DETERMINING THE EXTENT OF DE FACTO WATER RE-USE IN SOUTH AFRICA: THE CASE OF WASTEWATER TREATMENT PLANTS IN GAUTENG, KWAZULU-NATAL AND WESTERN CAPE PROVINCES

by

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I declare that the above dissertation is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

SIGNATURE

26 January 2022

DATE

DEDICATION

I would like to dedicate this dissertation to the following:

1. To Jesus Christ, my greatest encourager, the one who gave me the grace and the courage to start and complete my research.

2. My Parents Dumisani Campbell Swana and Gloria Ntombizandile Swana for their support and love. I am grateful to my father for his sincere wishes and prayers for me to prosper and for being a loving and supportive husband to our mother.

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ABSTRACT

The water quality of South African rivers is greatly impacted by insufficiently treated wastewater effluents (de facto reuse). Although de facto reuse serves as an alternative water supply it poses potential threats to human health and the environment. In this study therefore, the contribution of de facto reuse was determined for 6 wastewater treatment plants (WWTPs). Two methods were used to determine de facto reuse, *viz.* wastewater tracers (caffeine (CAF), lamivudine (LAM), and sulfamethoxazole (SULF)) and a geographic information system (GIS) based method. The wastewater tracers were selected based on their abundant use in food and medicine. Initially, the wastewater tracers were identified using ultra-high pressure liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) by their fragmentation patterns. After identification, the method was optimized and validated and then used to quantify de facto reuse. Subsequently, the wastewater traces were used to validate the GIS model results. The GIS model was developed using stream flow data and wastewater treatment locations to do spatial analysis for the WWTPs and the rivers they discharge to. Consequently, mass balance calculations were conducted based on the volumetric flow of the WWTPs and the stream flows thereby determining de facto re-use. In addition, the operation and maintenance (O&M) costs were predicted for the three Kwa-Zulu Natal WWTPs based on population equivalent (PE).

The target analytes were successfully identified by their fragmentation patterns. The obtained fragments corresponded with the fragments recorded in the United States Environmental Protection Agency's (EPA's) Estimation Program Interface. According to the optimization results, methanol (MeOH) is the most suitable solvent because it yielded higher signal-to-noise ratios for the analytes compared to acetonitrile (ACN) resulting in better sensitivity of the method. Solid phase extraction (SPE) efficiency results for CAF showed high recovery % in HLB cartridges compared to C-18 cartridges (103.75 and 56.98% respectively). In contrary, LAM had high recovery % in C-18 cartridges compared to HLB cartridges (100.71 and 32.91% respectively). In addition, low recoveries were obtained for SULF in both cartridges (31.74 and 20.05% respectively). Method validation results showed that the method was linear because the correlation coefficients (R^2) of the

calibration curves for all the analytes ranged from 0.9921-0.9984. Further, the results for matrix effect revealed that the sample matrix suppressed the ions of the target analytes because the matrix effect percentages were less than 100%. The method was also sensitive because of low limits of detection (LODs) (0.34, 0.06, and 0.04 µg/L) and limits of quantification (LOQs) (1.03, 0.17, and 0.14 µg/L) were obtained for CAF, LAM, and SULF, respectively. The results for repeatability and reproducibility demonstrated that the method is precise because the %RSD of the peak areas were < 4% and < 11% respectively. Additionally, the results proved that the method is precise because the mean recovery percentages were between 99.3% and 101.4%. In addition, the method was robust because the %RSDs of injection volumes and mobile phase flow rates were less than 7%. Method application results demonstrated that the concentrations of the target analytes were higher in winter (11.8-912.1 µg/L) compared to spring (0.5-10.6 µg/L).

The results for de facto reuse quantification proved that LAM is a more suitable tracer for quantifying de facto reuse than CAF and SULF because it yielded more reliable results. This is because LAM has a lower rate of degradation compared to CAF and SULF. De facto reuse trends were determined for WWTP1, WWTP2, WWTP3, WWTP4, and WWTP6 using a GIS model over a period of 10 years. The data was selected from 2009 to 2018 based on availability of monthly stream flow data. Out of all the WWTPs, WWTP1 had the highest percentages for de facto reuse (62.75-107.94%) throughout the 10 years due to its large design capacity (4.63 m^3/s). Consequently, the GIS-model and tracer method results were compared, and the results obtained using both methods followed a similar pattern (4.04-85.49 and 16.55-77.32 respectively). In contrary, the results obtained for WWTP3 (using the tracer method) were very high because of seasonal streamflow variations. A case study was conducted for the Jukskei river (one of the rivers mostly impacted by de facto reuse) and the assessment results demonstrated that the high levels of de facto reuse are a result of the large population served by WWTP1. Also, O&M costs were predicted for WWTP3, WWTP4 and WWTP5 and the results revealed that the O&M costs are influenced by the economies of scale (R 171.34, 5.53 and 1.69 respectively).

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LIST OF ABBREVIATIONS

ACN	Acetonitrile
ARVs	Anti-Retroviral Drugs
BGD	Billion Gallons per Day
CAF	Caffeine
CECs	Contaminants of Emerging Concern
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DPR	Direct Potable Reuse
DWA	Department of Water Affairs
DWS	Department of Water And Sanitation
DWTPs	Drinking Water Treatment Plants
ε°	Elution Strength
EC	Electrical Conductivity
EDCs	Endocrine Disrupting Chemicals
EPA	Environmental Protection Agency
ESI+	Positive Electron Spray Ionization Mode
EPI	Estimation Program Interface
FA	Formic Acid
GC	Gas Chromatography
GDP	Gross Domestic Product
GIS	Geographic Information System
HLB	Hydrophilic-Lipophilic-Balance
HPLC	High Performance Liquid Chromatography
IBU	Ibuprofen
IEC	Extracted Iron Chromatograph
IPR	Indirect Potable Reuse
LAM	Lamivudine
LC	Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry

LC-MS/MS	Liquid Chromatography with Tandem Mass Spectrometry
LHWP	Lesotho Highlands Water Project
LLE	Liquid Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MeOH	Methanol
MR	Mixing Ratio
MS/MS	Tandem Mass Spectrometry
m/z	Mass-to-Charge Ratio
N	Nitrogen
NAE	National Academy of Engineering
NAP	Naproxen
NHD	National Hydrography Data
NRC	National Research Council
NTU	Nephelometric Turbidity Units
O&M	Operation and Maintenance
P	Phosphorus
PE	Population Equivalent
POPs	Persistent Organic Pollutants
PPCPs	Pharmaceuticals and Personal Care Products
QA	Quality Assurance
Q-tof-MS	Quadrupole Time-of-Flight Mass Spectrometry
$\mathbf{R^2}$	Correlation Coefficient
RSD	Relative Standard Deviation
RWH	Rainwater Harvesting
SA	South Africa
SANS	South African National Standards
SAWA	South African Water Act
SD	Standard Deviation
S/N	Signal-to-Noise Ratio
SPE	Solid Phase Extraction

SS	Suspended Solids
SULF	Sulfamethoxazole
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
TSS	Total Suspended Solids
UHPLC	Ultra-High Pressure Liquid Chromatography
USGS	United States Geological Survey
WHO	World Health Organization
WRC	Water Research Commission
WRTT	Water Resilience Task Team
WWTP	Wastewater Treatment Plants

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Wastewater reuse is a necessary practice because it addresses the imbalance between water supply and economic demands of potable water. The reuse of wastewater for potable use is conducted in three ways, namely the direct potable reuse, indirect potable reuse and de facto reuse (Warsinger *et al.,* 2018). Direct and indirect potable reuse include using advanced treatment methods to treat wastewater to potable standards. On the other hand, de facto reuse is the unplanned discharge of insufficiently treated wastewater effluent into rivers used for potable water supply (Rice, 2014). De facto reuse occurs mainly because the conventional wastewater treatment processes were not designed to remove the new and emerging pollutants also known as contaminants of emerging concern (CECs). These CECs comprise persistent organic pollutants (POPs) and pharmaceuticals and personal care products (PPCPs) (Archer *et al*., 2017a). The presence of these CECs in surface water threatens human health and the environment. PPCPs can disrupt the endocrine functions, reduce the ability to resist bacteria and accelerate the growth of cancer in human beings (Raghav *et al.*, 2013). Further, it is assumed that the presence of POPs in the Hartbeespoort dam (Gauteng province, South Africa) is the cause of testicular abnormalities in male fishes (Wagenaar *et al.*, 2012). Therefore, more research should focus on finding economic ways to treat wastewater to acceptable standards, as this will result in the protection of human health and the environment.

1.2 PROBLEM STATEMENT

Although de facto reuse is an old practice that is common in many countries, only a few countries have quantified de facto reuse. Hence, one of the top ten research needs of the National Academy of Engineering (NAE) for human health, social and environmental studies is quantification of de facto reuse (Wang *et al.,* 2017). So far, the commonly used methods for de facto reuse quantification are wastewater tracers and a geographic information system (GIS) based method. The GIS based method is a cost effective and time saving tool that requires data collection and integration data in GIS software to acquire the intended output. The GIS method has only been used for quantifying de facto reuse in only two countries namely the United States and in China *(*Rice, 2014; Wang *et al.*, 2017). In China the predictive capabilities of the GIS model were limited because of limited data compared to the United States which has large data sets. Also, there a few countries that quantified de facto reuse using wastewater tracers such as Israel and the United States (Gasser *et al*., 2010; Rice, 2014). In addition, the method for wastewater tracers, is a costly and time consuming because it requires sampling, sample preparation (solid phase extraction (SPE)) and quantification in a Liquid Chromatography (LC). Therefore, wastewater tracers can only be used to quantify de facto reuse in a limited number of raw water sources.

1.3 JUSTIFICATION

The GIS-based model has the advantage of detecting and quantifying pollutants in surface and groundwater. Also, it can map polluted waterways (Johnson, 2016). A GIS does not require manual sample collection, therefore, it is time saving and cost effective (Martin *et al*., 2005). GIS software can model spatial data taking into account environmental changes and anthropogenic activities. Therefore, it can be used to investigate the sources of pollution. It is a good tool to use in case studies because it can map changes such as population growth, urbanisation, developments in communities etc. Besides, quantifying treated wastewater in raw water sources is useful because it can be used to predict concentrations of CECs in numerous raw water sources. In addition, when a GIS-based model is used to quantify de facto reuse, it is also important to validate its information with field studies (wastewater tracers). Some of the qualities for a good wastewater tracer is that the tracer must originate from households, the tracer must have low degradation and its concentration must be high in surface water (Gasser *et al*., 2010). Caffeine (CAF), lamivudine (LAM) and sulfamethoxazole (SULF) are amongst the mostly detected wastewater tracers in raw water quality studies of South African rivers (Archer *et al*., 2017b; Madikizela *et al*., 2017). Also, these compounds have low degradation in surface water and they also originate from households (Hillebrand *et al*., 2011; **US EPA, 2020)**. Therefore, these wastewater tracers can be used to validate the GIS-based model. De facto reuse quantification signifies the quality of water in raw water sources. Knowledge of the extent of de facto reuse is necessary to inform water treatment practitioners about the need to develop methods and water treatment processes that target the reduction of such CECs. To the best of our knowledge, no study has been conducted in South Africa that simultaneously quantified the extent of de facto reuse countrywide as well as drawn a map of where such indirect reuse is predominant.

1.4 AIM AND OBJECTIVES OF THE STUDY

This study, thus, aimed to quantify de facto reuse in selected cities of South Africa where there is a high rate of wastewater reuse. To achieve this aim, the following objectives were followed:

- To optimize and validate a liquid chromatography-mass spectrometry (LC-MS) method for 3 selected wastewater tracers.
- To assess the impact of seasonal variations on the concentrations of the wastewater tracers in raw water sources.
- To identify which of the 3 wastewater tracers is most suitable to predict the amount of treated wastewater in surface water.
- To develop a GIS model that will estimate the amount of wastewater effluent present in raw water sources and validating the model with a wastewater tracer.
- To evaluate spatial and temporal factors (seasonal variations) impacting on the extent of de facto reuse in the selected cities.
- To identify cities impacted by de facto reuse and perform a case study of priority de facto reuse water treatment plants and predict the operations and maintenance (O&M) costs.

1.5 DISSERTATION OUTLINE

Figure 1.1 provides a summary of the layout of this dissertation.

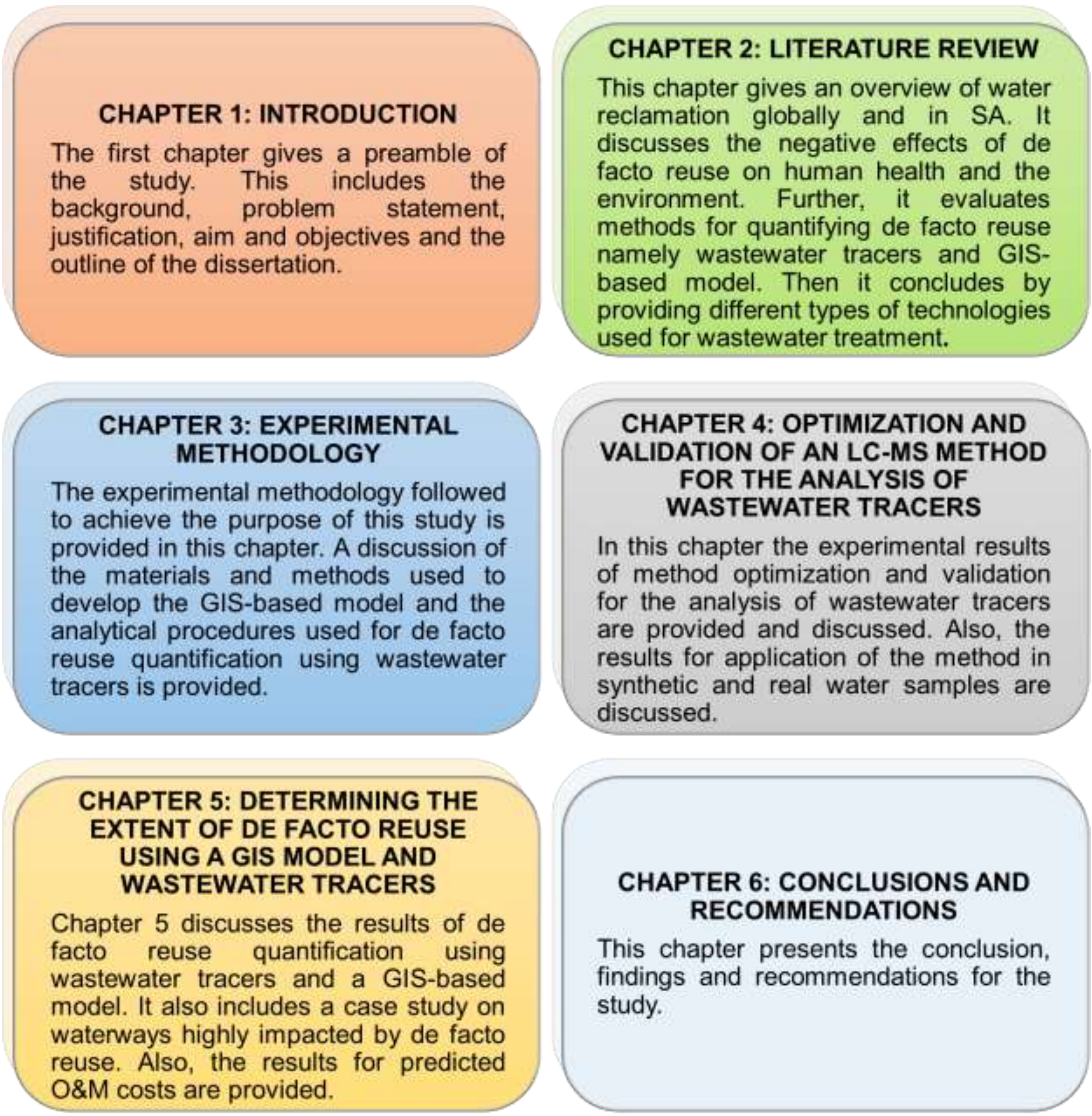

Figure 1.1 Dissertation outline

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CHAPTER 2
LITERATURE REVIEW

2.1 INTRODUCTION

Water is among the most essential resources for the survival of both humans and other living organisms (Rice, 2014; UNEP, 2009). Yet, due to the increased occurrence of droughts, worldwide population expansion, rapid industrialization and concurrent urban growth, and the agriculture sector's ever-increasing water demands, fresh drinking water has become a restricted resource (Lautze *et al*., 2014). This issue is exacerbated by deteriorating water quality caused by contamination from industrial wastewater discharges. In reality, water shortage caused by scarcity of water resources and deterioration of water quality is recognized as one of the most serious challenge affecting arid and semi-arid countries (Adewumi et al., 2010; Chaudhry et al., 2017; Roccaro and Verlicchi, 2018a).

2.1.1 Key factors contributing to water scarcity in South Africa.

South Africa is a semi-arid nation characterized by high variability in rainfall and high evaporation rates (DWA, 2013a). Drought, which is a recurring feature of South African weather, is another factor leading to water shortage (Rouault and Richard, 2003). From 2015 to 2017 the City of Cape Town was in the grip of a severe drought and water levels in the six main dams fell from 100% to 38% (Ziervogel, 2019). The Cape Town drought had serious implications; the City's water shortages reduced hotel occupancy by 10% in 2017, and the city’s economy, which is heavily reliant on tourism, suffered greatly. The drought also created a credit risk to Cape Town's debt rating, which was at the time at the lowest level of investment grade (i.e., Baa3). Aside from the national Gross Domestic Product (GDP), much-needed investment was impeded as rating agencies feared future downgrades. Additionally, the tourist and agricultural industries, which are the two largest water users in the entire Western Cape Region (i.e., the province where the City of Cape Town is situated), were the most affected by the drought. Other provinces, including

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Gauteng, the country's economic powerhouse, were also plagued by severe water restrictions, further harming the country's economy. On the bright side, the City of Cape Town was highly effective in reducing water use, and anecdotal facts show that the city is now considered as a global leader in drought management and methods for adapting to climate change.

Among these adaption steps was the formation of the Water Resilience Task Team (WRTT) in May 2017, directed by the Chief Resilience Officer and housed by the Mayor's Directorate (Ziervogel, 2019). The WRTT began by imagining three different scenarios. New normal (February–September 2017), demand control and zero (October–February 2018), and drought recovery (March 2018). The task team established a goal of obtaining 500 megaliters (ML) of non-surface water and lowering daily water use to 500 ML. While use did not fall overnight, at the peak of the zero-campaign day in early 2018, normal use was little under 500 ML. With respect to the rise, the plan was to speed up the provision of an additional 500 ML per day, beginning with the immediate first tranche, which involved the purchase of 100ML per day from the temporary desalination of nine small, containerized plants. The WRTT proposed a variety of strategies for complementing surface water and other water boost initiatives to escape the water crisis.

On the 16th of July 2017, the first desalination plant was introduced, and three temporary desalination plants were acquired in 2017 (16 ML per day overall) (Joubert and Ziervogel, 2019). During the emergency period, groundwater extraction was explored for the Atlantis, Cape Flats and Table Mountain Group aquifers, with 100 ML per day expected from these channels. The remediation of the Atlantis drainage scheme added 14 ML per day in January 2018 and 20 ML per day in January 2019. In 2018, 159 boreholes were bored into the Cape Flats aquifer producing 41 ML per day. Moreover, 60 ML per day was intended for direct drinking water re-use from six small re-use facilities. At the end of the day, a temporary re-use plant of 10 ML per day was contracted for a term of two years at the Zandvliet wastewater treatment plant. During the drought, flow restrictors were increased to target households consuming significant volumes of water. Treated effluent re-use systems were increased to maximize the volume of drinking water that may be discarded. Various outreach initiatives in both traditional and social media were launched to help people minimize their consumption, such as fixing household leaks, using 100 liters, then using 87 liters (September 2017), and 50 liters (early 2018), and sending messages using various kinds of media.

2.1.2 Water quality deterioration

The pollution of waterways by improperly treated effluents from WWTPs also adds to the decline of SA's quality of water (Edokpayi *et al*., 2017). Numerous investigations undertaken in several South African regions where CECs were identified in raw water sources confirm to this reality (Archer *et al*., 2017b; Madikizela *et al*., 2017; Matongo *et al*., 2015a; Momba *et al*., 2006; Skosana, 2015; Voulvoulis, 2018). The fundamental issue is that current treatment technologies were not intended to eliminate CECs, which primarily originate in residences. Endocrine disrupting chemicals (EDCs), PPCPs, nanomaterials, pathogens, and POPs are among the CECs (Bolong *et al.*, 2009; Murl, 2016; OW/ORD Emerging Contaminants Workgroup, 2008). These CECs have the potential to affect the endocrine system, diminish the ability to resist bacteria, and promote cancer progression in humans (Raghav *et al*., 2013). Furthermore, they hinder the growth of marine species, leading to death of aquatic organisms.

The concern is that most of South Africa's potable water is derived from contaminated rivers and streams, and this negatively affects human health and aquatic life (DWA, 2013b; Elliott *et al*., 2017; Momba *et al*., 2006; Pennington *et al*., 2017; Vidal-Dorsch *et al*., 2012). It is noteworthy, however, that various treatment methods have been adapted and improved specifically for the goal of eliminating contaminants such as particles, pathogens, natural organic matter, salts, and CECs from wastewater (Roccaro and Verlicchi, 2018; Warsinger et al., 2018). Adsorption, ozonation, activated carbon, and membrane technology are among the technologies used (Seow *et al*., 2016; Warsinger *et al*., 2018). Although these methods have been effective in improving the quality of water, they still have drawbacks such as a lack of appropriate adsorbents with high adsorption capacities and a shortage of commercial size columns (Sadegh and Ali, 2018).

Furthermore, ozonation may produce byproducts that cause cancer such as brominated byproducts and aldehydes (Rodriguez *et al*., 2009). The downside of activated carbon is that it does not efficiently remove substances that are not attached to carbon, such as nitrates, salt, and heavy metals, while membranes have the issue of fouling. Since some of the targeted chemical and biological pollutants are not entirely eliminated by some of these improved water treatment systems, ongoing study into the development of technologies that are more efficient is necessary (National Research Council, 2012a).

2.1.3 A case for wastewater reuse in South Africa

The key contributors to water shortages in South Africa, combined with competing water demands from the agricultural and industrial sectors (the largest consumers of water in South Africa), and limitations related to conventional wastewater treatment processes, have resulted in a significant number of South Africa communities lacking a sufficient supply of safe drinking water (Adewumi et al., 2010; DWA, 2012). As a result, wastewater reuse has been implemented as a technique in South Africa to overcome an imbalance between water availability and social and economic needs for safe drinking water (Okun, 2002). The plan implemented in South Africa is consistent with the policies of numerous other nations, which are aimed at wastewater reuse for agricultural and drinkable use (direct and indirect potable use), water conservation, and compensating for water shortages (Okun, 2002; Roccaro and Verlicchi, 2018).

2.1.4 De facto reuse and mapping

De facto reuse refers to the process of releasing inadequately treated wastewater effluent into waterways utilized for drinking water (Wiener *et al*., 2016). De facto reuse is popular in many European nations as well as other nations such as the United States and China (Rice, 2014; Wang *et al*., 2017). De facto reuse is commonly used to compensate for water limitations caused by climate change-induced raw water shortages (Wiener *et al*., 2016). In South Africa, de facto reuse was used to alleviate water scarcity and address concerns related to the lack of storage capacity for treated wastewater effluent (Skosana, 2015).

The level of de facto reuse in South Africa is unknown. As a result, there is a requirement in South Africa for the measurement and mapping of water bodies contaminated by de facto reuse. The mapping can be accomplished using a GIS, which is a low-cost technology utilized in several forms of water resource research (Rice *et al*., 2016; Schmid and Bogner, 2018; Wang *et al*., 2017). In the United States, wastewater tracers have also been used to evaluate wastewater effect and as a method for validating GIS-based models established for analyzing de facto reuse (Rice *et al*., 2016). This study outlines the state of de facto reuse in South Africa, as well as its harmful effects on human health and the environment. Furthermore, this review contains historical data on water reuse as well as current treatment technologies for safe drinking water reuse in the nation. GIS models are also mentioned as ways for quantifying de facto usage. CAF, a wastewater tracer found in South African water systems, may also be utilized in conjunction with GIS-models to analyze surface water contamination resulting from wastewater effluents.

2.2 A GLOBAL PERSPECTIVE OF WASTEWATER REUSE

Wastewater reuse is prevalent worldwide, and it is primarily intended for saving water and provide a sustainable water supply. Jimenez and Asano (2008) performed a global study on wastewater reuse and estimated a global rate of reuse of 5.55 billion gallons per day (BGD) (**Figure 1**). The United States appears to have the greatest rate of water reuse in the world, accounting for 45 percent of total worldwide reuse (Jimenez and Asano, 2008). Although wastewater reused for non-potable purposes is popular in many nations, only a few countries undertake planned potable reuse. Another issue influencing the limited execution of planned potable reuse is public disapproval of wastewater reuse (Ghernaout, 2019; Hartley, 2006).

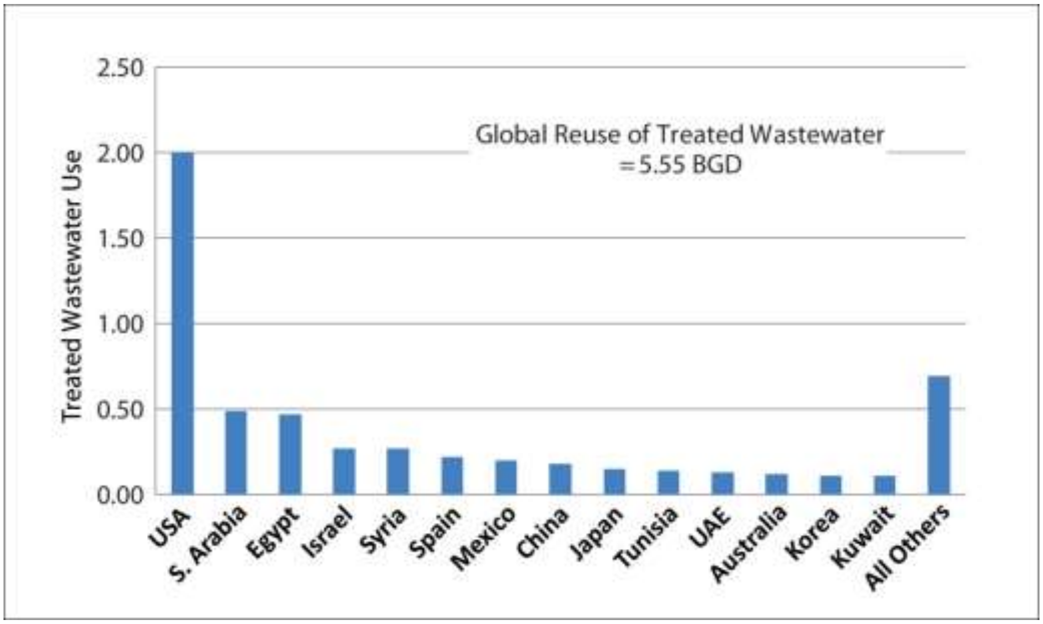

Figure 2.1: Global reuse estimate of treated wastewater (Jimenez and Asano, 2008)

Several studies on various water utilities throughout the globe have demonstrated that surface water is polluted by wastewater effluents. Numerous pollutants were found in research done in the Caribbean area (West Indies) utilizing a multi-residue solid phase extraction (SPE) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Edwards *et al*., 2017). The existence of artificial sweeteners, pharmaceuticals, steroid hormones, and pesticides was also discovered in the study by Edwards et al. (2017), with concentration levels ranging from 3.0 ng/L to 571 ng/L. In most situations, the existence of such contaminants in fresh water indicates that the water has been polluted by improperly treated wastewater effluents. Elliott et al. (2017) conducted similar research on 12 surface water supplies and sediments in the United States. While indole (0.0284 µg/L) and cholesterol (72.2 µg/L) were identified in the water sources, diphenhydramine (1.75 µg/L) and fluoranthene (20800 µg/L) were discovered in the sediments (Elliott *et al*., 2017). The majority of the contaminants investigated by Elliot *et al.* (2017) are caused by humans (e.g., indole is an organic compound found in feces). Similar investigations utilizing various approaches for the identification of contaminants in raw water sources have been performed in China (Wang *et al*., 2017), Malaysia (Al-Qaim et al., 2017), Israel

(Gasser *et al*., 2010), and Germany (Rossmann *et al*., 2014). In these investigations, wastewater tracers such CAF, antibiotics, chloride, and other CECs were used to examine surface water contamination caused by effluents from municipal wastewater treatment plants (WWTPs).

2.3 WATER SUSTAINABILITY OPTIONS IN SOUTH AFRICA

The department of water affairs (DWA) has proposed numerous possible strategies for increasing water supply in South Africa (DWA, 2011). Cloud seeding, rainwater harvesting (RWH), potable water reuse, and importing clean water from nearby countries such as Lesotho are among the strategies.

2.3.1 Cloud seedling

The problem of water resource depletion, both in quantity and quality, is significant; hence, there is an urgent need to investigate alternate methods for managing the water shortage. Cloud seedling is one method of increasing water supply. Cloud seedling is the process of causing rain to fall by distributing dry ice to the clouds. However, the type of clouds in some locations, such as the Western Cape Province, are not conducive to rainfall promotion.

