DEVELOPMENT OF A MATHEMATICAL MODEL FOR THE ASSESSMENT OF WATER QUALITY VARIATIONS DUE TO EMERGING CONTAMINANTS WITH SPECIAL EMPHASIS ON ANTIBIOTICS IN MSUNDUZI RIVER

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

The escalation of wastewater discharge into river bodies, fuelled by lifestyle changes, poses a substantial threat to human health and aquatic life. This surge in contamination is further exacerbated by the emergence of new contaminants, with antibiotics being particularly noteworthy among them. Antibiotics are a class of pharmaceutical agents designed for specific health conditions. They are typically prescribed for a limited duration and in specific doses. However, studies reveal that the human body metabolises only a fraction of ingested antibiotics, while the rest is excreted as waste. These remnants enter wastewater treatment plants through sewer systems and stormwater runoff. The effluent from these treatment plants is then discharged into receiving water bodies, contributing to widespread contamination. While substantial antibiotic doses offer health benefits over a short period, the continuous intake of smaller amounts can compromise the immune system. This becomes particularly significant in South Africa, where a considerable portion of the population faces compromised immune systems.

To address these concerns, it becomes imperative to investigate the fate of antibiotics in water channels and prevent their entry into water supply systems. However, existing water quality models face limitations due to their site-specific nature, operational complexity, and the demand for high-level skills and extensive datasets. These constraints hinder their application in regions with limited data, including many developing countries like South Africa. In response to these challenges, this project endeavours to develop a user-friendly and flexible water quality model explicitly tailored to simulate the movement of antibiotics along water channels. The envisioned model aims to overcome the limitations of current models and ensure adaptability to South African rivers. By doing so, it aspires to offer a valuable tool for effectively mitigating the entry of antibiotics into water supply systems.

AIMS

The following were the aims of the project:

1. To Identify sources and types of antibiotics within the Msunduzi Catchment
2. To quantify the extent of antibiotic pollution in the study area
3. To understand the fate and transport of antibiotics in surface water systems
4. To investigate the presence of antibiotics in wastewater treatment plants
5. To identify areas or hot spots of high antibiotic discharge into municipal wastewater
6. To develop a user-friendly, cost-effective, and time-efficient water quality model to predict the fate of antibiotics in water systems for effective water treatment and efficient water quality management

METHODOLOGY

The primary research approach for this study comprises scientific experimentation, testing, and modelling, including sample collection for data generation and quantitative analysis of dependent and independent variables for training and validating the proposed model. A literature review supported the primary research method, guiding the selection process of sampling, testing, and experimentation to create a model capable of tracing and detecting emerging contaminants (ECs), particularly antibiotics, in water bodies. The research methodology is detailed as follows:

Literature Review

The review analysed previous research on the fate and transport of ECs in water bodies. Subsequently, it explored the activities within and around the Msunduzi River catchment. The modelling processes and techniques essential for achieving the research aim were also investigated as a foundation for developing a model for tracing and simulating ECs in water bodies. A detailed review of the Water Quality Simulation and Assessment Model (WQSAM) was done to assess the performance of the proposed model. Additionally, the

INWARD tool for GIS/RS application in the study was explored to understand existing models better, identify their strengths, and pinpoint limitations.

Field Investigation and Experimental Procedure

The study area was identified and subdivided into grid cells using hydrological tools, with a digital elevation model providing detailed support for the model. Image classification utilised a Maximum Likelihood Algorithm (MLA), classified Land Use Land Cover (LULC) types, creating a LULC map. An experimental procedure was developed to explore the fate and transport of Emerging contaminants.

Sampling

Water samples were collected from 16 selected points along the Msunduzi River. All specimens were collected in sterile containers, stored in Styrofoam boxes with ice, and transported to laboratories for analysis. Sampling occurred over 12 to 18 months to capture variations in pollutant concentrations during the hydrological year, considering both dry and rainy seasons.

Sample Analyses

On-site tests included electrical conductivity, pH, and temperature, while pollutant concentrations were measured using appropriate methods and instruments. The physicochemical analysis included tests such as dissolved oxygen, TSS, turbidity, nitrates, and phosphorus. Antibiotic residues were quantified using solid-phase extraction (SPE) and analysed through liquid chromatography (LC-MS).

Motivation for Proposed Model Development

Several mechanisms lead to antibiotic degradation in the environment. Its behaviour depends on its interaction with environmental conditions, and its exit rate from the environment is far less than the entry rate (Ben et al., 2019; Chang et al., 2015; Van Boeckel, 2015; Kümmerer, 2009). Antibiotics remain in the water because standard wastewater treatment plants are not designed to remove these chemicals either in their natural state or metabolised form (Zhao et al., 2017; Cruzeiro et al., 2016; Ribeiro et al., 2015; Kolpin et al., 2002). Ribeiro et al. (2015) stated that routine chlorination of wastewater might transform specific pharmaceuticals (antibiotics) into more toxic compounds. Globally, no set regulations or standard experimental procedures exist for monitoring or analysing antibiotics or their residues in the environment (Polianciuc, 2020). While much research has focused primarily on the presence and concentration of antibiotics in WWTPs, surface water, and the environment, the factors that govern their fate and transport are unknown. Thus, the study of the hydrodynamics and in-stream pollutant transport processes cannot be ignored. Several water quality models have been developed to solve water quality challenges and validate water quality management decisions. However, with ECs, no specific universal model can be used considering varying climatic, geographic, and anthropogenic conditions (Li et al., 2014; Mamun and Saleh, 2014; Liu et al., 2011). The most frequently used water quality models are based on robust algorithms (Borah and Bera, 2004: 2003), making them cumbersome and site-specific, requiring extensive data sets and high-level operational skills. Thus, their use is limited in regions other than those in which they were developed (Li et al., 2014; Ongley et al., 2010). Moreover, available models are reasonably challenging to use in areas with limited data sets, as in most developing countries, including South Africa.

RESULTS AND DISCUSSION

Field- and laboratory experiments showed antibiotic residues and antimicrobial resistance genes in the Msunduzi River and wastewater treatment plants, including their effluents. The project team formulated a mathematical mass balance equation to develop a model to simulate the fate and transport of antibiotics in the River. Utilising the differential equation described in Brown and Barnwell(1987), the model considers steady-

state and varying flow conditions. The Python programming language was used to code and develop the front and back end of the Water Quality Modelling Tool.

CONCLUSIONS

Antibiotic residues and antimicrobial resistance genes were found in the Msunduzi River, in wastewater treatment plants and their effluents. A mathematical model is developed to simulate the fate and transport of antibiotics in the River. The significance of the developed model lies in its process-based nature, a distinctive feature that sets it apart from other water quality assessment tools. Unlike many traditional models that demand extensive datasets for operation, this process-based model is designed to be more economical in its data requirements. This characteristic not only streamlines its usage but also makes it highly adaptable to scenarios where comprehensive data may be scarce, a common challenge in the context of many developing regions. The operational aspect of the model is marked by its user-friendly interface. The user interface, constructed using Python programming, ensures that water quality managers and professionals with varying technical skills can interact with the tool effortlessly. However, the ease of operation does not compromise the model's reliability, the integrity of the output, or the accuracy and robustness required for effective water quality assessment. The urgency of addressing the presence of antibiotics in river systems cannot be overstated, particularly in the context of their role as contributors to antimicrobial resistance. The model becomes an essential component in the proactive management of water quality by providing a means to assess and predict the fate of antibiotics in river systems. The potential impacts of antibiotic residues on human and environmental health underscore the critical need for tools that enable decision-makers to understand and mitigate these risks. Therefore, the model becomes a valuable asset for those responsible for safeguarding water resources and ensuring safe and uncontaminated water for communal use. The developed mathematical model represents a technical and proactive response to a pressing environmental and public health concern. Its process-based approach, user-friendly interface, and focus on antibiotic contamination make it an indispensable tool for water quality managers and professionals providing safe water for communal use.

RECOMMENDATIONS

While this project has made significant strides in understanding the dynamics of water quality variations in the Msunduzi River, particularly concerning antibiotics, there are avenues for further studies that could enhance the depth of knowledge in this area. Recommendations for future research include the following:

- Model Validation
- Efficiency of Wastewater Treatment Technologies
- Multi-Temporal Studies
- Genomic Analysis
- Additional Emerging Contaminants
- Human Health Impact Assessment
- Community Engagement and Education
- Policy and Regulatory Assessment

By addressing these recommendations, future studies can contribute to the ongoing efforts to manage emerging contaminants in water bodies, ensuring sustainable and safe water resources for both human and environmental well-being.

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ACRONYMS & ABBREVIATIONS

ABR	Antibacterial Resistance
ACOLITE	Atmospheric Correction for OLI 'Lite'
ADE	Advection Dispersion Equation
ADZ	Aggregated Dead Zone
AGNPS	Agricultural Nonpoint Source Pollution Model
AMG	Antimicrobial Resistant Genes
AMR	Antimicrobial Resistance
AMZ	Aggregated Mixing Zone
ANSWERS	Areal Nonpoint Source Watershed Environment Response Simulation Model
ARCHER	Airborne Real-Time Cueing Hyperspectral Enhanced Reconnaissance
ASTER	Advanced Space-borne Thermal Emission and Reflectance Radiometer
AVIRIS	Airborne Visible/Infrared Imaging Spectrometer
CASI	Compact Airborne Spectrographic Imager
CDC	Center for Disease Control
CHRIS	Compact High Resolution Imaging Spectrometer
CIS	Cells in Series Model
CZCS	Coastal Zone Colour Scanner
DNs	Digital Numbers
DOS	Dark Object Subtraction
ECDPC	European Centre for Disease Prevention and Control
ECs	Emerging Contaminants
EPD-RIV1	One-Dimensional Riverine Hydrodynamic and Water Quality Model

DEFINITION OF SYMBOLS AND ABBREVIATIONS FOR THE MODEL

Symbols/abbreviations	Definition
A	Antibiotic concentration in the water column
As	Antibiotic concentration in the sediment
x	Distance/reach
t	Time
T1	Residence time in the first mixing zone
T2	Residence time in the second mixing zone
α	Residence time in the plug flow zone
POM	Particulate organic matter in the water column
POMs	Particulate organic matter in the sediment
DOM	Dissolved organic matter in the water column
DOMs	Dissolved organic matter in the sediment
Kb	The rate constant for biolysis of antibiotics
Kh	The rate constant for the hydrolysis of antibiotics
Kp	Rate constant for photolysis of antibiotics
Kd	Diffusion Constant
γ	The ratio of water depth to depth of sediment
Kr	Rate constant for resuspension
U	Advection velocity
m	Meter
ℓ	Litre
mℓ	Millilitre
mg	Milligram
h	Hour
d	Day
q	Rate flow from tributaries/wastewater treatment plant/point sources
K_s	The rate constant for settling
A_p	Particulate bounded concentration of antibiotics in the water column
Se	Antibiotic concentration from tributaries/wastewater treatment plant/point sources
V	Volume
∅	Porosity
K_{Pm}	Degradation constant for POM production from DOM photolysis-induced transformation
$K_p{}^*$	Rate constant for dissolved inorganic production from DOM
K_x	Hydrolysis of POM to DOM
M_x	Basal metabolism of algae
A_g	Algal biomass growth
S_d	Dissolved organic matter concentration from tributaries/wastewater treatment plant/point sources
ψ	Depth of movable sediment bed layer
μ	Coefficient for algal growth (day-1)
r	The Algael respiration rate (day-1)
K_{bw}	Biolysis of particulate organic matter in the sediment
K_{hw}	Hydrolysis of particulate organic matter in the water column
S_p	particulate organic matter from tributaries/wastewater treatment plant/point sources
K_{bs}	Biotransformation of particulate organic matter in the sediment

K_{hs}	Hydrolysis of particulate organic matter in the sediment
S_t	Total suspended solids from the external sources (mg/l)
Z	Hydrolysis coefficient for suspended solids
K_{h}	Rate constant for hydrolysis of suspended solids
Δx	Size of the hybrid unit
D_L	Dispersion
Pe	Peclet number

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Access to safe and clean water is a cornerstone for the survival of ecosystems and human populations. Unfortunately, contaminated water sources have been identified as significant contributors to severe health conditions affecting human and aquatic life (Gavrilescu et al., 2015; Deblonde et al., 2011). Factors such as population dynamics, human migration, socioeconomic development, and lifestyle patterns have led to increased wastewater generation, introducing a variety of emerging contaminants into water bodies (Murray et al., 2010; Kummerer, 2009; Barcelo and Petrovic, 2007). The escalating pollution of available water resources due to emerging contaminants is becoming a growing concern. Antibiotics, classified as pharmaceutical products, have gained attention among these emerging contaminants. Recent global studies, including investigations in South Africa, have reported the presence of antibiotic residues in water bodies (Ben et al., 2019; Danner et al., 2019; Chang et al., 2015; Matongo et al., 2015; Agunbiade and Moodley, 2014). The challenge posed by antibiotics extends beyond their essential role as pharmaceuticals for treating illnesses in humans and animals and their contribution to productivity in fish farming. Despite the extensive use of antibiotics in crop farming since the 1950s, their potential impact on environmental and water contamination has been underestimated, often attributed to limited data and the perception that the release of antibiotic residues into the environment from crop farming is minimal (Taylor and Reeder, 2020). As global concerns about water contamination from emerging contaminants, particularly antibiotics, continue to escalate, it becomes imperative to recognize and comprehend their presence and impact on effective water quality management.

Emerging contaminants (ECs) represent diverse substances that emanate from residential, industrial, and agricultural areas (Phillips et al., 2010; Parrot and Bennie, 2009). Previously, the fate of antibiotics in the environment received less attention, overshadowed by their perceived benefits (Scheurer et al., 2011; Lindberg et al., 2007; Fent et al., 2006; Pruden et al., 2006). However, detecting antibiotics in trace concentrations in water bodies and the alarming rise of drug-resistant bacterial strains have emerged as significant global concerns (Scheurer et al., 2011; Lindberg et al., 2007). The increasing recognition of antibiotic residues in water bodies and their potential role in antimicrobial resistance has shifted the narrative, prompting a re-evaluation of the environmental impact of these pharmaceuticals.

The health benefits of antibiotics are evident when administered in prescribed doses for short durations. However, prolonged exposure to small quantities, whether through water or other mediums, leads to antibiotic resistance and compromises the immune systems of consumers (Fouhse et al., 2019; Yang et al., 2017; Truter, 2015; Figueira et al., 2011; Essack, 2006). Antibiotics enter the aquatic environment through multiple pathways (Manzetti and Ghisi, 2014; Phillips et al., 2010; Parrot and Bennie, 2009). Notably, human and animal waste contributes to this contamination due to the irrational and excessive use of antibiotics in treating bacterial infections in humans and livestock. This practice results in significant antibiotic residues entering the environment (Ben et al., 2019; Hanna et al., 2018; Truter, 2015). When antibiotics are ingested by humans or livestock, a considerable portion (40 to 90%) of the dose remains unmetabolized and excreted in their active form. This excretion enters sewer lines and wastewater systems, creating a pathway for antibiotic residues into water channels (Polianciuc, 2020). The runoff from livestock farming and agricultural lands further contributes to the transport of antibiotic residues into water bodies (Danner et al., 2019; Benotti et al., 2009; Cooney, 2009). Another identified pathway is the improper and indiscriminate disposal of unused medicinal products into sewer and sewage systems (Sharma et al., 2016; Fick et al., 2015; Rizzo et al., 2013; Figueira et al., 2011). This intricate network of pathways emphasises the complexity of antibiotic entry into aquatic systems, necessitating comprehensive studies and effective management strategies to address this environmental concern.

Despite various wastewater treatment methods, the identification of substantial antibiotic concentrations in wastewater effluents suggests that conventional wastewater treatment plants (WWTPs) may not be efficient enough to eliminate antibiotics from the wastewater. Li et al. (2008) found elevated concentrations of Oxytetracycline, reaching 484 µg/L, more than 20 km downstream from a WWTP in the Xiao River, China. The study involved sampling raw wastewater, WWTP effluents, and Xiao River samples at four sites along the river for three consecutive days over two years, all during the non-rainy season (Li et al., 2008). Rodriguez-Mozaz et al. (2015) detected over 13 µg/L of Ciprofloxacin in hospital effluents near the Ter River in Spain, revealing mean influent antibiotic concentrations of 670 µg/d/inhabitant and a mean effluent concentration of 175 µg/d/inhabitant. Antibiotics in water sources were also identified in rivers in the USA by Gibs et al. (2013) and Karthikeyan and Meye (2006). Other countries where antibiotic residues in rivers have been observed include Japan (Murata et al., 2011), Australia (Bruce et al., 2010), and several European countries (Östman et al., 2017; Böckelmann et al., 2009). Furthermore, antibiotic contamination has been detected in various African countries, including Kenya (Madikizela et al., 2017; K'oreje et al., 2016), Nigeria (Olarinmoye et al., 2016), Tunisia (Moslah et al., 2018; Tahrani et al., 2016), and South Africa (Matongo et al., 2015; Agunbiade and Moodley, 2014, 2016). These findings underscore the widespread occurrence of antibiotic residues in water bodies globally, signalling a pressing environmental and public health concern.

Van Boeckel et al. (2015) estimated global antibiotic consumption, reporting 63,151 tons in 2010. They projected a 67% increase in antibiotic use by 2030, emphasising that, for some BRICS countries, the consumption rate could more than double (Van Boeckel et al., 2015; Pruden et al., 2013; Kinsella et al., 2009). According to the November 2018 report from the National Department of Health on Surveillance for Antimicrobial Resistance and Consumption of Antibiotics in South Africa, the 2015 estimate of antibiotic usage in South Africa was 21,149 standard units per 1000/population. This figure is notably higher than many other countries globally, indicating extensive antibiotic use (Leopold et al., 2014). These statistics are alarming considering the potential implications of high volumes of unmetabolized antibiotics re-entering the water supply system. This reintroduction poses significant risks, including the evolution and proliferation of drug-resistant bacterial strains, compromised immune systems, and associated health implications, as the World Health Organization emphasised in 2006 (WHO 2006). This situation, therefore, requires the development of a suitable model for tracing the presence of antibiotic residues in rivers and the water environment in general.

Mathematical models are relation estimators that describe the response of a receiving water body to input loads or variables with an anticipated water quality output (Teodosiu et al., 2009). The complexity of water quality modelling comes from the inability to represent the river processes, reactions, and system dynamics through the flow and input elements. Since flow drives water quality, water quality models rely on flow estimates from hydrological and systems models (Slaughter, 2018). However, complexities arise with the number of parameters, variables, modelling techniques, and input data. Hence, the more complex the model, the more complex and costlier its application for any given purpose, overwhelming water resource management and monitoring. Water quality managers would require a model that is easy to manoeuvre without tedious data or manipulation requirements and without compromising output efficiency and accuracy. Various water quality models and water resources management tools have been developed worldwide, especially in developed countries. Sometimes, they use specific default values of rate constants that may not be compatible with other regions. Hence, the models developed are for specific purposes, applicable to particular environments, and are modified when required for other scenarios.

1.2 PROJECT AIMS

The primary aim of this research is to investigate the fate and transport of antibiotics in surface water systems. The project seeks to develop a water quality modelling tool capable of predicting the fate of antibiotics in river systems. This tool is envisioned to enhance water treatment strategies, ultimately leading to more efficient water quality management. The overarching goal is to advance the understanding of the behaviour of antibiotics in surface water, with the ultimate aim of developing a practical tool that contributes to effective water treatment and quality control measures.

The specific objectives of the project include:

1. To Identify sources and types of antibiotics within the Msunduzi Catchment
2. To quantify the extent of antibiotic contamination in the study area
3. To understand the fate and transport of antibiotics in surface water systems
4. To investigate the presence of antibiotics in wastewater treatment plants
5. To identify areas or hot spots of high antibiotic discharge into municipal wastewater.
6. To develop a user-friendly, cost-effective, and time-efficient water quality model to predict the fate of antibiotics in water systems for effective water treatment and efficient water quality management

1.3 SCOPE AND LIMITATIONS

This project focused on tracing, monitoring, and modelling emerging contaminants in the Msunduzi River, a vital water resource in the Midlands of KwaZulu-Natal. The river is a tributary of the uMgeni River with Coordinates 29°37′14″S 30°40′36″E, and flows through Pietermaritzburg, providing water for domestic, agricultural, and industrial purposes in the Msunduzi Municipality. In response to a prior study revealing elevated concentrations of pharmaceutical residues in KwaZulu-Natal Rivers, the project specifically targeted four prevalent antibiotics to develop an effective monitoring tool. Standard water quality testing and analysis methods were employed to examine samples collected from various locations along the river. Additional samples from a wastewater treatment plant were analysed to assess the spatial distribution of antibiotic residues within the plant and their contribution to source water. Genomic studies were conducted to evaluate antimicrobial-resistant genes in the water system. However, the project faced challenges, including COVID-related restrictions and delays caused by floods and riots in KwaZulu-Natal. The extraction of antibiotic residues and Genomic studies proved time-consuming and very expensive, impacting the depth and number of analyses required; therefore, the planned metagenomic sequencing could not be completed as intended. Also, obtaining approval for sample collection from the wastewater treatment plant added further delays, limiting sampling and testing.

