version 4), which calculates the minimum, maximum, slope, EC50 value and 95% confidence limits. The EEq values of extracts with greater than a two-fold induction above the vehicle control will be interpolated from the estradiol standard curve and corrected with the appropriate dilution factor for each sample.

MDA-kb assay
Each well will receive 25; reaction buffer (25 mM glycylglycine, 15 mM MgCl2, 5 mM ATP, 0.1 mg/m; BSA, pH 7.8), followed by 25mM; 1 mM D-luciferin 5s later. The RLU will be converted to a fold induction above the vehicle control value. The curves for DHT and selected EC samples will be fitted (sigmoidal function with variable slope) using Graphpad Prism (version 4), then calculates the minimum, maximum, slope, EC50 value and 95% confidence limits.

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April 26, 2021