2.3.2 Rainwater harvesting

Another sensible approach, which is a historical tradition in rural regions, is the collecting of rainwater through house roofs to tanks (RWH), where it is stored and utilized for domestic purposes. This technique has always been practical for rural regions, and it is especially useful during dry seasons when rivers have little or no water. RWH is one of the feasible and advantageous methods for ensuring a reliable water supply.

2.3.3 Potable reuse

Another approach for increasing water supply and providing sustainable water resources is potable reuse. Furthermore, numerous nations have successfully adopted direct and indirect potable reuse utilizing modern treatment technologies like reverse osmosis. In South Africa, a mine water reuse plant exists that purifies mine water to drinking water standards using modern treatment processes, and the effluent can filter through the soil to supplement an aquifer or be utilized for drinking (DWA, 2011). Another plant, located near Middleburg, similarly purifies mine water to drinking standards and is utilized for potable water or aquifer augmentation (DWA, 2011).

2.3.4 Importing of raw water

South Africa is aiming to accommodate its water demand in part by importing water from Lesotho, a nation rich in water resources that borders South Africa. Lesotho launched the Lesotho Highlands Water Project (LHWP) in 1986 with the goal of exporting water to South Africa *via* a network of constructed dams, lakes, and tunnels (DWA, 2013b). The tunnels transfer around 780 million m^3 of water from these manmade lakes to South African rivers that supply the Vaal Dam in the Gauteng Province.

2.4 RECLAMATION OF TREATED WASTEWATER IN SOUTH AFRICA

2.4.1 Introduction of water reclamation in South Africa

Reclaimed water is wastewater that has been treated and reused for various purposes such as potable reuse, cooling water for industrial processes, feed water for the reboiler, agricultural purposes, irrigation of golf courses, recharging of aquifers and toilet flushing for businesses (Kandiah *et al*., 2019; Warsinger *et al*., 2018). Reusing treated wastewater instead of using pristine water saves water and thus offers a solution to water challenges faced by arid and semi-arid countries such as South Africa (Andersson *et al.*, 2020). Several countries are already benefiting from the reuse of treated wastewater for purposes of augmenting surface and groundwater to increase water supply. Although, water reclamation is a potential solution for mitigating water shortages, it also increases financial, technical and institutional challenges and raises health and safety concerns (National Research Council, 2012a). In addition, very few countries reuse treated wastewater for potable use due to negative public perceptions about such a practice. The idea of converting toilet to tap water has still not found a great deal of acceptance amongst the general public (Ghernaout, 2019; Hartley, 2006; Rice *et al*., 2016).

Reusing treated wastewater is an old practice in most dry regions (Bischel *et al*., 2013). Furthermore, numerous nations, including Singapore, Israel, Namibia, the United States, Australia, and other European countries, have already begun to adopt the reuse of treated wastewater for a variety of purposes. Regardless of the fact that some drinking water reuse projects have failed owing to public resistance, the majority of non-potable reuse initiatives have been viable (Po *et al*., 2003). Similarly, while there are certain downsides to water reuse, they are considerably exceeded by benefits such as reduced water scarcity, less coastal pollution, surface water conservation, nutrient recovery, surface and ground water augmentation, increased sustainability, and sustainable water resources (Po *et al*., 2003).

Water reuse was implemented in South Africa in 1956, after the introduction of the South African Water Act (SAWA) in 1954, which essentially authorized wastewater treatment to acceptable levels and release to the original surface water (Morrison *et al*., 2001). CECs had not yet been observed or were found in insignificant amounts in wastewater effluent streams at the time of the Act's implementation. Rapid population expansion and urbanization were followed by an increase in the use of PPCPs and other chemicals throughout time, which eventually led to an increase in CEC concentrations in wastewater effluent. To demonstrate this point, substantial amounts of CECs were identified in the final effluent of 80 percent of WWTPs in the Eastern Cape Province of South Africa that were still employing conventional water treatment processes (Mema, 2010). It goes without saying that larger levels of CECs offered a higher risk of diseases caused by de facto reuse.

2.4.2 South African types of water reclamation

Treated wastewater is reused in three ways: planned direct potable reuse (DPR), planned indirect potable reuse (IPR), and unplanned indirect potable reuse (de facto reuse) (**Figure 2**) (Giwa *et al*., 2016; Warsinger *et al*., 2018). When wastewater is processed to potable water standards utilizing highly advanced treatment methods and then directly fed to the downstream water of a drinking water treatment plant (DWTP) for distribution, this is referred to as DPR. IPR is the process of introducing advanced pre-treated wastewater into a raw water source that is raw water fed to a DWTP. Unintentional indirect potable reuse (de facto reuse) is the discharge of inadequately treated effluent into freshwater resources (Chaudhry *et al*., 2017; National Research Council, 2012b). De facto reuse is described as the unintended reclamation of poorly treated wastewater from an upstream WWTP (Rice *et al*., 2016). De facto reuse has been conducted for over a century, and it is often implemented when there is a restricted water supply (due to climate change) to compensate for the water deficit (Rice, 2014). Unlike in many other nations where planned potable reuse is conducted through improved wastewater treatment prior to reuse, water reclamation in South Africa is primarily unplanned indirect potable reuse (de facto reuse).

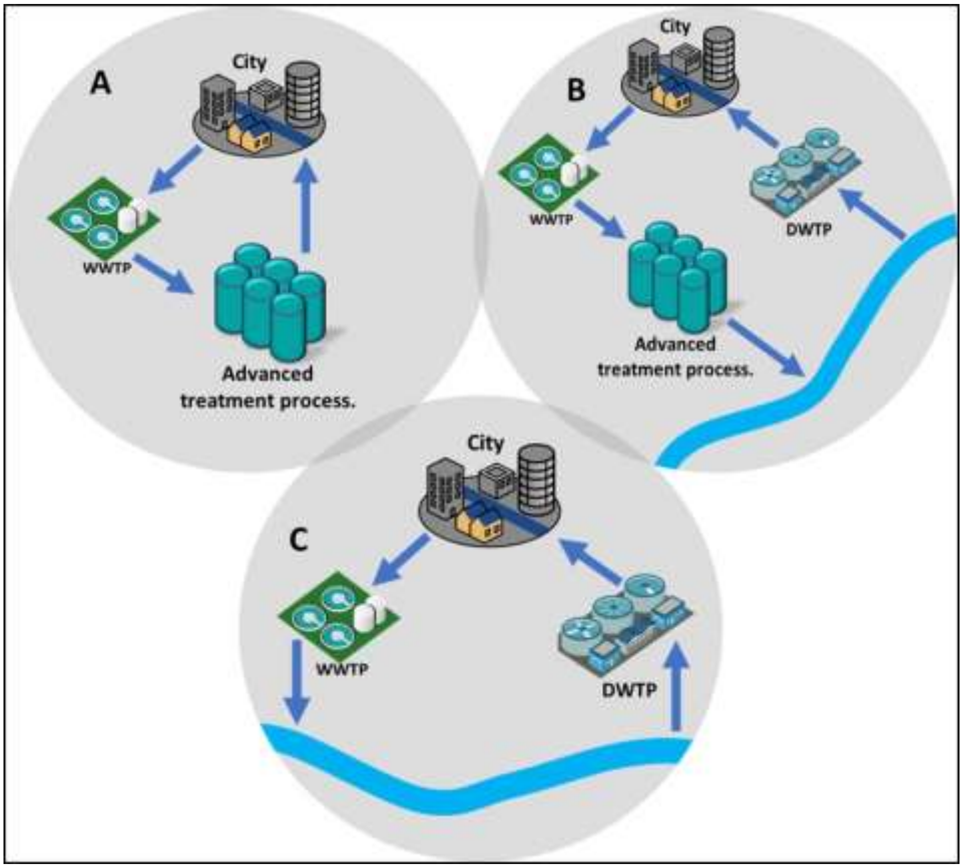

Figure 2.2: A schematic for wastewater reuse: A. DPR, B. IPR and C. de facto reuse.

2.5 DE FACTO REUSE IN SOUTH AFRICA

In South Africa, there is a scarcity of appropriate treatment technology capable of eliminating new and emerging contaminants from wastewater. Many South African raw water sources that provide raw water for ultimate potable use are severely contaminated with wastewater effluents because large volumes of wastewater are released to these water sources on a regular basis (Mema, 2010). Despite the fact that many rivers are heavily contaminated with raw sewage, South Africa relies on these toxic sources for raw water supplies. A recent review identified a number of new contaminants discovered in South African water bodies by other studies (Archer et al., 2017b). In several of the rivers where these contaminants were identified, DWTPs are located a few kilometers away from WWTPs that take raw water from the same river where the WWTPs release their effluents.

Multiple South African studies have discovered that the majority of South African rivers and dams are contaminated with various pollutants such as EDCs, PPCPs, and POPs due to wastewater effluent discharge on fresh water (Bolong *et al*., 2009; Murl, 2016; OW/ORD Emerging Contaminants Workgroup, 2008; Weber *et al*., 2006). Furthermore, several studies have indicated that the majority of the WWTPs in the Limpopo Province, one of South Africa's rural regions, do not adequately treat wastewater (Edokpayi, 2016). The low quality of wastewater discharge appears to be the result of inadequate wastewater treatment facilities, a scarcity of skills, poor planning, and fraud (Edokpayi *et al*., 2015). In another research done in South Africa's Gauteng Province, 55 CECs were found in WWTP influent, 41 in wastewater effluent, and 40 downstream and upstream of a river a few kilometers from a WWTP. Approximately 28 percent of the 55 CECs studied had a removal efficiency less than 50 percent, and 18 percent of the same CECs had a removal efficiency less than 25 percent (Archer et al., 2017a).

The Department of Water and Sanitation (DWS) initiated a program in 2008 to examine the performance of 831 South African WWTPs, and those that met the DWS's basic requirements were granted the Green Drop certification. This effort was launched to stimulate improvements in wastewater management quality, with the ultimate goal of safeguarding human health and the environment (Archer *et al*., 2017b). The WWTPs were assigned an ongoing risk rating based on an evaluation of their design capacity, operational flow in relation to design capacity, technical skill compliance, and final effluent quality in accordance with DWS criteria. Furthermore, the assessments were designed to provide annual evaluations of the plants' operating efficiency. In 2012, it was revealed that 323 of 831 WWTPs did not fulfill the DWS criteria, and 212 of the WWTPs were rated as high-risk. Furthermore, the design capacity of several of the WWTPs was undocumented, making assessing the effluent quality of those treatment facilities challenging (Archer *et al.*, 2017b). Although the 2013 evaluations demonstrated modest progress in risk rating compliance, 412 WWTPs were judged to be running at less than 50% efficiency (Archer *et al*., 2017b).

Paul *et al*. (2015) studied many rivers and dams in four South African provinces: Gauteng, Free State, Mpumalanga, and Kwa-Zulu Natal. The study aimed to quantify various anti-retroviral medicines (ARVs) in South African water bodies utilizing the SPE technique for pre-concentration and ultra-high pressure liquid chromatography (UHPLC) combined with mass spectrometry. ARV medicines are excreted and end up in wastewater because they are not entirely digested by the digestive system, making them tracers of the presence of wastewater in surface waterways. Furthermore, traditional wastewater treatment techniques do not entirely eliminate these chemicals, and they are released or discharged into surface water together with the wastewater effluent. As a result, when ARV medications are identified in surface water, they serve as indicators of wastewater discharge (de facto reuse). The average ARV medication concentrations measured in rivers and dams ranged from 26.5 to 430 ng/L (Wood *et al.*, 2015).

Non-steroid, anti-inflammatory, antibiotic, anti-retroviral, anti-epileptic, steroid hormone, and anti-malarial chemicals have also been discovered in South African surface water sources (Madikizela *et al*., 2017). These substances are both prescription and over-the-counter medications that end up in wastewater because they are not completely digested, and some of them are flushed unused. As a result, they end up in raw water sources *via* inadequately treated effluent discharges. Triclosan and ketoprofen were discovered in wastewater effluent and receiving surface water of the Mbokodweni river in 2014. (Madikizela *et al*., 2017). Whereas triclosan is an antibacterial compound present in toothpaste and liquid soap, ketoprofen is utilized as an analgesic in humans and animals. As result of its toxicity, the presence of triclosan in surface water is detrimental to aquatic creatures. Although triclosan is less hazardous to people, it leads to the synthesis of POPs, which can enter the food chain when wastewater is reused for agricultural reasons, causing harm to human health. Furthermore, when triclosan is measured at µg/L, it is known to be hazardous to fish, daphnia magna, and algae in the aquatic environment (Madikizela *et al*., 2014).

2.5.1 Rivers and dams in South Africa that have been reported to be impacted by de facto reuse.

In South Africa, many varieties of CECs have been discovered. **Table 1** shows the rivers and dams in South Africa that have been influenced by de facto reuse.

Table 2.1: Rivers and dams impacted by de facto reuse.

PROVINCE	RIVER	REFERENCE
Kwa-Zulu Natal	Mbokodweni River	Madikizela *et al.* (2017)
	Msunduzi River	Agunbiade & Moodley (2016)
	Inanda Dam	Wood *et al.* (2015)
	Umgeni River	Agunbiade & Moodley (2014)
	Mhlathuze River	Mema (2010)
Gauteng	Roodeplaat Dam	Wanda *et al.* (2017)
	Pienaars River	Wood *et al.* (2015)
	Vaal River	DWA (2011)
	Crocodile River	Wanda *et al.* (2017)
North West	Hartbeespoort Dam	
	Megalies River	
	Mkomazane River	Wanda *et al.* (2017)
Mpumalanga	Lipoponyane River	
	Renosterkop Dam	Wood *et al.* (2015)
	Kuils River	Swart & Pool (2007)
Western Cape	Eerste River	
	Orange River	Wood *et al.* (2015)
Free State	Gariep Dam	
	Vaal Dam	
	Kat River	Momba *et al.* (2006)
Eastern Cape	Tyume River	
	Tembisa Dam	
	Keiskamma River	Morrison *et al.* (2001)

2.6 IMPACT OF DE FACTO REUSE

Surface water contamination is mostly caused by wastewater effluent releases. To eradicate these developing contaminants from water, current approaches must be modified. The WWTPs are the primary source of CECs, and their presence in wastewater effluents poses a substantial hazard to human health and the environment (Edokpayi *et al*., 2017; Stackelberg *et al.*, 2004). Furthermore, the environmental effect of CECs is determined by their concentration in effluents as well as the amount and uniformity of wastewater effluent dumping into raw water sources (Akpor and Muchie, 2011). As a result, legal enforcement must be used to preserve the environment and human health, because many South African civilizations rely on these contaminated raw water sources for their water supply (DWA, 2012).

2.6.1 Environmental impact of de facto reuse

Specific variables in the aquatic environment, such as temperature and oxygen balance, must be met for aquatic animals to survive (Edokpayi *et al*., 2017). Any changes in survival circumstances can reduce aquatic animal production, growth, and life. Any wastewater effluent discharge has an effect on the oxygen requirement of surface water. When improperly treated wastewater is released into surface water, the dissolved oxygen (DO) of the surface water is reduced. This is because microorganisms that decompose organic compounds found in inadequately treated wastewater consume oxygen. The allowable DO limit in South Africa WWTPs is between 8 and 10 mg/L. However, when DO levels fall below 5 mg/L, they can have a negative influence on aquatic creatures. DO values with a mean range of 3.26 to 4.57 mg/L were recorded in a study done by Momba et al. (2006) on WWTP effluents of Buffalo City and Nkokonbe Municipalities (Eastern Cape Province of South Africa). An oxygen imbalance in surface water caused by improperly treated wastewater has a detrimental impact on aquatic species since oxygen is essential for maintaining aquatic life and low levels of DO limit productivity and development of aquatic organisms, ultimately leading to aquatic organism mortality (Edokpayi *et al*., 2017). Furthermore, numerous studies done in South Africa have found that DO levels in wastewater effluent are below permitted limits (Mema, 2010). This means that the aquatic life in South African water bodies is in jeopardy.

2.6.2 The effect of de facto reuse on human health

The reuse of wastewater effluent for diverse applications is accompanied by the danger of acquiring pathogens from surface water polluted by inadequately treated wastewater effluents. These dangers have both short-term (depending on human and environmental exposure) and long-term (depending on consistency in water reuse) consequences (Toze, 2006). Momba et al. (2006) discovered the presence of 21 bacterial species in water samples from raw wastewater, final effluent, and receiving surface water in the Eastern Cape towns of Buffalo City and Nkokonbe. Among the 21 bacterial species identified, 12 species, namely *Aeromonas hydrophilia, Enterobater cloacae, Escherichia coli, Klebsiella ornithinolytica, Mmorganella morganii, Pasteurella pneumoniae, Proteus mirabilis, Providencia rettgeri, Pseudomonas fluorescen, Salmonella spp., Serratia odorifera, Vibrio parahaemolyticus*, were detected in samples collected from the receiving surface water (Momba *et al*., 2006).

The National Research Council (NRC) assessed the danger of viruses, bacteria, and parasites (norovirus, adenovirus, salmonella, and cryptosporidium) linked with three different water reclamation scenarios (**Figure 3**) (National Research Council, 2012b). The first scenario (Scenario 1) is de facto reuse, whereas scenario 2 is wastewater effluent that is filtered by the soil and augments a drinkable aquifer. Scenario 3 is wastewater effluent that has gone through enhanced water treatment techniques such as reverse osmosis, micro filtration, and advanced oxidation and is allowed to flow through the soil to supplement an aquifer before being utilized for potable purposes. The risk assessment findings revealed that in all three situations, de facto reuse had the highest risk for all four illnesses. The risk of norovirus and adenovirus in Scenario 2 was less than 0.001, whereas the risk of salmonella and cryptosporidium was greater than 0.001 and 0.1, respectively. Furthermore, the NRC study indicated that when recycled water has been subjected to advanced treatment, the odds of getting infected by viruses, bacteria, and parasites are extremely low (below 0.000001).

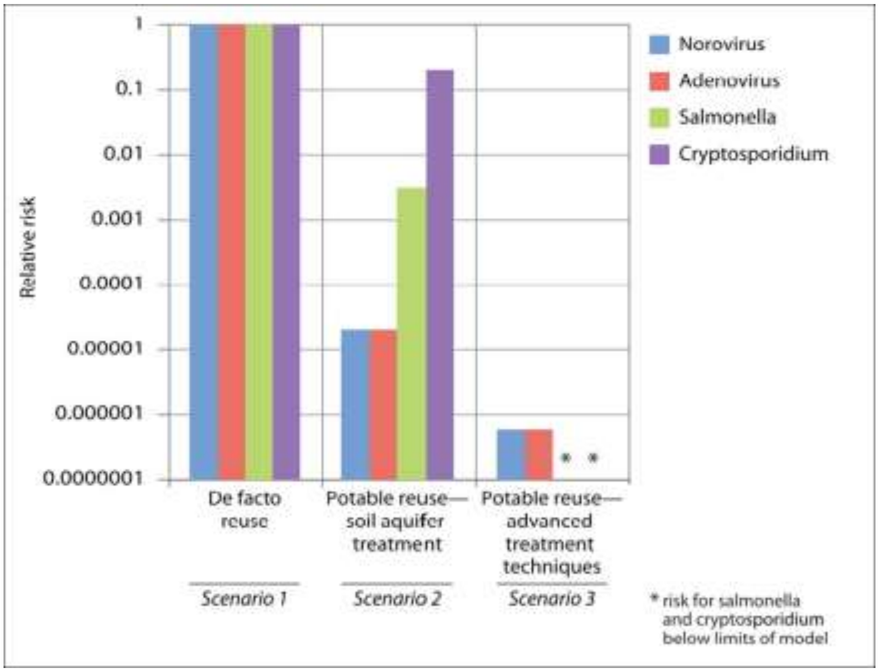

Figure 2.3: The hazard assessment of acquiring norovirus, adenovirus, salmonella, and cryptosporidium through three forms of water reclamation (Scenario 1, 2 and 3) (National Research Council, 2012b).

2.6.3 CEC impacts on human health and the environment

Emerging toxins in the environment have an impact on both human health and aquatic life. According to Kellock (2013), exposure of aquatic animals to these developing contaminants inhibits reproduction and development as well as the production of growth hormones. The presence of emerging contaminants in surface water also causes fish mortality, which has a detrimental impact on fish farming (Edokpayi *et al*., 2017). Furthermore, their presence in the environment endangers human health (**Table 2.2**) (Raghav *et al*., 2013).

Table 2.2: Effects of CECs to human health (Raghav *et al*., 2013).

CLASS OF CEC	EFFECTS TO HUMAN HEALTH
Prescribed drugs	Expedites cancer and harms organs.
Antibiotics	Affects the body's capacity to withstand sickness
Steroids	Disrupts the endocrine system
Disinfectants	Genotoxic, cytotoxic, cancer-causing
Solvents	Disrupts the endocrine system, damages the lever and kidney, respiratory impairment, causes cancer
Fire retardants	Disrupts the endocrine system, raises cancer risks
Reproductive hormones	Disrupts the endocrine system
Pesticides	Disrupts the endocrine system
Plasticizers	Disrupts the endocrine system and increases cancer risks
Industrial additives	Toxic to human, land, and aquatic ecosystems
Personal care products	Impairs the capacity to fight germs and alters the endocrine system.

2.7 DE FACTO REUSE DETECTION AND QUANTIFICATION METHODS

Despite the fact that de facto reuse is prevalent in many nations, only two countries, the United States and China, have measured it. The majority of research has instead concentrated on detecting and measuring CECs in surface water. Methods for quantifying de facto reuse, such as the use of GIS and wastewater tracers, have not been widely investigated or applied globally or in South Africa.

2.7.1 Geographic information system

Recent studies (Gasser *et al.*, 2010; Matongo *et al.*, 2015a; Rice, 2014; Wanda *et al*., 2017) show that wastewater tracers like CAF and carbamazepine are excellent indicators of the presence of wastewater effluents in surface water and may thus be used to assess water resource quality. However, the approach involving the use of tracers is costly and time-consuming since it needs sampling in rivers, sample preparation, SPE, and mass spectrometry connected to liquid chromatography (LC) or gas chromatography (GC) to measure wastewater tracer concentrations. Furthermore, the approach has limits in terms of maintaining the quality of water resources since measuring the amounts of contaminants in all raw water supplies is impractical (Rice, 2014). However, when combined with site-specific pollutant concentrations, the GIS approach can forecast pollutant amounts in all raw water sources (Rice, 2014).

A GIS is a computer-based system that captures, stores, manipulates, manages, and analyzes geographical data (Johnson, 2016). Following the construction of the necessary GIS-model, this tool may be used to estimate pollution concentrations in surface water and map contaminated waterways (Wu *et al*., 2005). GIS may also be used to display data such as river flow and the spatial connections between land features (Wu *et al*., 2005). A GIS may use spatial data to provide an all-encompassing perspective of a certain location (Martin *et al*., 2005). This integrated view is formed by combining sociologic, geographic, geologic, and ecological factors associated with the spatial aspects of water resource challenges and profiling them for decision making. For more than 20 years, GIS has been used to manage spatially allocated hydrologic modeling information. Furthermore, the advantages of implementing GIS in hydrologic inquiry include enhanced accuracy, reduced duplication, simpler map storage, greater flexibility, ease of information sharing, and higher product complexity (Ogden *et al.*, 2002).

GIS has four distinct uses in hydrologic applications: assessment, parameter determination, model set-up, and modeling. Several published studies have utilized GIS to estimate surface run-off, point and non-point pollution, water quality investigations, storm water modeling, and flood assessments (Rice, 2014). In the United States, an ArcGIS model was used to measure de facto reuse of raw water sources, and the findings indicated that de facto reuse had a 100 percent influence on some rivers (Rice *et al*., 2013). Using an ArcGIS, similar work was done in a river in China (Wang *et al*., 2017).

The amount of de facto reuse for the rivers was compared from 1998 to 2014, and the results indicated that the percentage of de facto reuse grew by 41% over the study period (Wang *et al*., 2017). It should be noted that data from certain measurement stations was unavailable, and de facto reuse was calculated using digital elevation mapping. The ArcGIS model's ability to forecast various scenarios contrasts strongly with scenarios in nations such as the United States, which have large databases for the creation of the GIS model.

2.7.2 GIS modelling for de facto reuse quantification

Rice et al. (2014) created a technique for modeling a GIS utilizing data from the United States Geological Survey (USGS), the National Atlas Web site, and the National Hydrography Data set (NHD). The data obtained comprised the locations of all WWTPs and DWTPs, as well as coordinate data for the DWTPs and WWTPs. Stream gauge data, as well as average, minimum, maximum, and percentile stream flows, were gathered. Data on topographic layers, city and state borders, and hydrography layers were also gathered. The information gathered was put into a GIS model. The GIS model was then utilized to turn regional level flow lines into a network utilizing network analysis capabilities from the Arc-GIS software after it had been programmed. The geographical study for DWTPs in relation to upstream WWTPs was performed using a GIS model. ArcHydro Tools was used to track the river flow upstream of the DWTP sites. When the upstream path was found, all of the WWTP discharges along the route were tallied together. For mass balance, the following assumptions were made: (a) the design flow was equal to the WWTP effluent discharge; (b) there was no loss in the WWTP effluent; and (c) there was perfect mixing in all surface water. Mass balance calculations were performed at DWTP intakes, assuming that the WWTPs were the only sources of wastewater in the surface water. As a result, the amount of de facto wastewater reuse was calculated by dividing the total of upstream discharge by the average stream flow of the nearest USGS stream gauge.

2.7.3 Advantages of employing a GIS model over traditional techniques

Since a GIS can map contaminated streams, GIS-based water quality studies are more efficient than manual sample collecting techniques. A GIS is also a low-cost and time-saving tool since it eliminates the need for manual sample collecting. It is capable of doing spatial analysis, which reveals the geographic locations of items and their distance from other objects (Martin *et al.*, 2005). Also, a GIS contains attribute data, which shows the characteristics of objects i.e. a GIS can provide the name of the land feature, activities done in the land feature and close to it. As a result, GIS can be used to analyze pollution sources, and it is a useful tool in case studies since it can track population changes and developments in a region. However, when using a GIS model to estimate de facto reuse, it is critical to confirm it using field investigations (e.g., wastewater tracers). While a GIS-model is a cost-effective and time-saving tool, it does have drawbacks. One of the drawbacks of a GIS model is that it necessitates large amounts of data for effective outputs, inadequate data limits the GIS model's predictive ability (Wang *et al*., 2017).

2.7.4 Wastewater tracers for quantifying de facto reuse

Although wastewater tracers are time-consuming and expensive, they are used to improve and validate the accuracy of the GIS-based technique. Rice *et al*. (2014) conducted research in the United States in which de facto reuse was calculated using GIS and sucralose was utilized as a wastewater tracer to verify the accuracy of the GIS analysis. Sucralose is an artificial sweetener that is included in a variety of products such as candy, soft drinks, and breakfast bars. Several researchers in the United States proposed it as a wastewater tracer (Rice, 2014). The criteria for a good wastewater tracer, on the other hand, include the tracer having a high concentration in the wastewater effluent (Gasser *et al*., 2010; Oppenheimer *et al*., 2011). Due to its abundance in wastewater effluents, CAF is regarded as an excellent wastewater tracer in South Africa. Archer et al. (2017a) reported CAF and SULF effluent values of 2077.5 and 1013.2 ng/L, respectively. Similar investigations have shown CAF and LAM concentrations in wastewater to be 397 and 184 ng/L, respectively (Archer et al., 2017a; Wood et al., 2015).

Because of its prevalence in polluted surface water, CAF is regarded a better tracer in South Africa than sucralose. Furthermore, CAF may be used to calculate the proportion of wastewater in surface water due to its good degradation in the environment (Hillebrand *et al*., 2011).

Gasser *et al*. (2010) quantified wastewater using alternate wastewater tracers. When carbamazepine and chloride were utilized as wastewater tracers to measure the ratio of wastewater in water sources, chlorine was discovered to be a significantly better tracer than carbamazepine for determining the quantity of wastewater in a raw water source. Using chloride and carbamazepine concentrations, the mixing ratios (MR) of wastewater in raw water were determined to be 0.84 and 0.63, respectively (Gasser *et al*., 2010). A research conducted in Germany employed CAF as a tracer to estimate the quantity of wastewater in surface water, and the results revealed that there was 0.4 percent of wastewater in the surface water (Hillebrand *et al*., 2011). **Table 2.3** lists the selection criteria for an effective wastewater tracer (Gasser *et al.*, 2010; Oppenheimer *et al*., 2011).

Table 2.3: Criteria for selecting an effective wastewater tracer (Gasser *et al.*, 2010; Oppenheimer *et al.,* 2011).

CHARACTERISTIC	DENOTATION
Specificity	The tracer must come primarily from residences.
Abundance	The concentration of tracer in the wastewater effluent must be high.
Background level	The tracer concentration in the neighbouring aquifer must be low.
Persistent level in source	The tracer must have a low degradability over a long period of time.
Conservative behavior	The tracer should not be volatile or undergo redox reactions.
Mobility	The tracer should be highly water soluble.
Degradation	The tracer must not deteriorate during transportation.
Proven method	The tracer analysis procedure must be verified.