While the developed model achieved its intended objectives, and the current focus on simulating water quality along the river reach provides valuable insights, it is essential to recognise that its applicability may be limited in scenarios requiring broader catchment-scale analysis. Although Geographic information system (GIS) was used in generating the land use map, delineating spatio-temporal variations of Water Quality parameters and Water quality mapping along the Msunduzi River in this project, future research could explore integrating GIS in the model at a two-dimensional catchment scale to understand variations within catchments better.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

The issue of water pollution has persisted for centuries and remains a pressing concern. Natural streams and rivers are essential global resources contributing significantly to utility and economic growth. Rivers are used as disposal sinks for municipal and industrial wastes, a practice that has become a significant threat to human lives and the eco-environment (Ramaswamy et al., 2011; Larsson et al., 2007). Over the past decades, the intentional or unintentional release of pollutants into rivers has compromised the water quality of many natural water bodies, rendering them unsafe for consumption and general use (Agunbiade and Moodley, 2014; Pal et al., 2014; Calderon-Preciado et al., 2011; Wintgens et al., 2008). Aquatic pollution due to antibiotics is rising and capable of causing adverse environmental and health issues. Emerging pollutants (EP) include substances not regulated by existing monitoring and release regulations (da Silva et al., 2013). Micro-plastics, pharmaceuticals and personal care products are among the emerging pollutants that enter the environment through human activities (Bell et al., 2013). A most pressing concern of EPs is the development of antimicrobial resistance (AMR) and genes (AMG). This situation has taken centre stage globally, with nations seeking out possible solutions to curbing AMR, considering that continual exposure to antibiotics may not be intentional but due to the continuous release of antibiotics into the environment from various sources at concentrations ranging from several ng L^{-1} to hundreds of µg L^{-1} annually (Yao et al., 2017; Nödler et al., 2012).

2.2 ANTIBIOTIC CONTAMINANT CONCERNS

Antibiotics are prevalent in many water systems in Africa due to the high prevalence of diseases such as tuberculosis, pneumonia, and diarrhoea due to compromised immunity occasioned by HIV infection, poor sanitation, and drug abuse (Faleye et al., 2018). Antibiotics have been used since 1928 with the discovery of Penicillin by Alexander Fleming, and Penicillin resistance was first reported in 1947 from hospital samples. Antibiotics are widely used antimicrobial substances that assist the immune system in fighting off harmful bacteria, thus preventing bacterial infections in humans and livestock when used correctly. According to research by various authors (Kirchelle 2018, 2019; van Bunnik and Woolhouse, 2017; Klein et al., 2017; van Boeckel et al., 2014, 2015; World Health Organization (WHO) 2011, 2015, 2017; Allen et al., 2010; Davies and Davies, 2010;), the global annual production of antibiotics is between 100 000-200 000 tons and is estimated to exceed 350 000 tons per annum by 2030. For several years, antibiotics have proven to be effective in treating and improving the lives of humans, while in agriculture, it has sustained the growth and health of poultry and livestock and promoted an increase in aquaculture (Zhang et al., 2013; Kümmerer, 2009). Globally, however, concerns have been raised in medical circles regarding the growing number of bacterial infections becoming resistant to antibiotics, which, according to Liu et al. (2019), leads to about 700,000 deaths globally. O'Neill (2014) supports this projection, stating that by 2050, antibiotic-resistant infections will be the leading cause of death globally.

Antimicrobial resistance to Antibiotics (AMR) occurs when microbes evolve and withstand the effects of antibiotics through several mutations (Lim et al., 2016; Laxminarayan et al., 2013, 2016), making them toxic and deadly. According to the Center for Disease Control (CDC), the main drivers of AMR are inappropriate, indiscriminate, and prolonged ingestion and exposure to antibiotics. This claim does not reflect only human use but also livestock and aquaculture farming due to the increasing demand for animal protein, leading to enhanced usage of antibiotics for veterinary use (Chollom et al., 2018). According to Sverdrup et al. (2020), of 167 000 metric tons of antibiotics produced in 2013, 131 000 metric tons were used for agricultural purposes globally. Recent studies show that the increasing use of antibiotics in human health and Agri and aquacultural practice translates to an increasing presence in surface water and other environmental matrices. Due to several factors, including the excretion of unmetabolised and sometimes parent compounds of antibiotics being released daily into the environment (Polianciuc, 2020).

In their study, Monahan et al. (2021) put the excretion rate of antibiotics in humans within the range of 8-95% and 17-90% for livestock. These numbers translate to possible elevated amounts of antibiotics released into wastewater from residential, commercial, and industrial areas, including hotspots of antibiotic use. The most important pathway of antibiotic residues into the environment, shown in Fig 2.1. is the sewerage and wastewater treatment plants. Conventional wastewater treatment plants (WWTP) do not remove antibiotic residues (Ahmed et al., 2015)), as they are not traditionally designed to remove pharmaceuticals (Bellotindos et al., 2015; Gothwal and Shashidhar, 2015). Various research has quantified antibiotics in different matrices that influence their distribution patterns and influx into surface water. Manure from the agricultural sector and sludge from WWTP (Martínez-Carballo et al., 2007; Chunhui et al., 2016) used as fertilisers provide a potent source of antibiotics in surface water through surface runoff (Kümmerer, 2009). Other sources include Hospital wastewater (Lien et al., 2016; Conte., 2017) and river sediments (Kim et al., 2007) through the hyporheic exchange process between the riverbed sediments and the overflowing river (Adu, 2021). Antibiotics enter water bodies as the parent compound or metabolite after partial metabolism and excretion (Gothwal and Shashidhar, 2015; Masse et al., 2014; Harris et al., 2012). Further, some processes within the WWTP can revert metabolites to their parent form by increasing their chemical concentration (Jelic et al., 2011). Sulfamethoxazole, the conjugate of N4-acetyl-sulfamethoxazole, is an antibiotic capable of retransforming into the original drug in water (Garcia-Galan et al., 2008).

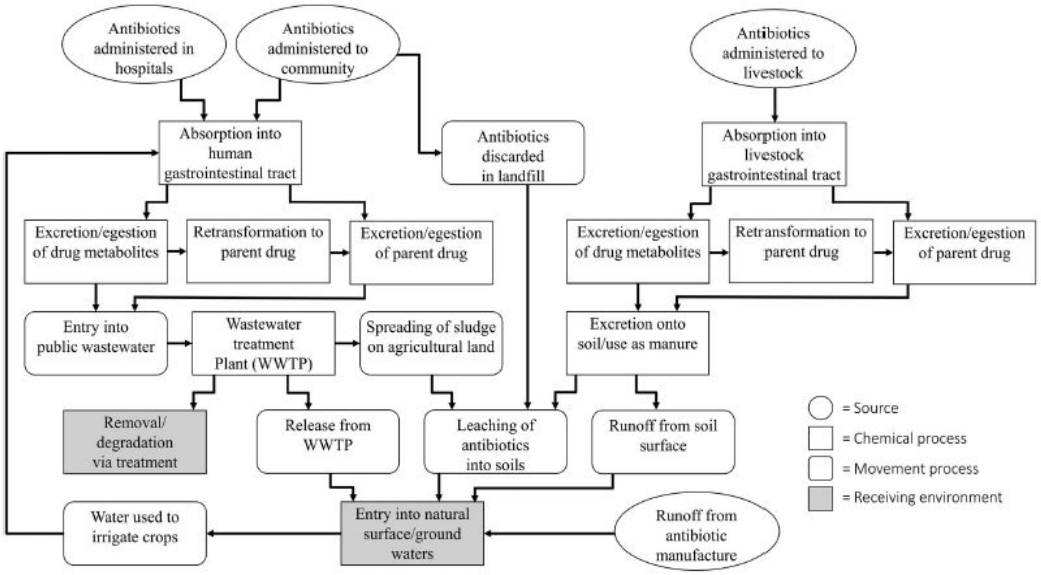


Figure 2.1 Antibiotics entry into surface water (Monahan et al., 2021).

2.3 GLOBAL OVERVIEW

Several studies (Danner et al., 2019; Mirzaei et al., 2019; Hanamoto et al., 2018; Agunbiade and Moodley, 2016; Matongo et al., 2015a; Richardson and Ternes, 2014) report the presence of antibiotic-resistant bacteria (ARB) in various water sources and the global challenge associated with antibiotic residues in rivers as the primary driver of ARB. In a 2018 report, the European Centre for Disease Prevention and Control (ECDPC) attributed 33 000 annual deaths in the EU to infections arising from ARB (Polianciuc et al., 2020; ECDPC, 2018). Similarly, CDC (2020) posited that antibiotic resistance is a primary challenge to public health in the U.S., accounting for more than 35 000 deaths annually. Researchers like Danner et al. (2019), Singh et al. (2019), and Šimatović and Udiković-Kolić (2019) raise concerns about rising cases of ARB in countries like Ireland, Greece, Germany, Netherlands, Norway, Sweden and Russia (Sverdrup et al., 2020). Zhang et al. (2012) investigated the presence of 13 antibiotics classified under four family groups of antibiotics (fluoroquinolones, trimethoprim, sulfonamides and macrolides) from ten river discharges into the Laizhou Bay

in China. The results showed high concentrations in hundreds of ng L^{-1} for all antibiotics investigated. Similarly, Li et al. (2016) examined 15 antibiotics belonging to families of chloramphenicol, sulfonamide, tetracycline and fluoroquinolone in the Gaoqiao mangrove area of China. Using LC-MS, antibiotic concentrations of 0.15 to 198 ng L^{-1} and 0.08 to 849 mg kg^{-1} were detected in surface water and sediment samples, respectively. Wang et al. (2017) detected four dominant antibiotics from the 13 antibiotics traced in the Honghu lake. The antibiotics identified belong to the tetracycline and sulfonamide family of antibiotics.

Research on the occurrence and fate of antibiotics in African rivers remains limited, and studies conducted so far reveal concerning findings. K'Oreje et al. (2012) conducted a study detecting antibiotics, namely Trimethoprim, Sulfamethoxazole, and Metronidazole, in Nairobi River water. The results highlighted the highest concentrations of contaminants, particularly Sulfamethoxazole and Trimethoprim, ranging between 20-50 µg L^{-1}. In a subsequent study by K'Oreje et al. (2016), two urban cities in Kenya were investigated by screening wastewater, surface water, and groundwater for 43 pharmaceutical products, including antibiotics. The study revealed elevated levels of antibiotics, including Metronidazole, Sulfamethoxazole, and Trimethoprim, across all the examined sites. Ngumba et al. (2016) extended the research by analysing surface water samples along the main rivers and tributaries of the Nairobi River basin. The selected sampling sites were located near recreational areas, wastewater treatment plants (WWTPs), and informal settlements. The study targeted antibiotics such as Trimethoprim, Sulfamethoxazole, and Ciprofloxacin. The results indicated variable levels, with Sulfamethoxazole ranging from <LOQ (Limit of Quantification) to 13 800 ng L^{-1} and a median concentration of 1800 ng L^{-1}. Trimethoprim values varied from <LOQ to 2650 ng L^{-1}, with a median of 327 ng L^{-1}, while Ciprofloxacin ranged from <LOQ to 509 ng L^{-1}, with a median concentration of 129 ng L^{-1}. These findings underscore the urgent need for more extensive research and proactive measures to address antibiotic contamination in African rivers.

Other global studies include Australia (Bruce et al., 2010), Canada (Saunders et al., 2016), China (Tang et al., 2015, Wang et al., 2019, Zhao et al., 2016), England (Lapworth et al., 2015, Wilkinson et al., 2016), Germany (Launay et al., 2016), India (Archana et al., 2016), Ireland (Monahan et al., 2021), Japan (Murata et al., 2011), Kenya (Madikizela et al., 2017), Nigeria (Olarinmoye et al., 2016), Russia (Sverdrup et al., 2020), Spain (Dahane et al., 2013, Gracia-Lor et al., 2012, Gros et al., 2012, Jelic et al., 2011), Tunisia (Moslah et al., 2018; Tahrani et al., 2016), Turkey (Aydin et al., 2013), USA (Fairbairn et al., 2016, Ferguson et al., 2013, Gibs et al., 2013, Maruya et al., 2016, Meador et al., 2016) and other European countries (Östman et al., 2017; Böckelmann et al., 2009). Researchers agree that intentional antibiotic misuse is not ARB's primary driver. Instead, it is unintentional due to environmental antibiotic contamination. In summary, although antibiotic residues detected in natural rivers are relatively low, the toxic levels from continuous ingestion are high enough to drive AMR.

2.4 ANTIBIOTIC USE IN SOUTH AFRICA

Essack et al. (2005) investigated the use and resistance of antibiotics in the KwaZulu-Natal public health system. The data was drawn from pharmacy records of two tertiary, five district and nine regional public hospitals. Penicillin was the most used antibiotic, with 371.17 divided daily doses per 1000 patient days, followed by Sulphamethoxazole and Erythromycin. In follow-up research, Essack et al. (2011) studied antibiotic consumption in private health facilities in South Africa. The data showed that the antibiotics most used were broad-spectrum oral penicillin, macrolide, penems, and carbapenems.

Katende-Kyende et al. (2006) conducted a comprehensive investigation into the most frequently prescribed antibiotics across nine random private healthcare clinics in South Africa, utilizing data collected between January 1 and December 31, 2001, from a study population of 83,655 patients (see Table 2-1). The findings revealed that the antibiotics most frequently prescribed belonged to the penicillin family group (38.17%), followed by sulphonamide (22.49%), antiprotozoals (9.88%), and tetracyclines (9.34%). In a subsequent study in 2015, Truter (2015) explored the trend of prescribing antimicrobial drugs to patients in community pharmacies across South Africa. The results indicated that a combination of amoxicillin and clavulanic acid

(54.69%) was the most frequently prescribed, followed by amoxicillin (18.15%), cefpodoxime (11.4%), and Cefuroxime (9.10%). Despite the limited available data on antibiotic use in South Africa (Essack et al., 2011), the literature suggests that penicillin is the most prescribed antibiotic, raising the possibility of its higher occurrence in the environment. Following penicillin, sulphamethoxazole and erythromycin are also noted as frequently prescribed. Consequently, the widespread usage of these antibiotics underscores the importance of monitoring their presence in the aquatic environment.

Table 2-1 Ten most prescribed antibiotics in South Africa and frequency of prescribing.

Rank	Prescribed Antibiotics	Prescribing frequency	
		n	%
1	Amoxicillin 250mg capsules	17368	23.26
2	Co-trimoxazole 480mg tablets	16261	21.77
3	Doxycycline 100mg capsules	8653	11.59
4	Erythromycin 250mg capsules	6511	8.72
5	Metronidazole 200mg tablets	6226	8.34
6	Co-trimoxazole 240mg/5mℓ syrup	5322	7.13
7	Ampicillin 250mg capsules	4432	5.93
8	Amoxycillin 125mg/5mℓ syrup	3701	4.96
9	Metronidazole 400mg tablets	3589	4.81
10	Phenoxymethyl penicillin 250mg tablets	2619	3.51
Total		74682	100.00

Source: Katende-Kyende et al. (2006)

2.4.1 Identified antibiotics in the aquatic environment of South Africa

Low- and middle-income countries, including South Africa, account for the global increase in antibiotic use due to the high incidence of diseases (Ndihokubwayo et al., 2013). Discharges from WWTPs and surface waters have been investigated to establish the presence of antibiotics in South African waters (Faleye et al., 2019; Fekadu et al., 2018; Matongo et., 2015a) Agunbiade and Moodley (2016) investigated the occurrence of eight pharmaceuticals from WWTP discharges, surface water and sediments along the Msunduzi River, KwaZulu-Natal, South Africa. Of the eight pharmaceuticals, three antibiotic drugs, ciprofloxacin, ampicillin, and nalidixic acid, were investigated. The authors reported ciprofloxacin concentrations of 27 µg L^{-1} and 14 µg L^{-1} in both influent and effluent from the WWTP but recorded minimal traces in the surface water. Matongo et al. (2015b) reported a high sulfamethoxazole concentration (59.28 µg/L) in the WWTP influent in Durban. As previously mentioned, Sulfamethoxazole is among the most ubiquitous antibiotics known to revert to its original active compound. Kanama et al. (2018) tested influent and effluent samples at WWTPs from two hospitals in the Northwest Province and reported tetracycline concentrations of 45.38 µg L^{-1} and 3.22 µg L^{-1}. Other antibiotics, namely azithromycin, ofloxacin, norfloxacin and erythromycin, have been detected in lower concentrations (<10 µg L^{-1} or 10 ng L^{-1}) in WWTPs in South Africa.

2.5 ANTIBIOTIC DETECTION PROCESSES

Detecting antibiotics in the environment poses a challenge due to their low concentrations (ng L^{-1} or µg L^{-1}), necessitating sensitive analytical methods (Seifrtova et al., 2009). Given the low analyte concentrations in samples, a preconcentration step is essential before detection analysis (Hao et al., 2007). Solid-phase extraction (SPE) has emerged as the preferred technique for aqueous sample matrices, replacing the

traditional liquid-liquid extraction (LLE) due to its improved selectivity, ease of operation, specificity, reproducibility, shorter sample preparation time, and lower organic solvent consumption (Seifrtova et al., 2009).

Following the preconcentration step, hyphenated chromatography methods are commonly employed. Liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) are widely accepted methods (Moodley et al., 2016; Richardson and Ternes, 2014; Hernández et al., 2014). LC-MS and GC-MS have proven effective for quantifying pharmaceutical compounds, with the choice between them depending on specific use cases. LC-MS is suitable for analysing polar, non-volatile, and acidic compounds in aqueous matrices, particularly in simpler matrices like tap water (Diaz-Cruz and Barcelo, 2006). On the other hand, GC-MS is preferred for quantifying trace compounds in more complex matrices, such as wastewaters and is limited for non-volatile compounds in the aquatic environment (Hao et al., 2007).

Matongo et al. (2005a, b) successfully used LC-MS to quantify pharmaceuticals in wastewater, demonstrating its effectiveness. While GC-MS is more cost-effective and readily available, it requires the derivatization of less volatile compounds, making it more time-consuming and expensive (Moodley et al., 2016; Gumbi et al., 2016). Therefore, the choice between LC-MS and GC-MS depends on the analytes' specific characteristics and the sample matrix's complexity.

2.6 ANTIBIOTIC-RESISTANT BACTERIA AND GENES IN WATER SYSTEMS

The global surge in antibiotic resistance and the detection of antimicrobial residues and resistant microbes in aquatic systems emphasise the need to understand the environmental dynamics of antibiotic resistance (Burgmann et al., 2018). A significant concern arises from antimicrobial-resistant microorganisms from human and animal waste (Tong and Wei, 2012; Burgmann et al., 2018). As previously mentioned, antibiotics persist in human and animal excretion, finding their way into wastewater treatment systems, identified as sources of pathogens and antimicrobial-resistant genes (AMRGs) (Okoh et al., 2007; Bouki et al., 2013).

Various studies have explored the effects of antibiotics on aquatic microorganism populations and the biogeographical processes within these communities (Yang et al., 2018). The distribution, adsorption, and degradation potential have also undergone scrutiny. However, despite the implementation of standard monitoring and preventive measures to address human exposure to specific antimicrobial-resistant pathogens, water systems are evolving into primary reservoirs for antibiotics, antimicrobial-resistant bacteria (ARB), and genes (ARGs) (Biyela et al., 2004).

2.7 FATE AND TRANSPORT OF ANTIBIOTICS IN WATER BODIES

Understanding the behaviour of antibiotics in water bodies is crucial for its effective management. Emerging contaminants, especially organic ones, often undergo adsorption on sediment particles and various transport processes. Simulation models serve as valuable tools for predicting the fate and transport of these contaminants, providing an alternative to expensive and time-consuming field and laboratory measurements. Numerous studies (Adu and Kumarasamy, 2020; Olowe and Kumarasamy, 2017; 2020; Kumarasamy, 2015; Kumarasamy et al., 2011; Wang and Chen, 1996; Leij and Dane, 1990; Fischer, 1979; Banks, 1974) have contributed to the development of pollutant transport models that simulate water quality along rivers while considering diverse pollutant transport processes. Since Mahloch's work in 1974, models have been developed to study antibiotic-resistant gene transfer using a mass action term at the bacteria and human level (Webb et al., 2005; Levin et al., 1979). Researchers have emphasized that emerging contaminants undergo processes such as sorption, dispersion, and decay, influencing their temporal and spatial concentrations (Lee et al., 2014; Leij and Dane, 1990; Van Genuchten, 1981). Mathematical models have become essential for decision support, especially in studying the impact of antibacterial drugs in the aquatic environment (Opatowski et al., 2011), prompting further development of models to simulate emerging contaminants, one of such emerging contaminants being antibiotics.