One of the most essential properties of a suitable wastewater tracer that may be used to measure de facto reuse, according to the requirements in **Table 2.3**, is its persistence in raw water (low degradation), because if the wastewater tracer degrades fast in raw water, the approach will provide false findings. As a result, **Table 2.4** compares some of the most critical physical and chemical parameters necessary for a suitable wastewater tracer for CAF, LAM, and SULF (US EPA, 2020). Although Hillebrand et al. (2011) suggested CAF as a good wastewater tracer due to its rapid degradation, **Table 2.4** shows that LAM degrades more slowly than CAF, making it a more reliable wastewater tracer for quantification of de facto reuse (biodegradation of LAM and CAF, respectively, 2.8438 and 2.7700 (weeks-months)).

Table 2.4: Physical and chemical characteristics of CAF, LAM, and SULF extracted from Estimation Program Interface (EPI) Suite Software (EPIWEB 4.1) (US EPA, 2020).

PROPERTIES	CAF	LAM	SULF
Molecular Formula	$C_8H_{10}N_4O_2$	$C_8H_{11}N_3O_3S_1$	$C_{10}H_{11}N_3O_3S_1$
Molecular Weight	194.19	229.26	253.28
CAS Number	58082	134678174	723466
Water Solubility at 25 ^{0}C (mg/L)	2632	9366	3942
Biodegradation (weeks-months)	2.7700	2.8438	2.4297
Half-Life in a river (hrs)	2.279×10^7	1.575×10^{14}	9.747×10^8
Total removal in WWTP (%)	1.85	1.85	1.88
Total biodegradation in WWTP (%)	0.09	0.09	0.09

2.8 INSTRUMENTAL ANALYSIS OF WASTEWATER TRACERS

2.8.1 Solid Phase Extraction

SPE is an effective sample preparation technique currently available for rapid cleaning and enrichment of sample analytes prior to chromatographic study (Liakh *et al*., 2019). Compared to conventional liquid-liquid extraction (LLE), SPE offers significant advantages in terms of simplicity, high throughput, robustness and, in most situations, improved cost-effectiveness (Wang *et al.*, 2015). It is used to simplify dynamic sample matrix, purify target analytes, reduce ion suppression in applications for mass spectrometry etc. SPE is used to separate a component in a sample prior to HPLC analysis. The analyte and some of the sample matrix components may be retained in the SPE material when the sample is slowly passed through the SPE cartridge or disk (Agunbiade and Moodley, 2016). A wash solvent can be used to selectively elute components from the SPE sorbent while leaving others, depending on the characteristics of the analyte and the SPE sorbent. The ultimate goal is to remove interferences from the analyte contained in the matrix, resulting in a solution that is mostly analyte **Figure 2.4**).

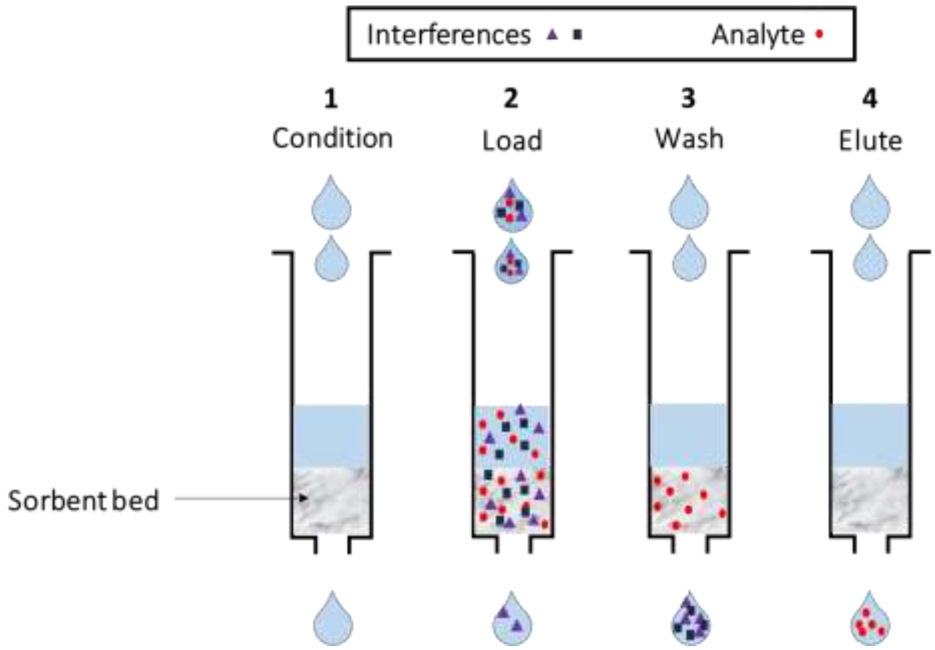

Figure 2.4: SPE stages

2.8.1.1 Different Solid Phase Extraction methods

The primary step in SPE is selecting a suitable adsorbent for separation. The sorbent must be selected based on the chemical properties of the target analytes and sample matrix. The three most common types of SPE cartridges are reversed phase (C-18), normal phase (silica), and ion exchange (anion or cation) (Liakh *et al.*, 2019) (**Figure 2.5**). The essential idea behind reverse phase SPE is that aliphatic fragments in oxylipins can interfere with non-polar stationary phases. Silica attracts polar molecules, which are often employed to clean the sample. Anion-exchange polymer-based resins selectively preserve oxylipins based on hydrophobic and anion-exchange interactions.

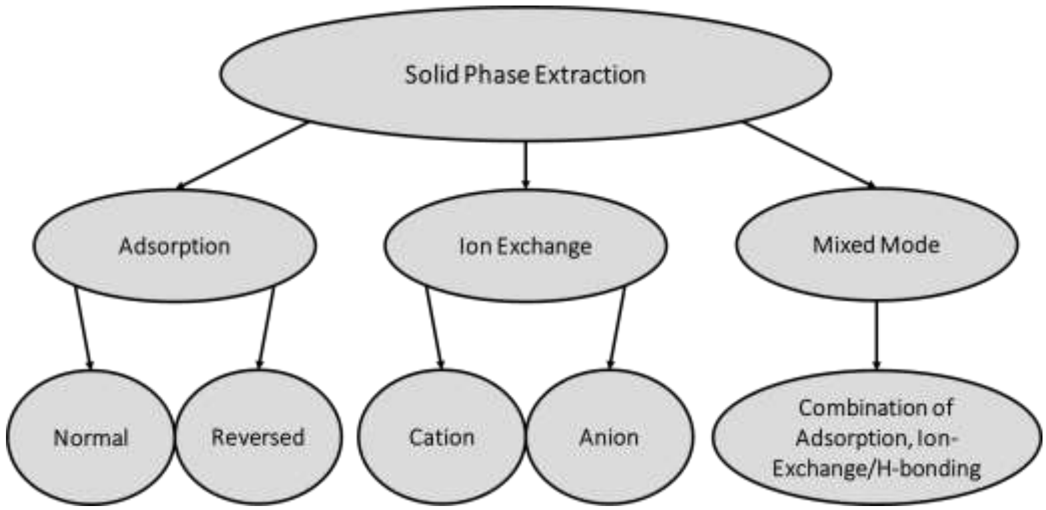

Figure 2.5: Different SPE methods

2.8.1.2 Solid Phase Extraction compared to Liquid Liquid Extraction

Liquid-Liquid Extraction (LLE) is a commonly used method of sample preparation for extracting of analytes of interest from aqueous samples (Andrade-Eiroa *et al*., 2016). It extracts the analyte by separating it into two immiscible solvents. However, LLE has drawbacks which involve expensive glassware (separatory funnels), vast and costly number of organic solvents required for separation which are not required in SPE. The SPE method is better suited for handling a large number of samples and has become a popular method of sample preparation due to its repeatability, reduced consumption of organic solvents, and ease of use. Moreover, SPE is compatible with automatic analysis systems (Wang *et al*., 2015).

2.8.2 High performance liquid chromatography

Over the years, high performance liquid chromatography (HPLC) has been a very flexible and effective analytical method used in qualitative and quantitative analysis of pharmaceutical compounds, dyes, biological samples (Malviya *et al*., 2010). Former investigations proved that the HPLC method is frequently used to determine PPCPs in raw water sources (Osunmakinde *et al*., 2013). The HPLC is highly sensitive and is able to detect PPCPs and their metabolites in very low concentrations (Archer *et al*., 2017a). Also, the HPLC method has more advantages than the GC because a GC requires derivatizing the analytes before analysis which makes the technique time consuming.

HPLC is advanced liquid chromatography format used for identifying and quantifying non-volatile liquid mixtures (Thammana, 2016). In this type of chromatography, the solvent is drained from a tank of solvents (mobile phases) and combined with a liquid sample. The mixture of solvent samples passes through a column of HPLC (stationary phase) and into a detector, where an electronic analysis is provided as a chromatograph. The waste is stored outside of the system in a waste bottle. The stationary phase is often a solid (small porous particles of surface-active substance) or a liquid placed onto inert solid support micro-particulate beads. The mobile phase is a solvent composition that flows under pressure through the stationary phase.

2.8.2.1 Different types of HPLC

There are diverse kinds of HPLC methods dependent on the stationary phase used namely, normal phase, reversed phase, size exclusion, ion exchange (Malviya *et al*., 2010). In Normal phase HPLC the separation is based on polarity where the stationary phase is polar, and the mobile phase is non-polar. In this type of HPLC polar analytes interact with the polar stationary phase causing them to have longer retention times. While non-polar analytes have shorter retention times. Reversed phase HPLC is the opposite or reverse of normal phase HPLC where the stationary phase is non-polar and the mobile phase is aqueous and partially polar (Madikizela *et al*., 2014). This chromatography is based on hydrophobic interactions caused by repulsive forces between a polar mobile phase, a comparatively non-polar analyte, and a non-polar stationary phase.

Size exclusion chromatography, commonly known as gel filtration chromatography, separates particles mostly based on their size (Malviya *et al*., 2010). It may also be used to determine the tertiary and quaternary structures of proteins and amino acids. This technique is commonly used for determining polysaccharides by molecular weight.

Retention in ion exchange chromatography is based on the attraction of solvent ions to charged sites associated to the stationary step. (Nyoni, 2010). This method of chromatography is commonly used in water purification, ligand-exchange chromatography, protein ion-exchange chromatography, carbohydrates and oligosaccharides high-pH anion-exchange chromatography, etc.

The HPLC has a range of uses in the pharmacy, forensic, environmental, and medical sectors (Malviya *et al*., 2010). It also assists in compound isolation and purification. In pharmaceutical applications it is used in drug stability control, dissolution tests, and quality control. The environmental applications include pollutant control and identification of pollutants in drinking water. HPLC is also used in forensics to analyse dyes, drug quantification in biological samples. It is also used the food industry for the analysis of fruit drinks, identifying polycyclic compounds in vegetables, analysing food preservatives. Also, in the medical sector it is used to identify neuropeptides and biological samples.

2.8.2.2 Reversed Phase HPLC Mechanism

In reversed phase chromatography the non-polar stationary phase interacts with the non-polar compounds. and are retained while polar compounds are eluted with the partially polar mobile phase (CHROMacademy, 2014; Thammana, 2016). Stationary phases in reversed phase are hydrophobic and chemically bound to the silica support particle surface **(Figure 2.6**). In most cases reversed phase chromatography is used in the analysis of pharmaceutical compounds and personal care products (Archer, 2018).

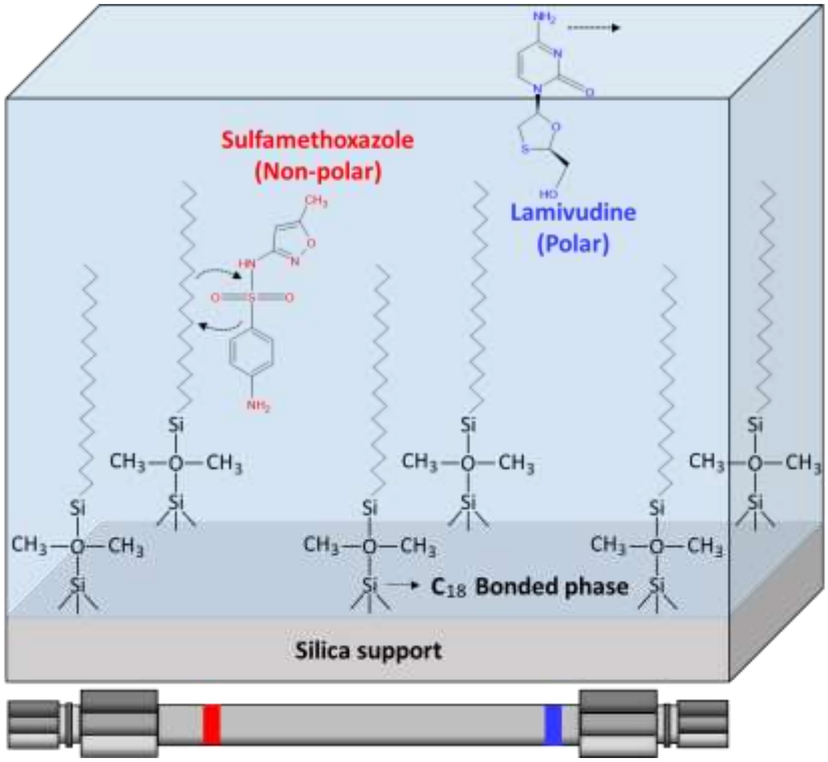

Figure 2.6: Reversed phase mechanism

The most widely used stationary phases are C_{18}, C_8, C_4, Cyano, Phenyl, Amino (CHROMacademy, 2014) (**Figure 2.7**). C_{18} column is highly hydrophobic and therefore, has increased retention for non-polar compounds compared to the other reversed phase columns.

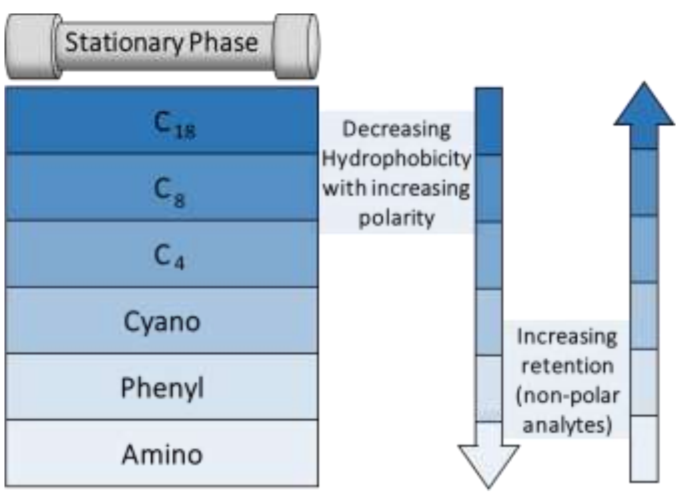

Figure 2.7: Commonly used stationary phases.

In the case of neutral analytes, the mobile phase is made up of water and an organic modifier that is utilized to modify analyte retention by reducing the polarity of the mobile phase (Varsha *et al*., 2012). Increased water content will repel hydrophobic or non-polar analytes from the mobile phase and into the non-polar stationary phase, where they will reside for some time before partitioning back into the mobile phase. In the presence of ionic analytes, extra additives like buffers or ion pairing reagents can be added to the mobile phase to monitor retention and repeatability. Generally, hydrophilic analytes are eluted before the hydrophobic analytes.

The hydrophobicity of the analyte molecule will be the key indication of retentivity in the reversed HPLC phase (Wood *et al*., 2015). Under normal conditions, hydrophobicity also stated as Log P, is a measure of how well the analyte differentiates between two immiscible solvents (typically octanol and water) (**Equation 2.1**). The greater the Log P value (between 0 and > 0), the more hydrophobic the molecule. Polar analytes that interact with silica surface silanol groups exhibit both adsorption and partitioning activity this can affect the peak form and increase the retention time.

$$LogP_{oct/wat} = Log\left(\frac{Solute_{octanol}}{Solute_{water}^{un-ionised}}\right) \qquad [2.1]$$

2.8.2.3 Mobile Phase Solvents for Reversed Phase HPLC

In reversed phase HPLC the mobile phase typically consists of water, aqueous buffer and an organic modifier (CHROMacademy, 2014; Varsha *et al*., 2012). In the analysis of ionizable compounds buffers and other additives can be present in the aqueous phase to monitor retention and peak structure. In reversed phase chromatography, water is the weakest solvent. As water is the most polar, it repels hydrophobic analytes to the stationary phase more than any other solvent, and therefore, has higher retention time. When the organic modifier is applied and the analyte is no longer highly repulsed into the stationary, it spends less time in the stationary phase because the organic modifiers are less polar, therefore this shortens the retention time. If more and more organic alteration is applied to the mobile phase, the retention time of the analyte will begin to decrease (Hopkins, 2019). Commonly used organic modifiers are water, methanol, acetonitrile, and tetrahydrofuran (**Figure 2.8**) (CHROMacademy, 2014). Also, the values of elution strength (ε°) which provide a measure of the relative strength of the elution are also shown in **Figure 2.8**.

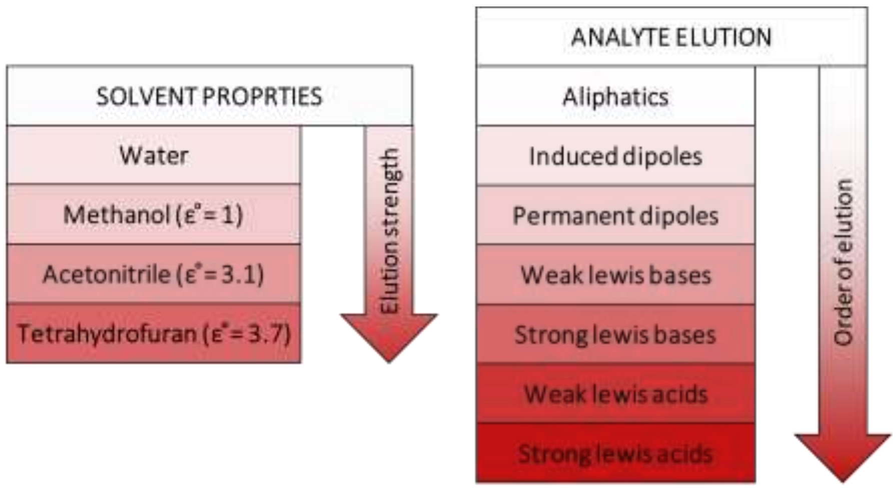

Figure 2.8: Commonly used organic modifiers and general order of elution for analytes.

2.9 MEMBRANE TECHNOLOGIES FOR POTABLE WASTEWATER REUSE APPLICATIONS

Advanced treatment methods now allow treated wastewater to be reused for drinkable purposes. Many nations, like Australia and Singapore, have been able to increase their drinking water supply during the last 20 years by utilizing membrane technology (Lautze *et al*., 2014). Furthermore, some studies have shown that improved membrane technology can filter municipal wastewater to drinking water standards.

Windhoek (Namibia's main city) was the first to implement DPR in 1968. (Du Pisani and Menge, 2013; Ghernaout, 2019; Wilcox *et al*., 2016). Windhoek's water supply was increased by 35% due to the water reuse treatment plants. The employment of modern treatment methods such as ultrafiltration and ozone in the removal of microorganisms, protozoa, EDCs, and organic materials at Windhoek has so far resulted in no health issues related to wastewater reuse for portable applications (Ghernaout, 2019). Potable reuse was not considered a viable solution in Australia until the onset of a six-year-long drought (2003-2009) (Rodriguez *et al*., 2009). Reclaimed wastewater was introduced as IPR, with wastewater being processed using modern membrane technology to supplement surface water supplies. However, near the end of the drought, potable reuse was discontinued.

The Water Factory 21 which was built in 1977 (a project in Orange County, California, USA) was the first to use reverse osmosis. The Water Factory 21 had a plant capacity of 19 megaliters per day (ML/day) for 27 years, and a new modern groundwater augmentation system with a capacity of 265 ML/day was only considered and deployed in 2007. (Warsinger *et al*., 2018). IPR is also used in several European nations, where reclaimed water contributes over 70% of the water supply during times of water scarcity (Rodriguez *et al*., 2009). In Belgium, an IPR project treated wastewater to drinking water standards using reverse osmosis and microfiltration, and the water was utilized to supplement an aquifer (Van Houtte and Verbauwhede, 2012). However, some herbicide was identified in the water treated by the microfiltration system that was below the water quality limits. As a result, the microfiltration treatment technology was phased out, and only reverse osmosis was employed beginning in 2004. England is also one of the countries that uses IPR, which it began in 1985 (Lazarova *et al*., 2001; Rodriguez *et al*., 2009). Microfiltration and ultra-violet disinfection are modern technologies utilized in England.

Singapore likewise uses IPR to address water scarcity issues. Singapore now has four water reuse treatment facilities known as the NEWater projects, which were put in place in 2003 (Ghernaout, 2019). NEWater projects treat wastewater to drinking water standards using modern treatment technologies such as microfiltration, ultrafiltration, reverse osmosis, and UV disinfection. These modern technologies have been shown to be successful in removing pollutants from wastewater such as organic matter, pesticides, EDCs, PPCPs, and herbicides (National Research Council, 2012a). Furthermore, the final water quality measurements match all of the Environmental Protection Agency (EPA) and World Health Organization (WHO) criteria, including turbidity of 0.5 nephelometric turbidity units (NTU), total dissolved solids (TDS) of 50 mg/L, and total organic carbon (TOC) of 0.5 mg/L.

Naghizadeh *et al*. (2011) purified municipal wastewater using a hollow fiber microfiltration membrane. The membrane was immersed in a bioreactor to investigate the removal of COD, total suspended solids (TSS), and turbidity at various retention durations. The study's findings demonstrated a high removal treatment efficiency, attributed to low COD, TSS, and turbidity of 9 mg/L, 1 mg/L, and 0.3 NTU, respectively (Naghizadeh *et al*., 2011). Another research used a hollow fiber microfiltration membrane connected to a biocathode microbial desalination cell to purify wastewater (Zuo *et al*., 2018). The final effluent's conductivity, COD, total nitrogen, and total phosphorus levels were determined to be within the respective water quality limits of 59.2 S/cm, 35.5 mg/L, 1.65 mg/L, and 0.14 mg/L. Despite its efficiency in removing contaminants from surface water, membrane technology is still prohibitively expensive (Herman *et al*., 2017).

2.9.1 Other technologies for wastewater treatment

It is vital that wastewater treatment plants operate properly since they represent the defining line between a healthy and contaminated environment. There are several major human health risks associated with water pollution caused by improperly treated effluents, including dilaceration of the reproductive system, which leads to ovarian cancer, breast cancer, and low sperm quality (Archer, 2018). As a result, water management authorities must invest in enhancing the operation of WWTPs since the consequences of reusing inadequately treated wastewater can be both short-term and long-term. Although there are new and better technologies, such as membrane technology, that may efficiently remove these contaminants, they are expensive. To achieve sustainable growth, the WWTPs chosen must have a technology type that is suited for a certain development, which may not be the finest technology available (WRC, 2016). The wastewater treatment process is divided into four stages: preliminary treatment, primary treatment, secondary treatment, and tertiary treatment. The sort of technology utilised in each phase is determined by population size and environmental constraints. **Figure 4** depicts the many types of technologies that may be utilised at various phases of the wastewater treatment process (WRC, 2016).

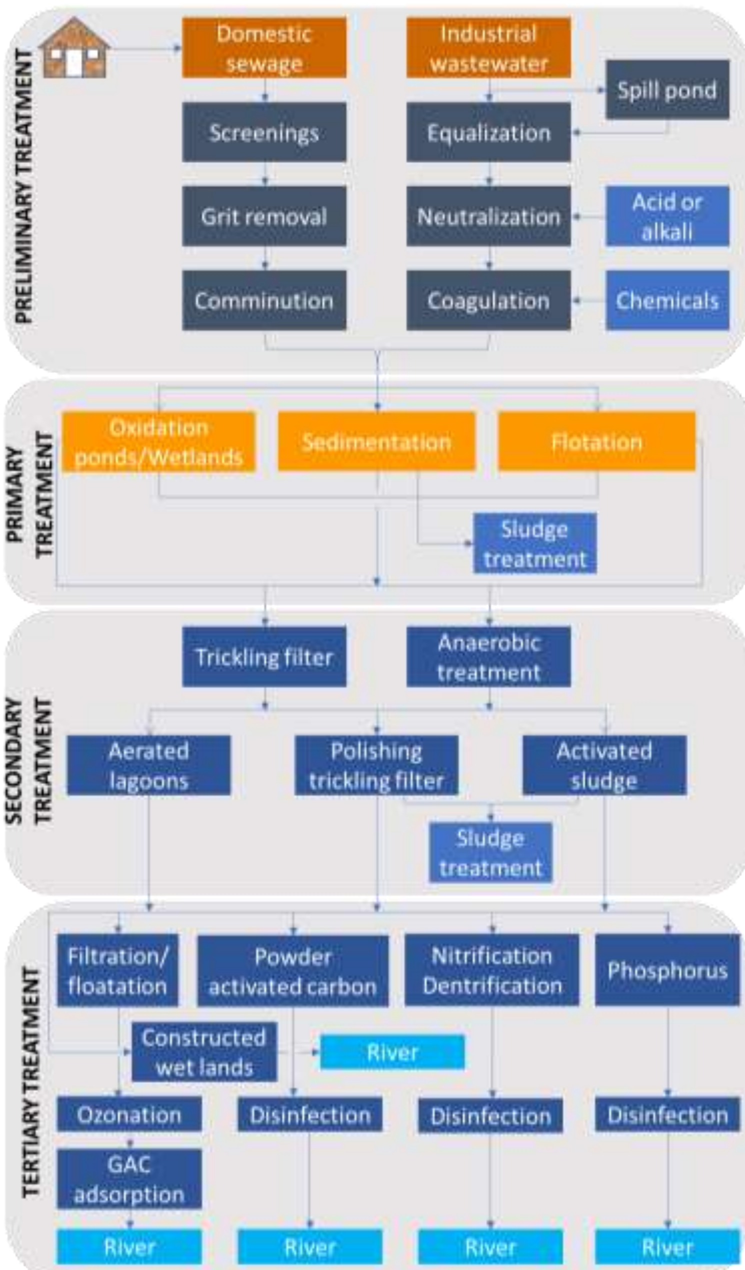

Figure 2.9: Different types of technologies for wastewater treatment (modified from WRC, 2016).

Van Der Merwe-Botha and Quilling (2012) conducted a review of several technology types in South Africa and categorised them as low, medium, and high based on ultimate effluent quality, capital and operational expenses, power use, and preservation needs (**Table 2.5**). The technologies are classified based on their stage in the WWTP (preliminary, primary, secondary, tertiary and sludge treatment).

Table 2.5: Technology and classification level (Van Der Merwe-Botha and Quilling, 2012)

LEVEL OF TREATMENT	TYPE OF TECHNOLOGY	GENERAL COMMENT ON TECHNOLOGY
PRIMARY	Primary settling	Low to medium
	Flow balancing	Low to medium
SECONDARY	Trickling filter	Low to medium
	Rotating biological filter	Medium
	Pasveer ditch	Medium
	Oxidation ponds	Low to medium
	Wetlands	Low to medium
	Extended aeration	Medium to high
	Biological nutrient removal/activated sludge	High
	Surface aeration	Medium to high
	Clarification	Low to medium
TERTIARY	Chlorine gas disinfection	Medium
	Maturation pond	Low
SLUDGE	Gravity thickening	Medium
	Thickening by dissolved air floatation	Medium to high
	Aerobic digestion	Medium to high
	Anaerobic digestion	Medium
	Belt press dewatering	Medium
	Solar drying beds	Low
	Centrifuge dewatering	Medium to high
	Composting	Low to medium
	Palletization	High
	Disposal to land	low

2.10 THE STATUS OF WASTEWATER TREATMENT PLANTS IN SOUTH AFRICA

The wastewater treatment technologies available in South Africa are trickling filters, activated sludge, wastewater ponds, rotating biological reactors, wetlands, membrane bioreactors, and aerobic granular activated sludge. **Figure 2.10** displays wastewater treatment methods commonly used in South Africa (DWA, 2012).

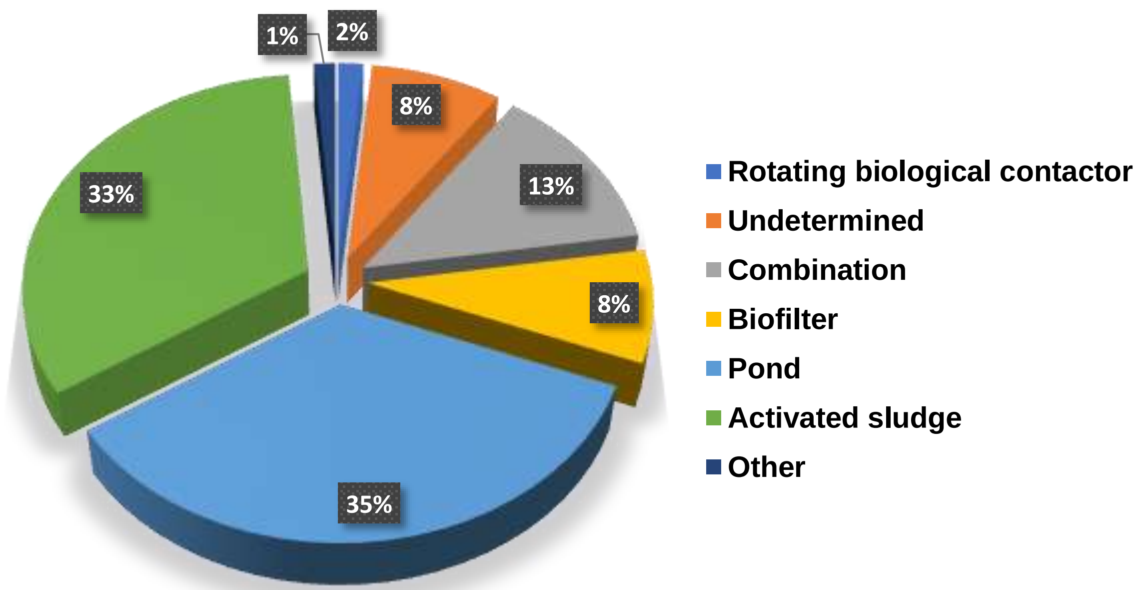

Figure 2.10: Wastewater treatment methods used in South Africa (DWA, 2012)

2.10.1 Factors to consider for wastewater treatment technology selection.

The main challenge in South Africa is that less attention was paid to long-term costs and the inability to sustain advanced technologies which is a result of lack of skilled workers, expansion costs, maintenances vs capital cost of recently developed processes (Jack *et al*., 2016). For the best application of the treatment technologies, the following should be considered:

- Authorization of necessities for land-water use.
- Size and local skills based on O&M systems.
- Finances to build the facility.
- Operating costs and consumer’s financial capacity
- Accessibility and cost of land
- Anticipated population growth
- Recovery opportunities (nutrients)

Some of the WWTPs in South Africa produce effluent that is not different from the influent (Van Der Merwe-Botha and Quilling, 2012). This is due to factors such as underbudgeting for WWTP maintenance, lack of knowledge for technology requirements and lack of visionary officials who are responsible enough to perform their duties.

2.10.2 Regulations for wastewater effluent quality

Water treatment plants that are properly monitored produce wastewater effluent that meets regulations, with up to 90% removal of pathogens and bacteria (Okeyo *et al*., 2018). The South African National Water Act introduced restrictions for discharging wastewater to surface water, which WWTPs must follow for environmental and human health safety (**Table 2.5**).

Table 2.6: Wastewater quality standards for discharging wastewater effluent to surface water (DWA, 2013c).

PARAMETER	GENERAL LIMIT
Faecal Coliforms	1000 cfu/100 mL
Chemical Oxygen Demand	75 (mg/L)
pH	5,5-9,5
Ionized and unionized Ammonia	3 (mg/L)
Nitrate	15 (mg/L)
Residual Chlorine	0,25 (mg/L)
Suspended Solids	25 (mg/L)
Conductivity	70- 150 (mS/m)
Phosphorous	10 (mg/L)
Dissolved Arsenic	0,02 (mg/L)
Dissolved Cadmium	0,005 (mg/L)
Dissolved Chromium (VI)	0,05 (mg/L)
Dissolved Copper	0,01 (mg/L)
Dissolved Cyanide	0,02 (mg/L)
Dissolved Iron	0,3 (mg/L)
Dissolved Lead	0,01 (mg/L)
Dissolved Manganese	0,1 (mg/L)
Mercury	0,005 (mg/L)
Dissolved Selenium	0,02 (mg/L)
Dissolved Zinc	0,1 (mg/L)
Boron	1 (mg/L)

2.11 METHODS FOR CALCULATING OPERATION AND MAINTENANCE COSTS

Current literature on O&M costs is broad. Nevertheless, because it illustrates a range of statistical methods and the types of costs discussed, the comparison of outcomes is minimal (Tsagarakis *et al*., 2003). Some research focused on overall quality parameters (i.e., the quality of the influent and effluent or the elimination of pollutants), some rely on the amount of wastewater being processed (Hernández-Sancho *et al*., 2015). Some base the O&M costs on the yearly data given by the treatment facility, namely personnel, maintenance, energy, disposal, chemicals and materials, and miscellaneous costs (Wendland, 2005). Other studies use the actual organic pollution load (PE) of the WWTPs, since the organic pollution load corresponds directly to operating costs and energy consumption (Kroiss and Lindtner, 2005). Several reports measure all cost factors for O&M while others estimate only energy consumption costs. According to Wendland, (2005) factors contributing to the O&M costs include design capacity, topography, and geographical position of the site (contributing to pumping energy costs). They also include composition of the raw wastewater and the discharge standard. Technologies and the preferred treatment method, including type of sludge treatment and method of disposal. Also, energy supply and recycle, level of automation, monitoring of the process, organization, and operation of the wastewater treatment facility.

Several authors have used cost functions in the form $y = ax^b$ to predict land use, construction, and O&M costs. Where a and b are calculated coefficients which are based on either real or analytical data and x represents the capacity of WWTPs based on PEs. These cost functions were developed from the data collected from WWTPs using the similar treatment processes. Further, these cost functions can then be used to predict the O&M costs for WWTPs that use similar treatment processes.

Tsagarakis et al. (2003) projected the life-cycle cost functions of wastewater treatment in Greece using the functional form $y = ax^b$. The variable y reflects the cost of land usage, construction, and O&M, while the variable x indicates the capacity of WWTPs in terms of PEs. Costs for treatment of sludge are also considered. All the numerical equations provided were developed from the evaluation of the statistical information of WWTPs falling on the three categories presented below. Estimates were provided for three kinds of primary and secondary treatment namely:

- **Conventional**: pre-treatment, primary clarifiers, aeration, secondary clarifiers, chlorination, sludge thickening and digestion, and mechanical dewatering.
- **Extended aeration with mechanical dewatering**: pre-treatment, aeration, secondary clarifiers, chlorination, sludge thickening and mechanical dewatering.
- **Extended air aeration with air drying**: pre-treatment, aeration, secondary clarifiers, chlorination, sludge thickening and drying beds.

Table 2.7: Cost functions for different activated sludge treatment processes (Tsagarakis et al., 2003).

WASTEWATER TREATMENT SYSTEM	COST OF LAND USE (L) (10^3 m^2)	CONSTRUCTION COST (C_c) (10^6 USD 10^{-3}PE)	ANNUAL O&M COSTS (C_a) (10^6 USD 10^{-3} PE)
Conventional	$L = 0.839x^{0.722}$ (R^2= 0.936)	$C_c = 0.116x^{0.954}$ (R^2= 0.935)	$C_a = 0.022x^{0.672}$ (R^2= 0.84)
Extended aeration with mechanical dewatering	$L = 0.764x^{0.810}$ (R^2= 0.84)	$C_c = 0.206x^{0.775}$ (R^2= 0.829)	$C_a = 0.0098x^{0.763}$ (R^2= 0.752)
Extended aeration with air drying	$L = 1.001x^{0.820}$ (R^2= 0.792)	$C_c = 0.153x^{0.727}$ (R^2= 0.808)	$C_a = 0.0083x^{0.801}$ (R^2= 0.874)

According to the data, extended aeration with natural air drying was by far the most cost-effective solution, followed by extended aeration with mechanical drying and conventional secondary treatment. The high O&M cost for conventional treatment regarding extended aeration treatment is related to increased energy costs.

Another study conducted in Spain assessed the cost functions (C in €/year) of seven different stages of wastewater treatment using the volume of treated wastewater (V in m^3/year), the age of the facility (A in years) and the efficiency of disposal of contaminants for the removal of suspended solids (SS), organic components (COD), nitrogen (N) and phosphorus (P), respectively (Hernández-Sancho *et al*., 2015). The generated cost functions are shown in **Table 2.8**.

Table 2.8: Cost functions for studied treatment processes (Hernández-Sancho *et al*., 2015).

TECHNOLOGY	COST FUNCTIONS	R^2
Extended aeration without nutrient removal	$C = 169.4844V0.4540\ e^{(0.0009A+0.6086SS)}$	0.61
Activated sludge without nutrient removal	$C = 2.1165V0.7128\ e^{(0.0174A+0.15122SS+0.0372BOD)}$	0.68
Activated sludge with nutrient removal	$C = 2.518V0.7153\ e^{(0.007A+1.455COD+0.15BN+0.243P)}$	0.73
Bacterial beds	$C = 17.3671V0.5771\ e^{(0.1006A+0.6932COD)}$	0.99
Peat beds	$C = 1\ 510.84V0.2596\ e^{(0.0171SS)}$	0.52
Biodisk	$C = 28.9522V0.4493\ e^{(2.3771SS)}$	0.81
Tertiary treatment	$C = 3.7732V0.7223\ e^{(0.6721COD+0.0195BN+0.7603P)}$	0.90

The cost functions in **Table 2.8** show the relationship between the cost of yearly operation and the treated volume, including the percentage of pollutants removed and the age of the plant. Depending on the technology, the volume being treated in all situations, the proportion of pollutants eliminated, and the age of the plant all have different effects. The use of these functions helps to identify the most appropriate technologies according to the volume of wastewater to be treated and the targets set for the removal of pollutants.

Dogot *et al*., (2010) modeled the costs of wastewater treatment using operating costs and investments. The approach used involved WWTPs and collection and sewer networks. The assessment was based on two sets of data collected from the Walloon Region in Belgium. The first category was 111 WWTPs with a capacity of 250 – 390 000 PE and the second data collection contains 314 WWTPs (> 390 000 PE.). The authors demonstrated that both expenditure and operational costs are influenced by economies of scale. The cost function derived was $y = 10{,}027^{-0.34}$ (R^2 = 0.75) where *y* was the unit cost per m^3/year and *x* is the design capacity of the WWTP. A similar study conducted by Hernández-Sancho *et al*., (2015), showed that not only the design capacity but also treatment methods have a major effect on the O&M cost. In this study cost function was $y= 899.8^{+0.44}$ (R^2 = 0.59) (based on the sum of WWTPs provided) where *y* is the O&M cost and *x* is nominal power.

2.12 COST OF WATER RECLAMATION SYSTEMS

Investing on water reuse projects is a difficult decision that is costly and beneficial in the present and future. Planned potable water reuse is generally more expensive than de facto reuse. However, planned potable water reuse remains less expensive than desalination. Water reuse prices vary greatly depending on location, water quality regulations, treatment methods, water dispersion system requirements, energy cost, subsidies, and a variety of other factors (Hosseinzadeh *et al*., 2017). In general, reusing wastewater for potable reuse is more expensive than reusing wastewater for non-potable usage (Ghernaout, 2019). Non-potable reuse needs less treatment, depending on the intended use of the reclaimed water. Furthermore, non-potable reuse can reduce the requirement for water reclamation operations. However, reusing wastewater for non-potable uses necessitates the installation of various pipe structures, which can be costly depending on where and how far the recovered water must be distributed. Water management authorities should consider non-financial costs and benefits of water reuse projects such as surface and ground water augmentation during dry seasons and high ecological effect when deciding on a more efficient water supply choice for their society (Herman *et al*., 2017).

2.13 CONCLUSION

The depletion of sustainable water supplies is a serious concern in South Africa and other arid and semi-arid nations. There is an imbalance between water supply and demand as a result of growing population growth, urbanization, the influence of climate change, and technological improvements in the country. Water pollution caused by the release of poorly treated wastewater effluent is another issue that is indirectly contributing to the loss of quality water resources (de facto reuse). Although de facto reuse is vital for augmenting surface water supplies when there is a scarcity of water, it poses substantial dangers to human health by exposing individuals to microorganism-induced diseases. Furthermore, CECs found in de facto wastewater effluents impede aquatic animal development and reproduction. As a result, environmental and health rules should be strictly implemented to guarantee that many South African communities who rely on polluted raw water sources for their water supply are effectively safeguarded. The importance of de facto reuse quantification is that it will allow identification of possible health concerns associated with recycling inadequately treated wastewater. Furthermore, information on the level of de facto reuse is required to advise water treatment facilities on the necessity to create procedures and water treatment trains aimed at reducing CECs from wastewater. As a result, tools like GIS-based approaches for quantifying and mapping SA water bodies contaminated by de facto wastewater reuse are required. Water management agencies can also utilize such a model to make well-informed judgments on water quality issues. Because of its abundance in surface water and high stability, LAM is the ideal wastewater tracer for verifying the GIS model in South Africa. LAM is an ideal wastewater tracer for verifying the GIS model in South Africa because of its abundance in surface water and its stability.

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CHAPTER 3
EXPERIMENTAL METHODOLOGY

3.1 INTRODUCTION

This chapter outlines details of the methodology followed to achieve the aim and objectives of the study. Materials and methods used to optimize and validate a method for analyzing wastewater tracers are discussed in this chapter. Also, the methods used to quantify de facto reuse using wastewater tracers and a GIS-based model are also discussed.

3.2 MATERIALS AND METHODS

3.2.1 Materials

All chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich, SA. The chemicals include CAF ($C_8H_{10}N_4O_2$, ≥99.9%), SULF ($C_{10}H_{11}N_3O_3S$, ≥98.9%), LAM ($C_8H_{11}N_3O_3S$, ≥98.9%) standards, methanol (MeOH) (CH_3OH, ≥99.9%), Liquid chromatography mass spectrometry (LC-MS) grade, acetonitrile (ACN) (C_2H_3N, ≥99.9%) LC-MS grade, formic acid (FA) (CH_2O_2, ≥99.0%) LC-MS grade and ammonium hydroxide solution (NH_4OH) (28% NH_4 in H_2O ≥ 99.99%). All the chemical standards were purchased in powder form and were prepared in 100% MeOH. Whatman membrane glass microfiber (GF/C) filters (0.7 µm and diameter 47 mm) were purchased from Sigma-Aldrich, SA. Ultrapure water for LC-MS was generated at the UNISA laboratory, Florida Campus. Supel^{TM} swift, 200mg, 6mL HLB cartridges and Supelclean^{TM}, 200mg, 6mL C-18 cartridges were bought from Sigma Aldrich (Johannesburg, SA). ACQUITY UPLC BEH C-18 1.7 µm (2.1x100mm column) was purchased from MICROSEP (Johannesburg, SA). Unless explicitly stated otherwise, all chemicals were used without further purification.

3.2.2 Collection of water samples

A grab sampling technique was used to collect water samples from 5 WWTPs (WWTP1, WWTP1, WWTP2, WWTP3, WWTP4, and WWTP5) and in rivers they discharge to (Jukskei, Diepsloot (Jukskei tributary), crocodile, Msunduzi, Ilovu, and Donga river respectively) (**Figure 3.1**). The sampling was conducted in two provinces of South Africa, viz Gauteng, and Kwa-Zulu Natal. For quality assurance (QA) measures, all the samples were collected in triplicates in all the sampling points (FEM, 2009). Water quality parameters such as pH, Electrical conductivity (EC), DO and TOC were measured on site for all the sampling points. The samples were collected in 1 L borosilicate Schott bottles wearing nitrile gloves to avoid contamination and were immediately stored in dry ice in a cooler box at -4 ^{o}C and SPE was conducted within 24 hours of sampling. Samplings were conducted in winter (June) and spring (October) to assess the impact of seasonal variations on the concentrations of the analytes in the wastewater effluents. Assessing seasonal variations of the concentrations of wastewater tracers in surface water is among the most important details in the analysis of wastewater tracers (Wanda *et al*., 2017). Previous studies have shown that seasonal variations in wastewater tracer concentrations can be linked to the flow conditions of the river and the local climate. Further, in rainy seasons, rainwater washes away some of these emerging pollutants used in the agricultural processes by surface runoff to the rivers (Sun *et al.*, 2018).

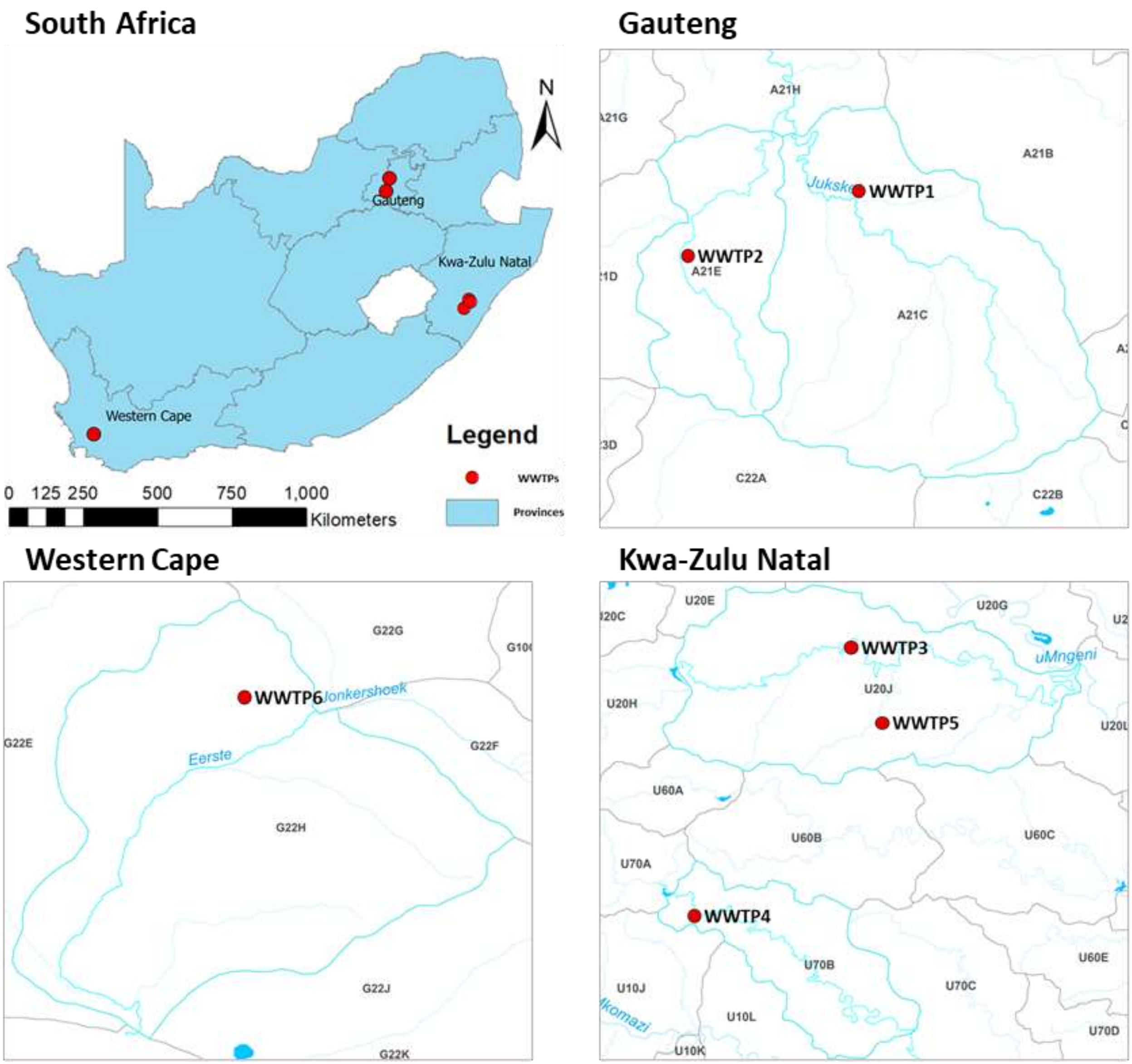

Figure 3.1: ArcGIS map showing sampling sites (extracted from water resources of South Africa).

The concentrations of the wastewater tracers were quantified for 5 WWTPs. Water samples were collected from the wastewater effluents and the rivers receiving the effluents (upstream and downstream). The studied WWTPs and rivers are described in **Table 3.1.**

Table 3.1: Table of description

WWTP	LOCATION	ACTIVITY	PROVINCE
WWTP1	Johannesburg	Agricultural, industrial and residential	Gauteng
WWTP2	Johannesburg	Residential and industrial	Gauteng
WWTP3	Pietermaritzburg	Industrial and residential	Kwa-Zulu Natal
WWTP4	Richmond	Agricultural	Kwa-Zulu Natal
WWTP5	Pietermaritzburg	Agricultural and residential	Kwa-Zulu Natal
WWTP6	Stellenbosch	Agricultural and residential	Western Cape

*Due to sensitivities of WWTP data, the names of the WWTPs have been coded.

Table 3.2 shows the geographic coordinates of the sampling points for the WWTPs and rivers.

Table 3.2: Sampling point GPS co-ordinates.

SAMPLING AREA	LATITUDE	LONGITUDE
Jukskei river upstream	-26.03001	28.10887
Diepsloot river (Jukskei tributary)	-25.94756	28.00052
WWTP1	-25.95220	27.97473
Jukskei river downstream	-25.95329	27.96171
Crocodile river upstream	-26.01554	27.83502
WWTP2	-26.00998	27.83282
Crocodile river downstream	-25.98942	27.84290
Ilovu river upstream	-29.883958	30.265750
WWTP4	-29.885438	30.266341
Ilovu river downstream	-29.883697	30.268342
Msunduzi river upstream	-29.59688	30.43912
WWTP3	-29.602118	30.430884
Msunduzi river downstream	-29.59838	30.44051
Donga river upstream	-29.6846	30.46398
WWTP5	-29.684052	30.465233
Donga river downstream	-29.68429	30.46452

3.2.3 Solid phase extraction

SPE is a sample preparation technique used to extract, clean and preconcentrate the sample. The SPE approach also allows the separation of target analytes from interfering substances. Preconcentrating the samples is necessary because in real water samples, the concentrations of these analytes may be below the detection limit of the ultra-high-pressure liquid chromatography (UHPLC) method.

The water samples were first filtered with 0.7 µm GF/C filter papers to get rid of suspended particles. Then transferred to 100 mL volumetric cylinders where they were spiked with 50 µL of a 1µg/L mixed standard (containing CAF, LAM and SULF). After the samples were spiked, the sample pH was adjusted to 8 by adding 10 µL of NH_4OH (to enhance SPE recoveries). The HLB and C-18 SPE cartridges were connected to a SPE manifold, conditioned with 5 mL of MeOH and 5 mL of LC-MS grade water at a flow rate of 1 mL/min. After conditioning, the SPE cartridges were dried under vacuum for 5 min, and then 100 mL of sample was loaded from each sampling bottle at 0.8 mL/min to maximize the interface between the sorbent and the sample matrix. After sample loading the SPE cartridges were dried under vacuum at 5 mL/min for 30 minutes and then the samples were eluted with 5 mL MeOH at 1 mL/min. The collected samples were evaporated to dryness using N and stored at -22 ºC. The samples were then reconstituted with 1 mL of 50:50 (H_2O: MeOH) prior to UHPLC analysis.

The extraction efficiency of the method was studied prior to application in real water samples. This was achieved by determining the recovery percentages of the target analytes in synthetic samples. The recoveries were studied for the HLB and C-18 cartridges, where synthetic samples with known concentrations of target analytes were prepared and preconcentrated using the cartridges (HLB and C-18 cartridges). The concentrations of the analytes were quantified in a UHPLC using a calibration curve. Further, the recoveries were calculated using the measured concentrations and the nominal concentrations (**Equation 3.1**).

$$\%\,Recoveries = \left(\frac{Nominal\ concentration}{Measured\ concentration}\right) \times 100 \qquad [3.1]$$

3.2.4 Preparation of standard solutions for calibration curve plots

Stock solutions (1000 mg/L) of the analytes (CAF, LAM and SULF) were prepared by dissolving 1 mg of each analyte in 1000 µL of HPLC grade MeOH using 1.5 mL HPLC vials. The vials were vortexed until the analytes were completely dissolved. Consequently, a mixed standard solution (100 mg/L) was prepared by adding 100 µL of each stock solution to a 700 µL solution of H_2O:MeOH (50:50). The prepared 1000 mg/L stock solutions were kept in the fridge at -20ºC for further analysis within a month. For construction of a calibration curve plot, 10 dilute aliquots (10-100 µg/L) were prepared from the 100 mg/L mixed standard solution and the H_2O:MeOH (50:50) solution. The 10-100 µg/L equidistant standards were analyzed with the collected water samples (WWTP effluent and river water) in triplicates with the blank in between to avoid carryover (Kaila, 2016).

3.2.5 Ultra High-Pressure Liquid Chromatography Mass Spectrometry analysis

LC-MS is a method for separating, identifying, and quantifying mixtures in a sample based on their molecular structure and composition (Malviya *et al*., 2010). This consists of a stationary phase, which is a solid or a liquid held up on a solid, and a mobile phase, which is a liquid. The mobile phase transports the sample to the stationary phase. Components having greater interactions with the stationary phase will travel along the column more slowly than components with weaker connections (**Figure 3.2**).

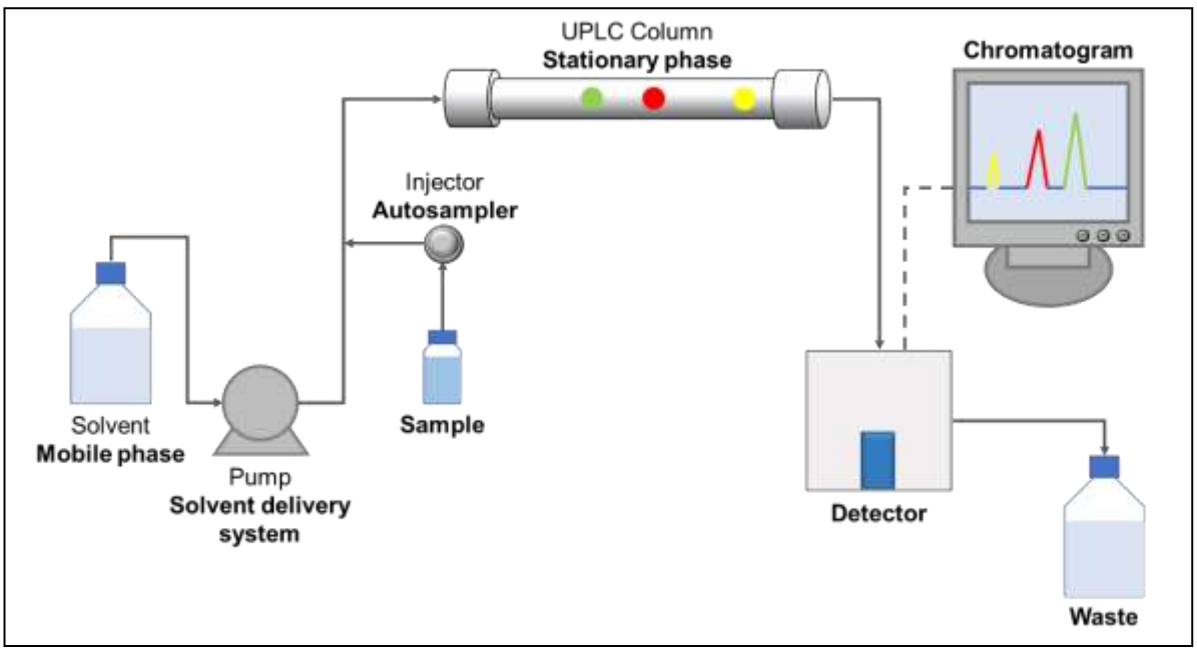

Figure 3.2: General working mechanism of a high-pressure liquid chromatography.

A UPLC BEH C_{18} column is reverse phase non-polar column consisting of 18 carbon atoms chemically bonded to particles of silica (Žuvela *et al*., 2019). In this type of chromatography there are strong interactions between the polar solvent and polar particles in a sample passing through the stationary phase. Thus, there are less interactions between the stationary phase and the polar particles. This implies that the polar molecules will be eluted first with reduced retention times. However, non-polar particles will have longer retention times because of the strong interactions between non-polar column and non-polar particles.

In this study a UHPLC coupled with a quadrupole time of flight mass spectrometry (Q-tof-MS) (DIONEX Ultimate 300 by Thermo Scientific) was used to separate and quantify compounds of interest in WWTP effluent and surface water samples. A UPLC BEH C-18 1.7 µm, 2.1x100 mm column was used for stationary phase. The mobile phase consisted of mobile phase A (a polar inorganic solvent) and mobile phase B (a polar organic solvent), both mobile phases were modified with 0.1% FA. Mobile phase A comprised of LC-MS grade water and two organic solvents were tested for mobile phase B viz. MeOH and ACN. To achieve the purpose of the study the target analytes were first identified and confirmed by studying their fragmentation patterns. After identification, the method was optimized and validated. The LC-MS conditions were as follows; the final injection volume was 10 µL and the mobile phase flow rate was 0.3 min/L with a total run time of 14 min and the column temperature was 30 ^{0}C. Mass spectrometry was conducted in positive Electron Spray Ionization (ESI+) mode with the capillary voltage at 4.5 kV, dry heater at 220 ^{0}C, dry gas at 8.0 L/min and nebulizer pressure at 1.8 bar. The masses were calibrated with sodium formate (mass range from 50 to 1500) and the calibrant was infused before each sample run to re-calibrate and compute the accuracy of the masses.

3.2.6 LC-MS-MS analysis

Mass spectrometry was conducted by injecting a 1000 µg/L synthetic solution consisting of CAF, LAM and SULF in a UHPLC to identify the mass spectrum (relative intensity (%) over the mass-to-charge ratio (m/z)) of each analyte. The fragmentation pathways of the analytes were also studied in tandem mass spectrometry (MS-MS) mode using data analysis software to confirm the target analytes, and the analysis was conducted in ESI+ mode. The obtained fragments were then compared to those that are recorded in data bases such as the United States Environmental Protection Agency's (EPA's) Estimation Program Interface Suite (EPISuite) (US EPA, 2020).