2.8 WATER QUALITY MODELLING

2.8.1 Water quality models

Water quality models are essential for investigating and understanding the various processes impacting water quality and facilitating efficient water quality management scenarios (Beer and Young, 1983; Fischer, 1967; Taylor, 1953; 1954). However, existing models often focus on steady-state 1-D transport of constituents governed by advection and dispersion processes (Ogata and Banks, 1961). The Fickian-based Advection Dispersion Equation (ADE) model is commonly used for conservative pollutants, but practical limitations arise in natural streams due to the model's limited assumptions (Ghosh et al., 2004, 2008; Ghosh, 2001; Van Genuchten and Jury, 1987; Chatwin and Allen, 1985; Chatwin, 1980; Fischer et al., 1979; Day and Wood, 1976; Sooky, 1969; Fischer, 1967, 1968; Thackston and Krenkel, 1967). Alternative models, such as the Cells-in-Series (CIS) model (Wang and Chen, 1996; Young and Wallis, 1993; Beven and Young, 1988; Van Ommen, 1985; Yurtsever, 1983; Stefan and Demetracopoulos, 1981; Beltaos, 1980; Banks, 1974; Bear, 1972) and the Aggregated Dead Zone (ADZ) model (Beer and Young, 1983), incorporate an advective time delay, providing an improved simulation of solute transport. However, they come with limitations, including inadequate simulation of the advection component, particularly for longitudinal dispersion (Ghosh et al., 2004, 2008; Ghosh, 2001; Rutherford, 1994; Stefan and Demetracopoulos, 1981). The Hybrid Cells in Series (HCIS) model addresses these limitations by incorporating an advective time delay, providing an improved simulation of solute transport (Ghosh et al., 2004, 2008; Ghosh, 2001). The HCIS model, conceptualized to simulate advection and dispersion, closely matches the ADE model under certain conditions. However, when dealing with non-conservative or reactive pollutants, these models may fail to simulate pollutant transport adequately (Ghosh et al., 2004, 2008; Ghosh, 2001). For pathogenic pollutant transport and antibacterial resistance, sorption processes need to be considered in addition to advection and dispersion (Cameron and Klute, 1977).

Simulating nonequilibrium sorption processes alongside advection and dispersion is challenging due to the complexity of adsorption and desorption processes (Ghosh, 2001; Ghosh et al., 2004, 2008). Although the numerical solution has limitations, the HCIS model proves valuable for simulating the transport of conservative solutes in rivers (Ghosh, 2001). Several investigators have formulated equations to describe pollutant transport, considering first-order exchange between different phases of the flow medium (De Smedt et al., 2005; Worman et al., 2002; Worman, 1998; Runkel, 1998; Czernuszenko and Rowinski, 1997; Runkel and Chapra, 1993; Runkel and Broshears, 1991; Bencala et al., 1990; Bencala and Walters, 1983; Nordin and Troutman, 1980; Hays et al., 1966).

The continual evolution of pollutant loading, interaction, mutations, and dynamics in waterways necessitates ongoing improvements and developments in water quality management tools. Accurate calibration is essential for using a model with confidence. Although water quality models have made significant contributions, ongoing research and model development are imperative to tackle the intricate challenges of pollutant transport, particularly in unique environments such as South Africa. The subsequent section provides more detailed descriptions of models specifically developed to accommodate the unique characteristics of South African rivers.

2.8.2 Water quality systems assessment model (WQSAM)

The Water Quality Systems Assessment Model (WQSAM) is a water quality management tool developed to address the scarcity of models that adequately simulate the variabilities in South African rivers (Slaughter et al., 2018). Recognized for its simplicity and suitability for data-scarce regions, WQSAM is particularly beneficial for managing water quality in large river basins. The model utilizes flows generated from the Water Resources Yield Model (WRYM) or the Water Resources Modelling Platform (WReMP) for water quality simulations (Slaughter et al., 2018).

WQSAM is structured with a modular design, facilitating the maintenance and updates of its various modules within other applications (Slaughter et al., 2012). These modules include the in-stream fate module, baseflow separation module, point and diffuse source modules, reservoir module, and monthly-daily flow disaggregation module. The operating system of WQSAM caters to southern African regions, with specific system model setups in place. However, utilising WQSAM for catchments outside of South Africa necessitates interfacing with other models that link to internationally applied systems models, such as the Water Evaluation and Planning (WEAP) model. This adaptability enhances WQSAM's applicability beyond its regional focus.

2.8.3 Integrated water resources decision support system (INWARDS)

Association for Water and Rural Development (AWARD) developed the Integrated Water Resources Decision Support System (INWARDS) specifically for the Olifants River Catchment (ORC) in response to the pressing water quality management needs in South Africa. INWARDS is a management and response-orientated tool equipped with features designed to directly or indirectly influence strategic and operational decisions, providing crucial support for key management stakeholders (Hugo and Sharon, 2019).

The toolset within INWARDS includes a near real-time water quality system, a decision support software system for water quality and quantity compliance against benchmarks, and early warning system Support. The real-time water quality components help users access water quality data for dissolved oxygen, electrical conductivity, pH, and temperature. INWARDS provides a robust, flexible, and 'user-friendly' platform with good visualisation options. The water quality interface of INWARDS was developed to support DWA's water management system (WMS). Therefore, to access WMS, users must connect to the DWS gateway. This makes remote access a challenge for users. (Hugo and Sharon, 2019). However, this reliance on the DWS gateway presents a challenge for users' remote access (Hugo and Sharon, 2019).

2.8.4 Hybrid cells in series model (HCIS)

The Hybrid Cells in Series (HCIS) model, initially conceptualised by Ghosh (2001), is a mixing cells-based model designed to describe pure advection with time delay, incorporating dispersion when assuming thorough mixing within each cell. Initially developed to simulate the fate and transport of point source conservative pollutants in streams and rivers, the HCIS model has undergone significant enhancements to broaden its applicability. Kumarasamy et al. (2013; 2011) expanded the model by incorporating pollutant sorption and decay processes, while Kumarasamy (2015) included de-oxygenation and reaeration components for simulating dissolved oxygen. Olowe and Kumarasamy (2017) extended the model's capabilities by integrating the first-order kinetic equation to simulate nutrients such as ammonia, nitrite, and nitrates. Recent developments by Adu and Kumarasamy (2020a; 2020b) and Adu (2021) have incorporated non-point source components, reaction kinetics of pollutants, and a hyporheic exchange module for simulating contaminants from diffuse sources. These developments highlight the HCIS model's flexibility in adopting different scenarios and modules for water quality simulations.

The validation of any model is demonstrated in its ability to handle diverse scenarios and water quality variables. However, existing models face challenges when dealing with patterns of pollutant clouds, especially antibiotics in water bodies. Predicting in-stream solute flux necessitates a model that balances complexity with data availability, offering simplicity and ease of interpretation (Schoups et al., 2008). Therefore, there is a need for a model that effectively monitors Emerging Contaminants (ECs) in South Africa's water bodies, considering factors like adaptability to developing countries, user-friendliness, cost-effectiveness, and time efficiency. Table 2.2 presents local and international models, elucidating their descriptions, applications, strengths, and limitations. It is crucial to emphasise that the choice of a model should hinge on its capacity to address specified objectives accurately.

Table 2-2 Summary of Water Quality Models

Model	Description	Applications	Strengths	Limitations	References
WQSAM	-It is a spatially distributed model -It is run within the SPATSIM modelling framework -The model uses water quantity (flow data) from water quantity system models, i.e. WRYM or WReMP -The critical process modelled include uptake of nutrients by algae and macrophytes, decomposition, sedimentation and chemical speciation -Temperature modelling is given much consideration as the key driver of processes affecting water quality -Reservoirs are modelled as completely stirred tank reactors (CSTR)	-Physical and chemical water quality -Eutrophication -Microbial water quality -Land use/land cover (non-point sources) and nutrient loads	-It is a simple decision support system tool and can be used in water resource management -It uses a modular structure for various water quality and quantity components -It aggregates monthly data to daily time steps, which closely mimics water quality as affected by transient hydrological events -Disaggregation of monthly flows to daily flows eliminates the necessity of simulating daily hydrology in the model and thus reduces computational run time -Suitable for data-scarce catchments and thus suitable for use in developing countries - It allows for coupling L-1inking with other models - Ability to separate flow into surface flow (quick flow), interflow and groundwater flow components	-Microbial quality simulations are restricted to point sources and catchment contribution is not included -It was developed for South African catchments -Stratification in reservoirs is not considered -Possibility of carry-over of errors from the water quantity system models, e.g. WRYM -Simulation of non-conservative parameters such as nutrients (ammonia and phosphate) is not adequately represented in the model, especially under extreme hydrological events - The CSTR modelling approach limits the applicability to catchments, shallow reservoirs and lakes The current version does not simulate dissolved oxygen, a critical water-quality parameter.	(Slaughter et al., 2012) (Slaughter et al., 2014) (Slaughter 2017) (Slaughter & Mantel, 2017) (Slaughter et al., 2017) Slaughter & Mantel, 2018)
INWARDS	-It is an IWRM decision support system - It was developed and applied for IWRM in Olifants River Catchment	-Integrated suite for water quantity and quality and reservoir operations monitoring	-It enables sharing of information across departments on a common platform and minimises the silo (sectoral) approach to water management -It helps in real-time monitoring of water quality and quantity -The potential for modification to use remote-sensed data	-Currently a platform for data sharing and analysis and not for modelling -The real-time monitoring of data requires the installation of sensors (as is currently done) to capture water quality data -It monitors only four water quality parameters, i.e. DO, EC, pH and temperature	(Retief and Pollard 2019)
HEC-RAS model	Solves the one-dimensional	sediment transport/mobile	Modelling non-prismatic channels	It cannot simulate antibiotics	(Abed et al., 2021) (Bai et

Model	Description	Applications	Strengths	Limitations	References
	Saint-Venant unsteady flow equations for hydrodynamic modelling, applies the one-dimensional steady-state and solves the dispersion equation using numerical methods (explicit method) for water quality simulation. The model requires hydraulic, geometric, and water quality data.	bed computations, and water temperature/water quality modelling	and flow fluctuation is possible, and computation time is short.	Implementation of the model requires the support of expert The model's result will contain an error because it uses a numerical/approximation method	al., 2022) (Fan et al., 2009)
MIKE-11	MIKE 11 is a one-dimensional model comprised of rainfall runoff, hydrodynamic and advection-dispersion The Model has modules that simulate water quality, flow, and sediment transport in rivers, estuaries, irrigation systems, and other water bodies. Those modules can be used in combination or as stand-alone. The advection-diffusion and ECOLAB modules simulate first-order decays of pollutants and water quality dynamics in rivers, channels, and reservoirs.	Used to simulate salinity, pollutant decay (phosphorus, ammonia), and dissolved oxygen (biochemical oxygen demand, chemical oxygen demand)	MIKE-11 allows simulating flow variability or unsteady flow conditions, as well as pollutants in any surface water, including pollutant transport and metabolism It can simulate multiple water bodies at the same time or individual water bodies	Representing the model is complex, and calibration requires detailed data/information. The model also does not simulate alkaline, inorganic carbon, and antibiotics. MIKE 11 does not simulate the denitrification process in rivers Calibration of the model requires channel cross-section data to reach the limits. This requires a long computational time to produce the result.	(Beck, 1988); (Benedini and Tsakiris, 2013); (Ziemińska-Stolarska and Skrzypski, 2012)

Model	Description	Applications	Strengths	Limitations	References
HCIS	- It is a conceptual model that simulates water quality parameters through a series of cells conceptualised in three zones, i.e. plug flow and two thoroughly mixed reservoir zones. -It is a process-based mechanistic model Initially developed for conservative contaminants, it has been modified to simulate non-conservative parameters, e.g. DO, BOD and nutrients. - First-order kinetics is assumed as appropriate for non-conservative pollutants while incorporating advection and dispersion transport -The critical parameters in the model are advection time, residence time, number of cell length units and decay rate	-Physical and chemical water quality parameters in rivers -water quality through porous media	-Adding a plug-flow component helps adequately simulate the advective transport of solutes. -Conversion of second-order PDE to first-order ODE enables analytical solutions to be obtained for the parameters of interest and improves its simplicity -The model is flexible and thus can accommodate the addition of water quality parameters -The ability to incorporate adsorption processes in the simulation process provides adequate room for modelling removal of pollutants through adsorption, which occurs in natural streams via sediments or other river bed materials, and potential for use in modelling surface water – groundwater interactions -It requires low input data requirements and is thus suitable for data-scarce environments	-It is a steady-state model and thus may not be suitable for simulating dynamic (unsteady flow) conditions -Increasing the number of parameters increases the model complexity -Estimation of the model parameter (dispersion coefficient, unit length) can be impacted by a pre-determined empirical equation that does not have a specific parameter to incorporate for the determination of these parameters	(Ghosh *et al.,* 2004) (Kumarasamy *et al.,* 2011) (Kumarasamy *et al.,* 2013) (Olowe and Kumarasamy 2017) (Kumarasamy 2015) (Chabokpour 2019) (Chabokpour 2020) (Chabokpour and Samadi 2020) (Adu and Kumarasamy 2020) (Adu 2021)

Table 2-3 Review of environmental antibiotic fate simulation models

Model	Pollution Source	Environment (medium)	Type	Pollutant modelled	Risk Assessment	Strengths	Limitations	Sources
PhATE	Point	Stream River Lakes Reservoir	1D	Pharmaceuticals (antibiotics)	Screen-level risk assessment	Provides a range of load emission scenario	Highly sensitive to WWTP removal efficiency, affecting all predictions in the catchment It does not consider in-sewer removal Limited geographic scope Coarse-resolution descript segment (~16 km)	(Aldekoa et al., 2016)
GREATER	Point and diffuse	Rivers	1D	Pharmaceuticals (antibiotics), nutrient	Screen-level risk assessment	It enables the study of potential risk management scenarios It provides a statistical distribution of pollutants The stochastic simulation enables accounting for uncertainties in the input data Efficient emission/source calculation Considers removal of a compound during sewer transport	The laborious pre-processing steps to set up the database and fill in the required data for the parameters Calculates spatially steady-state concentrations susceptible to temporal fluctuation Emission pattern calculation influenced by the WWTP bypass flow	(Capdevielle et al., 2008) (Aldekoa et al., 2016) (Lämmchen et al., 2021)
QUAL-2E	Point and diffuse	Streams Rivers	1D	Dissolved oxygen, organic nutrients, algal concentration, antibiotics	-	Simulation of point and nonpoint source pollutants Provides simulation of non-uniform flow	Cannot model the temporal variability of flow The model gives a good simulation of narrow rivers (susceptible to water depth), as deep rivers have different stratification and mixing rates. It cannot simulate the effect of toxic organic compounds and heavy metals. It is inappropriate for waterbodies exhibiting significant lateral variations.	(Bai et al., 2022) (Ziemińska-Stolarska & Skrzypski 2012)
QUAL-2K	Point and diffuse	Streams Rivers	1D	Pharmaceuticals (antibiotics), conventional parameters	-	Enables to divide the river into unevenly spaced segments Simulation of the effect of generic pathogens, total inorganic carbon, and light extinction	It is inappropriate for waterbodies exhibiting significant lateral variations. It does not consider the effect of sedimentation	(Aldekoa et al., 2016) (Zhi et al., 2022)
Global FATE	Point	Rivers Lakes Reservoirs	2D/3D	Pharmaceuticals (antibiotics)	-	Efficient resolution to represent small streams Worldwide geographic scope	Cannot simulate flow variabilities Requires extensive data and external hydrological pre-processing step	(Font et al., 2019)
WASP	Point and diffuse	Rivers Reservoirs Lakes Estuaries	1D/2D/3D	Pharmaceuticals (antibiotics), conventional parameters	-	It enables analysis of the significance of individual mechanisms	It is challenging to obtain segment/site-specific data to calibrate fate mechanisms.	(Han et al., 2022) (Wool et al., 2020) (Noutsopoulos et al., 2019)

Model	Pollution Source	Environment (medium)	Type	Pollutant modelled	Risk Assessment	Strengths	Limitations	Sources
		Coastal areas Wetlands				It includes a sediment diagenesis module for remineralisation It provides a sensitivity analysis	It has a limitation in modelling concentration gradients in the mixing zone for wide channels with poor mixing conditions. Cannot simulate high-flow events It requires external hydrodynamic models for flow information	(Arlos et al., 2014)
AQUASIM	Point	Streams Rivers Lakes Reservoirs	2D/3D	Pharmaceuticals (antibiotics), conventional parameters	-	Efficient vertical mixing representation and temperature profiling	It assumes uniform horizontal mixing in lakes and reservoirs	(Reichert, 1994) (Nieto-Juárez, 2021)
iSTREEM	Point	Streams Rivers	1D	Pharmaceuticals (antibiotics)	Conservative risk assessment	simplicity of simulation	Suitable for simple simulation Require pre-processed data It does not consider in-sewer removal	(Kapo et al., 2016) (Ferrer and Deleo, 2017)
QWASI	Point and diffuse	Lakes	Multimedia Fugacity (air, sediment, and water)	Pharmaceuticals (antibiotics) and organic pollutants	Screening-level risk assessment	Efficient and advanced modelling of lake temperature stratification Modelling of ice melt in lakes	Result influenced by choice and calculation of fugacity factor Depends on uniform mixing condition Requires exclusive half-life degradation data	(Wang et al., 2020) (Chen et al., 2018) (Liu et al., 2017) (Kim et al., 2017)
ePiE	Point	Streams Rivers Lakes Reservoirs Estuaries	1D	Pharmaceuticals (antibiotics)	-	Ease of application	Suitable for narrow rivers It does not consider in-sewer removal	(Austin et al., 2022) (Ragas 2019) (Oldenkamp et al., 2018)
EUSES	Point	Streams Rivers Marine	Multimedia Fugacity (air, Water, Sediment. Soil and groundwater)	Organic chemicals Pharmaceuticals (antibiotics)	Conservative risk assessment	Simulation in multimedia, including groundwater pollution Simulation of exposure through the food chain Allows estimation of media-specific degradation	Provides steady-state concentration susceptible to temporal fluctuation Extensive data requirement Intensive pre-processing of model parametrization for site-specific simulation	(Spaniol et al., 2021) (Arnot et al., 2010) (Berding & Matthies, 2002) (Kawamoto et al., 2000) (Vermeire et al., 1999) (Berding & Schwartz, 1996)

2.8.5 Water quality modelling considering antibiotic resistance

The primary transport equation is developed from a simple mass balance concept, which is presented in equation 1 as:

$$M_a = M_{in} - M_o \pm R \quad [1]$$

The following partial differential equations were developed to simulate various constituents, including susceptible and resistant bacteria.

First-order decay:

The organism undergoes degradation, which follows the first-order variation (equation 2).

$$\frac{\partial C}{\partial t} = -k_1 C \quad [2]$$

Metals:

Metals are utilised by bacterial cells for growth up to a certain quantity, represented by the equation 3.

$$\frac{\partial M}{\partial t} = -m \frac{\mu\, n}{\gamma}\Big|_{resistant} \quad [3]$$

Where μ is the specific growth rate, γ is the yield coefficient, n is resistant bacteria culture, and m is mass reduced due to bacterial growth.

Organic matters:

Dissolved organic matter is an essential parameter in simulating bacteria, and this takes a similar first-order differential form as metals, which means that the mass reduction of dissolved organic matter depends on bacterial growth.

Susceptible and Resistant culture:

The primary purpose of this model is to identify the factors that govern the processes involving resistant and susceptible or sensitive bacteria. Studies (Baker et al., 2016; Liu et al., 2015) considered E. coli in their study.

The following partial differential equations 4 and 5 simulate both sensitive and resistant bacteria cultures.

$$\frac{\partial n_{Susceptible}}{\partial t} = \left(f\ \mu\ n|_{Susceptible}\right) - k_d n_{Susceptible} + r_t \frac{n_t}{n+n_{resistant}} + k_s n_{segregation} \quad [4]$$

$$\frac{\partial n_{resistant}}{\partial t} = \left(f\ \mu\ n|_{resistant}\right) - k_d n_{resistant} + r_t \frac{n_t}{n+n_{resistant}} - k_s n_{segregation} \quad [5]$$

Where f is a factor, k_d is the death rate of bacteria, r_t is the resistant gene transfer rate, n_t is total resistant gene transfer, and n is susceptible culture.

Deterministic transport model:

Antibiotic-resistant bacteria cultures in the aquatic environment follow the Fickian-based advection-dispersion equation with the above parameters for considering bacterial settling, growth and death, segregation, and genomic transfer processes. A second-order partial differential equation 6 represents this model.

$$\frac{\partial n_{rC}}{\partial t} = -u\frac{\partial n_{rC}}{\partial x} + D_L\frac{\partial^2 n_{rC}}{\partial x^2} - k_d n_{rC} + (f\ \mu\ n|_{rC}) + r_t\frac{n_t}{n+n_{rC}} - k_s n_s \quad [6]$$

Where u is flow velocity and D_L dispersion coefficient, r_C represents the resistant culture of bacteria.

Antibacterial resistance development is a significant threat to public health. Simulation of ABR using mathematical models is beneficial to manage such impacts. Further, the models can present knowledge output for evaluating the impact of ABR in the aquatic environment. Several elements, including external factors, present the impact of ABR as a multifactorial problem. Understanding the model's framework, type, genetic composition, and host environment is necessary. This is also understood from the review of previous studies. There is a need to develop further ABR models that consider highly complex and multilevel interactions. This study currently only focuses on deterministic models, not stochastic ones, as the proposed model is deterministic. However, the project will also look at stochastic models during model development for performance evaluation. This project aims to develop a conceptual-based model using the mixing cells concept.