3.2.7 Method optimization

Irrespective of whether a method is new or well-developed, validation of the method is in many cases necessarily preceded by method optimization (FEM, 2009). Method optimization is conducted to improve or maximize the efficiency of a method for the target analytes. The original method was developed by Wood *et al*. (2015) using a UHPLC, for mobile phase A and B water and ACN were used both containing 0.1% FA. The stationary phase was a Zorbax Eclipse C8 XDB, 3.0 × 50 mm, 1.8 mm column and the sample injection volume was 15 µL. The mobile phase flow rate was 0.4 mL/min, and the column temperature was 22^{0}C. Method optimization was performed in UHPLC-Q-tof-MS equipped with a UPLC BEH C-18 1.7 µm, 2.1x100 mm column. The mobile phase used to carry the sample to the stationary phase was composed of mobile phases A and B. Where mobile phase A was composed of LC-MS grade water with 0.1 % FA. For mobile phase B two solvents were tested namely ACN with 0.1% FA, and MeOH with 0.1% FA. The flow rate of the mobile phases was set to 0.3 mL/min and the injection volume of the sample was 5 µL and the column temperature was set to 30^{0}C. The analysis was conducted in positive ESI mode.

3.2.8 Method validation

Method validation is a necessary procedure for demonstrating that an analytical method is suitable for its intended purpose. This was achieved by studying the linearity, range, specificity, LOD, LOQ, precision, and robustness of the method.

3.2.8.1 Linearity

Linearity is the capacity of a method to acquire results in a specific range that are directly proportional to the concentration of the analytes in the sample. Linearity of the method was evaluated by preparing 10 different dilute aliquots (10-100 µg/L) from the mixed standard solution of 100 µg/L. Each of the dilute mixed standards (i.e. the 10-100 µg/L standards) were injected sequentially and in triplicates with a sample blank in-between under same conditions. Then a calibration plot was constructed using quant analysis software.

3.2.8.2 Range

The range of an analytical method is the difference between the lower and higher concentrations of the analytes in a sample. The range of calibration standards for the analytes was chosen based on the LOQ and reported average concentrations of the target analytes found in African water bodies.

3.2.8.3 Specificity

Specificity is the capability to distinguish between the target analytes and other compounds that are present in a sample matrix. There are three methods used to evaluate matrix effects viz, signal-based method, concentration-based method, and the calibration graph method. The signal-based method uses the signal of the analyte in the solvent and in a spiked extract of the sample (post-extraction spiked matrix). Also, the concentration-based method uses the concentration of the analyte in the solvent and in post-extraction spiked matrix. The calibration graph method uses the slope of the calibration curve in the solvent and in the spiked sample (matrix matched calibration graph). The calibration graph method was chosen because it evaluates matrix effects over a wide concentration range. The matrix effect percentage was calculated based on the slopes of the calibration curves of the solvent (MeOH and water), river and wastewater effluent samples (matrix matched calibration curves) (**Equation 3.2**). When the matrix effect percentage is less than 100% the ions of the analytes are supressed, and when the matrix effect percentage is above 100% the ions are enhanced.

$$\%ME = \left(\frac{Slope_{matrix-matched}}{Slope_{solvent}}\right) \times 100 \qquad [3.2]$$

3.2.8.4 Limit of detection and Limit of quantification

The limit of detection (LOD) is the lowest concentration of an analyte that can be accurately detected by an instrument and the limit of quantification (LOQ) is the lowest concentration that can be reliably quantified by the instrument. The LOD and LOQ for CAF, LAM and SULF were determined using linear regression analysis obtained from the calibration curves of each analyte. The LOD and the LOQ were determined based on the standard deviation (SD) of the y-intercept and the slope of the regression line of the calibration curve (**Equation 3.3** and **Equation 3.4**). The analysis was conducted in triplicates to ensure accuracy.

$$LOD = 3.3\left(\frac{SD}{slope}\right) \qquad [3.3]$$

$$LOQ = 10\left(\frac{SD}{slope}\right) \qquad [3.4]$$

3.2.8.5 Precision

Precision is the closeness of results for experiments carried out in the same operating conditions. Method precision was assessed by determining the relative standard deviation (RSD) percentage for repeatability and reproducibility of the method (**Equation 3.5**).

Method repeatability was assessed by injecting a 90 µg/L standard six times the same day under the same flow rate, mobile phase, column temperature and pump pressure. Method reproducibility was also assessed by injecting a 90 µg/L standard six times the same day under the same operating conditions and repeating the same analysis on a separate day.

$$\% \, RSD = \left(\frac{SD}{Mean}\right) \times 100 \qquad [3.5]$$

3.2.8.6 Robustness

Method robustness measures the ability of the method to remain stable under deliberate varying conditions. Robustness proves the reliability of the method during normal application (Vidushi and Meenakshi, 2017). Robustness of the method was tested by calculating the % RSD of varied sample injection volumes (5, 10 and 15 µL) and mobile phase flowrates (0.2, 0.25 and 0.3 µL/min).

3.3 MATERIALS AND METHODS (GIS-MODEL)

3.3.1 Data collection and modelling

The GIS system was built using the (Rice, 2014) approach with minor modifications. Data for water resources was collected from various sources, such as the DWA databases, the Water Research Commission (WRC), Water Resources of South Africa and the Department of Geography (UNISA, Florida Campus). The collected data included locations, coordinate data, name and design capacity of WWTPs (collected from WRC). Data for stream gauges, was collected from the DWA via their website. In addition, municipal and provincial boundaries and hydrography layers were collected from the Department of Geography and Water Resources of South Africa. Subsequently, the data was imported into an ArcMap 10.6.1 software with provincial boundaries as a base layer for the model as illustrated in (**Figure 3.3**). The reference coordinate system for all layers was set to the World Geodetic System 1984 (WGS 1984). After programming the ArcMap software, spatial analysis for rivers that are close to WWTPs was carried out using the GIS model.

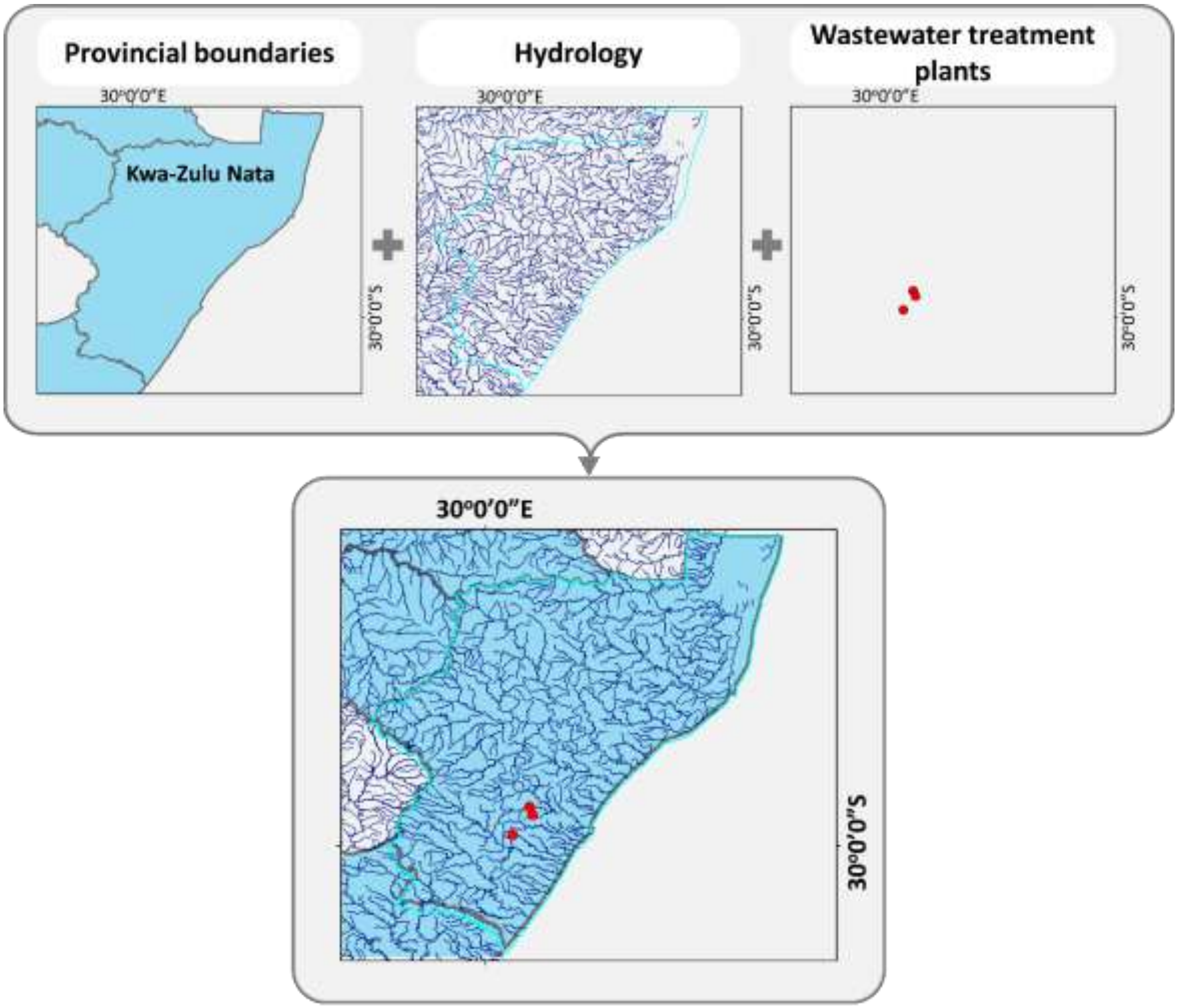

Figure 3.3 GIS vector layers showing provincial boundaries, stream networks and WWTPs.

3.3.2 GIS Model Validation

Herein, the GIS model will be validated with field studies where concentrations of wastewater tracers including (CAF, LAM and SULF) will be used to determine the contribution of the WWTPs to the selected rivers. The results obtained from the model will be compared to the results obtained using the wastewater tracers.

3.3.3 Calculation models for GIS-model and wastewater tracers

According to the DWS there are 1363 registered WWTPs in South Africa (DWS, 2015). Out of the 1363 at least 1226 of the WWTPs do not comply with all the final wastewater effluent standards. Hence, this study focused on 6 WWTPs from 3 provinces of SA. The remaining WWTPs will be analysed in the future. **Figure 3.4** is a representation of a de facto reuse scenario showing the mass balance inputs and outputs. The GIS-model calculations were based on mass balance of the volumetric flows (wastewater effluent flow (Q_x) and the average stream flow (Q_z) as shown in the scenario). In addition, the wastewater tracer calculations were based on the concentrations of the wastewater tracers in WWTP effluent (C_x) and in the river (upstream (C_y) and downstream (C_z)). The calculation model used in this study differs from the calculation model used in the study conducted in the US (Rice, 2014). The study conducted in the US was focused on the upstream wastewater effluent discharge contributions to the raw water intakes of downstream DWTPs, whereas the current study is focused on the contribution of individual WWTPs to raw water supplies.

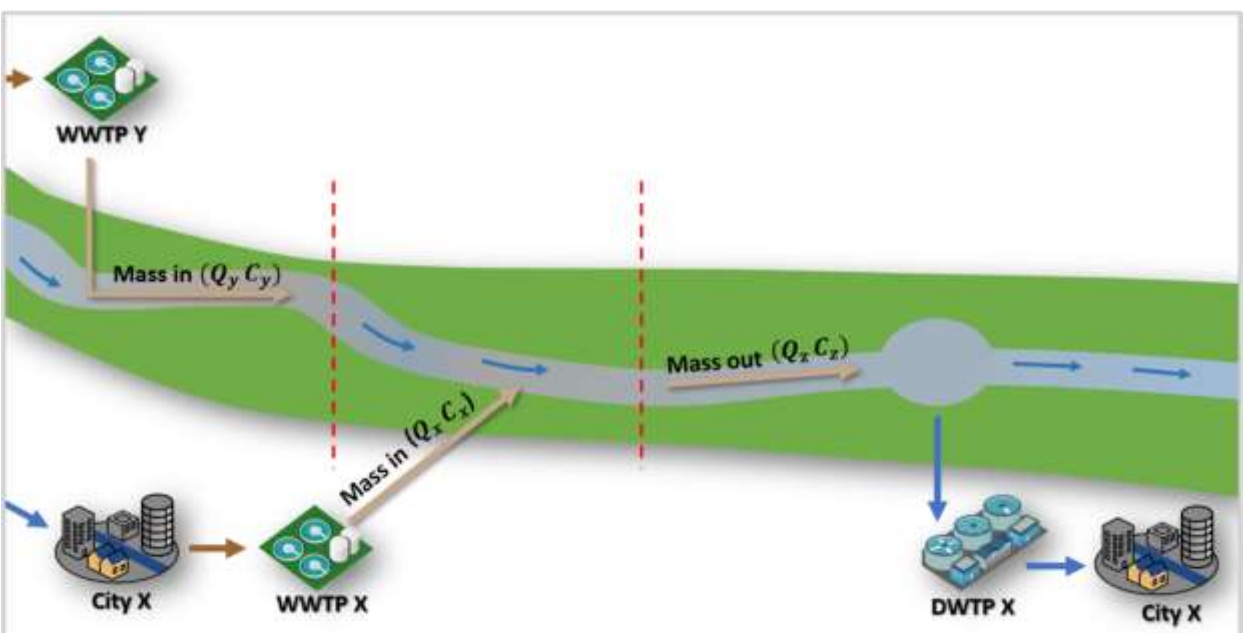

Figure 3.4: De facto reuse scenario

3.3.3.1 Mass balance calculations

Therefore, de facto reuse quantification using GIS-model and wastewater tracers was achieved using **Equation 3.8** and **Equation 3.9**, derived from the overall mass balance and component mass balance (**Equation 3.6** and **3.7** respectively).

Overall mass balance:

$$Q_y + Q_x = Q_z \qquad [3.6]$$

Component balance:

$$Q_y C_y + Q_x C_x = Q_z C_z \qquad [3.7]$$

Quantification of de facto reuse:

$$GIS - model;\ De\ facto\ \% = \frac{Q_x}{Q_z} \times 100 \qquad [3.8]$$

$$Wastewater\ tracers;\ De\ facto\ \% = \frac{C_z - C_y}{C_x - C_y} \times 100 \qquad [3.9]$$

Where:

Q_y is is the river flow before effluent discharge;

Q_x is the wastewater effluent flow;

Q_z is the river flow after effluent discharge;

C_y is the concentration of wastewater tracer in the river before effluent discharge;

C_x is the concentration of wastewater tracer in the effluent; and

C_z is the concentration of wastewater tracer in the river after effluent discharge.

For **Equation 3.8 and Equation 3.9**, the following assumptions were made:

i. The plant design flow is the same as the discharge flow of the WWTP.
ii. There is perfect mixing of wastewater effluent and the river.
iii. The rate of change of properties in the river with time is insignificant

3.4 METHODS FOR PREDICTING OPERATION AND MAINTENANCE COSTS

The O&M costs were estimated using cost functions developed by Tsagarakis *et al.*, (2003). The cost functions used to estimate the O&M costs were based on three categories, conventional, extended aeration with mechanical dewatering and extended aeration with air drying. Where conventional processes included pre-treatment, primary clarifiers, aeration, secondary clarifiers, chlorination, sludge thickening and digestion and mechanical dewatering. The process for extended aeration with mechanical dewatering was similar to conventional process but it did not have digestion. Also, extended aeration with air drying had a similar process with conventional but used drying beds rather than mechanical dewatering. The cost functions were generated from evaluating WWTPs O&M costs and categorising them according to the type of treatment methods used (**Figure 3.5**). Factors considered to evaluate the O&M costs included personnel, energy, chemicals, maintenance, sludge treatment and disposal costs. The cost functions were in the form of $y=ax^b$ where a and b are calculated coefficients and y and x are the O&M costs and PE respectively.

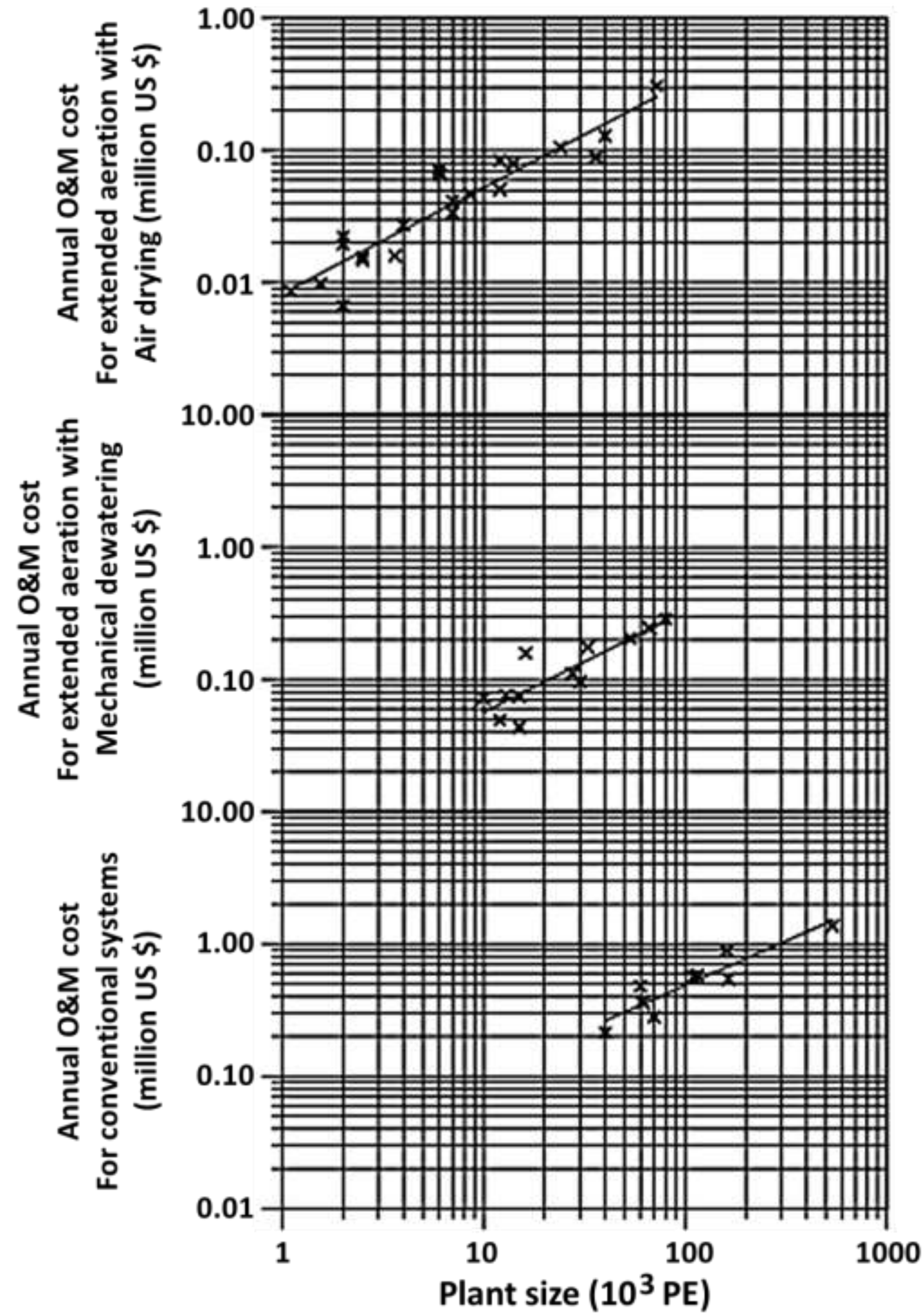

Figure 3.5: Annual O&M costs (Tsagarakis *et al*., 2003)

Therefore, the O&M costs were estimated for three WWTPs in Kwa-Zulu Natal (WWTP3, WWTP4 and WWTP5) categorising them according to their treatment processes. The population served by the WWTPs was taken from the 2011 census and the formulas and R^2 for the cost functions are shown in **Table 3.4.**

Table 3.4: Cost functions for conventional and extended aeration with air drying.

TREATMENT PROCESS	WWTPS	COST FUNCTION	R^2
Conventional	WWTP3	$C_a = 0.022x^{0.672}$	0.84
Extended aeration with air drying	WWTP4	$C_a = 0.0083x^{0.801}$	0.874
	WWTP5	$C_a = 0.0083x^{0.801}$	0.874

3.5 REFERENCES

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CHAPTER 4
OPTIMIZATION AND VALIDATION OF AN LC-MS METHOD FOR THE ANALYSIS OF WASTEWATER TRACERS

4.1 INTRODUCTION

An LC-MS method developed by Wood *et al*. (2015) for quantifying CECs in surface water was optimized and validated for CAF, LAM and SULF. The three analytes of interest are amongst the commonly detected CECs in surface water, and they can be used as wastewater tracers. This chapter discusses the findings for method optimization and validation for the wastewater tracers. Also, the findings for effects of seasonal variations on the concentrations of the wastewater tracers in surface water are discussed.

4.2 METHODOLOGY

The detailed methodology on method optimization and validation is discussed in chapter 3 from section **3.2.3-3.2.8**, page **67-73**.

4.2.1 SPE efficiency

The extraction efficiency of HLB and C-18 SPE cartridges was studied using a Waters Extraction Manifold. Briefly, synthetic water samples with initial analyte concentrations of 50 µg/L and 100 µg/L were prepared in triplicates and the pH was adjusted to 8 (section **3.2.3** page **67**). The extraction was conducted using HLB and C-18 SPE cartridges and the results were compared. The findings revealed the recoveries for CAF obtained using C-18 cartridges were higher than the recoveries obtained using HLB cartridges (**Figure 4.1**). Similar results were recorded on a review on SPE methods for caffeine compiled by Khalik and Abdullah, (2017) where the recovery percentages were 68 % and > 90 % for HLB and C-18 cartridges respectively (extraction solvents: MeOH and water). On the contrary, the findings for LAM showed that the recoveries obtained using HLB cartridges were higher compared to the C-18 cartridge results. Similar results were also obtained in the studies conducted by (Matta *et al.*, 2012) (92.267±5.01% for HLB cartridges). Further, the findings showed low recoveries for both the C-18 and HLB cartridges for SULF. These results were not comparable to results obtained from other studies, this could be because for some compounds the efficiency is higher when the pH of the sample is low, these was observed in the study conducted by Semreen *et al*., (2019).

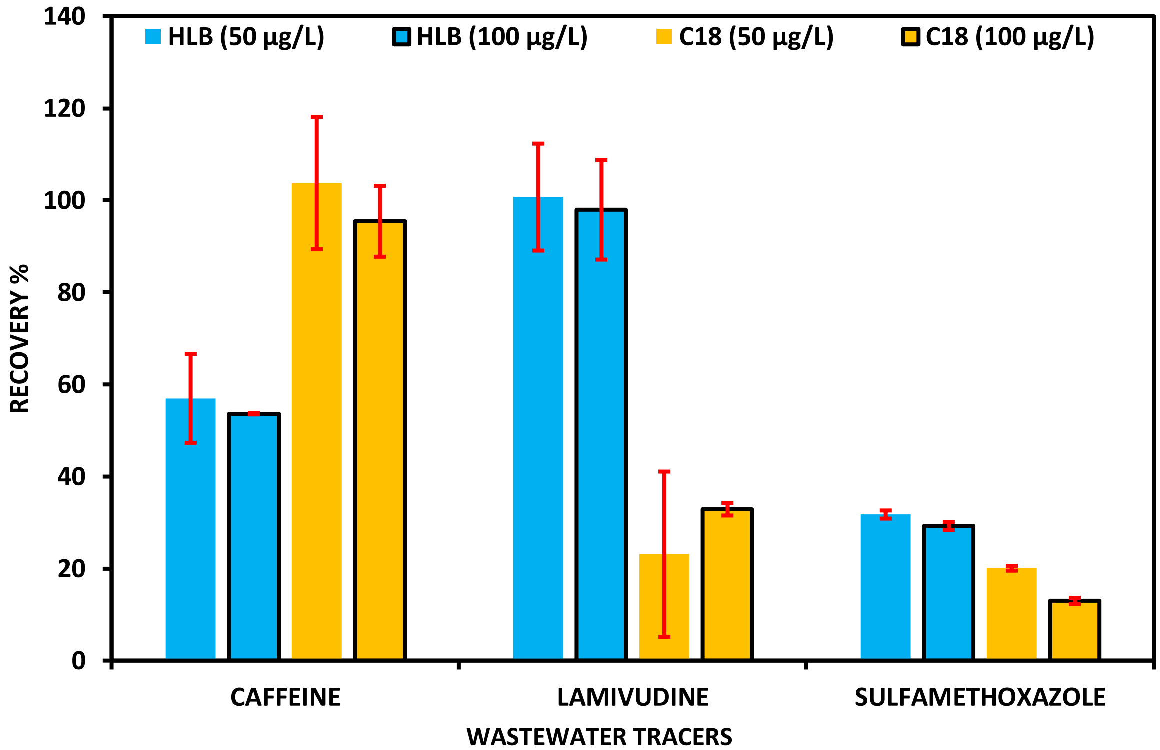
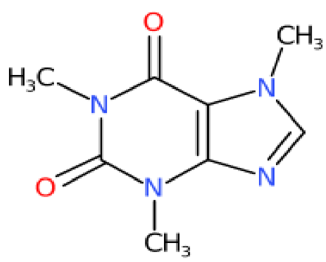
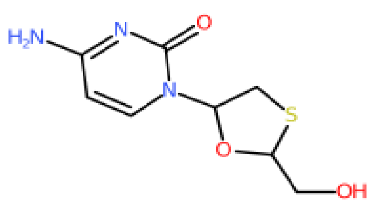
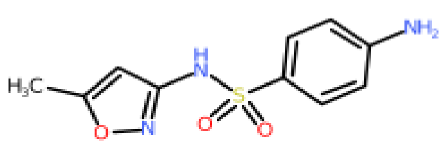


Figure 4.1: Extraction efficiency for HLB and C18 SPE cartridges

4.2.2 LC-MS-MS analysis

Before the method was optimised, the target analytes were first identified using tandem mass spectrometry (MS-MS) at positive ESI mode (see section **3.2.6,** page **69**). The analytes were confirmed by their fragmentation patterns and the fragmentation profile showed the protonated molecular ions of CAF, LAM and SULF with their fragments (**Table 4.1**). The obtained fragments were contrasted with the fragments recorded in the United States EPA's EPISuite data base (US EPA, 2020) and also with those obtained in other similar studies. The fragments for CAF resulted from loss of H_2O, CO, CH_3OH, CH_3CHO, OH, CH_2=C=O, CO_2 and CH_3. Identical results were also generated from the study conducted by Bianco *et al*., (2009). The fragments for LAM are a result of loss of H_2O, NH_3 and HNCO, the same results were obtained by Bedse *et al*., (2009). In addition, the fragments for SULF resulted from the loss of $C_4H_5N_2O$ and O=S=O these results correspond with the results obtained by Trivedi *et al*., (2021).

Table 4.1: LC-MS/MS fragmentation results for CAF, LAM and SULF.

COMP. NAME	MOL. FORMULA	MASS (g/mol)	FRAGMENTS		
			Formular	Mass (g/mol)	Peak m/z
CAF	$C_8H_{10}N_4O_2$	194.08	$[C_3H_4N_2]+$	68.03691	68.036
			$[C_3H_6N_2\text{-}H]+$	69.04474	69.0437
			$[C_4H_4N_2]+H+$	81.04474	81.0438
			$[C_4H_4N_2+H]+H+$	82.05257	82.0514
			$[C_4H_6N_2]+H+$	83.0604	83.0594
			$[C_4H_6N_2O\text{-}H]+$	97.03965	97.0389
			$[C_5H_7N_3\text{-}H]+$	108.05564	108.0546
			$[C_5H_4N_2O]+H+$	109.03965	109.0386
			$[C_5H_7N_3]+H+$	110.0713	110.0701
			$[C_5H_7N_2O]+$	111.05531	111.0541
			$[C_5H_4N_3O]+H+$	123.04272	123.0414
			$[C_6H_7N_3O]+H+$	138.06621	138.0646
			$[C_7H_{10}N_4]+H+$	151.09786	151.0962
LAM	$C_8H_{11}N_3O_3S$	229.052	$[C_3H_4N_2]+H+$	94.03998	94.0399
			$[C_4H_2N_2O]+H+$	95.02399	95.024
			$[C_4H_6OS\text{-}H]+$	101.00558	101.0054
			$[C_4H_4N_3O+H]+H+$	112.05055	112.0504
SULF	$C_{10}H_{11}N_3O_3S$	253.052	$[C_5H_4]+H+$	65.0386	65.0376
			$[C_3H_4NO+H]+H+$	72.04441	72.0436
			$[C_6H4]+H+$	77.0386	77.0377
			$[C_5H_6N]+$	80.0495	80.0486
			$[C_6H_6N]+$	92.0495	92.0484
			$[C_4H_5N_2O+H]+H+$	99.05531	99.0542
			$[C_4H_5N_2O_3S]+$	161.00154	160.9999
			$[C_5H_5N_2O_3S+2H]+H+$	176.02503	176.0258

4.2.3 Method Optimization

The method was optimized by comparing two solvents MeOH and ACN for mobile phase B (MeOH with 0.1%FA and ACN with 0.1%FA) both in ESI+. The original method optimized is provided section **3.2.7** page **70**. The results in **Table 4.2** show that with ACN the analytes were eluted earlier which resulted in lesser retention times compared to MeOH. This is because ACN is less polar therefore, it has higher elution strength (ε°=3.1) than MeOH (ε°=1) (CHROMacademy, 2014). However, with MeOH higher peak areas and (S/N) were observed compared to ACN, this has also been observed in previous studies (Zhou, 2005). Therefore, MeOH was selected as the best solvent for mobile phase B because the (S/N) determines the LOD and improves the specification of the method (Wells *et al*., 2011).

Table 4.2: Peak areas and S/N ratios at different mobile phase B solvents

MOBILE PHASE B	ANALYTES	RT±SD (min)	AV. PEAK AREA	AV. S/N	RESULT
ACN:0.1% FA	LAM	0.90±0.00	1756.1	13.4	Rejected
	CAF	3.77±0.01	34435.15	150.7	
	SULF	5.11±0.01	32219.05	110.3	
MeOH:0.1% FA	LAM	0.95±0.01	35515	25.3	Accepted
	CAF	5.00±0.01	336425	185	
	SULF	5.46±0.01	333206.5	250.45	

4.2.4 Method Validation

4.2.4.1 Linearity and range

The linearity of the method was determined by injecting dilute mixed standards (*n*=10, 10-100 μg/L) of CAF, LAM and SULF in triplicates. An average of two data sets (bracketed average) was used to obtain a line of best fit. The results show that the method is linear because the R^2 is greater than 0.99 for all the analytes (**Figure 4.2**).

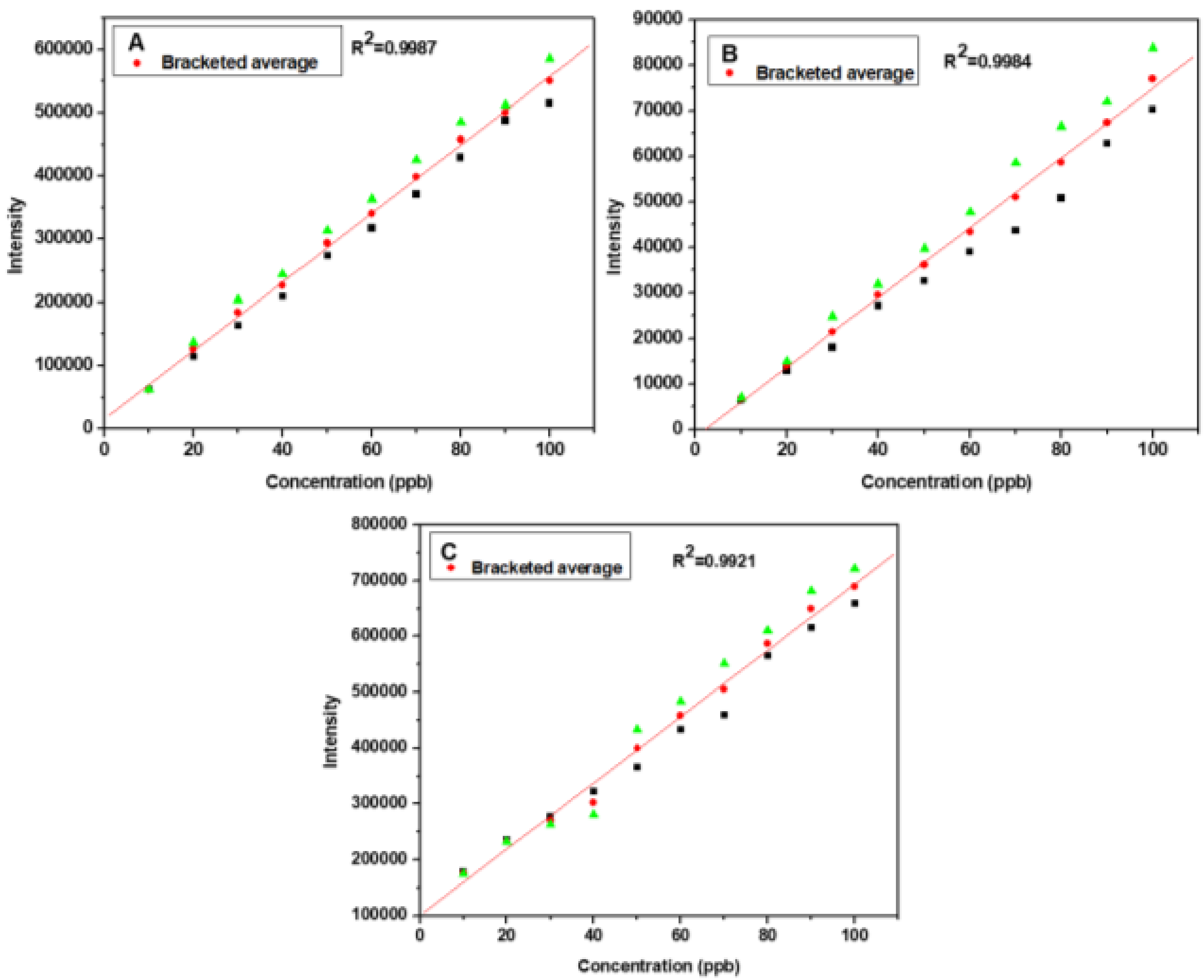


Figure 4.2: Linearity and range for CAF (**A**), LAM (**B**) and SULF (**C**)

4.2.4.2 Matrix effect, LOD and LOQ

The matrix effect percentage was calculated based on the slopes of the calibration curves of the solvent (MeOH and water), river and wastewater effluent samples (matrix matched calibration curves). The calculation methods used to calculate matrix effect are provided in section **3.2.8.3** page **71**. According to the results obtained for CAF, LAM and SULF in both the river and wastewater effluent samples the ions for the analytes were supressed (**Table 4.3**). According to Fang *et al*., (2015) the matrix interferences occur in the ion source in the LC-MS system where they either charge or desolvate the analyte. This results in a change in peak area, peak shape, and retention time.

The LOD and LOQ were determined based on the SD and slope of the calibration curve. According to the results low LOD's and LOQ's were obtained for the analytes (in the solvent) and were within the range (µg/L) of reported results (Al-Qaim *et al*., 2017; De Oliveira *et al*., 2019; Ngumba *et al*., 2016) (**Table 4.3**). Also, it was observed that matrix interferences also affect the LOD and LOQ by either decreasing or increasing it. As shown in **Table 4.3** the LOD and LOQ for CAF is lower in the matrix matched calibration curves (calibration curves for river and wastewater effluent) than in the solvent. On the contrary, the LOD and LOQ for LAM and SULF were higher in the matrix matched calibration than in the solvent.

Table 4.3: Matrix effect, LOD and LOQ results

MATRICES	ANALYTES	LC-MS			
		LOD (µg/L)	LOQ (µg/L)	LINEAR EQUATION	MATRIX EFFECT %
Solvent	CAF	0.34	1.03	*y = 2038x + 114553*	100.00
	LAM	0.06	0.17	*y = 516.16x + 716.98*	100.00
	SULF	0.04	0.14	*y = 3894.8x + 2653.2*	100.00
River	CAF	0.18	0.56	*y = 847.01x + 243856*	41.56
	LAM	0.09	0.28	*y = 376x + 582.39*	72.85
	SULF	0.36	1.09	*y = 3468.6x + 3962.2*	89.06
Wastewater effluent	CAF	0.19	0.57	*y = 1191.2x + 80384*	58.45
	LAM	0.17	0.51	*y = 171.91x + 2344.8*	33.31
	SULF	0.35	1.07	*y = 12.431x + 1577.4*	0.32

4.2.4.3 Precision

The precision and accuracy of the method was determined by injecting a dilute mixed standard solution (90 µg/L containing CAF, LAM and SULF) (*n=6*) on the same day and the same analysis was repeated on a separate day. The relative standard deviation (RSD) percentage was determined from the peak areas (section **3.2.8.5**, page **72**). Further, the mean recovery percentage was calculated based on the nominal and measured concentration of the analytes. According to the results for repeatability and reproducibility the method is precise because the RSD percentage of the peak areas were < 4% and < 11% respectively (**Table 4.4**). Similar results were obtained in the studies conducted by Al-Qaim *et al*., (2017) (for LAM and SULF) and Ngumba *et al*., (2016) (for CAF). The results also reveal that the method is accurate because the mean recovery percentages are between 99.3-101.4%.

Table 4.4: Repeatability and reproducibility

		REPEATABILITY		
CONC. (µg/L)	**ANALYTES**	**RT±SD (min)**	**MEAN RECOVERY (%)**	**RSD (%)**
90	LAM	0.94±0.00	99. 3	3.35
	CAF	4.98±0.01	101.4	3.53
	SULF	5.43±0.01	101.3	3.17
		REPRODUCIBILITY		
CONC. (µg/L)	**ANALYTES**	**RT±SD (min)**		**RSD (%)**
		Day 1	**Day 2**	
90	LAM	0.94±00	0.96±0.02	10.45
	CAF	4.97±00	5.01±0.01	5.07
	SULF	5.42±00	5.47±0.01	6.61

4.2.4.4 Robustness

Method robustness was evaluated by varying injection volumes (5, 10 and 15 µL) and mobile phase flow rates (0.2, 0.25 and 0.3 µL/min). For each analysis a 1 mg/L standard was injected six times and then % RSD was calculated from the average peak areas and the standard deviations. The % RSD for injection volumes ranged from 0.01% to 4.40% and the % RSD for mobile phase flowrates ranged from 0.13% to 5.69% (**Table 4.5**). Therefore, the method was robust because all the % RSDs were within the accepted limit (20%) (UNODC, 2009)

Table 4.5: Method robustness

INJECTION VOLUME		
ANALYTES	**AVERAGE RT±SD (min)**	**%RSD**
5µl		
LAM	1.51±0.01	3.82
CAF	2.27±0.01	0.01
SULF	2.36±0.01	0.20
10µl		
LAM	0.98±0.02	0.73
CAF	2.29±0.01	0.13
SULF	2.38±0.02	0.27
15µl		
LAM	0.96±0.00	4.40
CAF	2.30±0.00	2.12
SULF	2.39±0.01	0.22
MOBILE PHASE FLOWRATE		
0.2µL/min		
LAM	1.13±0.01	5.69
CAF	2.41±0.01	1.05
SULF	2.49±0.01	1.10
0.25µL/min		
LAM	1.40±0.02	1.58
CAF	2.81±0.01	0.88
SULF	2.88±0.01	0.35
0.3µL/min		
LAM	0.97±0.01	0.73
CAF	2.29±0.01	0.13
SULF	2.37±0.01	0.27

4.2.5 Method application in real water samples

After the method was optimized and validated, it was then applied to real water samples collected from five WWTP effluents (WWTP1, WWTP2, WWTP3, WWTP4 and WWTP5). Also the samples were collected from the rivers receiving the effluents namely Diepsloot (tributary of Jukskei) Jukskei, Crocodile, Msunduzi, Ilovu and Donga River. Then the concentrations of the tracers were compared for the different sampling sites. Further, the effects of seasonal variations on the concentrations of CAF, LAM and SULF were assessed. The methods for sample preparation and quantification are provided in section **3.2.3-3.2.4**, page **67-68**.

4.2.5.1 Variations of tracer concentrations

A general outlook of the results for tracer concentrations was deliberated for all the sampling sites. Also, the concentrations of the wastewater tracers were evaluated for the sampling sites. In comparison to all the sampling sites, Diepsloot river reported high concentrations of CAF, LAM, and SULF (367.66±6.63, 342.72±18.42, and 912.10±1.12 µg/L respectively) (**Figure 4.3**). Diepsloot is a small river that is usually contaminated by raw sewage resulting from pipe bursts. Diepsloot river is a tributary of the Jukskei river, it joins the Jukskei river just before WWTP1's discharge point. As a result of the polluted water coming from the Diepsloot river, the Jukskei river upstream also had high concentrations of the tracers (27.07±4.01, 39.40±15.48, and 1.97±0.16 µg/L, CAF, LAM and SULF respectively). Moreover, the wastewater effluent from WWTP1 also contributes to the increase in tracer concentrations in the Jukskei river downstream (63.35±11.01, 44.03±15.89, and 4.00±0.68 µg/L, CAF, LAM and SULF respectively). Furthermore, compared to the rest of the WWTPs, the concentrations of CAF, LAM, and SULF were greater in WWTP1 (72.99±11.00, 25.07±8.90, and 7.80±0.57 µg/L respectively) and WWTP3 (96.36±8.08 and 0.10±0.10g/L only CAF and SULF were quantified). This could be because they both have the largest design capacities, thus, they serve a larger population compared to the rest of the WWTPs.

For each sampling site, the concentrations of LAM, CAF, and SULF were compared. In most of the sampling sites, the concentrations for SULF (0.10±0.10-912.10±1.12 µg/L) and CAF (0.30±2.05-367.66±6.63 µg/L) were higher than LAM (0.54±0.38-342.72±18.40 µg/L). This could be because SULF and CAF have a wide range of applications, hence, their concentrations are high (Wilkinson *et al*., 2022). SULF is an antibiotic used to treat bacterial infections such as urinary tract infections, bronchitis, and prostatitis. CAF, on the other side, is used in coffee, tea, soft drinks, chocolate, cigarettes, and some medications (Walter 2022). While, LAM is specifically used to treat the human immunodeficiency virus (HIV) and the hepatitis B virus (Garcı´a-Trejo *et al*., 2021).

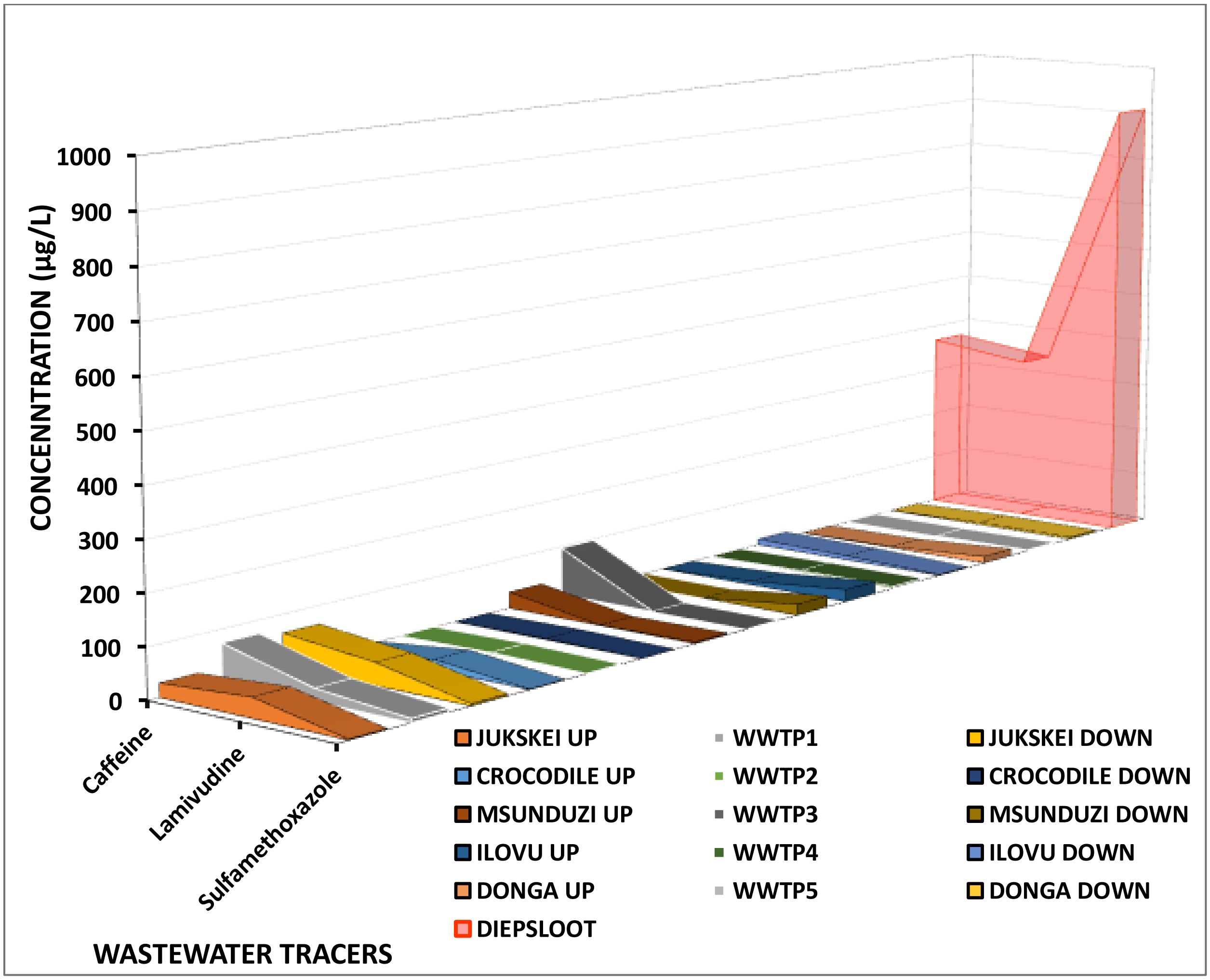


Figure 4.3: Variations of wastewater tracer concentrations.

4.2.5.2 Assessing seasonal variations

The sampling was conducted in winter (June) and spring (October) to assess the effects of seasonal variations on the concentrations of the analytes in the wastewater effluents and rivers. According to the results in winter the concentrations of the analytes were very high in most of the rivers and wastewater effluents compared to the concentrations that were measured in spring (**Figure 4.4 and Figure 4.5**). One of the contributing factors to an increase in concentration in winter is that the percentage of the wastewater effluents in the streams is higher in winter compared to spring because there are low rainfalls in winter. Another contributing factor to the increased concentrations in rivers and effluents in winter is that there is a high consumption of some of these compounds in winter e.g. caffeine consumption in winter is higher than in spring. As a result the concentration of CAF (11.8-96.4 μg/L) is higher in some of the effluents and rivers compared to LAM (2.4-44.0 μg/L) and SULF (0.1- 24.5 μg/L) except for Diepsloot river. The concentrations of CAF, LAM and SULF were extremely high in Diepsloot river (367.7, 342.7 and 912.1 μg/L respectively) because it is one of the rivers highly polluted by raw sewage resulting from sewage pipe bursts. A recent study conducted in Germany where seasonal changes of the concentrations of the emerging pollutants in surface water were studied obtained similar results (Corrêa *et al*., 2021). The author concluded that high concentrations of the emerging pollutants were observed in rivers with low stream flows during dry periods. When there is low rainfall, the river cannot dilute the concentrations discharged by the wastewater effluents. Although in some cases the increase in concentration is a result of agricultural, livestock and industrial activities near the raw water sources (Sun *et al*., 2018).

The concentrations of the wastewater tracers measured in spring were lower than the concentrations measured in winter. This is because in spring the rainfall is higher than in winter and this results in diluted concentrations of the wastewater tracers in the rivers. The concentrations for CAF, LAM and SULF in the wastewater effluents and rivers ranged from 2.1-9.2, 0.5-4.1 and 1.7-10.6 μg/L respectively in spring.

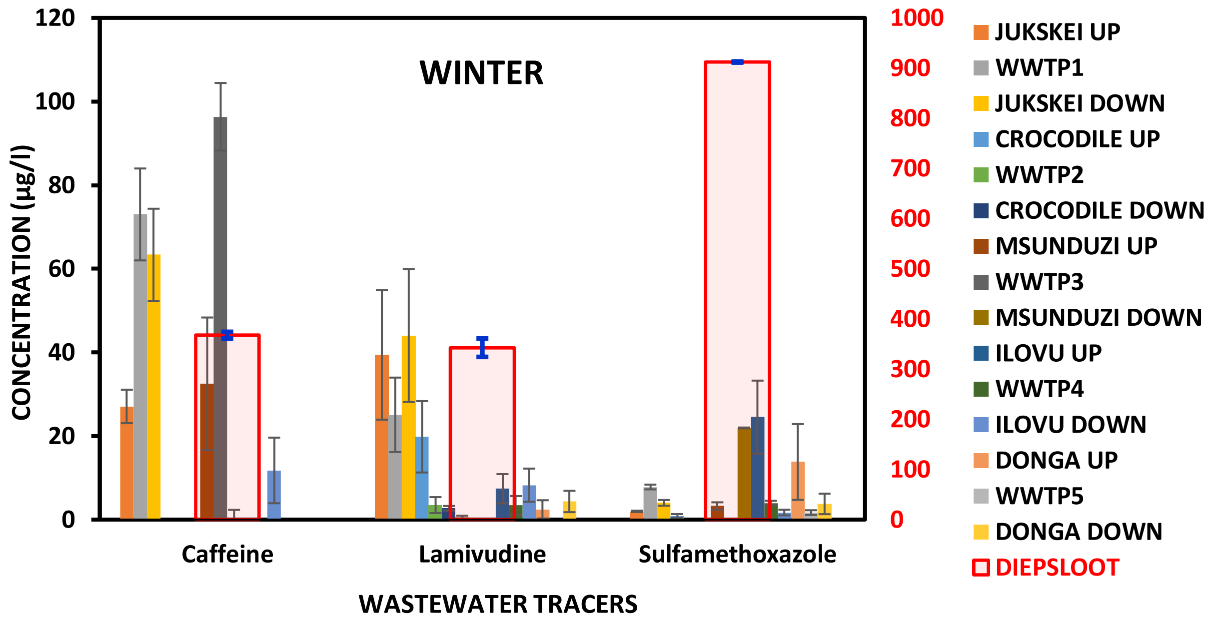


Figure 4.4: Concentrations of wastewater tracers in raw water sources and wastewater effluents in winter.

Figure 4.5 shows concentrations for CAF, LAM and SULF measured in spring the same WWTPs and rivers studied in winter.

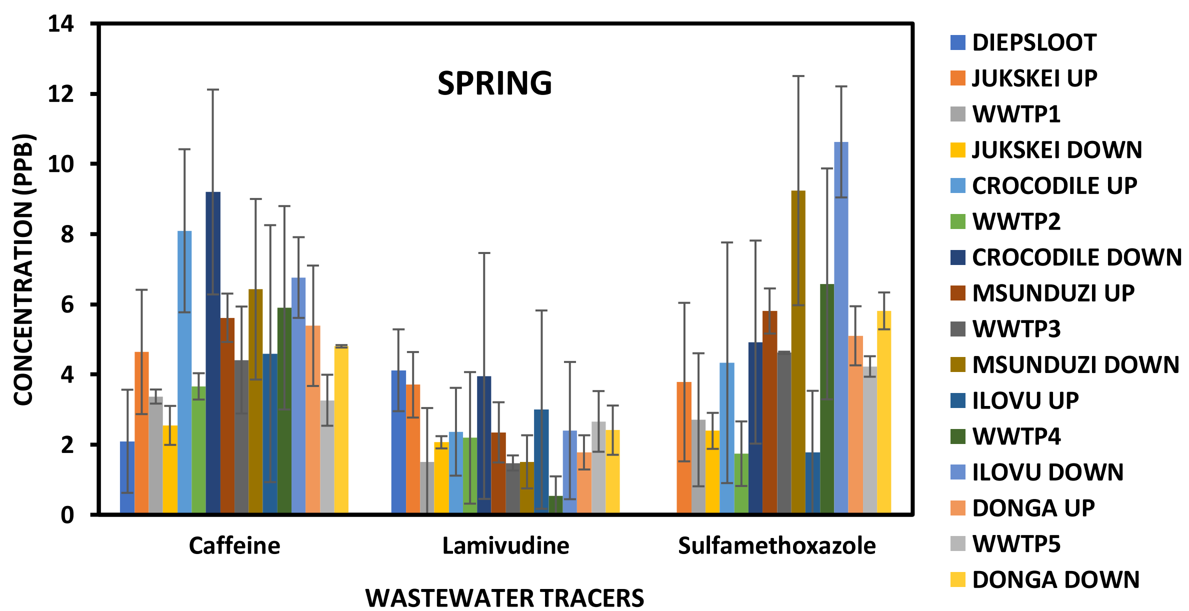


Figure 4.5: Concentrations of wastewater tracers in raw water sources and wastewater effluents in summer.

4.3 CONCLUSION

The optimization experiments have proven that MeOH is more efficient than ACN because it has higher (S/N) and peak area than ACN. Method validation results were obtained based on the linearity, precision, sensitivity, specificity, and robustness of the method. Also, the method is linear because $R^2 > 0.99$ for all the analytes. The results for matrix effect revealed that the sample matrix supressed the ions of the target analytes because the matrix effect percentage is less than 100%. Also, low LODs and LOQs were attained and within the reported concentration range (µg/L). It was also observed that the sample matrix affects the LODs and LOQs by either decreasing or increasing it. The results for repeatability and reproducibility demonstrated that the method is precise because the RSD percentage of the peak areas were < 4% and < 11% respectively. Also, the results revealed that the method is accurate because the mean recovery percentages are between 99.3% and 101.4%. The results for robustness showed that the method is robust because the % RSD for injection volume and mobile phase flowrate were less than 6%.

After optimization validation, the approach was applied to real water samples to assess the levels of occurrence of CAF, LAM, and SULF at various sampling sites. The findings demonstrated that Diepsloot river had the highest levels of the tracer concentrations compared to other sample sites. The data also indicated that SULF and CAF had higher concentrations in majority of the sample sites than LAM due to their multiple applications. This resulted from sewage contamination caused by pipe leakages. Seasonal variations of concentrations of target analytes in wastewater effluents and river were assessed. The findings demonstrated that the concentrations of the target analytes were very high in winter compared to the concentrations that were measured in spring. This is because the percentage of the wastewater effluents in the streams is higher in winter compared to spring because there are low rainfalls in winter.

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CHAPTER 5
DETERMINING THE EXTENT OF DE FACTO REUSE USING A GIS MODEL AND WASTEWATER TRACERS

5.1 INTRODUCTION

Chapter 5 describes the results of de facto reuse quantification using wastewater tracers and a GIS-based model. It also includes findings obtained using a GIS-model for seasonal variations. In addition, a case study on waterways highly impacted by de facto reuse is discussed and estimates of O&M costs for selected WWTPs are discussed.

5.2 METHODOLOGY

The detailed methodology for determining the extent of de facto reuse is discussed in chapter 3 (section **3.3.1-3.4**, page **73-77**). Briefly, the percentage of de facto reuse was determined for 6 WWTPs from Gauteng and Kwa-Zulu Natal and the Western Cape provinces using wastewater tracers and a GIS-method. The method for wastewater tracers was applied to all the WWTPs except WWTP6. As well as the GIS-method was applied to all the WWTPs excluding WWTP5 due to unavailability of data. The studied WWTPs and the rivers they discharge to are listed in **Table 5.1**.

Table 5.1: WWTPs and rivers studied.

WWTPs	PROVINCE	DESIGN CAPACITY (m^3/s)	RECEIVING WATER
WWTP1	Gauteng	4.63	Jukskei river
WWTP2	Gauteng	0.39	Crocodile river
WWTP3	Kwa-Zulu Natal	0.87	Msunduzi river
WWTP4	Kwa-Zulu Natal	0.2×10^{-2}	Donga river
WWTP5	Kwa-Zulu Natal	0.17×10^{-1}	Ilovu river
WWTP6	Western Cape	0.23	Eerste river

5.2.1 Quantification of de facto reuse using wastewater tracers.

The percentage of de facto reuse was determined using three wastewater tracers namely CAF, LAM and SULF (section **3.3.3**, page **75**). De facto reuse percentage was calculated based on the mass balance of the concentrations of the analytes in the rivers before and after the discharge points of the WWTPs and in the wastewater effluent using **Equation 5.1.**

$$De\ facto\ \% = \frac{C_z - C_y}{C_x - C_y} \times 100 \qquad [5.1]$$

Where:

C_y is the concentration of wastewater tracer in the river before effluent discharge;

C_x is the concentration of wastewater tracer in the effluent; and

C_z is the concentration of wastewater tracer in the river after effluent discharge.

The results for the percentage of de facto reuse were calculated using the concentrations of CAF, LAM and SULF as shown in **Figure 5.1**. According to the findings, the percentages obtained using CAF were not reliable because some of the percentages were overpredicted. As shown in **Figure 5.1** the results obtained for WWTP1 and WWTP4 were > 160%. Also, the results obtained using SULF were incorrect because for WWTP1, WWTP3 and WWTP4 the percentage of de facto reuse was over predicted (129.42, 287.94, and 174.8% respectively). In contrast, de facto reuse percentage results obtained using LAM were reliable because percentages for de facto reuse were all less than 100% and were the expected results. In general, the percentage for de facto reuse is expected to be high for WWTPs with large design capacities and low for WWTPs with small design capacities unless. Further, de facto reuse percentage increases as the stream flow decreases. To demonstrate this point, WWTP1 and WWTP5 (discharging to Jukskei and Donga river respectively) have high percentages of de facto reuse (85.49 and 98.24 % respectively). This is due to WWTP1 having a very large design capacity (4.63 m^3/s) and discharging more than 400 000 000 L/d. Conversely, WWTP5 although it has a very small design capacity (0.2×10^{-2} m^3/s), it discharges to a very small river hence the percentage for de facto reuse is high (98.24 %). Additionally, the percentage for de facto reuse determined for WWTP3, was also reliable because WWTP3 has a large design capacity (it discharges a large volume of effluent to the river) therefore the percentage for de facto reuse was high (76.83 %). The percentage for de facto reuse obtained for WWTP2 was low (16.55 %) because WWTP2 is a medium sized WWTP with a design capacity of 0.39 m^3/s discharging to one of SA's largest river (Crocodile river). Therefore, because of the large size of the river the percentage of the effluent discharged by WWTP2 to the river will be low. Also, WWTP4 has low percentage of de facto reuse (24.02 %) because it has a small design capacity (0.17×10^{-1} m^3/s).