2.9 SUMMARY

The literature review underscores the pervasive presence of antibiotics in rivers, highlighting their potential role in fostering bacterial resistance. Recognizing the intricate pathways through which antibacterial resistance can spread, the study emphasizes the severe threat emerging contaminants pose to water bodies. This prompts the need for effective water quality management strategies, mainly by applying mathematical models. Existing water quality models are discussed in detail, examining their complexity, nature, simulation methods, and consideration of processes and parameters. The review advocates for collective global collaboration to address the escalating detection of emerging contaminants in water bodies. Overall, the literature review serves as a foundation for understanding the occurrence, configuration, and impacts of antibiotic residues, emphasising the importance of water quality models in this context.

CHAPTER 3: REMOTE SENSING AND GEOGRAPHIC INFORMATION SYSTEMS APPLICATION IN WATER QUALITY MONITORING

3.1 INTRODUCTION

Developing a suitable Water resource management model requires continuous and accurate monitoring. The traditional water quality assessment methods are limited in their spatial and temporal coverages, and as such, they are time-consuming and costly. In this regard, satellite-observed data provide good spatial and temporal coverages relevant to water resources assessment and, when adequately undertaken, are time and cost-effective (Wang and Yang, 2019; Okin, 2011; Landgrebe, 1999). GIS and remote sensing have been used to map land use and pollutants in surface water bodies for several years (Dunca, 2018; Sheffield et al., 2018; Fataei, 2011; Wei et al., 2009). The National Aeronautics and Space Administration (NASA) has been monitoring the Earth's resources from space with multispectral scanners that collect data sets in about five to ten bands of relatively large bandwidths (70-400 nm) since the 1960s (Sheffield et al., 2018; Landgrebe, 1999). These multispectral scanners generated data for suitable land use mapping, but the data has been limited for adequately evaluating water quality. Since the mid-1980s, multispectral and hyperspectral scanners (both space and airborne systems) have been deployed to improve the sensors' spectral resolution, allowing water quality assessment.

There are two critical aspects in water quality assessments using remote sensing: mapping and identifying the source of pollutants and mapping the surface water quality temporally and spatially. The former is much easier to map as most satellite products, after proper data analyses, provide land use maps for the catchment that drains into the target surface water body under investigation (rivers, Lakes, reservoirs and estuaries) and from which the pollutant source could be identified with some targeted water quality sampling for validation purposes. However, mapping water quality from satellite products is limited due to the spatial and spectral resolution of data from the multispectral scanners and, thus, inadequately evaluating water quality until the mid-1980s (Mbuh et al., 2016; Mbuh, 2019). Development in space and airborne multispectral and hyperspectral sensors means that the spatial and temporal monitoring of surface water quality is becoming a reality.

3.1.1 Pollutant source tracing using remote sensing and GIS

A significant difficulty in assessing and modelling surface water quality is identifying the sources of pollutants and the associated type and concentration of water quality parameters. The different types of land use that contribute to the pollutant load of a particular surface water body can be mapped using satellite data analyses and interpretation. For this, several satellite products exist, including, among others, Landsat, Sentinel, Moderate Resolution Imaging Spectroradiometer (MODIS), Advanced Space-borne Thermal Emission and Reflectance Radiometer (ASTER), and High-resolution imagery (Gorelick et al., 2017). Landsat 5 Thematic Mapper (TM), Landsat 7 Enhanced Thematic Mapper Plus (ETM+), and Landsat 8 Operational Land Imager (OLI) are the latest series of Landsat satellites. Since the operational period for each satellite is different, a combination of three satellites is usually used. Landsat 5 TM was launched in March 1984 and decommissioned in June 2013. Landsat 7 ETM+ and Landsat 8 OLI were launched in April 1999 and February 2013 and are still operational (USGS, 2018). The above satellite sensors have 30 m spatial resolution, limiting the identification of relatively small land uses that are point sources of contaminants.

Satellite images acquired from various sensors are classified to create land-use types for further validation. Satellite Image classification processes are grouped into supervised and unsupervised image classifications (Reynolds et al., 2016; Rodriguez-Galiano et al., 2012; Vorovencii, 2012; Richards, 2006). Supervised classification is the most common method to classify satellite images (Vorovencii, 2012; Richards, 2006).

Supervised image classification is based on the premise that prior knowledge of the land use classes within the area of interest is known (Vorovencii, 2012; Richards, 2006). The users determine the land use and cover types to be investigated, and representative pixels for each type are identified. This process is known as training, and the training information is obtained either from field surveys or from existing aerial photography, maps, satellite imagery information or a combination of these (Berhane et al., 2018; Vorovencii, 2012; Richards, 2006). Once the pixels are trained, a classifier is used to classify the rest of the images. Several classifiers are available, including the maximum likelihood, minimum likelihood, support vector machine, and random forest (Li et al., 2014; Mellor et al., 2012; Senf et al., 2012). Training data is an essential component in the classification and accuracy of remotely sensed data.

The accuracy of classified satellite imagery is assessed using validation techniques based on an error matrix for each image classified or by comparing it with the latest land use data generated from another high-resolution satellite data interpretation (NASA, 2018).

3.1.2 Water quality mapping using multispectral and hyperspectral remote sensing

Optical indicators of water quality can improve the ability of scientists and resource managers to monitor water bodies' quality in a timely and cost-effective manner. The spectral signature changes in the water can be measured through remote sensing techniques and can be related to a water quality parameter using empirical or analytical models (Gholizadeh et al., 2016; Shafique et al., 2001, 2002). Thus, surface water quality can be monitored and mapped based on different water quality parameters and reflectance characteristics. The potential water quality parameters that may be mapped from spectral characteristics include, among others, Temperature, Chlorophyll a, total suspended solids, total phosphorus and related nutrients, and turbidity.

3.1.2.1 Image resolution

Remotely sensed images derive valuable information from their spatial, spectral, radiometric, and temporal components, contributing to the interpretation of surface materials and conditions (Smith, 2012). The resolution of an image, as produced by the sensor system, is defined by these components. Spatial resolution, defined as the fineness of spatial detail in an image, is influenced by sensor design and its altitude above the surface (Campbell and Wynne, 2011; Smith, 2012). A smaller pixel size corresponds to a higher spatial resolution. Spectral resolution refers to a sensor's ability to measure different wavelength intervals, with its bandwidth determining sensitivity to various spectral ranges (Smith, 2012). The sensor's capacity to revisit and collect data from the same scene also contributes to spectral resolution. Images are categorised as panchromatic, multispectral, and hyperspectral in increasing order of spectral resolution. Radiometric resolution signifies a sensor's ability to record multiple brightness levels (Campbell and Wynne, 2011). In satellite images, radiometric resolution is quantified in bits, where a higher number of bits corresponds to a higher radiometric resolution for a spectral sensor (Campbell and Wynne, 2011; Smith, 2012).

3.1.2.2 Visible to middle infrared image bands of Landsat multispectral sensors

Wavelengths recorded by sensors are termed bands, and their number varies with remote sensing sensors. However, the display of remotely sensed data is limited to only three bands (Acharya, 2015). The Landsat program (Landsat 1-8) has been one of the best and most accessible sources for monitoring and mapping land surface and cover since 1972 (Acharya, 2015). Landsat-7 ETM+ and Landsat-8 Oli are currently operational Landsat satellites. Landsat-7 ETM+ has 8 bands shown below, of which 7 bands have a 30 m spatial resolution, and the panchromatic band has a 15 m spatial resolution.

- Blue Band (0.45-0.52 µm):- since this band has a short wavelength, light penetrates deeper than other bands (Horning, 2004). Thus, this band can fully penetrate shallow water bodies (Smith, 2012).

- Green Band (0.52-0.60 µm):- this band differentiates yellow and green vegetation as it provides peak visible reflectance for green vegetation (Smith, 2012).
- Red Band (0.63-0.69 µm):- this band is strongly absorbed by chlorophyll, which gives vegetation a green colour. Therefore, green vegetation would appear darker in this band compared to other visible bands. Different plant types can be differentiated based on absorption strength. The band is also used for the determination of soil colour.
- Near-infrared (0.76-0.90 µm): green vegetation is brighter in this band than in any visible bands (Smith, 2012). Water nearly absorbs all light at wavelength and appears very dark, making it suitable for defining land/water interface (Horning, 2004).
- Middle infrared (1.55-1.75 µm): strongly absorbed by snow, water and ice and reflected by clouds. It is, therefore, useful when differentiating snow and clouds (Smith, 2012).
- Middle infrared (2.08-2.35 µm): similar to the Middle infrared (1.55-1.75 µm) band but includes an absorption feature that can be absorbed by only clay minerals, and material consisting of clay appears darker than in the TM 5 band. The increasing light scattering in water is due to increasing suspended load (Papoutsa et al., 2013; Han, 1997).

Landsat 8 OLi include all Landsat 7 bands and an additional coastal (0.433-0.453 µm), Cirrus (1.360-1.390) and two thermal infrared sensors (TIRS) (10.30-11.30 and 11.50-12.50 µm) bands (Archaya, 2015). The coastal band is a deep blue band used for coastal water studies, and Cirrus bands improve cirrus cloud contamination detection (Archaya, 2015). TIRS bands provide improved thermal mapping and soil moisture estimation.

3.1.2.3 Satellite sensors

Space-borne sensors can be classified based on their spatial resolution into three categories (Abdelmalik, 2016):

- High-resolution sensors like IKONOS, Quickbird, and Worldview Series have less than 10 m spatial resolution.
- Moderate-resolution sensors include the Landsat series (15-20 m), ASTER (15-90 m), and Sentinel-2 (10-60 m).
- Regional to Global resolution sensors include Terra MODIS (250-1000 m) and SeaWiFS (1130 m).

Satellite sensors characterized by coarse spatial resolution present limitations in mapping smaller water bodies, as observed with sensors like Sea-viewing Wide-Field-of-View (SeaWiFS) and Coastal Zone Colour Scanner (CZCS) (Liu et al., 2003). In instances where several features are smaller than the pixel size of a sensor, such as a narrow river with a width of less than 10 m, a pixel may combine spectral signatures of multiple features, making it challenging to distinguish between water and the land surface (Sivanpillai and Miller, 2010; Zou et al., 2006). On the other hand, sensors from the IKONOS, Quickbird, and Worldview series offer high resolution, ranging from 1 m to 4 m and 1.5-2 m, respectively (Abdelmalik, 2016). These high-resolution sensors facilitate the detection of lesser features with increased accuracy. However, it is essential to note that access to these high-resolution images typically involves costs and is not freely available to the public.

The Landsat series of satellites, which includes the Landsat Thematic Mapper (TM), Landsat Multispectral Scanner (MSS), and a combination of TM and Enhanced Thematic Mapper (ETM+), has been extensively utilised for the estimation of surface water quality parameters (Khattab and Merkel, 2014; Nas et al., 2010; Khorram et al., 1991; Khorram, 1981). These sensors have effectively estimated various surface water quality parameters across multiple studies. The latest iteration of the Landsat series, Landsat 8 Operational Land Imager (OLI), offers several improvements, including narrower spectral bands, increased radiometric resolution from 6 to 12 bits, enhanced geometry precision, improved calibration, and superior signal-to-noise characteristics (Roy et al., 2016). Landsat 8 OLI exhibits heightened sensitivity to colour, brightness, and suspended material, with an additional two bands from Landsat-7 ETM providing superior spectral wavelength

resolution compared to its predecessors. Due to these advancements, Landsat 8 OLI has become the most popular and widely used Landsat satellite for surface water quality studies (Acharya et al., 2016).

Advanced Space-borne Thermal Emission and Reflection Radiometer (ASTER), an instrument onboard the TERRA satellite, has 14 spectral bands in total: three in the visible and near-infrared (VNIR) region, six in the short-wave infrared (SWIR) and five thermal regions (Yamaguchi and Naito, 2000). It can capture images at higher spatial resolutions, as high as 15 m in the VNIR band. It, therefore, can detect smaller surface features than Landsat images (Sivanpillai and Miller, 2020); despite being limited to only three spectral bands in VNIR regions, Sivanpillai and Miller (2020) compared Landsat Thematic Mapper (TM) and ASTER data. They indicated that ASTER has significantly higher accuracy in identifying clear, green and turbid surface water bodies than Landsat TM. ASTER sufficiently distinguishes narrow and elongated water bodies' spectral values (Sivanpillai and Miller, 2020).

The Sentinel-2A and Sentinel-2b are operational systems launched on 23 June 2015 and 7 March 2017, respectively. It has a temporal resolution of 5 days and a spatial resolution of 10 m in blue/green/red/NIR band (490 to 665 nm), which is an improvement from both Landsat 8 and ASTER data based on the temporal resolution of 16 days and their spatial resolution (Chen et al., 2017). The high spatial resolution of the Sentinel-2 satellite allows for characterising smaller water bodies with higher accuracy than ASTER and the Landsat series sensors.

The Sentinel-2 imagery comprises 13 spectral bands, spanning from Visible and Near-Infrared (VNIR) to Shortwave Infrared (SWIR). Guo et al. (2021) conducted a study on the effectiveness of Sentinel-2 in estimating water parameters, highlighting that the three bands B3 (559.8-595.8nm), B4 (664-695.6nm) with 10 m spatial resolution, and B5 (704.1-719.1nm) with 20 m spatial resolution were particularly influential in retrieving total phosphorus, total nitrogen, and chemical oxygen demand. In a comparative study by Bande et al. (2018) focusing on turbidity and chlorophyll parameters in Vaal Dam, Landsat 8 OLI and Sentinel-2 were compared, with Sentinel-2 demonstrating superior results for both parameters. It is noteworthy that all the mentioned satellite sensors provide multispectral imagery.

3.1.2.4 Water quality assessment and mapping using Hyperspectral sensors

Hyperspectral remote sensing, with its higher spectral resolution encompassing up to 224 bands covering wavelengths from 400 to 2500 nm and spatial resolution of about 30 meters, has become available for earth sciences applications, including water quality monitoring (Guo et al., 2020; Li et al., 2017; Gitelson et al., 2011; Brando and Dekker, 2003). Hyperspectral imagery consists of several spectral bands, each less than 10 nm wide, offering enhanced spectral information for precise water quality analysis. Notable hyperspectral sensors include FTHSI on MightySat II, Hyperion on NASA EO-1, airborne visible/infrared imaging spectrometer (AVIRIS), Airborne Real-Time Cueing Hyperspectral Enhanced Reconnaissance (ARCHER), Hyperspectral Digital Imagery Collection Experiment (HYDICE), PROBE-1, Compact Airborne Spectrographic Imager (CASI), and HyMap (Mbuh, 2019; Mbuh et al., 2016; Hadjimitsis and Clayton, 2011). The very high spectral resolution of hyperspectral sensors provides a significant advantage over multispectral sensors, enabling exceptional differentiation of objects based on their spectral response in narrow bands (Hadjimitsis and Clayton, 2011; Landgrebe, 1999). This spectral information has proven valuable in estimating dissolved organic matter, chlorophyll, and total suspended matter concentrations from optical remote sensing technologies (Hakvoort, 2002). However, the operation of hyperspectral data is currently limited to airborne platforms, such as the Airborne Visible Infrared Imaging Spectrometer (AVIRIS), due to the high cost and limited spatial coverage. Space-borne hyperspectral data, including examples like Hyperion and Compact High-Resolution Imaging Spectrometer (CHRIS) data, remains experimental (Guo et al., 2020; Halme et al., 2019; Lunetta et al., 2009). Consequently, comprehensive surface water quality investigation is presently constrained to the use of multispectral imagery.

3.1.3 Satellite data processing for water quality assessment

3.1.3.1 Atmospheric Correction

Recorded signals from sensors in the electromagnetic spectrum contain two types of brightness: the brightness due to atmospheric interference, referred to as noise, and the actual reflectance from surface materials (Abdelmalik, 2016). For accurate and precise reflectance signatures of surface materials, which are essential for water quality assessment, atmospheric correction is necessary (Abdelmalik, 2016; Tyagi and Bhosle, 2011; Chander et al., 2009; Lillesand et al., 2004; Liang et al., 2001).

Various methods have been employed for atmospheric correction of satellite images in inland water quality studies. These methods include Dark Object Subtraction (DOS) (Gilmore et al., 2015), Fast Line-of-Sight Atmospheric Analysis of Hypercubes (FLAASH) (Abdelmalik, 2016), Atmospheric Correction for OLI 'Lite,' ACOLITE (Yunus et al., 2020), and Provisional Landsat-8 Surface Reflectance Algorithm (L8SR) (Allam et al., 2020). In a study evaluating different atmospheric correction methods for estimating total suspended matter concentrations using Landsat 8 OLI, Bernado et al. (2017) found that the L8SR correction exhibited the best performance. FLAASH produced better estimations of water quality parameters when in-situ atmospheric conditions were available, while other methods failed to produce accurate results (Bernado et al., 2017).

3.1.3.2 Regression analysis

The estimation of water quality parameters through satellite image interpretations involves correlation and regression analysis of in-situ measured water quality parameters, field spectrometry, and spectral reflectance recorded by sensors at the exact location, date, and time of satellite overpass (Nas et al., 2010; Lim and Choi, 2015; Mushtaq and Nee Lala, 2016; Avdan et al., 2019). Initially, the image undergoes radiometric correction to determine actual surface reflectance by converting digital numbers (DNs) to spectral radiance and then to reflectance using techniques proposed by USGS (2013) (Mushtaq and Nee Lala, 2016; Lu et al., 2002; Yang and Lo, 2000; Quaidrari and Vermote, 1999; Chavez, 1996; Moran et al., 1992; Markam and Barker, 1986; Lillesand et al., 1983). The statistical method chosen generates equations for modelling, and the coefficient of determination between reflectance values and in-situ water quality parameters is calculated (Mushtaq and Nee Lala, 2016). The selection of the modelling equation considers three factors: the adjusted Square Correction Coefficient (R^2), the probability value (p), where p should be less than 0.05, and the Standard Error Estimation (SEE), with lower values indicating higher accuracy (Abdelmalik, 2016). Crucial in satellite data analysis is choosing suitable regression methods and independent variables that result in a high coefficient of determination (R^2) value (Mushtaq and Nee Lala, 2016).

The reflectance data bands, converted from DNs for each station, are typically chosen as independent variables, while the water quality parameters at the same station serve as dependent variables (Mushtaq and Nee Lala, 2016; Abdelmalik, 2016; Alparslan et al., 2007). The reflectance band with the highest coefficient for each parameter is selected for modelling (Abdelmalik, 2016). Various regression models such as Linear, Logarithmic, Inverse, Quadratic, Cubic, Compound, Power, and growth fits are tried and identified to determine the best-fitting regression model (Mushtaq and Nee Lala, 2016; Abdelmalik, 2016). The selected best regression model enables the derivation of equations suitable for each variable, allowing for the estimation of water quality parameters from reflectance at any point within the studied water body. This approach facilitates spatial and temporal water quality mapping.

3.2 INVESTIGATING THE SPECTRAL CHARACTERISTICS OF WATER BODIES

Spectral characteristics of water are defined by the amount of scattering determined by light transmittance. Maximum light transmittance in clear waters occurs from 0.44 µm to 0.54 µm, peaking at 0.48 µm (Campbell and Wynne, 2011). In the near-infrared region, sunlight in clear water is strongly absorbed and nearly reaches zero (Malinowski et al., 2015). The presence of sediments or any particulate matter other than chlorophyll

changes the spectral properties of water, increasing the overall brightness in the visible region and shifting the maximum reflectance from the blue region towards the green (Campbell and Wynne, 2011;Wezernak et al., 1976). In the region of 0.4 µm to 0.5 µm, increasing particulates other than chlorophyll has a negligible effect on the reflectance spectrum. Therefore, suspended solids are generally measured using the red spectral band (Wezernak et al., 1976). In the near-infrared region, clear waters are completely dark, whereas turbid water has a spectral response of nearly 5%, making it possible to differentiate between turbid and clear water (Bartolucci et al., 1977).

3.2.1 FieldSpec Spectroradiometer

Field spectrometry is the quantitative measurement of the field's radiance, irradiance, reflectance or transmission. The FieldSpec spectroradiometer is designed explicitly for field environment remote sensing to acquire visible near-infrared (VNIR) and short-wave infrared (SWIR) spectra. It is a compact, portable, and precision instrument with a spectral range of 350-2500 nm and a rapid data collection time of 0.1 seconds per spectrum. It involves the collection of accurate spectra and requires an awareness of the influences of:

- Sources of illumination.
- Atmospheric characteristics and stability.
- Winds.
- Instrument field-of-view.
- Sample viewing and illumination geometry.
- Instrument scanning time.
- Spatial and temporal variability of the sample characteristics.

Field spectroscopy measures the reflectance properties of water bodies, providing detailed measurements of the spectral characteristics in the natural environment, allowing for more precise image analysis and interpretation, which, through remote sensing, detects a process or material of interest in the water body.

CHAPTER 4: STUDY AREA, SITE SELECTION AND LAND USE CLASSIFICATION

4.1 INTRODUCTION

Defining the study area and land use classification lays the foundation for exploring the environmental dynamics. The study area captures the diverse natural features, human settlements, and anthropogenic activities. The rationale behind the selection is crucial in ensuring a representation of the complexities inherent in the chosen area. Subsequently, site selection becomes paramount, involving identifying specific locations within the study area that offer comprehensive insights into the varying environmental variables. Finally, land use classification is a fundamental tool for categorising varied land types to establish the spatial distribution of human activities. These foundational considerations set the stage for unravelling the intricate relationships between land use patterns and water quality outcomes, contributing to a holistic understanding of environmental processes within the chosen region.