De facto reuse percentage for the selected WWTPs was calculated based on the mass balance of the concentrations of the wastewater tracers (CAF, LAM and SULF) in the wastewater effluents and the rivers they discharge to. The results for de facto reuse percentage obtained using CAF and SULF were inconsistent because CAF and SULF have a higher rate of degradation because their half-lives in river are 2.279×10^7 and 9.747E$\times 10^8$ hours respectively (US EPA, 2020). When a compound is unstable in water it will yield incorrect mass balance results because when a compound degrades, its concentration decreases. In addition, the results obtained using LAM were reliable because it has a low rate of degradation (half-life in a river is 1.575×10^{14} hours) (US EPA, 2020). Although all these compounds are good wastewater tracers (because they can be detected and quantified in surface water at low concentrations (μg/L**)),** accurate quantification of de facto reuse requires a stable compound in surface water.

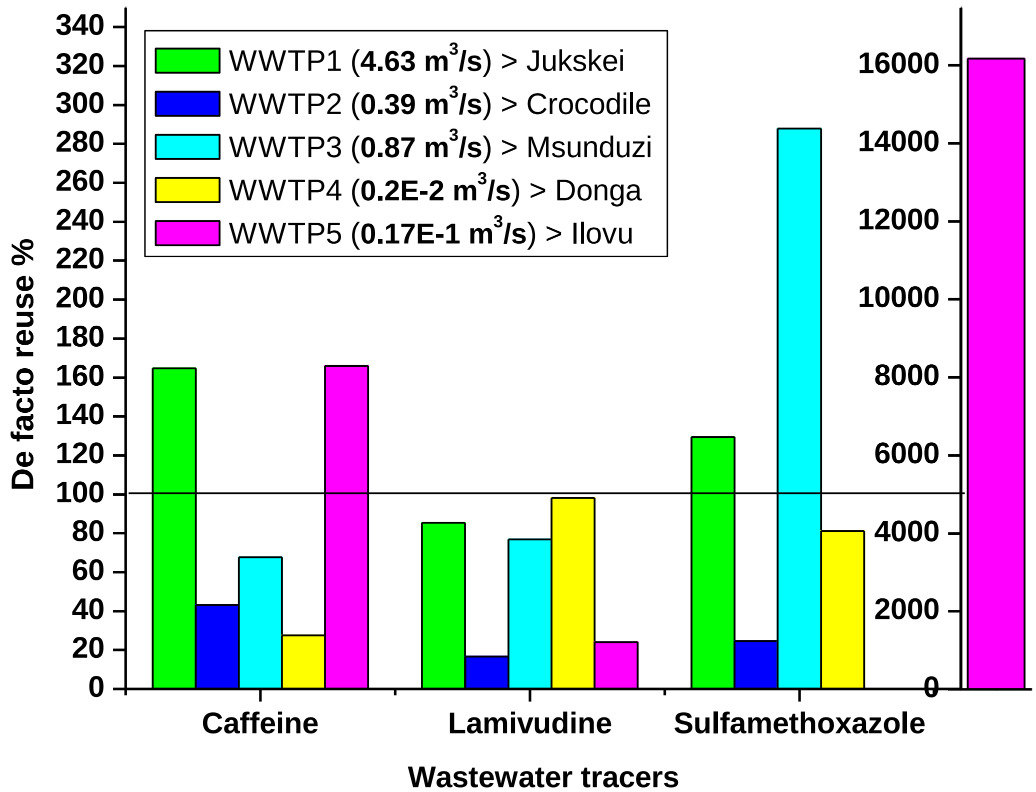

Figure 5.1: De facto reuse quantification using wastewater tracers.

5.2.2 Quantification of de facto reuse using GIS-model.

The percentage of de facto reuse was also determined using a GIS-based model for WWTP1, WWTP2, WWTP3, WWTP4, and WWTP6. Design capacities, yearly stream flow data and vector layers were collected from the DWA, Water Resources of South Africa and WRC databases (section **3.3.1**, page **73-74**). The data was incorporated to an ArcMap 10.6.1 software to do spatial analysis for the WWTPs and the rivers they discharge to. Then the percentage for de facto reuse was calculated using **Equation 5.2**.

$$De\ facto\ \% = \frac{Q_x}{Q_z} \times 100 \qquad [5.2]$$

Where:

Q_x is the wastewater effluent flow;

Q_z is the river flow after effluent discharge.

A yearly average of de facto reuse trends (from 2009 - 2018) was determined for WWTP1, WWTP2, WWTP3, WWTP4, and WWTP6 using a GIS-model (**Figure 5.2**). Throughout the 10 years WWTP1 had the highest de facto reuse percentage (ranging from 62.75 - 107.94 %). In addition, WWTP2 has the lowest discharge percentage (ranging from 1.69 - 6.69 %) throughout the 10 years. The results obtained for WWTP3 and WWTP4 were low (ranging from 12.58 - 39.59 % and 4.28 - 51.13 % respectively). From 2009 - 2015 the percentage for de facto reuse from WWTP6 ranged from 9.14 - 30.98 %. Further, from 2016 - 2017 the percentage for de facto reuse increased significantly (92% and 60% respectively) due to a draught which began in 2015 and ended in 2017. According to Ziervogel, (2019) the water levels in the rivers and dams were significantly low, therefore the contribution of the wastewater effluent in the river was more than the raw water supply of the river.

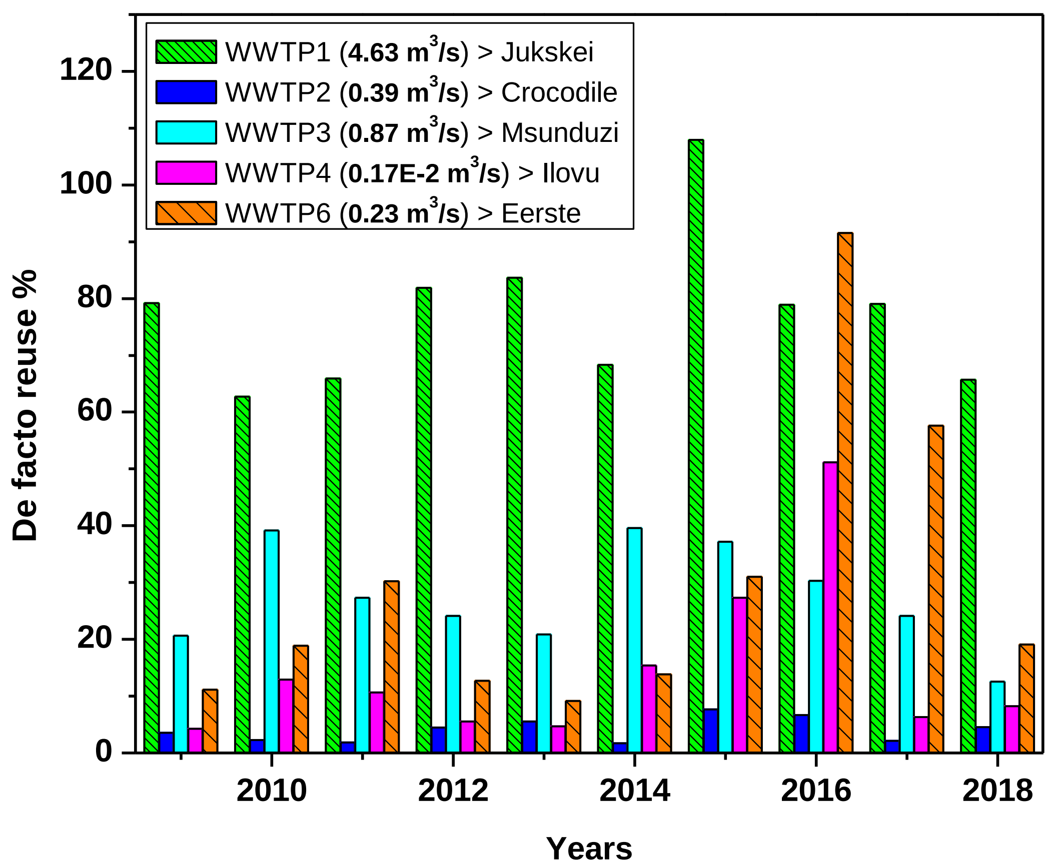

Figure 5.2: De facto reuse trends from 2009-2018.

5.2.3 Validation of GIS model with LAM

The results for de facto reuse percentage obtained using LAM were compared to the results obtained using a GIS-model (**Figure 5.3**). Due to unavailability of stream flow data (for 2019) matching the same date when field studies were conducted for LAM, the results for de facto reuse obtained using LAM were compared to the 10-year average obtained using a GIS-model. As shown in **Figure 5.3** both methods follow a similar pattern for all the WWTPs. In both methods, WWTP1 has a high percentage for de facto reuse (85.49 % (LAM) and 77.32 % (GIS-model)). The results for WWTP2 obtained using LAM and GIS-model were both low (4.04 and 16.55 % respectively). Further, low de facto reuse percentages were obtained for WWTP4 in both methods (14.65 and 24.02 % for LAM and GIS-model respectively). Although the results follow the same pattern for both methods there was a huge gap in the results obtained for WWTP3. The percentage for de facto reuse was high (76.83 %) when using LAM and low when using the GIS-model (27.60 %). This could be influenced by high stream flows in Msunduzi river during summer, because the higher the stream flow the less the percentage of the wastewater effluent in the river.

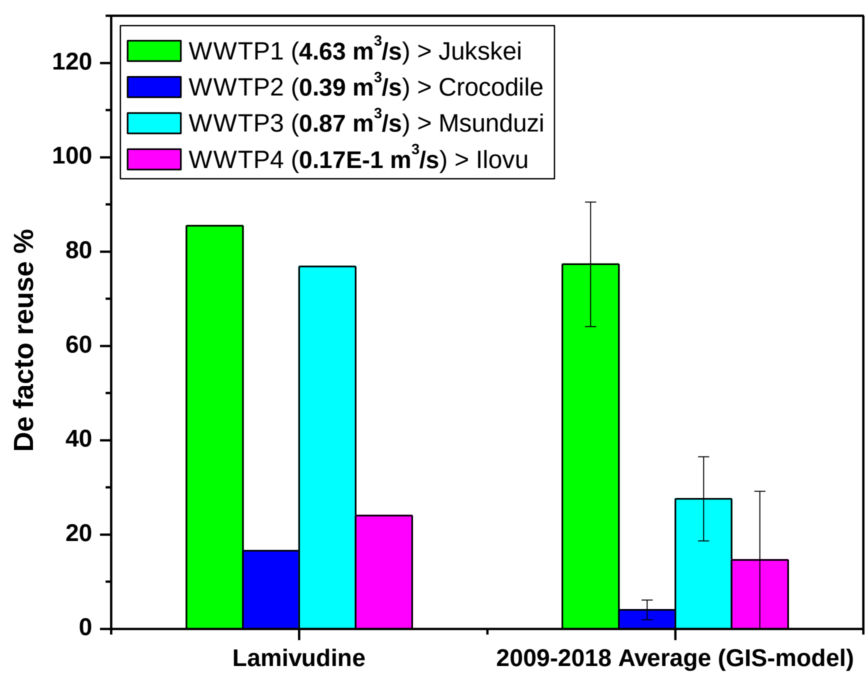

Figure 5.3: Validation of GIS-model with LAM.

5.2.4 Effects of climate change and seasonal variations

The extent of de facto reuse can also be influenced by climate change or seasonal variations. Usually, during summer the stream flows are high because of increased rainfalls, this results in low percentage of de facto reuse. Further, in winter there are low stream flows resulting in a high percentage for de facto reuse. Therefore, seasonal variations for de facto reuse percentage from 2009-2016 were compared for WWTP1, WWTP2, WWTP3, WWTP4, and WWTP6 using a GIS-model. According to the results shown in **Figure 5.4** the percentage for de facto reuse in summer was lower than winter for WWTP1, WWTP2, WWTP3, and WWTP4. On the contrary, the percentages for de facto reuse obtained for WWTP6 were high in summer and low in winter. This is because WWTP6 is in the Western Cape province where the climate is Mediterranean with hot, dry summers and mild, humid winters therefore, rainfall is low during summer and high during winter.

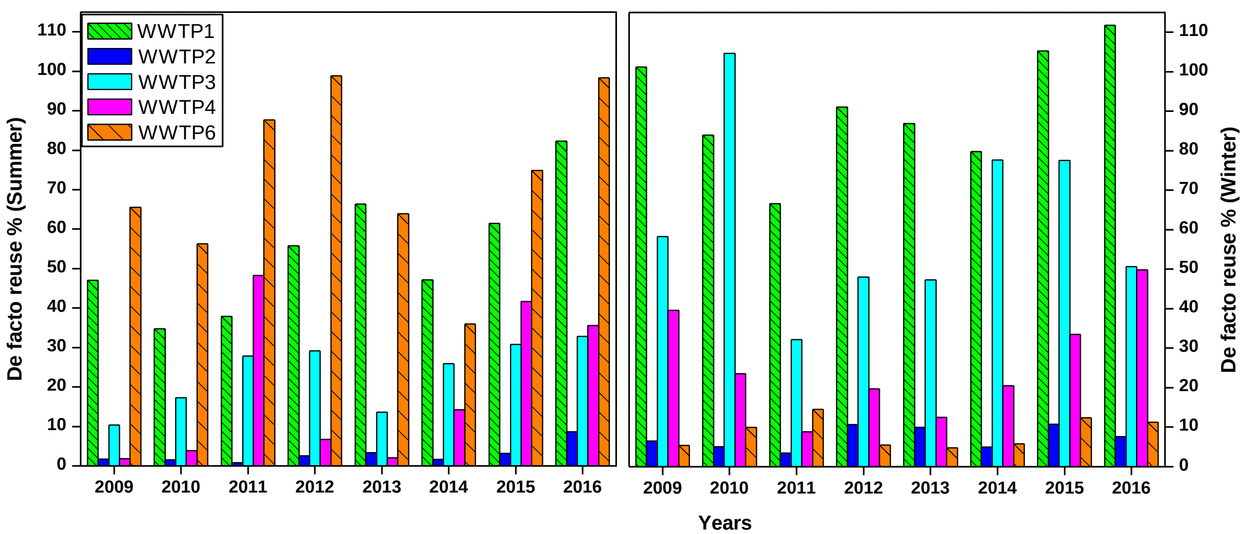

Figure 5.4: Seasonal variations in de facto reuse trends.

5.2.5 Case study

5.2.5.1 Description of the river

A case study was conducted in one of the rivers mostly impacted by de facto reuse and other forms of pollution viz the Jukskei river. The Jukskei river is located in the A21C Quaternary catchment (**Figure 5.5**). The catchment area is 760km^2 and it includes 5 major perennials namely, Little-Jukskei river, Braamfontein spruit, Sandspruit, Upper-Jukskei river, and Modderfontein spruit (Mitchell *et al*., 2014). The Jukskei river flows from the northern side and through highly populated and industrialized areas of the Gauteng province before it flows to the crocodile river (Sibali *et al.*, 2010). It is one of Gauteng's large rivers highly polluted with industrial, mining, raw and treated sewage, agricultural chemicals and chemical discharges (Jardine-Da Silva, 2016). Water quality of the Jukskei river is also influenced by the water coming from its tributaries (Rimayi *et al*., 2019).

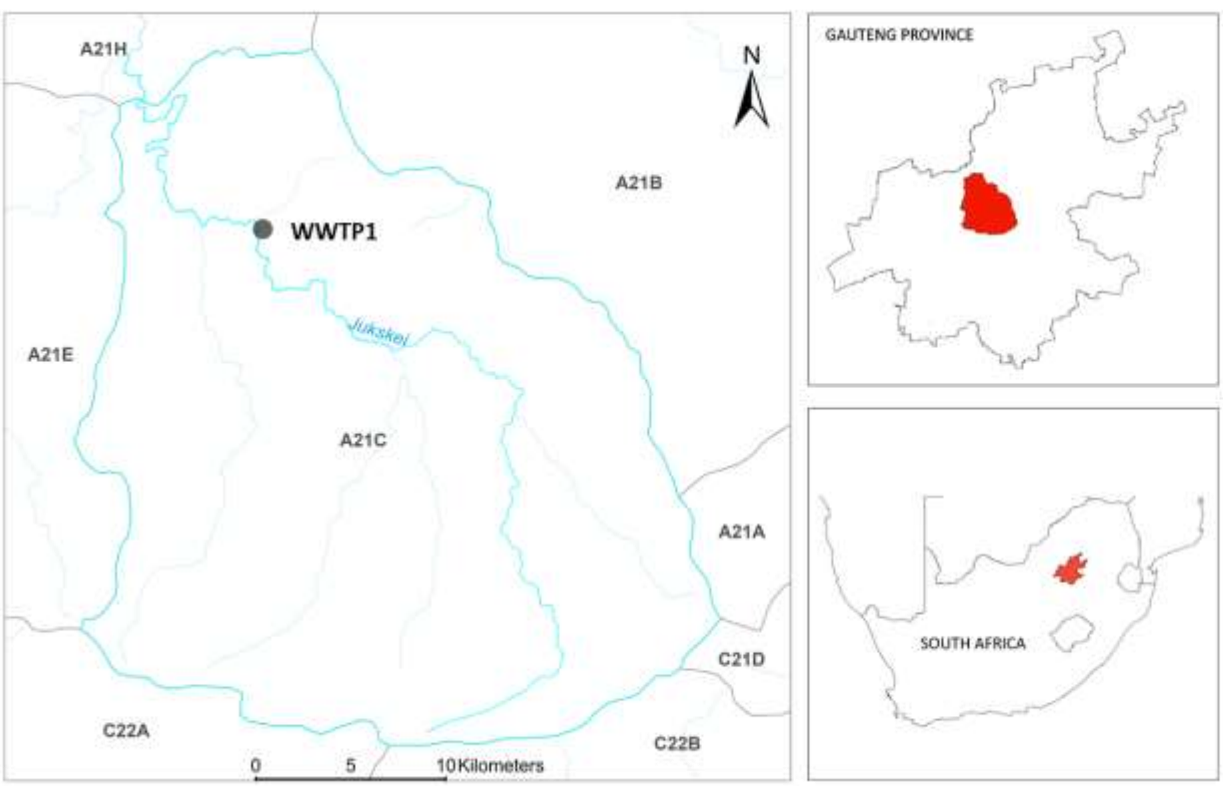

Figure 5.5: GIS map layer of the Jukskei river catchment (extracted from water resources of South Africa).

5.2.5.2 Factors contributing to high levels of de facto reuse in the Jukskei river.

One of the contributing factors to high levels of de facto reuse is urbanization and population growth. Population growth and urbanisation increase the demand for fresh water supply resulting in large amounts of wastewater generated. Gauteng is the smallest province of South Africa and yet has the largest share of the total population (Gauteng Provincial Government, 2020). One of the contributing factors to increasing population in Gauteng is that it is the economic powerhouse of SA. It accounts for more than 34% of the GDP of South Africa (Gauteng Provincial Government, 2020). Cross-border and internal migration is also the reason for population growth in Gauteng. Also, the Gauteng province has the highest rate of migration relative to the rest of the provinces of South Africa (Gauteng Provincial Government, 2020). As a result of its dense population WWTP1 was built and it has been reported to be the largest in the continent (Kamika *et al*., 2014). WWTP1 has been functioning for more than 50 years, primarily it began with one unit, and it now has 5 units. The WWTP treats more than 400 million litres per day (ML/d) of domestic wastewater from the north of the Hillbrow ridge, including Alexandra, Randburg, Sandton and several regions of Midrand and Roodeplaat and discharges to the Jukskei river (Kamika *et al*., 2014).

5.2.5.3 Other factors contributing to pollution of the Jukskei river.

The Jukskei river is one of the mostly studied rivers because it is amongst highly polluted rivers in Gauteng (Rimayi *et al*., 2019). According to Rimayi *et al*., (2019) more than 200 emerging compounds were detected in Jukskei and Hennops river. Amongst the compounds detected were pesticides, PPCPs, drugs of abuse and their metabolites. Mitchell *et al.*, (2014) reported increased levels of Phosphates and Ammonia in the Jukskei river during the period of 2012-2013. Other additional factors contributing to pollution of the Jukskei river are industrial, mining, raw and treated sewage, chemical discharges, and agricultural chemicals (Jardine-Da Silva, 2016). According to Jardine-Da Silva, (2016) several challenges in the Jukskei river result from increasing urbanization in Alexandra Township. Resettling policies have been executed to manage the population of the area, however, the population was constantly increasing due to the availability of affordable housing. As a result, further pollution of the Jukskei river comes from poor sanitation and doing laundry along the riverbanks.

Some of the pollution in the Jukskei river results from water coming from its tributaries such as Diepsloot river. Diepsloot river is highly polluted with raw sewage from sewage pipe burst coming from Diepsloot. The river continually flows with raw sewage and joins the Jukskei river before the point of discharge of WWTP1. Therefore, water quality parameters such as DO, EC, pH, and TOC were measured. The measurements were conducted onsite upstream of the Jukskei river (before it is joined by Diepsloot river), in Diepsloot river, in WWTP1 effluent and downstream of the Jukskei river (**Figure 5.6**). According to the results the DO was very low (3.9 mg/L) in Diepsloot river which also affected the DO levels downstream of the Jukskei river (5.07 mg/L) because in the wastewater effluent the DO levels were within the limit (8.3 mg/L). Also the EC for Diepsloot river was very high compared to that of the wastewater effluent (0.73 and 0.53 mS/cm respectively) although they were both beyond the limit for water quality standards. All pH measurements were within the water quality limit, however, in Diepsloot river the pH was to some extent lower than the values in the wastewater effluents and in the Jukskei river (upstream and downstream). Moreover, high TOC levels were measured in Diepsloot river (5.94 mg/L) compared to the levels in the Jukskei river (upstream and downstream were 2.09 and 2.16 mg/L respectively) and effluent from WWTP1 (2.3 mg/L). Therefore, some of the pollution in the Jukskei river comes from its tributaries.

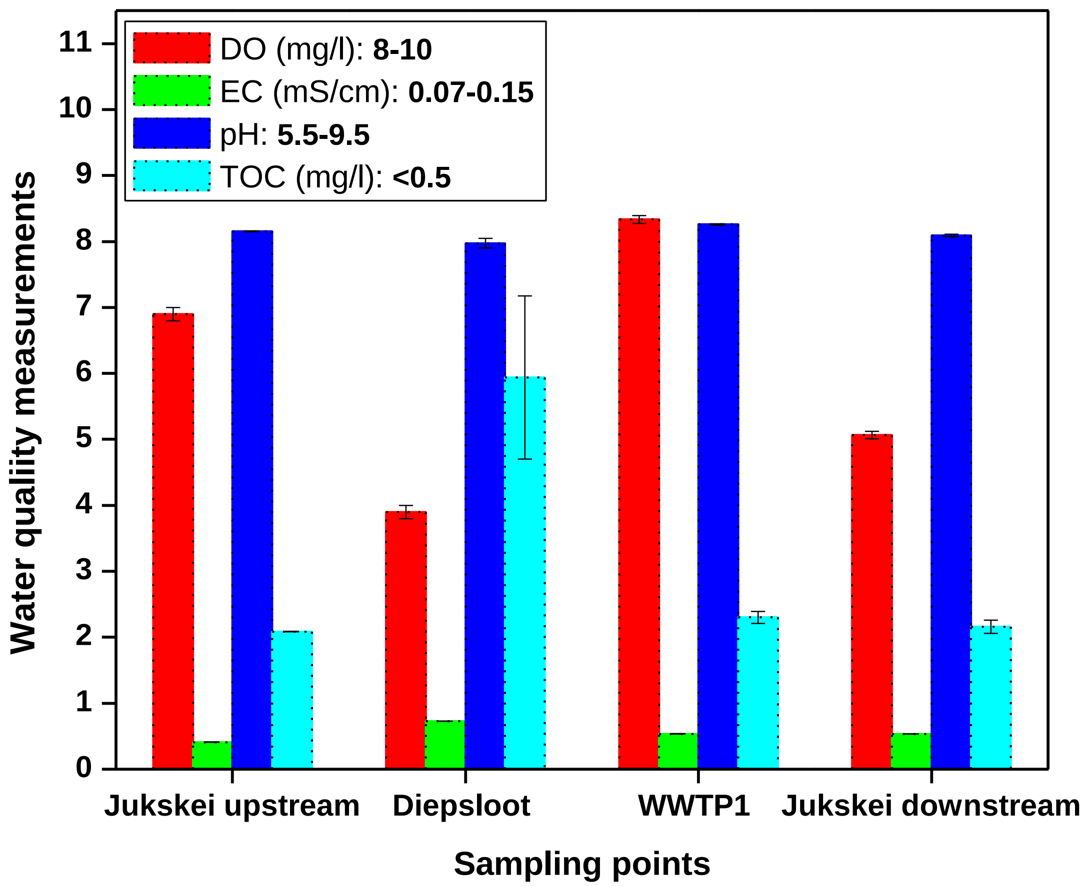

Figure 5.6: Water quality measurements in Jukskei river, Diepsloot river and WWTP1 effluent.

5.2.6 Operation and Maintenance costs of a Wastewater treatment plant.

The sustainability of WWTPs was assessed by calculating O&M costs. O&M costs are those costs related to the operation, maintenance, and management of the wastewater treatment facility. O&M costs can amount to 50% of the overall annual costs, therefore, they are important for deciding on the efficiency of the process and technologies used.

The O&M costs were predicted for the three WWTPs in Kwa-Zulu Natal (WWTP3, WWTP4 and WWTP5). The calculations were based on PE (expressed as 120 g chemical oxygen demand per person/day) because organic pollution loads are better associated with operational costs and energy usage. According to the findings for a PE of 617 772, the O&M costs for a conventional activated sludge at WWTP3 are R 171.34 per person per year. The results show that O&M costs for conventional activated sludge processes are high compared to activated sludge processes with extended aeration with air drying (**Table 5.2**). Large-sized conventional activated sludge processes need higher O&M costs per PE compared to extended aeration processes because they need to hire more personnel per PE and require more energy. Also, more professionals are hired requiring higher wages. WWTP4 and WWTP5 require low O&M costs (R 5.53 and 1.69 per person per year, respectively) because of economy of scale.

Table 5.2: Estimated O&M costs.

TREATMENT PROCESS	WWTPS	PE	COST FUNCTION	R^2	O&M COSTS R (person/year)
Conventional	WWTP3	617 772	$C_a = 0.022x^{0.672}$	0.84	171.34
Extended aeration with air drying	WWTP4	3 349	$C_a = 0.0083x^{0.801}$	0.874	5.53
	WWTP5	764	$C_a = 0.0083x^{0.801}$	0.874	1.69

5.3 CONCLUSION

De facto reuse was determined using CAF, LAM and SULF and the results were compared. The results revealed that LAM is a suitable wastewater tracer for quantification of de facto reuse because LAM has a low degradation rate. A yearly average of de facto reuse trends (10 years) was determined using a GIS-model and the results generated were precise because they corresponded with the design capacities of the WWTPs and the size of the rivers they discharged to. The results obtained using LAM and GIS-model were similar, except for results generated for WWTP3. The huge gap in the results for WWTP3 is influenced by high summer stream flows in Msunduzi river because the higher the stream flow the less the percentage of the wastewater effluent in the river. In addition, a case study was conducted for one of the rivers highly impacted by de facto reuse (the Jukskei river). The findings showed that the high levels of de facto reuse are a result of population growth and urbanization (resulting from high rate of a cross-border and internal migration) because these factors influence the design capacity and discharge volume of

WWTPs. O&M costs were also predicted for the three WWTPs in Kwa-Zulu Natal based on PE. The results showed that the O&M costs are high for a conventional activated sludge WWTPs (WWTP3) than extended aeration with air drying WWTPs (WWTP4 and WWTP5) due to economy of scale.

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CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The aim this study was to quantify de facto reuse in selected cities of SA. To achieve this the study set out to develop an LC-MS method for detecting and quantifying concentrations of three wastewater tracers in surface water. After the wastewater tracer method was optimized and validated, a GIS-based method was developed to quantify de facto reuse and validated using the wastewater tracers. Further, a case study for a city highly impacted by de facto reuse was conducted.

The following conclusions were thus drawn from the study methodology:

- For method optimization, two solvents (MeOH and ACN) with 0.1 % FA were utilised as mobile phase B solvents. When ACN was employed as the mobile phase B solvent, the analytes displayed lower retention times when compared to MeOH. However, MeOH produced greater (S/N) for the analytes than ACN, hence MeOH was elected as the ideal solvent for the analytes. Method validation was determined by evaluating the linearity, specificity, LOD, LOQ, accuracy, and robustness of the method. The calibration curves demonstrated high linearity ($R^2 > 0.99$) for all analytes. The data for matrix effect revealed that the sample matrix suppressed the ions of the target analytes because the matrix effect percentage was less than 100%. In addition, low LODs and LOQs were observed, and were within the reported concentration range (g/L). It was also observed that the sample matrix influenced the LODs and LOQs by either decreasing or increasing them. Findings for repeatability and reproducibility demonstrated that the method was precise because the %RSDs of the peak areas were less than 4% and 11%, respectively. In addition, the findings showed that the method is accurate, with mean recovery percentages ranging from 99.3% to 101.4%. The findings on robustness displayed that the method is robust because the %RSDs for volume injection and mobile phase flow rate were less than 6%.

- After the method was validated, it was applied to real water samples to determine the levels of occurrence of CAF, LAM, and SULF at different sampling sites. The results showed that the tracer concentrations were higher in the Diepsloot river compared to other sampling sites. This was due to sewage pollution resulting from pipe bursts. Furthermore, due to their many uses, SULF and CAF showed higher concentrations in the majority of the sample locations than LAM. Also, seasonal fluctuations in the concentrations of the target analytes were assessed for the different sampling areas. The findings demonstrated that the concentrations of the target analytes were higher in winter than in spring. This was because the proportion of wastewater effluents in the streams was higher in the winter than in the spring due to decreased rainfall. During low rainfall seasons, rivers are unable to dilute the quantities of wastewater, and this results in higher concentrations of the wastewater tracers in the rivers.

- Further, de facto reuse was successfully determined using wastewater tracers and a GIS-model. The results for de facto reuse quantification using wastewater tracers, LAM is a more suitable tracer because it is more stable in river water, therefore, it yielded an accurate mass balance.

- De facto reuse trends were also examined (from 2009-2018) using a GIS-model, with the findings revealing that WWTP1 had the highest proportion of de facto reuse compared to WWTP2, WWTP3, WWTP4, and WWTP6. In addition, there was a significant rise in de facto reuse in WWTP6 (from 2016–2017) that was a result of drought that began in 2015 and ended in the beginning of 2018. This demonstrated that the GIS model can be used to quantify de facto reuse.

- The results for de facto reuse obtained using LAM were compared to the GIS-model to validate the GIS-model. According to the findings, both methods followed a similar pattern, WWTP1 had the highest percentage and WWTP2 had the lowest de facto reuse percentage in both methods. Although, both methods followed a similar pattern, there was a huge gap in the results obtained for WWTP3. The huge gap could be a result of high stream flows in Msunduzi river during summer because the higher the stream flow the less the percentage of the wastewater effluent in the river.

- Seasonal variations for de facto reuse trends were compared from 2009-2016 (based on monthly stream flow availability). According to the results the percentage for de facto reuse in summer was lower than winter for all the WWTPs except WWTP6. De facto reuse percentages obtained for WWTP6 were high in summer and low in winter because the climate in WWTP6 is Mediterranean, with low summer rainfalls and high winter rainfalls.

- The investigations of the case study demonstrated that the causes for a high percentage of de facto reuse in WWTP1 are due to population growth and urbanization. Population growth and urbanization influence the design capacity and discharge volume of WWTPs. In addition, the O&M costs were also predicted for the three WWTPs in Kwa-Zulu Natal based on PE and the results showed that the O&M costs are influenced mainly by economies of scale.

6.2 RECOMMENDATIONS

- Future research should focus on pharmacotoxicology and long-term effects of de facto reuse to human health and the environment. In addition, more research should be focused on developing economic methods that can be combined with the current conventional treatment methods to reduce the CEC loads in wastewater effluents.

- Also, de facto reuse should be determined for all the rivers in South Africa using a GIS-model as this will assist in the knowledge of the quality of water resources at national level. Further, there are numerous CECs to be explored that can be used as wastewater tracers for validating the GIS-model, that are more stable in surface water and can yield more accurate results.

- Future studies can focus in using a GIS method to predict concentrations of pollutants in raw water supplies, because the current methods used to quantify CEC concentrations are costly and time consuming.

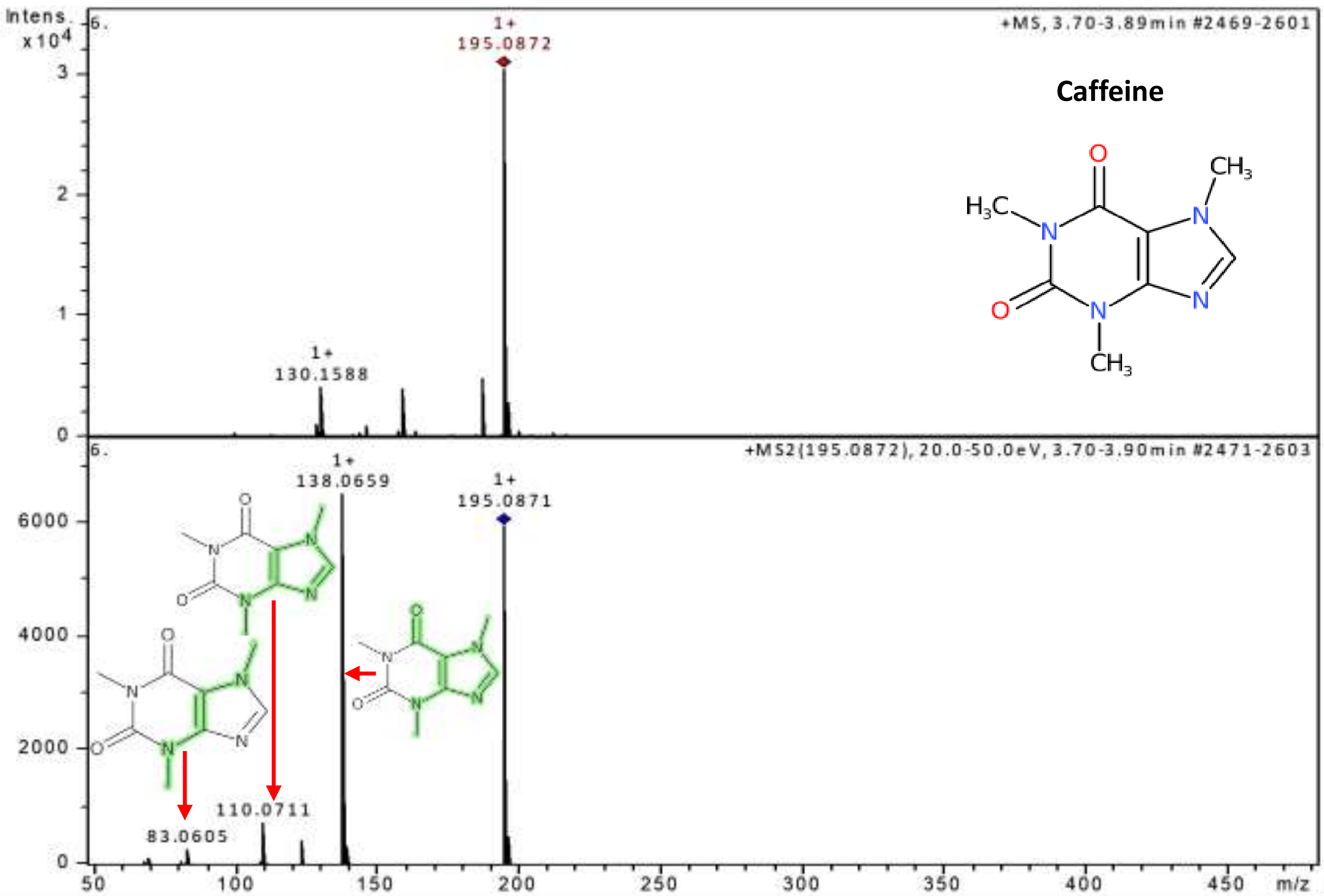

Figure A1: MS-MS spectra for CAF and its fragments (ESI+ mode).

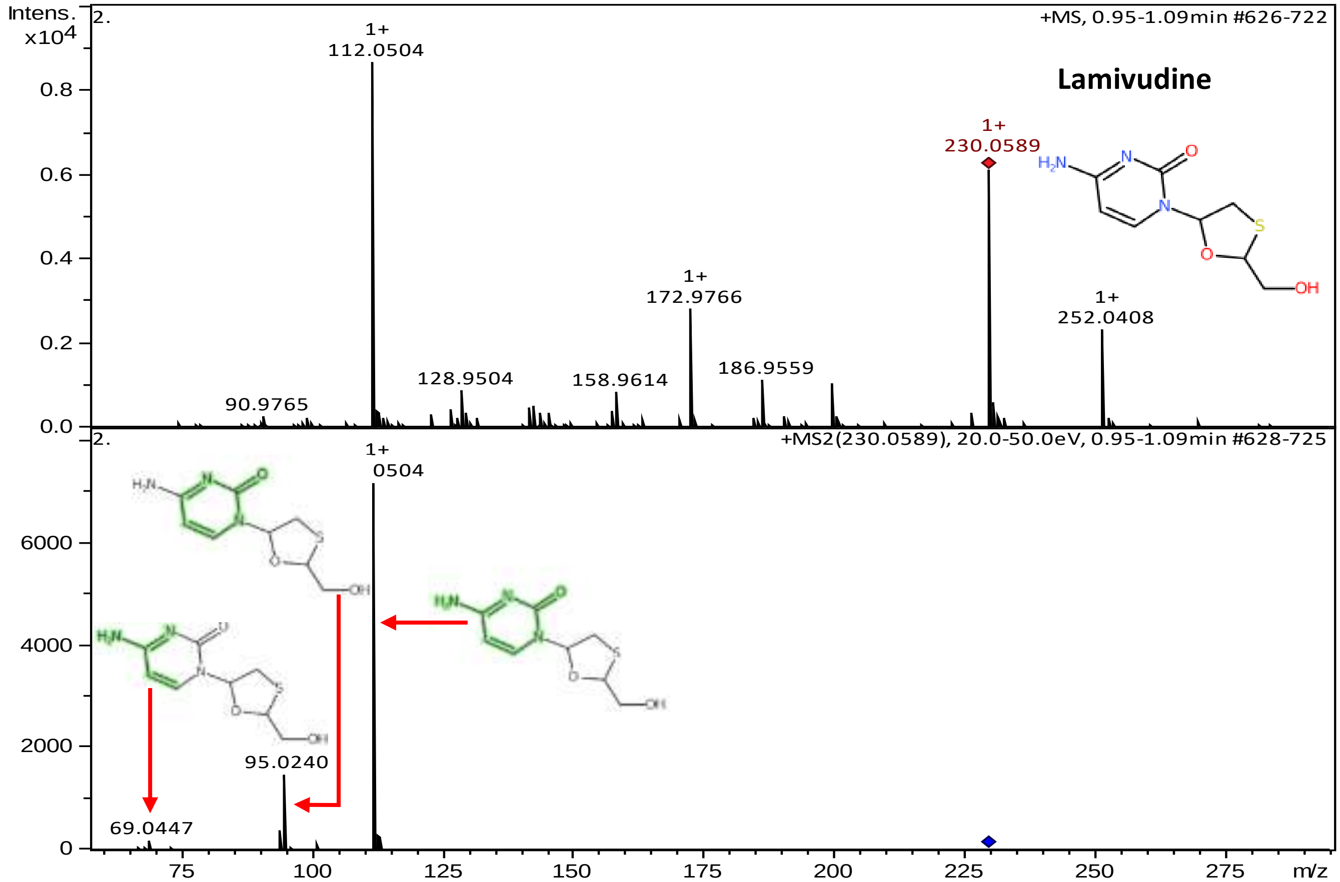

Figure A2: MS-MS spectra for LAM and its fragments (ESI+ mode).

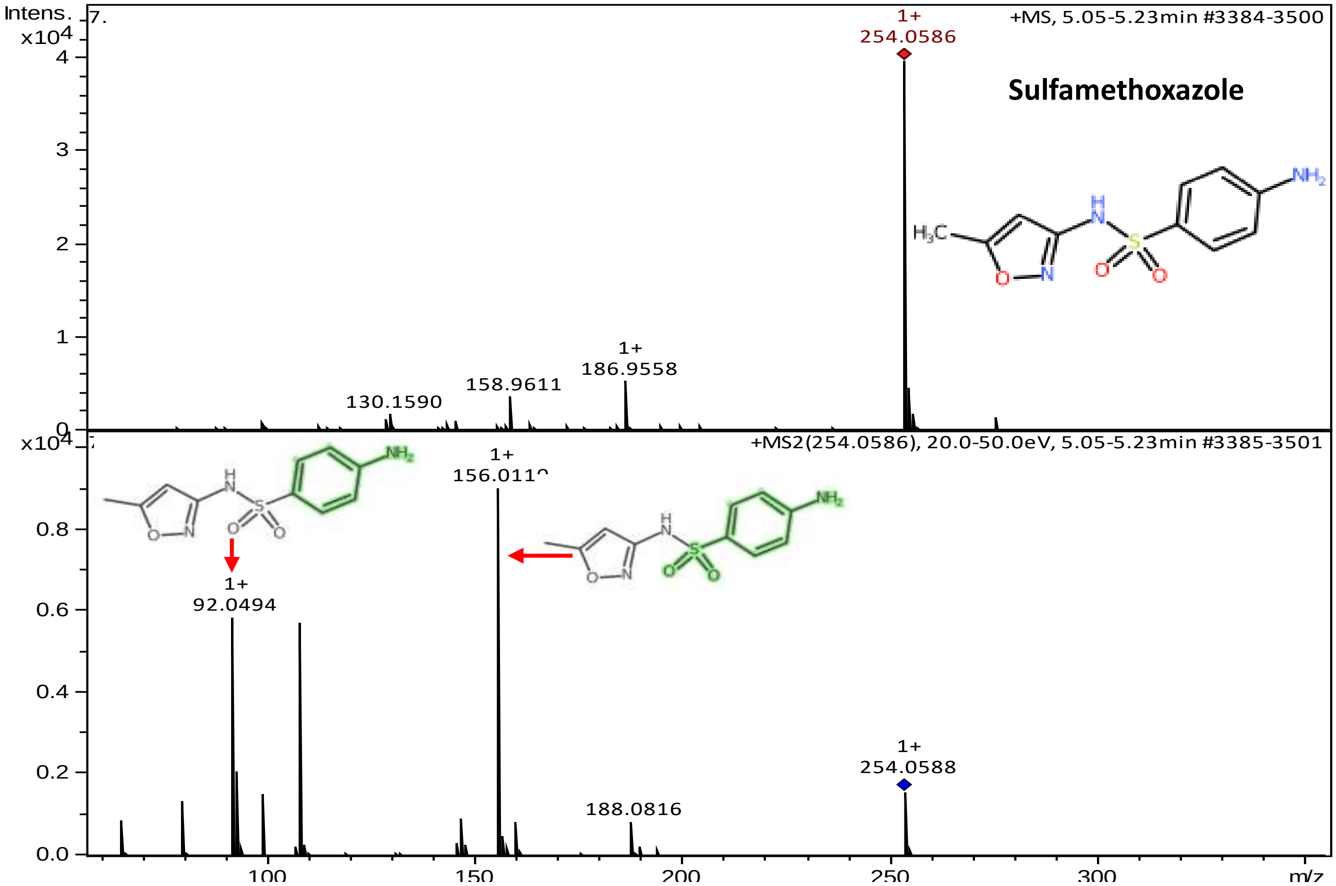

Figure A3: MS-MS spectra for SULF and its fragments (ESI+ mode).

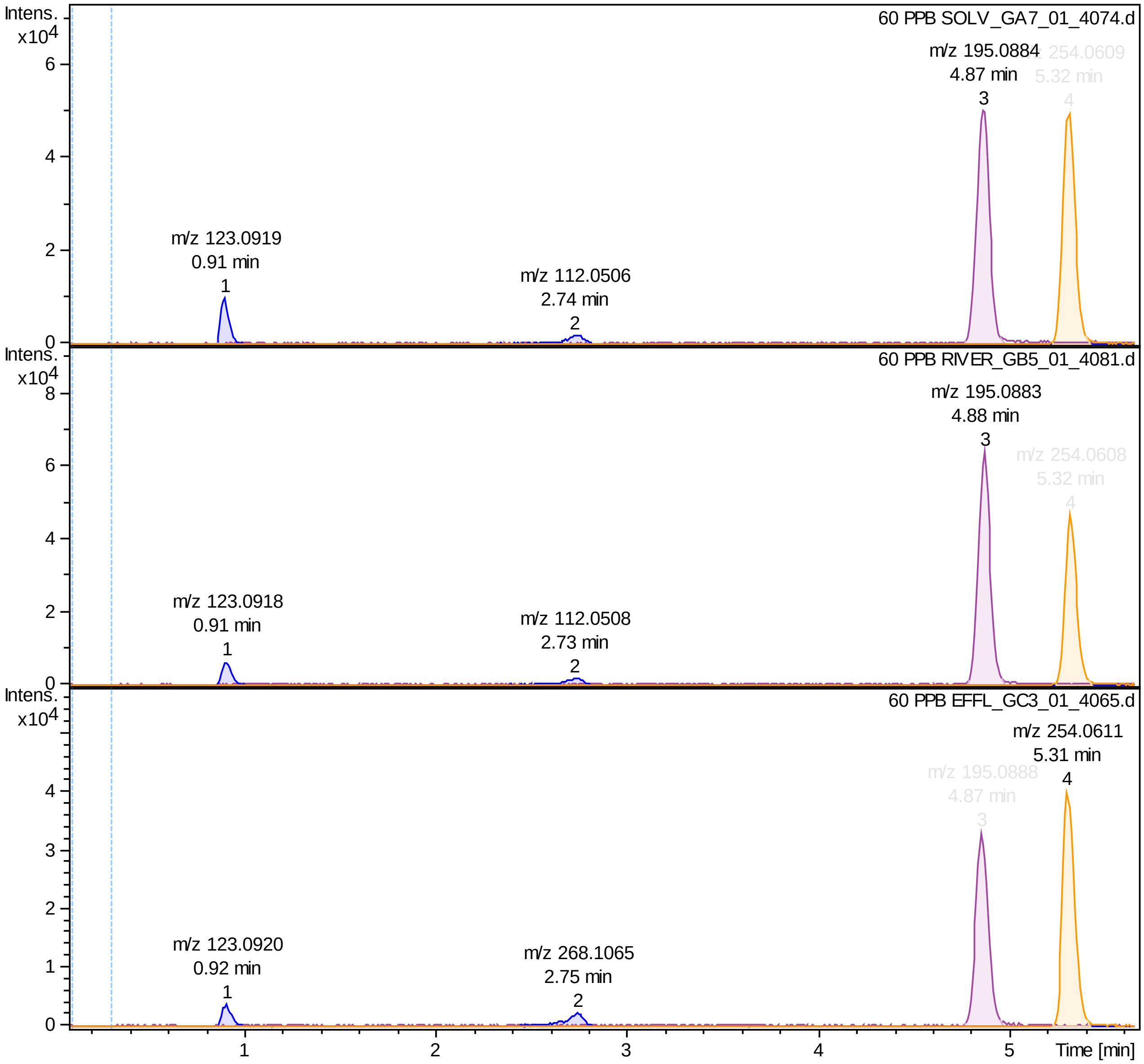

Figure A4: Extracted ion chromatographs for CAF, LAM and SULF in solvent, river and wastewater effluent.

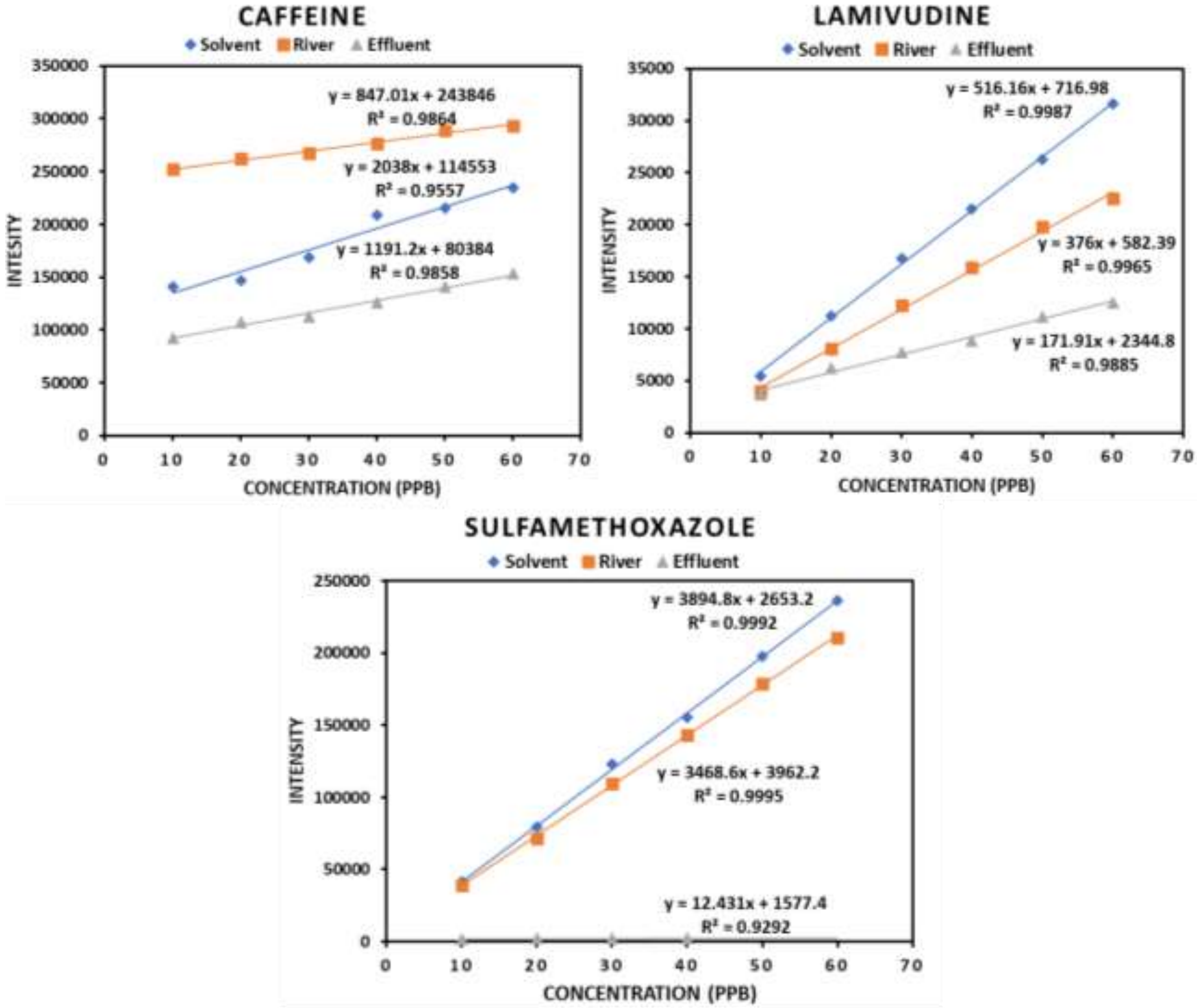

Figure A5: Solvent and Matrix matched (river and effluent) calibration curves for CAF, LAM and SULF.

Table A.1: Onsite water quality parameter measurements (*n=3*).

SAMPLING POINT	DO±SD (mg/l)	TDS±SD (ppm)	COND.±SD (mS/cm)	pH±SD	TEMP.±SD (0C)
WWTP1	8.48±0.04	352±3.46	0.54±0.00	7.79±0.01	21.37±0.21
Diepsloot river	4.53±0.49	-	0.77±0.00	7.52±0.01	20.60±0.10
Jukskei upstream	6.90±0.10	-	0.40±0.00	8.02±0.01	21.13±0.21
Jukskei downstream	5.20±0.26	291.00±1.00	0.45±0.00	7.66±0.01	18.30±0.00
WWTP2	8.10±0.10	373.67±0.58	0.56±0.00	7.69±0.01	21.40±0.10
Crocodile upstream	8.37±0.06	136.00±1.73	0.21±0.00	7.72±0.01	21.13±0.06
Crocodile downstream	8.50±0.10	299.67±0.58	0.44±0.00	8.00±0.01	21.33±0.06
WWTP3	5.17±0.31	412.33±0.58	0.62±0.00	7.26±0.01	17.40±0.00
Msunduzi upstream	6.37±0.40	117.67±0.58	0.18±0.00	7.48±0.01	18.00±0.17
Msunduzi downstream	5.23±0.12	185.33±1.15	0.28±0.00	2.97±3.87	18.00±0.00
WWTP4	5.17±0.21	197.33±1.53	0.30±0.00	7.63±0.01	17.77±0.46
Ilovu upstream	4.97±0.31	50.50±0.35	0.08±0.00	7.45±0.02	18.23±0.06
Ilovu downstream	7.07±0.31	55.43±0.32	0.08±0.00	7.50±0.01	18.00±0.00
WWTP5	9.20±0.20	226.67±0.58	0.34±0.00	7.34±0.01	18.57±0.12
Donga upstream	8.23±0.51	393.33±0.58	0.59±0.00	7.08±0.04	18.20±0.10
Donga downstream	4.77±0.25	394.33±0.58	0.59±0.00	7.75±0.01	18.30±0.00

Table A.2: Supplementary information for rivers studied.

Station Number	Place	Catchment Area(km²)	Latitude	Longitude
Stations in EERSTE river				
G2H011	Eerste River @ Macassar	395	-34.07118	18.77021
G2H015	Eerste River @ Faure	342	-34.03083	18.74777
G2H019	Eerste River @ Stellenbosch	176	-33.94369	18.84354
G2H020	Eerste @ Fleurbaai	183	-33.9498	18.83854
G2H040	Eerste @ Klein Welmoed	328	-34.00277	18.76305
Stations in MSUNDUZE river				
U2H011	Msunduze River @ Henley Dam	176	-29.64708	30.25975
U2H022	Msunduze River @ Inanda Loc.	881	-29.66086	30.63616
U2H041	Msunduze River @ Hamstead Park	534	-29.60772	30.45025
U2H058	Msunduze River @ Masons Mill	327	-29.63072	30.35322
Stations in ILOVU river				
U7H002	Lovu River @ Illovo	936	-30.0967	30.8231
U7H007	Lovu River @ Beaulieu Estate	114	-29.86244	30.24416
Stations in JUKSKEI river				
A2H023	Jukskei River @ Nietgedacht	686	-25.95444	27.96256
A2H040	Jukskei River @ Waterval	199	-26.03194	28.11188
A2H042	Jukskei River @ Lone Hill	409	-26.00583	28.03302
A2H044	Jukskei River @ Vlakfontein	798	-25.8955	27.93481
Stations in KROKODIL river				
A2H001	Krokodil River @ Hartbeespoort	2909	-25.73386	27.85969
A2H012	Krokodil River @ Kalkheuwel	2551	-25.81056	27.90983
A2H015	Krokodil River @ Buffelshoek	23940	-24.67313	27.3955
A2H018	Krokodil River @ Donkerpoort	1070	-24.63452	27.31638
A2H022	Krokodil River @ Welgegund	2616	-25.79777	27.89552
A2H025	Krokodil River @ Hardekoolbult	21349	-24.93391	27.548
A2H037	Krokodil River @ Buffelshoek	23762	-24.66422	27.3755

Station Number	Place	Catchment Area(km²)	Latitude	Longitude
		Stations in KROKODIL river		
A2H045	Krokodil River @ Vlakfontein	653	-25.89275	27.91483
A2H048	Krokodil River @ Krokodilpoort	4691	-25.57342	27.75411
A2H050	Krokodil River @ Zwartkop	148	-25.99142	27.84211
A2H051	Krokodil River @ Van Wyks Restant	109	-26.03303	27.84269
A2H052	Krokodil River @ Krokodildrift	4355	-25.64613	27.78413
A2H059	Krokodil River @ Vaalkop	12674	-25.20631	27.55794
A2H060	Krokodil River @ Nooitgedacht	20627	-25.06303	27.52
A2H078	Krokodil River @ Kalkheuwel	2551	-25.80777	27.91025
A2H132	Krokodil River @ Haakdoringdrift	22270	-24.69508	27.409
X2H004	Krokodil River @ Nelspruit	3929	-25.45059	30.9645
X2H006	Krokodil River @ Karino	5097	-25.46977	31.08813
X2H013	Krokodil River @ Montrose	1508	-25.44863	30.71177
X2H016	Krokodil River @ Tenbosch Kruger National Park	10365	-25.36386	31.95572
X2H017	Krokodil River @ Thankerton Van Graan se dam Kruger NP	8811	-25.43837	31.63452
X2H032	Krokodil River @ Weltevrede	5380	-25.51419	31.22452
X2H033	Krokodil River @ Sterkdoorn	998	-25.37726	30.44615
X2H046	Krokodil River @ Riverside	8473	-25.39888	31.61055

Table A.3: Supplementary information for de facto reuse quantification.

AVERAGE DE FACTO REUSE %					
Year	**WWTP1**	**WWTP2**	**WWTP3**	**WWTP4**	**WWTP6**
2009	79.17	3.51	20.68	4.28	11.16
2010	62.75	2.25	39.18	12.95	18.87
2011	65.89	1.85	27.35	10.66	30.24
2012	81.91	4.44	24.09	5.55	12.67
2013	83.63	5.57	20.89	4.70	9.14
2014	68.30	1.69	39.60	15.40	13.83
2015	107.94	7.68	37.18	27.29	30.98
2016	78.89	6.69	30.28	51.14	91.54
2017	79.04	2.14	24.13	6.33	57.63
2018	65.68	4.53	12.58	8.25	19.10
AVERAGE STREAM FLOWS FOR RECEIVING WATER (m^3/s)					
Year	**Jukskei**	**Crocodile**	**Msunduzi**	**Ilovu**	**Eerste**
2009	5.85	11.20	3.64	0.41	2.07
2010	7.38	17.47	1.92	0.13	1.23
2011	7.03	21.28	2.75	0.16	0.77
2012	5.65	8.86	3.60	0.31	1.83
2013	5.54	7.07	3.60	0.37	2.53
2014	6.78	23.24	1.90	0.11	1.67
2015	4.29	5.12	2.02	0.06	0.75
2016	5.87	5.88	2.48	0.03	0.82
2017	5.86	18.40	3.12	0.27	0.40
2018	7.05	8.69	5.98	0.21	1.21