4.2 DESCRIPTION OF STUDY AREA

The Msunduzi River, situated in the KwaZulu-Natal (KZN) Province of eastern South Africa (Figure 4.1), is a significant tributary of the uMgeni River. Stretching from the western KwaZulu-Natal midlands through Pietermaritzburg, the Msunduzi River is a vital feeder into the uMgeni River. It is a principal water source in the KwaZulu-Natal region. Spanning a catchment area of 875 km^2 and a tributary length of 115 km, the Msunduzi River traverses diverse landscapes, including highly industrialised zones, informal development areas, forests, wetlands, and agricultural and urban areas. Runoff from these varied surroundings contributes to the river's recharge. Serving a population of approximately 300,000 people, the Darvil WWTP discharges its effluents into the Msunduzi River, compounding its pollution loading (Matongo et al., 2015). The catchment experiences a subtropical climate marked by hot and wet summers and dry and cold winters and is characterised by seasonal rainfall that occurs between October and March, which contributes 80% of the total annual rainfall.

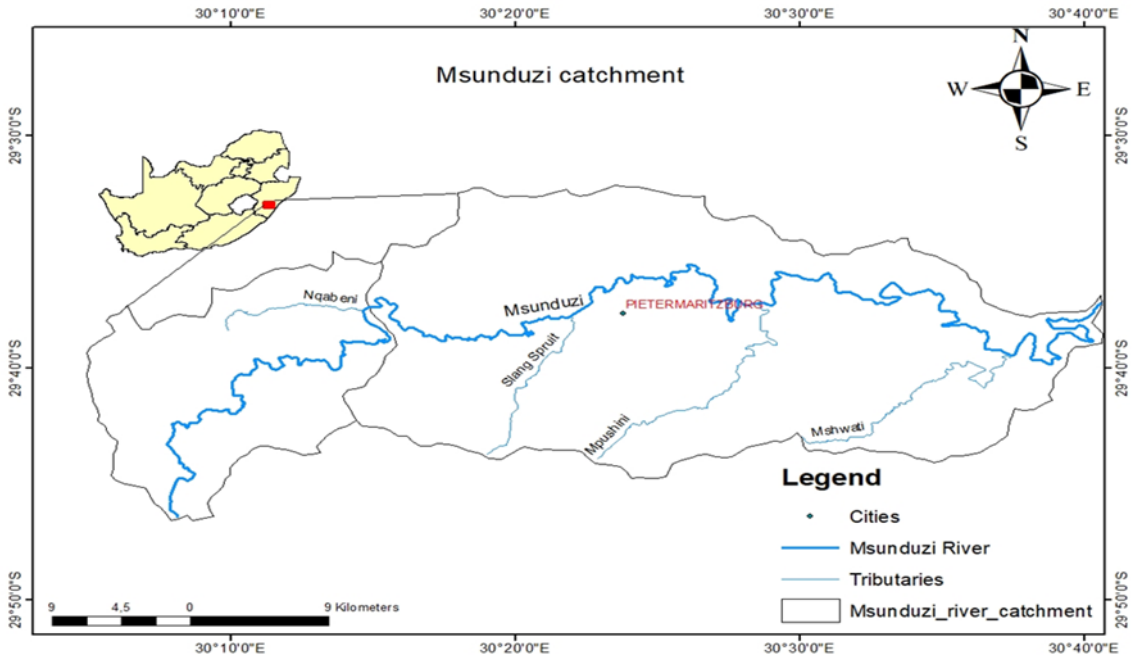


Figure 4.1 Location of the study area in KwaZulu-Natal, South Africa.

4.3 LAND-USE IDENTIFICATION AND CLASSIFICATION

The presence of pollutants in South African waters is increasing. Identified sources of these pollutants include medical waste from health facility establishments and Industries, urine and faecal matter, homes, hospices, WWTPs and rural and informal settlements lacking adequate sanitary facilities (Gumbi et al., 2017). Others include discharges from wastewater treatment plants and the extensive use of antibiotics for agriculture. As stated in the preceding sections, the Msunduzi River runs through highly industrialised areas, agricultural areas, and the discharge point of the Darvill wastewater treatment plant. Further, the river receives runoff from rural communities and the municipality along its course. Therefore, a land-use map (see Figure 4.2) of the Msunduzi catchment area was developed to identify land-use activities relating to antibiotic use and applications within the catchment that would likely serve as a pollution contributor to the river. The Landuse map was used to establish suitable sample collection sites.

4.3.1 Land-use mapping and classification

Human activities cause changes to the environment and ecosystems. Recent damage to the ecosystem has been attributed to land use activities, including agriculture, building construction, urban expansion, forest timber extraction, mining activities, and industrial growth. These activities are driven by human needs and associated wants (Shende et al., 2015). Determining and mapping the various anthropogenic land-use activities within a catchment area is imperative to understanding the possible sources and pollution trends of rivers resulting from surface runoff. Land use maps describe the arrangements and activities within a specified catchment and input how the land is used and modified due to human activities (Disperati et al., 2015). Image classification is a remote sensing process for producing Land use maps (Campbell and Wynne, 2011). Several elements are needed to ensure that the classification process is efficient. The main elements include the user's knowledge and proficiency of the processes involved, the accuracy of the classification process and the availability of quality satellite imagery and field observed validation and training information.

4.3.1.1 Mapping sources of contaminants

A relationship between land use and water quality parameters is examined to identify sources of surface water quality issues. Multivariate statistics and GIS are employed for this analysis (Li et al., 2016). Sources of water quality parameters are reviewed from literature relevant to the region. For instance, potential sources of pollutants include WTTPs, hospitals, agricultural regions, animal farming (Martinez, 2009), and urban informal settlements (Nadimpalli et al., 2020). Transmission routes for these pollutants, such as surface water networks (Lakes, Estuaries, and rivers), are mapped and analysed using GIS (Chique et al., 2019). Features are manually identified within Google Earth imagery and batch-exported into ArcMap to create a shapefile of feature classes (Chique et al., 2019).

Following image classification into land-use categories, land-use data is extracted using GIS spatial analysis functions and overlaid with the catchment boundary (Wang and Yin, 1997). The percentage of each land use is calculated. Water quality parameters are statistically compared with spatial variation in land use data, and the strength of a potential relationship is analysed using Pearson’s correlation, with higher correlation values serving as a reference for relative importance (Wang and Yin, 1997). Principal component analysis (PCA) is conducted for further analysis to identify potential sources of environmental stress (Li et al., 2016).

4.3.2 Land use classification of Msunduzi Catchment

Identifying the various land classes within the Msunduzi River catchment is critical to effective land use classification and mapping. The Intergovernmental Panel on Climate Change (IPCC, 2003) suggests a mixture of six land classes reasonably mapped using remote sensing. The classes include grassland, forest, agriculture, human settlement, water bodies and a general group of other lands. The Msunduzi River

catchment used in this study has been classified into water bodies, settlements, industrial areas, bare land, agricultural lands, roads, shrubs, and forests. For simplicity, bare land and grassland are grouped as bare land. The land uses are mapped and classified based on careful processing of the latest cloud-free sentinel-2B image acquired on 20 October 2021.

4.3.3 Selection of training sites

Supervised image classification was applied using maximum likelihood classification as it is the most used classification technique and yields good results (Eastman, 2003). Supervised classification requires users to identify the areas of interest called training sites from existing maps and site visits (Richards and Jia, 2006; Rwanga and Ndambuki, 2017). The area of each class is calculated relative to the total study area and pixel count. The three steps used for classification are 1) defining training sites, 2) extracting their signature, and 3) supervised classification. These steps were followed in the preparation of the Msunduzi River catchment land use map (Figure 4.2).

4.3.4 Validation

Accuracy assessment and validation of the classification process are essential to ensure the correct pixel selection and classification of each land-use class. For accuracy assessment, pixels chosen for the assessment were identified and compared to the area's latest existing land use map and through field verification

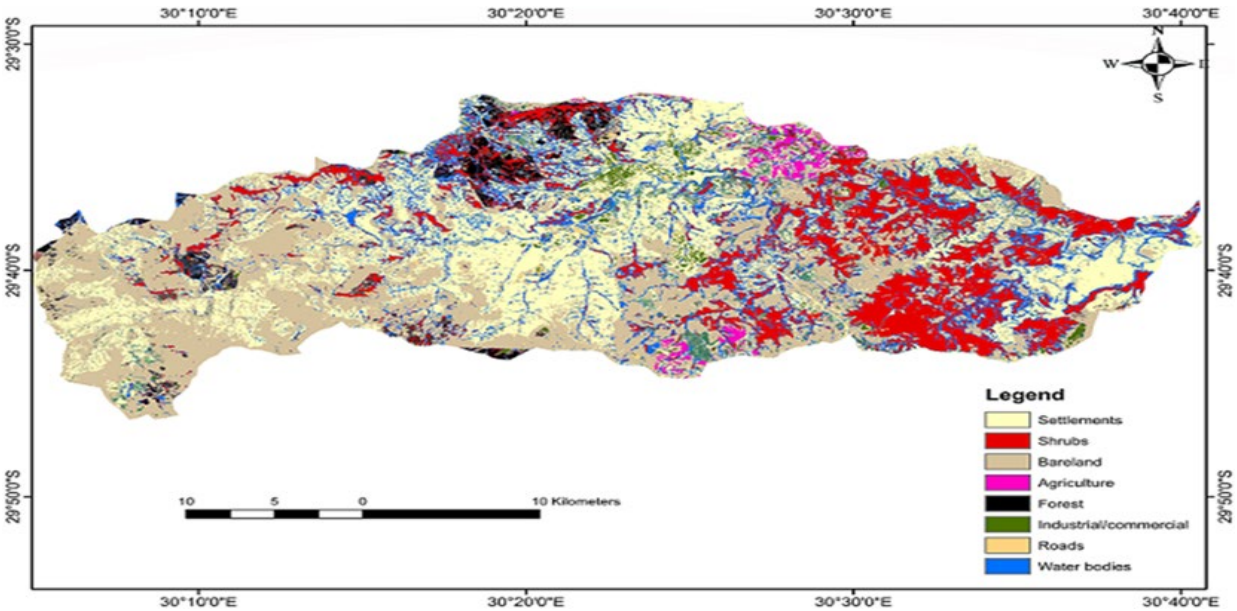


Figure 4.2 Land Use Map of the Msunduzi Catchment, KwaZulu-Natal, South Africa

CHAPTER 5: SAMPLE COLLECTION AND LABORATORY ANALYSIS

5.1 WATER SAMPLING SITE LOCATIONS

Surface water samples were collected from 16 sampling sites (Figure 5.1). The sites were selected to represent various land use activities, including industrial, agricultural, residential, urban parks, and WWTPs along the Msunduzi River catchment. Water sampling locations within the catchment were identified and selected using the produced land use map representing all land use activities. The Global Positioning System (GPS) receiver (Ramadas and Samantaray, 2018) was used to locate and fix the coordinates of the 16 sampling sites along the river. Once the coordinates were fixed, a recce exercise was carried out to ascertain the accessibility and safety of the selected sampling points.

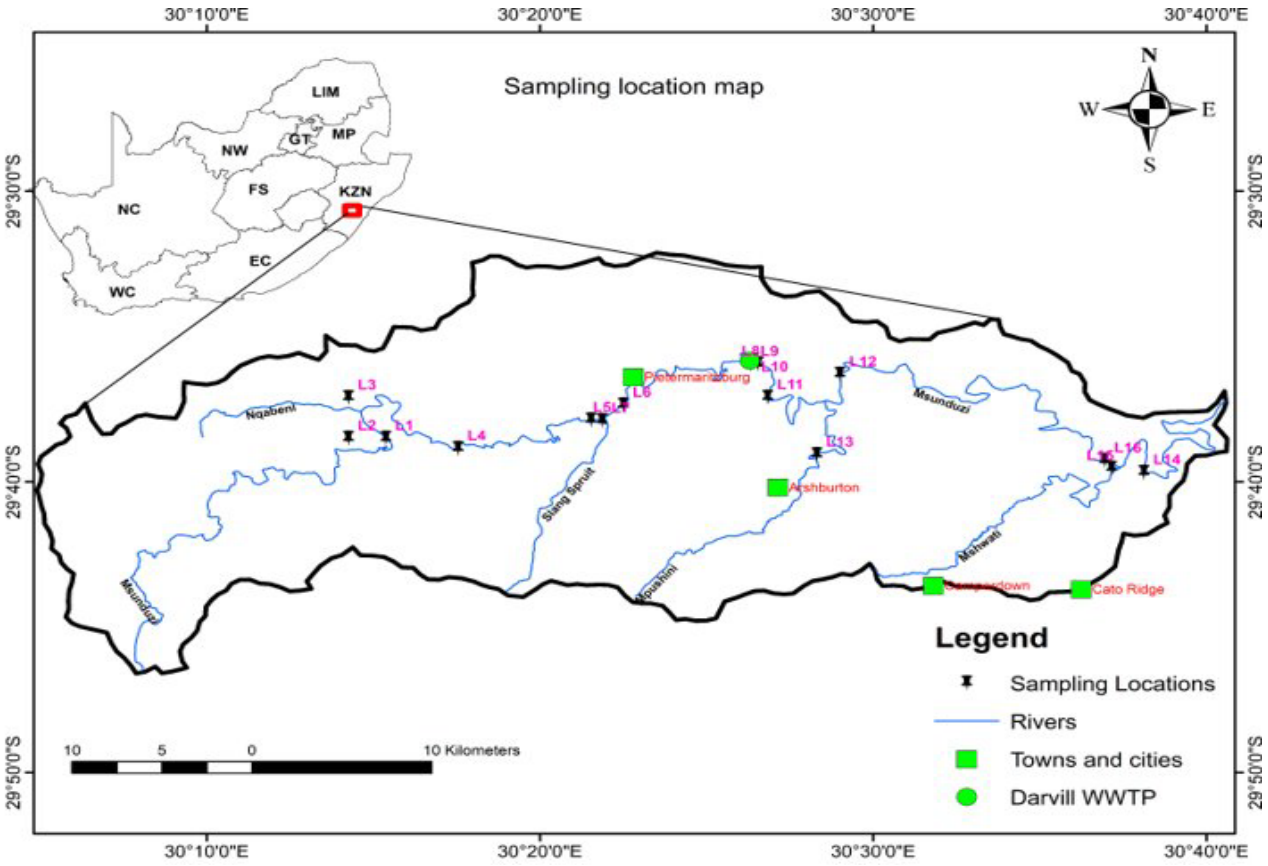


Figure 5.1 Sampling Locations along the Msunduzi River, KwaZulu-Natal, South Africa.

Samples were collected from surface water along the Msunduzi River and its tributaries, wastewater effluents from a WWTP, and the imminent injection of the discharge from the WWTP into the river. The coordinates for each sampling location are presented in Table 5.1.

Table 5-1 Coordinates of sampling sites along the Msunduzi River, South Africa.

S/No	South	East	Location	Abbreviation	Description
1	29.64169	30.25631	Msunduzi Town	MT	
2	29.64169	3023749	Nqabeni tributary	NT	
3	29.61837	30.23751	Car wash stream	CWS	
4	29.64755	30.27233	Below Mabane tributary	BMT	
5	29.63135	30.35887	PMB Industrial effluent	PIE	Kwapata and Mvubukazi streams
6	29.6226	30.375	Camps Drift	CD	
7	29.63136	30.36443	Wilgerfontein River	WR	
8	29.59909	30.44254	River water before effluent release	BER	
9	29.59725	30.43886	Darville WWT effluent	DWWE	
10	29.3549	30.2625	River water after effluent release	DER	1km downstream of the effluent discharge
11	29.61822	30.44724	Gripthorpe	GRP	Bayne's Spruit tributary
12	29.60502	30.48338	Kayeni Agricultural area	KAA	Ashburton Commercial Farm
13	29.65112	30.47177	Mpushini River tributary	MRT	
14	29.6613	30.63542	Duzi Bridge	DB	Farm and Informal settlement
15	29.3932	30.3709	Mshwati River tributary	MKT	Informal settlement
16	29.3932	30.3657	Table mountain	TB	Near the joining of the Umgeni River

5.2 SAMPLE COLLECTION

Samples were collected from 16 designated sites using sterilized 500 mℓ amber glass bottles. Before use, these bottles underwent a thorough cleaning process involving washing with DynaChem soap, rinsing with tap water and Milli-Q ultra-pure water, and a subsequent wash with acetone to eliminate polar and nonpolar compounds. To ensure sterility and prevent biotic transformation by microbial and enzymatic activities, the sample bottles were sterilized in a steam sterilizer at 125°C for 15 minutes, followed by exposure to air steam laminar flow.

Duplicate samples (n=2) were collected from each site at a depth ranging from 10 to 20 mm below the water surface. Following collection, each sample bottle was promptly covered with aluminium foil and secured with bottle caps. To maintain sample integrity, the bottles were stored in ice boxes at appropriate temperatures during transportation to the laboratory for testing. Subsequently, the samples were stored in a dark, cold room at 4°C in preparation for the solid phase extraction (SPE) process, which was conducted within a week of sample collection.

Preliminary sampling was undertaken as a control measure and to establish baseline values for nutrient and pollutant loading at the selected sites. In situ measurements of selected parameters were performed using a portable Hanna multi-parameter field probe to maximize data accuracy and ensure data integrity. The physical and chemical characteristics of the sampling sites during the collection period are detailed in Table 5.2.

Field parameters

During sampling, selected parameters were measured in situ to enhance data accuracy and maintain integrity. These parameters include temperature, alkalinity, electrical conductivity, Total Dissolved Solids (TDS), pH, Eh (Redox potential), and Dissolved Oxygen (DO) (Wilde, 2008). All field parameters, excluding alkalinity, were measured using a portable Hanna multi-parameter field probe. The physical and chemical characteristics of the sampling sites during the collection period are detailed in Table 5.2.

An on-site titration method was employed using a 0.02 N HCl solution for alkalinity determination. The titration process continued until reaching 7 and 4.3 endpoints. This on-site determination of alkalinity adds a comprehensive dimension to the water quality assessment at the selected sites, providing valuable information for the overall understanding of environmental conditions.

Transportation and preservation

Cation and anion samples were filtered on-site using a 0.45 µm filter. Additionally, cation samples underwent acidification to achieve a pH below 2, utilizing ultra-pure nitric acid. All collected samples were stored in cooler boxes to maintain sample integrity during transportation.

For samples sent to external laboratories, an additional precaution was taken. The samples were sealed in polystyrene cooler boxes containing ice bricks, ensuring they remained at suitable temperatures throughout transportation. This meticulous approach to sample handling and transportation aims to preserve the quality and reliability of the analytical data obtained from external laboratories.

5.3 LABORATORY ANALYSIS

Major cation, trace metal, and major and minor anion samples underwent analysis. The analysis employed various techniques: major cations were analysed using an Inductively Coupled Plasma atomic emission spectrometer (ICP-AES), trace elements were analysed using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS), and anion samples (including Chloride, Sulphate, Nitrate, Nitrite, Fluoride, Bromide, Ammonia, and phosphate) underwent analysis using a Dionex Ion Chromatography (IC).

The field and laboratory measurement results for various physicochemical parameters are presented in Table 5.2. These parameters exhibit variation along the river flow, with spatial trends complexly influenced by the catchment's intricate land use and the onset of summer during sampling. Preliminary findings suggest elevated concentrations of known contaminants associated with land-use settings, such as nitrate, Na, Cl, and certain trace metals.

The results presented in Table 5.2 played a crucial role in identifying the subsequent sampling location for antibiotics and nutrients. These findings contribute to a more comprehensive understanding of the water quality dynamics in the studied environment.

Table 5-2 Physicochemical characteristics of the Msunduzi River during the sampling.

Parameter	MT	NT	CWS	BMT	PIE	CD	WR	BER	DWWE	DER	GRP	KAA	MRT	DB	MKT	TB
Spring																
PH	7.0	7.7	5.8	6.1	7.7	8.1	7.7	7.7	6.7	7.5	7.6	7.8	7.6	7.9	7.2	7.7
T (°C)	20.4	24.1	18.8	14.3	16.4	17.9	15.4	15.9	22.4	23.3	22.0	22.7	24.5	20.0	28.9	22.4
EC (µs/cm)	8464.0	2.0	14.0	77.0	88.0	90.0	93.0	151.0	232.0	415.0	236.0	426.0	415.0	243.0	265.0	586.0
TURB (FNB)	4232.0	1.0	7.0	38.0	44.0	45.0	47.0	75.0	116.0	207.0	118.0	213.0	207.0	121.0	133.0	293.0
ORP (mv)	168.6	293.5	264.6	233.5	195.9	191.9	205.4	32.5	120.2	142.6	109.1	139.3	142.6	147.8	132.2	137.5
F (ppm)				0.1			0.1								1.1	
Cl^- (ppm)	11.3	44.5	10.7	60.7	23.4	25.6	35.5	42.6	88.0	29.4	41.3	8.7	195.5	10.7	419.1	41.7
$NO3^-$ (ppm)	2.0	5.4	3.6	7.5			1.0	1.4	3.3	3.4	3.1	1.9	1.6	1.8	17.2	7.8
$SO42^-$ (ppm)	3.1	21.1	5.5	19.9	20.7	17.0	19.4	24.1	41.2	15.6	21.8	2.7	28.7	3.0	111.0	17.9
$PO4^-$ (ppm)									0.1							
Autumn																
PH	7.4	8.3	8.1	8.2	7.6	7.5	7.6	7.6	7.6	8.0	7.8	7.6	7.7	7.6	7.7	7.9
T (°C)	18.1	19.6	23.1	22.7	22.9	24.3	24.7	25.9	27.7	25.1	23.2	23.4	23.8	23.5	23.5	23.8
EC (µs/cm)	70	90.0	96.0	89	96	105	413.0	247.0	547.0	413	253.0	252.0	669.0	253	871.0	230.0
TURB (FNB)	3.1	4.5	5.8	28.6	49.3	52.5	109.7	323.4	34.1	0.0	77.2	71.0	161.9	94.8	134.2	11.5
ORP (mv)	309.0	201.6	222.7	223.1	252.2	268.4	66.7	185.4	196.5	171.1	180.3	202.2	187.8	178.8	188.1	195.6
F (ppm)															0.6	
Cl- (ppm)	5.4	8.6	9.8	10.1	19.7	21.4	36.0	21.0	63.8	25.1	23.6	24.6	100.5	29.4	329.5	21.9
NO3- (ppm)	3.0	5.5	5.7	6.2	8.6	6.8	6.5	6.8	4.7	6.5	6.4	7.8	0.3	9.6	3.8	2.1
SO42- (ppm)			2.3		2.5	2.0	3.5	2.5	6.8	3.0	2.7	2.7	3.7	3.1	30.7	3.1
PO4- (ppm)																
Winter																
PH	6.0	7.2	7.0	7.2	6.9	7.0	6.9	7.2	7.6	7.4	6.4	7.0	6.7	6.6	6.9	7.1
T (°C)	12.2	16.1	15.5	14.3	14.5	16.1	15.5	15.5	16.7	19.6	23.3	18.0	28.9	26.5	28.0	15.9
EC (µs/cm)	119	110	111	88	90	98	39	229	240	388	234	245.8	295	273	274	125
TURB (FNB)	46.2	63.5	29.3	210.8	6.0	133.5	82.0	30.4	12.0	17.0	11.6	204.4	60.5	15.4	9.4	0.0
ORP (mv)	301.0	344.7	327.9	293.7	346.6	324.0	305.5	280.4	246.6	262.7	226.3	2.2	241.3	273.2	254.9	244.2
F (ppm)													0.1		1.2	
Cl^- (ppm)	12.4	11.1	13.0	14.5	35.1	31.3	48.5	30.7	88.1	41.0	38.0	40.3	176.9	69.3	493.5	45.8
$NO3^-$ (ppm)	7.3	6.0	7.3	6.9	7.9	10.9	16.4	12.8	16.2	15.3	12.1	11.9	2.7	13.5	22.7	12.3
$SO42^-$ (ppm)	2.3	2.4	6.0	3.3	15.4	11.9	24.1	12.5	40.9	17.5	18.0	17.1	31.4	25.2	37.4	20.4
$PO4^-$ (ppm)									3.1							

5.3.1 Chemicals and Reagents

The selection of antibiotics for investigation was grounded in the consumption patterns observed in South Africa. Erythromycin (ERY), Sulfathiazole (STZ), and Tetracycline (TTC) emerged as the most frequently used antibiotics between 2014 and 2020, based on studies by Alabi & Essack (2022) and Mthombeni et al. (2014). While tetracycline and erythromycin have been previously studied, sulfathiazole and penicillin-G (PNC) occurrences in South African waste and surface water have not been investigated.

All antibiotic standards used in the study were procured from Merck (South Africa). The solvents, including HPLC-grade acetic acid (95%), methanol (99.8%), and acetone (98.5%), were obtained from Sigma-Aldrich (Merck, South Africa). Whatman filter paper with a 0.45-µm filter diameter was sourced from Sigma-Aldrich (Merck, South Africa), and laboratory reagent water was generated using a water purification system (Milli-Q ultra-pure water, 18Ω). Hydrophobic-Lipophilic Balance (HLB) solid phase extraction (SPE) cartridges (Oasis PRIME HLB 6CC, 20 mmPE, 60 mg, 5 mℓ) were acquired from Waters Microsep, Pty Ltd (South Africa). The chemical structures and characteristics of the selected antibiotics are detailed in Table 5.3. The careful selection of antibiotics, coupled with the use of high-quality standards and analytical reagents, ensures the accuracy and reliability of the study's findings.

Table 5-3 Targeted antibiotics and their physical and chemical properties.

Antibiotics	Log Kow (pKa)	Solubility (mg/L)	Structure	Molar mass	Use
Erythromycin	3.06 (8.89)	4.2	C37H67NO13	734	Treat bacterial infection
Penicillin-G	1.83 (2.74)	210	C16H18N2O4S	334	Antibacterial
Sulfathiazole	0.05 (7.2)	2370	C9H9N3O2S2	255	Inhibit bacterial infection
Tetracycline	-1.37 (3.30)	231	C22H24N2O8	444	Antibacterial

5.3.2 Stock solutions

From each therapeutic antibiotic reagent (Tetracycline, Penicillin-G, Sulfathiazole, and Erythromycin), a crystal weighing 0.01 g was measured. Subsequently, a stock solution with a concentration of 100 mg L^{-1} was created by dissolving the weighed crystal in 10 mℓ of methanol and milli-Q water in a 50:50 volume ratio. The resulting stock solution was stored in a cold, dark room at a temperature of 4°C until extraction.

For calibration purposes, multi-standard solutions at various concentrations were prepared using methanol and dilution from the previously created stock solution. This meticulous process ensures accurate and standardized concentrations for calibrating the analytical instrumentation, contributing to the precision and reliability of subsequent analyses.

5.3.3 Sample extraction

In the sample preparation process, 0.45 µm Whatman filter paper was used for filtration, and extraction of the targeted analytes was achieved using Oasis HLB SPE cartridges, following established methodologies (Babić et al., 2006; T. Li et al., 2020; Motsoane et al., 2021; Wallace & Aga, 2016). The SPE Supeclo manifold and HLB SPE cartridges were employed for the extraction process. Cartridges were conditioned with 5 mℓ powdered methanol and 5 mℓ powdered milli-Q ultra-pure water at a flow rate of 1 mℓ min^{-1} before loading the

samples. The pH of the filtered samples was adjusted to 4 using acetic acid, and 400 mℓ of the pH-adjusted samples were loaded into the conditioned cartridges.

After loading, the solid phase extract was subjected to 30 minutes of drying under a -70 kPa manifold vacuum. The analytes were then eluted with 10 mℓ of methanol and 5 mℓ of n-hexane/acetone, each at a flow rate of 2 mℓ min^{-1}. The elutes were subsequently dried under a manifold vacuum before being reconstituted in 1 mℓ of methanol. This sample preparation process ensures the extraction of targeted analytes with high efficiency and precision, laying the groundwork for subsequent analytical procedures.

5.3.4 Antibiotic separation and quantification

The quantification of analytes was performed using LC-MS, a technique that has gained prominence in the detection of antibiotics in the aquatic environment due to its versatility and effectiveness (Na et al., 2013; Patel et al., 2019; Wu et al., 2022; Yang et al., 2021). LC-MS, renowned for its suitability in analyzing organic compounds, provides rapid separation and analysis of antibiotics in water samples (Li et al., 2020; Wallace & Aga, 2016). This robust technique employs the physical and chemical properties of different components in a sample for separation and analysis, ensuring high sensitivity and selectivity, particularly for trace-level antibiotics in surface waters, detectable down to parts per billion (ppb).

The liquid chromatography and separation procedures were conducted using the SHIMADZU LC-MS-2020, with the Shimadzu Shim-Pack GIST-HP 3 µm C18, 4.6 x 150 mm column. Analyte identification and quantification were performed in both positive and negative ion modes. The mobile phase consisted of 0.1% formic acid in milli-Q water at 30°C (Mobile phase A) and 0.1% formic acid in acetonitrile (Mobile phase B). A gradient elution method was employed, and the mobile phase compositions are detailed in Table 5.4. The column temperature was maintained at 35°C, and a 20 µl injection volume was used. The column was allowed to calibrate for 5 minutes before each injection. The analysis duration was 40 minutes, with retention times falling between 10 and 24 minutes for the four detected analytes. This LC-MS methodology ensures accurate and efficient quantification of antibiotics in the water samples.

Table 5-4 LC-MS parameters and gradient composition used during the quantification of antibiotics.

Chromatograph	SHIMADZU LCMS-2020		
Column	Shim-Pack GIST-HP 3µm C18		
Injection Volume (µl)	20		
Temperature (°C)	30		
Flow rate (mℓ min-1)	0.25		
Gradient Composition	Time (min)	%A	%B
	0	95	5
	25	10	90
	27	10	90
	32	95	5
	37	95	5

CHAPTER 6: ANTIBIOTIC RESIDUES IN MSUNDUZI RIVER

6.1 OCCURRENCE AND CONCENTRATION OF DETECTED ANTIBIOTICS

All target analytes, including Tetracycline, Sulfathiazole, Penicillin-G, and Erythromycin, were detected in surface and wastewater effluent samples (Milić et al., 2013). Tetracycline was the most frequently detected, followed by Sulfathiazole and penicillin-G, while Erythromycin was less frequent. Higher concentrations of these analytes were observed in samples collected during the spring compared to those collected in autumn and winter (Milić et al., 2013).

In wastewater effluent samples, the concentrations of the investigated antibiotics were in the range of 138.03-1756.51 ng L^{-1} for Tetracycline, <LOQ for Penicillin-G, 120.74-5613.58 ng L^{-1} for Sulfathiazole, and 52.14-142.63 ng L^{-1} for Erythromycin (Milić et al., 2013). Elevated concentrations were mainly observed at the WWTP effluent point and the discharge into the Msunduzi River. During the spring season, the maximum concentrations were 5613.58 ng L^{-1} for Sulfathiazole, 1756.51 ng L^{-1} for Tetracycline, and 142.63 ng L^{-1} for Erythromycin, while Penicillin-G was detected below the limit of quantitation (143.98 ng L^{-1}) (Milić et al., 2013). In river water samples, concentrations ranged from 158.42 to 1290.43 ng L^{-1} for Tetracycline, 143.98 to 503.30 ng L^{-1} for Penicillin-G, 112.68 to 1151.25 ng L^{-1} for Sulfathiazole, and 52.14 to 106.63 ng L^{-1} for Erythromycin (Milić et al., 2013). The maximum concentrations were observed 1 km downstream of the WWTP effluent discharge for Tetracycline (1290.43 ng L^{-1}) and Sulfathiazole (1151.25 ng L^{-1}) (Milić et al., 2013). However, the maximum concentration of Penicillin-G (503.30 ng L^{-1}) and Erythromycin (106.63 ng L^{-1}) within the Msunduzi River water sample was observed in samples collected at its tributaries, namely, Wilgerfontein River (upstream tributary located in Pietermaritzburg city) and Mshwati River (downstream tributary), respectively (Milić et al., 2013).

The detection of these antibiotics at relatively higher concentrations is attributed to the characteristics of each antibiotic and the prevailing human activities and land use practices in the studied catchment. For instance, Sulfathiazole, widely used in animal husbandry, was frequently detected in areas with significant informal settlements and commercial livestock farming. Its poor sorption properties and slow degradation rate in WWTP contribute to its persistence in the environment. Penicillin-G, a commonly used antibiotic in veterinary and human medicine, exhibited low-level detection in wastewater samples due to its high rate of biodegradation and hydrolysis. The swift transformation of Penicillin-G in the environment results in its low presence in the samples. These findings highlight the complex interplay between antibiotic properties, land use practices, and environmental fate.

The persistence of Sulfathiazole in the environment is attributed to its poor sorption properties, resulting in a low removal rate in wastewater treatment plants (WWTP) due to low sorption to treatment sludges (Liu et al., 2019). This antibiotic exhibits a relatively slow degradation process with a half-life of more than 50 days in the pore water of sediments (Milić et al., 2013). Unlike other antibiotics, such as tetracyclines and macrolides, sulfathiazole experiences a relatively lower attenuation rate under normal environmental conditions (Tamtam et al., 2008). This slower degradation risks the aquatic biosystem, contributing to its persistence in the environment.

Penicillin-G, one of the most influential antibiotic families used in veterinary and human medicine, was detected in river water within the range of 143.98 to 503.30 ng L^{-1}. However, its detection in treated wastewater effluent samples was observed below the quantitation limit (Li et al., 2008). Penicillin-G is characterised as a weak base and hydrophilic, exhibiting poor stability of the β-lactam ring in the aquatic environment (Li et al., 2008). The β-lactam ring of Penicillin-G is susceptible to hydrolysis under acidic and alkaline conditions, by reaction with weak nucleophiles such as water or metal ions, or through enzymatic activity, similar to acidic hydrolysis

(Li et al., 2008). Consequently, Penicillin-G detection in surface water samples is considered a real-time occurrence due to its vulnerability to environmental conditions.

Moreover, the low-level detection of Penicillin-G in wastewater samples is attributed to its high rate of biodegradation in various solid matrices and hydrolysis (Li et al., 2008; Pirt, 1990). Hydrolysis, particularly the hydrolysis of the β-lactam ring, is a dominant attenuation mechanism for Penicillin-G. The unstable structure of the β-lactam ring is highly influenced by factors such as pH and heat, leading to its conversion into various by-products, including penicilloic acid, penicilloaldehyde, penicillamine, penicilloic, and isopenillic acid (Yang et al., 2021). Consequently, the presence of Penicillin-G in environmental samples is low due to its rapid transformation through the easy hydrolysis of the β-lactam ring.

Tetracycline, a widely used antibiotic in both human and veterinary applications, serves various purposes, including pain relief for individuals engaged in high-energy physical activities. Its broad administration, particularly as feed additives in animal farming, contributes to its prevalence (Milić et al., 2013). The detection of tetracycline in concentrations ranging from 137.07 to 684.11 ng L^{-1} in tributaries (BMT, PIE, WR, and MRT), industrial effluent (PIE), and suburban informal settlement areas (MT and DB) underscores the likelihood of its presence in the water system through wastewater discharge and diffused sources like runoff from informal settlements. A notably high concentration of tetracycline, 1756.51 ng L^{-1}, was identified in wastewater effluent samples during the spring season. This persistence can be attributed to tetracycline's resistance to conventional wastewater and sludge treatment systems (Oharisi et al., 2023). However, the concentration decreased to 1290.43 ng L^{-1}, 1 km downstream of the effluent discharge point, indicating factors such as dilution, sorption, and hydrolysis that contribute to the removal of antibiotics from the water column (Chen et al., 2020; Fernández-Calviño et al., 2014; Wei et al., 2019). Tetracyclines, being hydrophobic compounds, tend to sorb into sediments and experience partial degradation through photolysis (Yang et al., 2021). The low concentrations observed in the surface water of the river at various sampling points can be attributed to the compound's high affinity for adsorption and lower tendency for desorption from different sediment components due to its multiple functional groups (Fernández-Calviño et al., 2015). This complex interplay of factors highlights tetracycline's intricate fate and transport mechanisms in aquatic environments.

The concentration of Erythromycin in this study ranges from 52.14 to 106.63 ng L^{-1} in surface water and is detected at a concentration of 142.63 ng L^{-1} in treated wastewater effluent samples. Erythromycin is observed in the main Msunduzi River, tributaries (BMT and MRT), and industrial effluent (PIE), suggesting that human activities, such as medical treatment in hospitals and pharmaceutical production, are significant sources of Erythromycin along the Msunduzi River. Erythromycin exhibits stability against hydrolysis and sorption but is sensitive to photodegradation through cladinose ring cleavage; additionally, bacterial species, specifically Ochrobactrum sp. Strain contributes to Erythromycin degradation (Li et al., 2022; Yang et al., 2021), which may account for its low-level detection in wastewater samples. Interestingly, Erythromycin is more frequently observed during winter than in spring (Table 6.1), indicating a role for photolysis in its environmental occurrence. The observed concentrations of these analytes may not solely be attributed to anthropogenic activities but could also result from naturally occurring bacteria. The Actinomycetes group and Streptomycetes have been identified as contributors to the environmental load of β-lactam and tetracycline antibiotics (Kümmerer, 2009; Mikulik et al., 1983; Pala-Ozkok et al., 2019). Le et al. (2014) demonstrated that polyketide synthase genes (PKS I/PKSII) are responsible for the natural production of macrolide antibiotics, such as Erythromycin and Tylosin (Le et al., 2014).

Furthermore, the presence of anions and metallic ions may influence the occurrence and persistence of antibiotic residues. Cl^-, NO_2^-, and NO_3^- abundance may inhibit the photodegradation of Sulfathiazole (Bai et al., 2021; Tang et al., 2021). NO_3^- inhibits the indirect photodegradation of Sulfathiazole by reducing the steady-state concentration of excited reactive intermediates, which are sensitizers for the compound's removal. NO_3^- acts as a source of Hydroxyl radical Oxidant (HO) photosensitizer. The photolysis of nitrate generates nitrite ions, which are crucial for masking HO sensitization (Benedict et al., 2017; Zafiriou, 1979).

On the other hand, Br^- has been reported to promote the degradation of tetracycline, while Cl^- may inhibit tetracycline degradation by scavenging the reactive form of SO_4^{2-} and forming inactive chlorine species (Zhang et al., 2022). Tetracyclines are often found at low levels in the aquatic environment due to cations like calcium, facilitating their precipitation and accumulation in solids/sediments. The concentration of the investigated antibiotic analytes from both surface and wastewater samples is presented in Table 6-1. All the analytes, Erythromycin, Sulfathiazole, Penicillin-G, and Tetracycline, were detected at a wavelength of 254 nm and in the positive ion mode (Figure 6.1). The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated using 3 and 10 times the signal-to-noise ratio obtained from the chromatographic analysis, respectively, as shown in Table 6.2.

6.2 SPATIAL DISTRIBUTION AND SEASONAL VARIATION OF DETECTED ANTIBIOTICS IN THE MSUNDUZI RIVER

The targeted antibiotics were detected more frequently during the spring (36.4%) than in the autumn (30.3%) and winter (33.3%) seasons. This may be linked to antibiotic detection being enhanced during dry water periods. The increased detection of antibiotics throughout the spring (dry periods) can be ascribed to antibiotic persistence due to a lack of dilution by rainfall. Furthermore, antibiotic-persistent sites (e.g. dissolved organic materials, algae, suspended solids) are more abundant during dry than rainy seasons. Tetracycline and Sulfathiazole were more frequently detected than Penicillin-G and Erythromycin during both seasons. The spatial variability of antibiotics from the results shows that anthropogenic activities like industries, aquaculture, commercial farms, grazing land, and informal settlements have a significant impact on the upper reach of the Msunduzi River (MT, NT, CWS, BMT, PIE, CD, WR, BER, DWWE, and DER). These human activities produced a sizable amount of antibiotic residue at the river's upper reach, exhibiting significantly higher detection and concentration of the targeted antibiotics than the lower reach (Figure 6.2).

Penicillin-G residues were detected at the Mabane tributary streams (BMT) and Wilgerfontein tributary (WR). Sulfathiazole was also observed in the Mabane tributary (BMT), Wilgerfontein tributary (WR), and Msunduzi river before Darville WWTP effluent release. Kwapata and Mvubukazi tributary streams and Wilgerfontein tributaries drain waste from industrial effluents and household waste, contributing antibiotic residue to the Msunduzi River. Further, MRT, KAA, and MKT drain land that agricultural farms and suburban developments mainly occupy. Thus, these target antibiotic residues from the tributaries might be related to antibiotic usage, which could cause continuous antibiotic residual sources in the Msunduzi River.

Antibiotic pollution of the Msunduzi River is primarily caused by the effluent from the Darville WWTP, as shown in Figure 6.2. Effluent from the Darville Wastewater treatment plant is found to be a hotspot and the primary source of high antibiotic residual contamination in the river. Therefore, proper treatment and monitoring of waste discharge releases from industries and municipal wastewater treatment plants are essential. Additionally, it must be noted that similar to the temporal variations, the location (spatial distribution) is essential in characterising antibiotic pollution in a river. The comparison is made between the results obtained and antibiotic concentrations from other countries obtained in the literature (See Table 6.3)

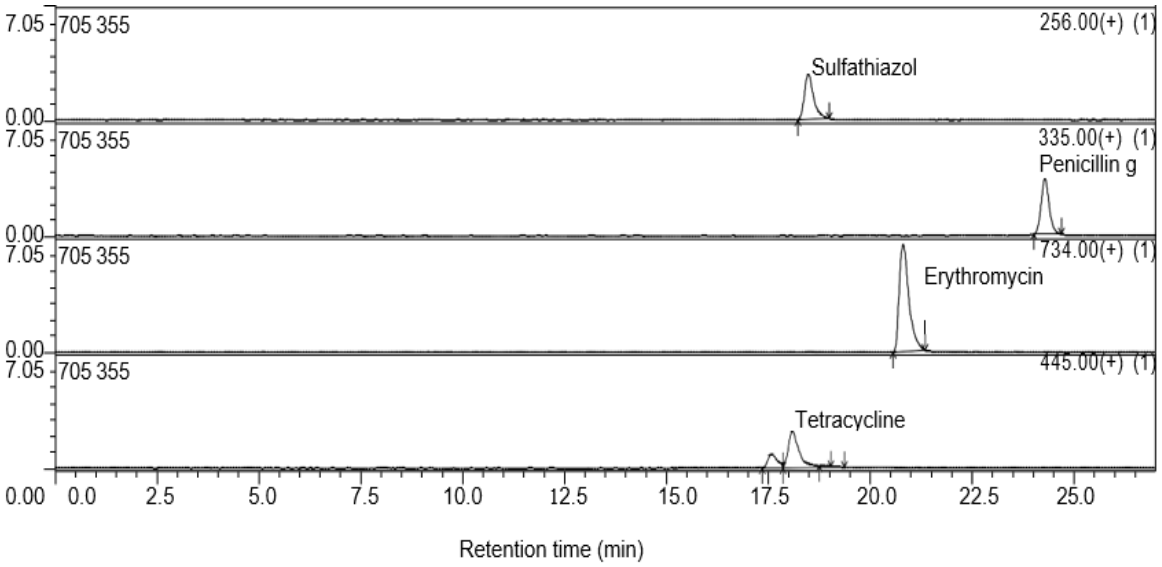
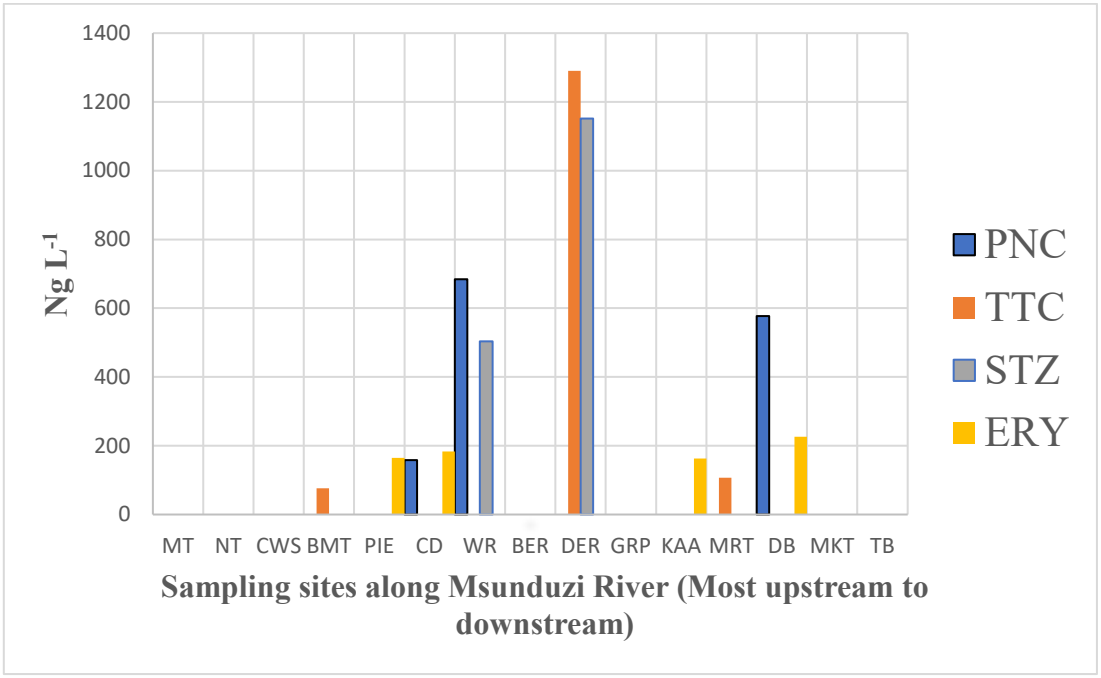


Figure 6.1 Mass extract of the target antibiotics using a mass spectrometer detector



ND observations are set to zero to generate the plot.

Figure 6.2 Spatial distribution of detected antibiotics along the Msunduzi River from the most upstream (MT) to Downstream (TB).

Table 6-1 The concentration of analyzed antibiotics (ng L-1) at each sampling site along the Msunduzi River.

Antibiotics	MT	NT	CWS	BMT	PIE	CD	WR	BER	DWWE	DER	GRP	KAA	MRT	DB	MKT	TB
Spring																
PNC	ND	ND	ND	ND	ND	ND	<LOQ	<LOQ	ND	ND	ND	ND	ND	ND	ND	ND
TTC	ND	ND	ND	ND	<LOQ	158.42	ND	<LOQ	1756.51	1290.43	ND	ND	<LOQ	577.63	ND	ND
ERY	ND	ND	ND	ND	ND	ND	ND	ND	142.63	<LOQ	ND	ND	ND	ND	ND	ND
STZ	ND	ND	ND	ND	164.64	183.47	ND	ND	5613.58	1151.25	ND	162.88	ND	ND	ND	ND
Autumn																
PNC	ND	ND	ND	<LOQ	ND	ND	503.30	ND	ND	ND	ND	ND	ND	ND	ND	ND
TTC	ND	ND	ND	<LOQ	ND	ND	<LOQ	ND	1519.65	720	ND	ND	ND	ND	ND	ND
ERY	ND	ND	ND	<LOQ	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	106.63	ND	ND	ND
STZ	ND	ND	ND	<LOQ	ND	ND	ND	ND	<LOQ	ND	ND	ND	ND	226.18	ND	ND
Winter																
PNC	ND	ND	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND
TTC	ND	ND	ND	ND	ND	<LOQ	684.11	ND	138.03	ND	ND	ND	ND	<LOQ	ND	ND
ERY	ND	ND	ND	76.3	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND	ND	ND	ND	ND
STZ	<LOQ	ND	<LOQ	ND	ND	ND	ND	<LOQ	120.74	<LOQ	ND	ND	<LOQ	ND	ND	ND

ND = Not Detected; LOQ = Limit of Quantification

Table 6-2 Mass detection and separation parameters for the analysis of target analytes.

Compound	Linear range	Retention time	Precursor ion	LOD	LOQ	R^2
Tetracycline	100-10000	18	$445[M+H]^+$	40.22	137.07	0.996
Penicillin-G	10-10000	24	$335[M+H]^+$	43.19	143.98	0.892
Erythromycin	1-100000	21	$735[M+H]^+$	15.64	52.14	0.997
Sulfathiazole	10-10000	18	$256[M+H]^+$	37.4	112.68	0.961

Table 6-3 Analysis of the levels of antibiotics from this study and earlier investigations reported from other countries.

Antibiotics	Concentration (ng L^{-1})		Country	Citation
	Current study	Previous studies		
Wastewater effluent				
Tetracycline	1756.51	853	Vietnam	(Han et al., 2019)
		1420	Korea	(Minh et al., 2009)
Erythromycin	142.63	275	Egypt	(Abou-Elwafa Abdallah et al., 2019)
		1187	Tunisia	(Moslah et al., 2018)
		2350	Korea	(Sim et al., 2011)
		48520	Vietnam	(Han et al., 2019)
Penicillin G	<LOQ	11	Canada	(Guerra et al., 2014)
		13500	Korea	(Sim et al., 2011)
Sulfathiazole	5613.58	350	USA	(Karthikeyan and Meyer, 2006)
		16	Canada	(Guerra et al., 2014)
		5000	Korea	(Cardoso et al., 2014)
		600	Australia	(Watkinson et al., 2009)
Surface water				
Tetracycline	1290.43	50	Nigeria	(Oluwatosin et al., 2016)
		138	Tunisia	(Moslah et al., 2018)
		120	Pakistan	(Khan et al., 2013)
		138	Vietnam	(Han et al., 2019)
		430	China	(Ju et al., 2023)
Erythromycin	106.63	1000	Nigeria	(Oluwatosin et al., 2016)
		61	Egypt	(Abou-Elwafa Abdallah et al., 2019)
		741	Vietnam	(Han et al., 2019)
		17	China	(Ju et al., 2023)
		310	Pakistan	(Khan et al., 2013)
Penicillin G	503.30	668	China	(Zhou et al., 2022)
		250	Australia	(Watkinson et al., 2009)
Sulfathiazole	1151.25	253	Korea	(Ji et al., 2010)
		4610	Korea	(Awad et al., 2014)

6.3 ENVIRONMENTAL RISK ASSESSMENT

A screening-level risk characterisation assesses the likelihood of environmental hazards resulting from a specific antibiotic in the aquatic ecosystem. This involves comparing the detected concentration of the antibiotic in the river with the no-effect concentration (threshold level) known as the Predicted No-Effect Concentration (PNEC). The PNEC represents the concentration at which antibiotics cause no adverse effects on the environment and non-target aquatic organisms.

The Risk Quotient (RQ) method, per the European Commission technical document (European Commission, 2003), is commonly used to evaluate the ecological risk of antibiotics. The screening-level risk assessment for the antibiotics analysed in this study was conducted using the RQ formula (equation 7):

$$RQ = \frac{MEC}{PNEC} \quad [7]$$

Here, the predicted or measured environmental concentration (MEC) of the detected antibiotic is divided by the PNEC values reported for aquatic species (Hu et al., 2018; Manickum and John, 2014; Nieto-Juárez et al., 2021; Straub et al., 2019). The PNEC is estimated from toxicology test data of aquatic organisms (e.g. algae,

fish, protozoa, and crustaceans), based on the Minimum Inhibition Concentration (MIC), which includes No-Observed-Adverse-Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL) or EC_{50} in laboratory studies. EC_{50} is the concentration of the measured antibiotic in the aquatic environment that causes an effect on 50% of exposed aquatic organisms. The PNEC is usually estimated by applying an Assessment Factor (AF) to account for variability and uncertainties in the ecotoxicology data, as shown in equation (8):

$$\mathrm{PNEC}_{aquatic\ organism} = \frac{\mathrm{MIC}}{\mathrm{AF}} \quad [8]$$

When the MEC is greater than PNEC, i.e. (MEC/PNEC > 1), a potential risk is suspected, and the observed antibiotics are considered to have an ecological risk based on the sensitivity of the ecological receptor. In detail, RQ < 0.1 assumes that the risk is insignificant, 0.1 ≤ RQ ≥ 1 indicates low risk, 1 ≤ RQ ≥ 10 suggests moderate risk, and RQ > 10 signifies high risk. The PNEC data used in this study is obtained from Mheidli et al. (2022).

In this study, an ecological risk assessment was conducted for the target antibiotics (see Table 6.4). Using toxicity thresholds from relevant literature (Chen et al., 2018a; Chen et al., 2018b; Mheidli et al., 2022; Nieto-Juárez et al., 2021), calculations were based on parameters like No Observed Effect Concentration (NOEC), Lowest-Observed-Effect-Concentration (LOEC), and the concentration affecting 10% of organisms (EC10) (Nieto-Juárez et al., 2021). The average antibiotic concentration in surface water served as Minimum Effect Concentration (MEC), and Minimum Inhibition Concentration (MIC) values were used for algae, fish, and daphnids, applying assessment factors from Mheidli et al. (2022).

Results indicate that among the investigated antibiotics, only tetracycline poses a high risk for fish but shows a low risk for algae and daphnids. Erythromycin presents a moderate risk for algae (RQ values of 2.286 and 1.6), while Sulfathiazole and Penicillin G show no immediate observed ecological impact. It is important to note that even trace levels of these antibiotics in surface water can harm the ecology. Additionally, the sensitivity to toxicity may vary among species based on genomic characteristics, potentially differing between countries. Consequently, future studies should include a specific investigation into ecological risk assessment.

Table 6-4 Risk quotients from the detected concentration of targeted antibiotics in Msunduzi River.

Antibiotics	MEC (ng L^{-1})	Organism	MIC (ng L^{-1})	SF	PNEC (ng L^{-1})	RQ
TTC	686.118	Algae	50000 (NOEC)[a]	10	5000	0.114
		Fish	500 [b]		50	11.43
		Daphnids	10000 [b]		1000	0.571
PNG	503.3	Algae	$6.51*10^{10}$ [b]	1000	$6.51*10^{6}$	0.000008
		Fish	$2.05*10^{13}$ [b]			0
		Daphnids	$1.32*10^{11}$ [b]			0.000004
ERY	91.465	Algae	2000 [b, c]	50	40	2.286
		Fish	10^{8} [b]		$2*10^{6}$	0.00004
		Daphnids	220000 [b]		4400	0.02
STZ	377.684	Algae	$1.31*10^{7}$ (NOEC) [a]	1000	13100	0.02
		Fish	$5*10^{8}$ [b]		500000	0.0007
		Daphnids	$2.2*10^{8}$ [b]		220000	0.0017

[a] (Nieto-Juárez et al., 2021)
[b] (Mheidli et al., 2022)
[c] (H. Chen et al., 2018)

6.4 FATE AND TRANSPORT OF ANTIBIOTICS IN A WASTEWATER TREATMENT PLANT

6.4.1 Antibiotic Fate and Impact on Wastewater

The widespread use of antibiotics has led to their presence in various environmental compartments, including wastewater. Discharging antibiotic-contaminated wastewater into the river system poses a significant environmental and human health challenge. Antibiotics are introduced into wastewater primarily through multiple pathways, such as domestic sewage, hospital discharges, and industrial effluents, and attributed to the incomplete metabolism and excretion of these pharmaceuticals by humans and animals. Wastewater from these sources and more contains various pollutants, including antibiotics. In healthcare settings, the disposal of expired or unused medications contributes significantly to the presence of antibiotics in wastewater.

Additionally, pharmaceutical manufacturing plants release antibiotics into wastewater during the production process. The heightened levels of antibiotics in wastewater have raised concerns due to their potential impact on the environment and public health. The discharge of antibiotic-contaminated wastewater into natural water bodies can have detrimental effects on aquatic ecosystems. Antibiotics may induce antibiotic resistance in environmental bacteria, contributing to the global emergence of antibiotic-resistant strains. Furthermore, antibiotics in surface water may pose risks to human health by consuming contaminated water or aquatic organisms.

The diverse antibiotics commonly found in wastewater include fluoroquinolones, sulfonamides, tetracyclines, and macrolides. In understanding the Fate of antibiotics within a wastewater treatment system, a crucial aspect of investigation involves the collection of samples from the wastewater treatment facilities to detect the presence of antibiotics at different stages of the treatment process and understand the disparities in concentrations, along with the contributing factors to these variations. The target antibiotics were penicillin, tetracycline, erythromycin, and sulfamethoxazole. The methodology incorporated solid-phase extraction as a sample preparation component, followed by determining the relative concentrations of these antibiotics using LC-MS. The ensuing sections furnish valuable insights into the antibiotic levels identified in the wastewater samples and their subsequent Fate within the wastewater treatment system.

Wastewater treatment plants (WWTPs) play a crucial role in reducing the levels of antibiotics in effluents before their discharge into receiving water bodies. However, despite advancements in wastewater treatment technologies, challenges remain in effectively eliminating antibiotics from wastewater. The complex nature of antibiotic mixtures, the potential for the formation of antibiotic-resistant bacteria in treatment plants, and the persistence of certain antibiotics are ongoing concerns. Conventional treatment processes may not effectively remove all antibiotics, leading to residual concentrations in the treated effluent. Factors such as the physicochemical properties of antibiotics, treatment plant design, and operational conditions influence their fate during wastewater treatment.

While the impact of antibiotics on wastewater treatment efficiency has been acknowledged, there is limited evidence on how these processes degrade antibiotics and their potential impact on receiving natural water bodies (Uyak and Toroz, 2014). In the case study conducted by Brown et al. (2006), the identification of regions with elevated antibiotic concentrations in influent highlights the significance of targeted sampling in sewer lines that receive hospital influent, as well as strategic points along the sewer line leading to the wastewater treatment plant (WWTP). The study underscores the necessity of assessing antibiotic concentrations before undergoing treatment processes within the WWTP and as part of a pollution model applied to a natural water body.

6.5 MATERIALS AND METHODS

6.5.1 Chemicals and Reagents

Standards for penicillin, tetracycline, erythromycin and sulfamethoxazole were purchased and stock solutions of 1 000 ppm, 10 ppm, 1 ppm, 100 ppb, 10 ppb, 1 ppb, 0.10 ppb and 0.01 ppb created from the respective standards. Preparing stock solutions for penicillin, tetracycline, erythromycin, and sulfamethoxazole involved a systematic procedure to ensure accurate concentrations for calibrating the LC-MS equipment. The following method was employed:

- 0.01 g of each antibiotic standard was measured and added into separate flasks.
- 10 mℓ of methanol was added to each flask.
- The stopper was secured onto each flask, and the mixture was shaken well.
- The mixture was left to stand for approximately 10 minutes.
- Using a syringe, 1 mℓ of each antibiotic was extracted and added to a new 100 mℓ flask.
- Methanol was added to the 100 mℓ mark of the flask, and the mixture was hand-shaken well.
- The mixture was allowed to stand for 5 minutes.
- This mixture was the stock solution for the 1 000 ppm mixture. To get the 10 ppm mixture, 1 mℓ of the 1 000 ppm stock solution was extracted using a syringe and placed into a new 100 mℓ flask.
- Methanol was added to the flask, hand-shaken vigorously, and then left to stand for 5 minutes. This then formed the 10 ppm mixture.
- To get the 1 ppm mixture, 10 mℓ of the 100 ℓ mixture was extracted using a syringe and placed into a new 100 mℓ flask.
- Methanol was added to the flask, hand-shaken well, and then left to stand for 5 minutes. This then formed the 1 ppm mixture.
- The above method was used to obtain solutions for the 100 ppb, 1 ppb, 0.10 ppb and 0.01 ppb antibiotic standards.
- The solutions were stored in a fridge at 4 ºC until required for analysis.

This method ensured the creation of accurately calibrated antibiotic solutions at various concentrations, facilitating the reliable calibration of the LC-MS equipment for detecting target antibiotics in different sample matrices.

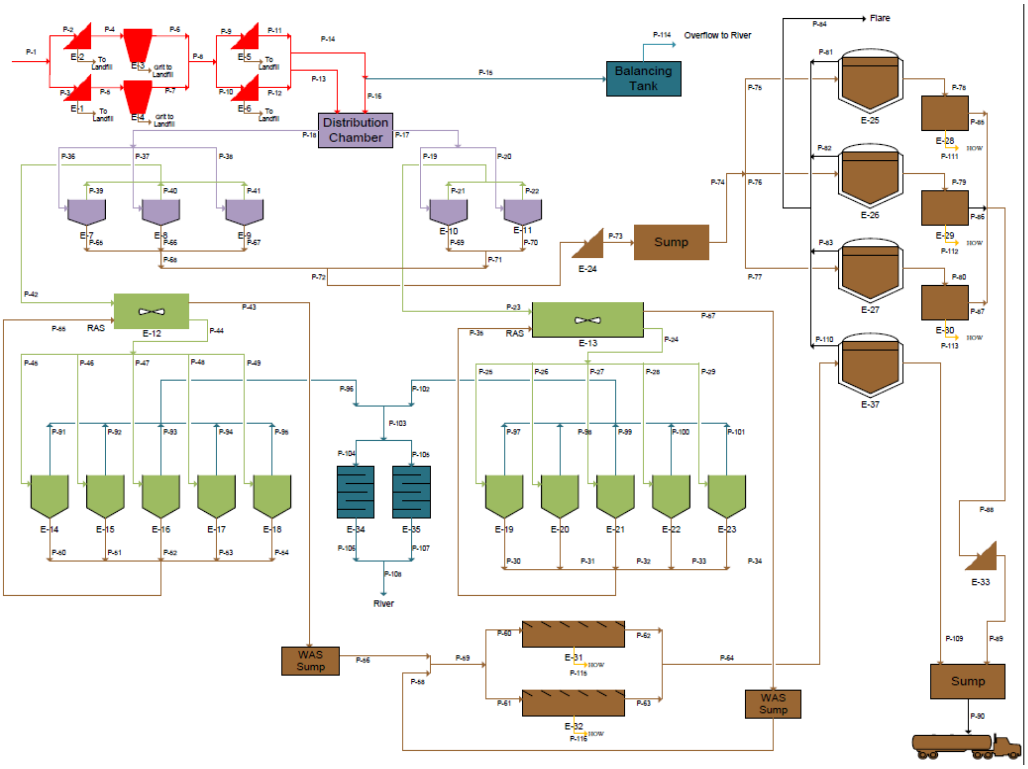
6.5.2 Sample Collection and Preparation

6.5.2.1 Sample Collection

Wastewater samples were collected by using grab sampling in 0.50 ℓ glass amber bottles from a depth of approximately 20 mm from the surface of the water. For each of the sampling sites mentioned below, two samples were collected.

Sampling was conducted at Amanzimtoti Wastewater Works (see Fig 6.3). Liquid samples were collected at the following positions: the Inlet to the treatment works, the Outlet to the primary settling tanks, the inlet to the aeration basin and the Final effluent point (See Fig 6.4) for the process flow diagram for Amanzimtoti Wastewater Works. Once collected, the samples were stored on ice and transported to a laboratory, where they were stored at 4^{o}C until sample preparation took place.

Figure 6.3 Location of Amanzimtoti Wastewater Treatment Plant



EQUIPMENT LIST	
E-1/2	Screens 1 & 2
E-3/4	Degritters 1 & 2
E-5	Standby Manual Screen
E-6	Screen
E-7-9	Primary Settling Tanks 4, 5, 6
E-10/11	Primary Settling Tanks 1, 2
E-12	Aeration Basin (EAST)
E-13	Aeration Basin (WEST)
E-14-18	Clarifiers 6, 7, 8, 9, 10
E-19-23	Clarifiers 1, 2, 3, 4, 5
E-24	Mechanical Screen
E-25-27	Primary Digesters 1, 2, 3
E-28-30	Secondary Digesters 1, 2, 3
E-31-32	DAFs 1, 2
E-33	Screen
E-34	East Contact Tank
E-35	West Contact Tank
E-36	WAS Primary Digester

CAPACITY	
E-7-9	31.1 ML/d
E-10/11	20.8 ML/d
E-12	29.1 ML/d
E-13	29.1 ML/d
E-14-18	21.15 ML/d
E-19-23	21.15 ML/d
E-25-27	44.8 ML/d
E-28-30	4500 m3
E-31-32	29.6 ML/d
E-34	720 m3
E-35	512 m3

LOADING	
Total Organic	24203 kg/d
Total Hydraulic	27000 kL/d

STREAM SUMMARY	
P-1-3	Raw Sewage
P-4-20/36-38	Screened Sewage
P-21-23/39-42	Primary Effluent
P-24-29/44-49	Mixed Liquor
P-30-35/50-55	Return Activated Sludge
P-65-77	Primary Sludge
P-43/56-61	Waste Activated Sludge
P-62-64	DAF Sludge
P-78-80/109	Primary Digester Sludge
P-85-89	Secondary Dig Sludge
P-81-84/110	Methane/CO_2 Gas
P-91-105	Secondary Effluent
P-106-108	Final Effluent
P-111-113	Supernatant Liquor
P-114	Overflow to River
P-115-116	DAF Underflow

Figure 6.4 Amanzimtoti Wastewater Works Process Flow Diagram

6.5.2.2 Sample Preparation

The methodology for liquid sample preparation followed by Matongo et al. (2015) has been adopted for this study and used as follows:

1. Wastewater samples were first filtered using Whatman Econofilt reinforced glass fibre filter paper (47 mm diameter).
2. The wastewater samples were then filtered again using Clear Right 0.45 µm filter membrane (47 mm diameter).
3. Hydrophobic-Lipophilic Balance (HLB) cartridges were prepared with 5 mℓ methanol and equilibrated with 5 mℓ of distilled water adjusted to a pH of 4.20 with acetic acid.
4. 100 mℓ of the filtered wastewater sample was added to the cartridge, and the flow rate was maintained at 4 mℓ/ minute.
5. The solid phase was then dried, and the analytes eluted ten times with 1 mℓ of methanol and five times with 1,0 mℓ of acetone, each with a flow rate of 2 mℓ / minute.
6. Eluents were then evaporated and reconstituted with 1 mℓ of methanol.

6.6 ANALYSIS OF RESULTS

samples were collected in the Winter and Spring seasons from three locations within the Amanzimtoti WWTP. Furthermore, as mentioned previously, the first point of sample collection is the Inlet to the WWTP line, the second is the Outlet to the Primary Settling Tank and Inlet to the Aeration Basin, and the third sample collection point is located at the Final Effluent point of the WWTP.

Sample Point 1: Inlet to Works

Samples were collected at the inlet of the wastewater treatment plant. For samples collected in Winter, the results yielded 11 peaks, with detection times ranging from 4,496 minutes to 28,626 minutes, as seen in Figure 6-5 and Table 6-5. Peaks were observed from 4,496 min to 7,330 min. At the same time, a wander is observed between 5 and 6 minutes. A wander can be due to contamination in the carrier gas, column, or injector (All Chromatograms are presented in Appendix C). Tables 6-5 to 6-10 are presented.

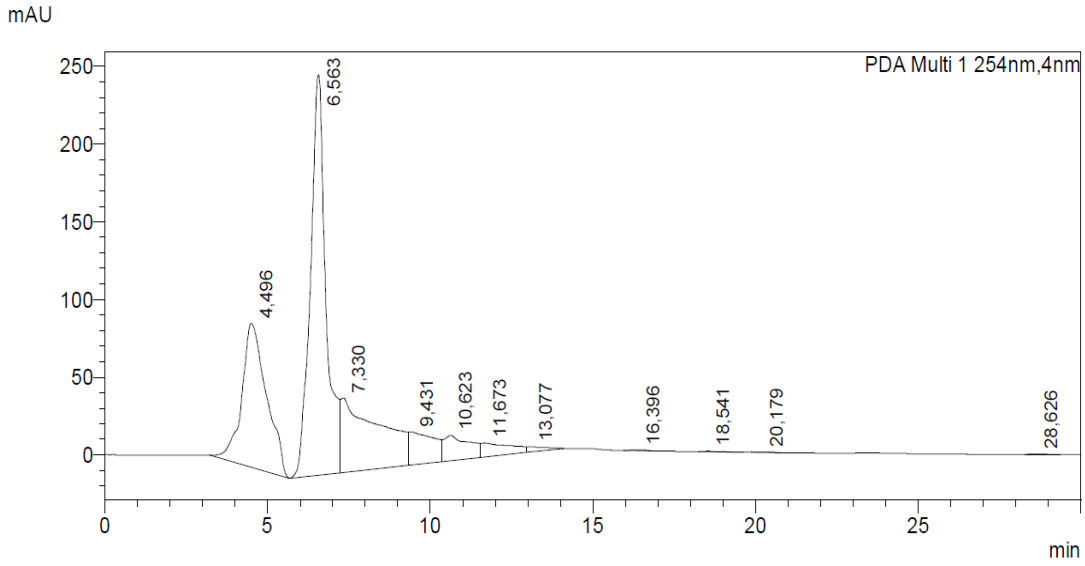


Figure 6.5 Chromatogram for Sample collected at the inlet of the WWTP in Winter

Table 6-5 Results for Samples collected at the inlet of the WWTP in Winter

PDA Ch1 254 nm

Peak Number	Ret. Time	Area	Height	Noise	S/N	Peak Start	Peak End
1	4,496	4923280	92768	61,03	1520,06	3,232	5,664
2	6,563	8520767	257682	61,03	4222,31	5,685	7,221
3	7,330	3901004	47867	61,03	784,34	7,221	9,333
4	9,431	1093081	21177	61,03	346,99	9,333	10,357
5	10,623	871988	16199	61,03	365,43	10,357	11,531
6	11,673	541910	8987	61,03	147,25	11,531	12,960
7	13,077	124197	3367	61,03	55,17	12,960	14,091
8	16,396	18670	678	61,03	11,11	15,947	16,939
9	18,541	12822	328	61,03	5,37	18,251	19,616
10	20,179	9730	283	61,03	4,64	19,883	20,864
11	28,626	7986	231	61,03	3,79	28,288	29,344
Total		20025435	449565				

Table 6-6 Results for Samples collected at the inlet of the WWTP in Spring

PDA Ch1 254 nm

Peak Number	Ret. Time	Area	Height	Noise	S/N	Peak Start	Peak End
1	1,270	8620	284	35,99	7,89	0,939	1,941
2	5,094	1980554	29863	35,99	829,85	3,509	5,707
3	6,510	1788079	53105	35,99	1475,72	5,888	7,776
4	7,346	1276620	28512	35,99	792,31	6,869	8,160
5	7,883	392364	17551	35,99	487,71	7,776	9,120
6	8,534	851940	15891	35,99	441,59	8,160	9,451
7	9,269	235611	11971	35,99	332,65	9,120	10,325
8	9,750	532932	11074	35,99	307,72	9,451	11,072
9	10,661	365123	10347	35,99	287,54	10,325	12,469
10	11,502	270305	5402	35,99	150,11	11,072	13,632
11	13,493	5362	324	35,99	8,99	13,216	14,635
12	13,960	40187	1535	35,99	42,65	13,632	22,485
13	21,841	19703	604	35,99	16,79	21,333	
Total		7767399	186463				

Sample Point 2: Outlet to Primary Settling Tank and Inlet to Aeration Basin

Table 6-7 Results for Samples collected at the Outlet to the Primary Settling Tank/ Inlet to the Aeration Basin Spring

PDA Ch1 254 nm

Peak Number	Ret. Time	Area	Height	Noise	S/N	Peak Start	Peak End
1	4,595	2969450	44592	40,87	1091,05	3,360	5,696
2	6,487	3473337	113314	40,87	2772,52	5,760	6,955
3	7,347	2006365	37852	40,87	926,15	6,955	8,139
4	8,363	1009201	23732	40,87	580,65	8,139	8,992
5	9,160	347416	16137	40,87	394,84	8,992	9,355
6	9,473	296641	15238	40,87	372,84	9,355	9,685
7	9,892	408213	14441	40,87	353,33	9,685	10,176
8	10,661	767838	15759	40,87	385,58	10,176	11,211
9	11,548	545278	9212	40,87	225,39	11,211	12,395
10	12,663	227499	5305	40,87	129,80	12,395	13,184
11	13,533	96510	3484	40,87	85,24	13,185	13,643
12	13,992	207236	6910	40,87	169,08	13,643	14,773
Total		12355045	305975				

Table 6-8 Results for Samples collected at the Outlet to the Primary Settling Tank/ Inlet to the Aeration Basin in Winter

PDA Ch1 254 nm

Peak Number	Ret. Time	Area	Height	Noise	S/N	Peak Start	Peak End
1	4,506	6684378	123683	70,93	1743,83	3,232	5,675
2	6,556	10303080	320512	70,93	4518,94	5,717	7,189
3	7,348	2137585	78163	70,93	1102,03	7,189	7,733
4	7,949	1556598	58023	70,93	818,08	7,733	8,224
5	8,383	2592415	47066	70,93	663,59	8,224	9,280
6	9,492	1386467	35196	70,93	496,23	9,280	9,952
7	10,182	856129	34353	70,93	484,34	9,952	10,379
8	10,652	1358128	40437	70,93	570,13	10,379	11,061
9	11,312	1794773	28063	70,93	395,66	11,061	12,395
10	12,563	683070	17518	70,93	246,99	12,395	13,077
11	13,498	601285	21170	70,93	298,47	13,077	13,621
12	13,956	1614836	40383	70,93	569,37	13,621	14,784
13	14,880	668327	12942	70,93	182,47	14,784	15,861
14	16,149	258652	8022	70,93	113,10	15,681	16,405
15	16,514	213554	7611	70,93	107,31	16,405	16,917
16	17,284	324722	6477	70,93	91,32	16,917	17,973
17	18,790	323447	4317	70,93	60,86	17,973	19,936
18	21,888	34297	1420	70,93	20,02	21,280	21,975
19	22,365	89693	2229	70,93	31,42	21,973	23,179
20	24,747	5091	195	70,93	2,75	24,491	25,195
Total		33486526	887779				

Sample Point 3: Final Effluent

Table 6-9 Results for Samples collected at the WWTP Effluent Point in Winter

PDA Ch1 254 nm

Peak Number	Ret. Time	Area	Height	Noise	S/N	Peak Start	Peak End
1	4,549	7818990	153862	70,14	2193,74	2,560	5,685
2	6,555	14187359	368744	70,14	5257,50	5,707	8,085
3	8,355	2952765	69063	70,14	984,70	8,085	9,344
4	9,442	613711	25940	70,14	369,85	9,344	9,760
5	9,883	876628	22492	70,14	320,68	9,760	10,507
6	10,675	840115	15937	70,14	227,22	10,507	11,541
7	11,690	633000	10667	70,14	152,08	11,541	12,949
8	13,077	160765	4209	70,14	60,01	12,949	14,176
9	16,175	72582	2511	70,14	35,80	15,787	16,917
10	17,989	7909	375	70,14	5,35	17,717	18,240
11	18,550	24154	580	70,14	8,28	18,240	19,616
12	20,214	15129	441	70,14	6,29	19,893	20,907
13	28,710	9851	298	70,14	4,25	28,373	29,451
Total		28212959	675119				

Table 6-10 Results for Samples collected at the WWTP Effluent Point in Spring

PDA Ch1 254 nm

Peak Number	Ret. Time	Area	Height	Noise	S/N	Peak Start	Peak End
1	4,581	2566544	36117	52,87	683,16	3,413	5,696
2	6,523	2554100	82815	52,87	1566,46	5,845	6,933
3	7,359	1395442	27537	52,87	520,87	6,933	8,992
4	13,982	14381	699	52,87	13,22	13,675	14,517
5	16,433	4771	186	52,87	3,52	16,181	16,907
Total		6535239	147354				

6.7 SUMMARY

Between Sample Point 1 and 2 collected in Winter, there is an increase of 67.22% while for the samples collected at the exact sampling locations in Spring, the peak is 59.06%. The increase in respective concentrations indicates that a source of contamination is present within the treatment works between the inlet to the works and the inlet to the aeration basin. This source of contamination is assumed to come from the sludge within the primary settling tank.

Between Sample Points 2 and 3 in Winter, there is a decrease of 15.75%; in spring, the concentration is 47.10%. The decrease in respective concentrations indicates that the treatment processes between Sample Points 2 and 3 degrade the antibiotic concentrations. Environmental factors, such as UV light and temperature, could also aid in the degradation of antibiotics.

When considering the variations in the concentrations of antibiotics at Winter from the inlet to the works at Sample Point 1 to the final effluent discharge at Sample Point 3, there is an increase of 40,89%, which indicates that the treatment works itself is a source of antibiotic contamination. Conversely, for samples collected in Spring, there is a decrease in antibiotic concentrations of 15.86% observed between Sample Points 1 and 3. Further investigations are required into trends of the weather patterns during the testing period to determine if external factors such as rainfall and photolytic degradation could cause a significant decrease as observed. These results also need to be corroborated with the statutory testing data of the treatment works to determine the plant performance at the time of sampling.

CHAPTER 7: ESTROGENIC ACTIVITY IN RIVER WATER AT SELECTED POINTS OF THE MSUNDUZI RIVER

7.1 INTRODUCTION

Ubiquitous toxic contaminants in the aquatic environment are of growing concern as they have been implicated in many chronic diseases with severe adverse effects on human health and the environment. Some of these contaminants are identified as Endocrine disrupters. Endocrine-disrupting chemicals (EDCs) are released into surface water and the environment through sewage effluent and from sources such as agricultural and pharmaceutical activities (Falconer et al., 2006; Slabbert et al., 2008; Burkhardt-Holm, 2010). Further, due to the prevalence and ease of entry of EDCs in the aquatic environment, EDCs have been detected in raw and treated water at varying concentrations in several countries. Water treatment processes may not effectively remove these contaminants from the source water, leading to potential risks to human health and the environment. With limited information on the estrogenic activity occurring within the water environment, it is imperative to understand this concept and its associated risks.

7.1.1 Sample Collection and Analysis

Sterilised amber bottles were used to collect water samples in triplicates from 16 locations along the Msunduzi River using the grab sampling method. All standard procedures were followed in collecting the water samples in 500 mℓ bottles. The bottles were filled to the brim, and the covers were lined with foil to avoid sample contamination. The samples were transported to the laboratory, processed and stored until extraction.

7.2 ANTIMICROBIAL-RESISTANT GENES

7.2.1 Sample Collection and Analysis

The following processes were followed to analyse and estimate microcontaminants and estrogenic activity in the water environment.

a. Measurement of Antibiotic Concentrations: The concentrations of antibiotics present in the water samples were measured to assess their levels and potential impact.

b. Estimation of Microbial Community: The microbial community within the water samples was characterised to understand the composition and diversity of microorganisms.

c. Antibiotic Susceptibility/Resistance Testing: Bacterial strains isolated from the water samples were tested for antibiotic susceptibility and resistance. This analysis aimed to determine the response of the bacterial strains to various antibiotics.

These experiments collectively provided valuable insights into the presence of microcontaminants, the estrogenic activity, and the interaction between microbial communities and antibiotics in the water environment.

7.2.1.1 Estimation of microbial communities.

The microbial communities in the water samples collected were measured using the metagenomics approach. The genomic DNA of the total microbial community was extracted using DNeasy Power water kit (Qiagen, Cat

No:1490-100NF) following the instructions in the manual. The concentration and quality of the DNA samples were determined using the nanodrop spectrophotometer and agarose gel electrophoresis, respectively. Fifty (50) microlitres of 20ng/µl concentration samples were sent to Inqaba Biotech, Pretoria, for metagenomic16S long-read sequencing using PacBio system. The raw data obtained was analysed using taxa and genus assigning tool KRAKEN2 (Wood and Salzberg, 2014)

The analysis of the data resulted in generating a large set of tables and graphs. Most of the taxa were labelled as unclassified in all the samples. The other taxa were *Enterobacterales, Acinetobacter ursingii, Polynucleobacter, Hyphomicrobiales, Proteobacteria, Gammaproteobacteria, Opitutus terrae, Candidatus Profftella armature, Roseomonas sp., Streptomyces sviceus, Anaerostipes, Microbacterium chocolatum, Sphingomonadales and Humisphaera borealis*.

Most of the bacteria detected were reported to have virulence effects and may be involved in causing certain diseases. These bacteria may be adapted as a multidrug resistance strain and hence need to be removed from the wastewater during treatment before reuse. The data generated will be published as a manuscript in the highly reputed journal in the research area under the title, “The metagenomic analysis of microbial community of water samples collected from major rivers in KwaZulu-Natal province of South Africa: Deciphering the possible cause of Multi drug resistance”.

7.2.1.2 Antibiotics susceptibility/resistance testing.

The susceptibility of bacterial strains to antibiotics was assessed using the disk diffusion technique. Pure bacterial strains were obtained from water samples through an enrichment process and subculturing on Nutrient Agar media. Following overnight growth at 30°C, a 100-microlitre culture was spread on Mueller Hinton agar plates. Antibiotic discs (Ampicillin 10 mcg, Erythromycin 10 mcg, Erythromycin 30 mcg, Tetracycline 30 mcg, and Sulphamethoxazole 25 mcg) were placed on the culture, and the plates were incubated overnight at 30°C. Growth observations were made, and the diameter of the zone of clearance was measured and recorded in centimetres. Data analysis was conducted using IBM SPSS v29.

108 and 150 bacterial strains were isolated from the first and second batches at 17 locations, respectively, based on colony size, type, and colour. The isolates exhibited varying sizes of the zone of clearance in the presence of different antibiotic discs. Figure 7.1 depicts the percentage of resistance and susceptible isolates. In contrast, Figure 7.2 illustrates the severity of resistance and susceptibility (diameter of the zone of inhibition, cm) of isolates for antibiotics, regardless of the sample collection location (first collection). Figure 7.3 presents the percentage of resistant and susceptible isolates, while Figure 7.4 displays the severity of resistance and susceptibility (diameter of the zone of inhibition, cm) of isolates for antibiotics, irrespective of the sample collection location (second batch collection).

The Antibiotic resistance was assessed using fixed concentrations of 10 mcg of Ampicillin, 10 mcg of Erythromycin, 30 mcg of Erythromycin, 30 mcg of Tetracycline, and 25 mcg of Sulphamethoxazole. It is important to note that strains may exhibit susceptibility below these concentrations. Technically, antibiotic resistance cannot be reliably determined solely based on threshold concentrations. If a strain is resistant to a high concentration, it is likely resistant to lower concentrations. Threshold concentrations are primarily used to gauge susceptibility. The classification into categories such as mild, moderate, serious, and severe was based on the diameter size of the zone of clearance (cm), as detailed in Table 7.1. Table 7.2 presents the Pearson correlation results between the antibiotic parameters detected in water samples and the resistance and susceptibility of the isolates, while Table 7.3 includes the significance values of these correlations.

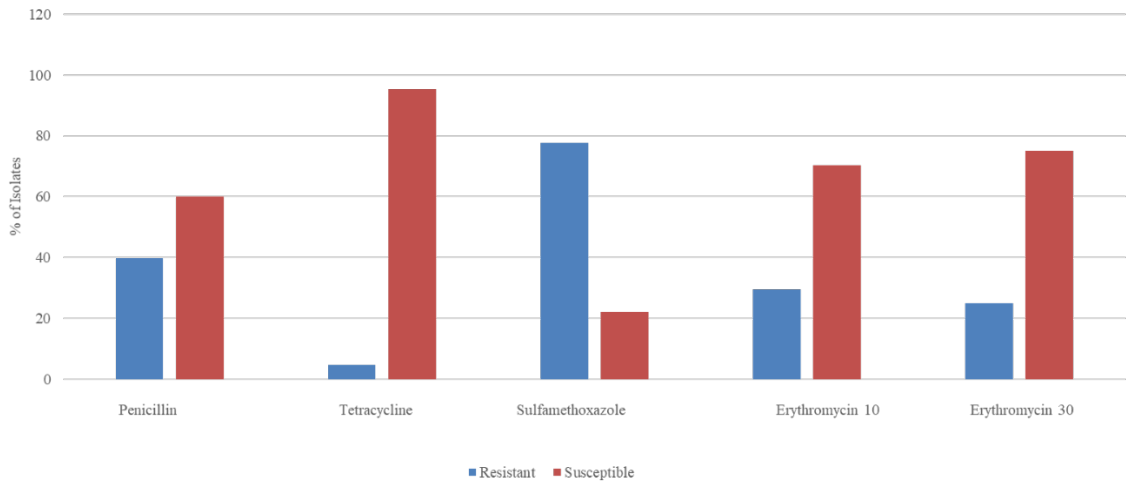


Figure 7.1 Isolates resistant or susceptible to antibiotics tested irrespective of location of sample collection (First collection)

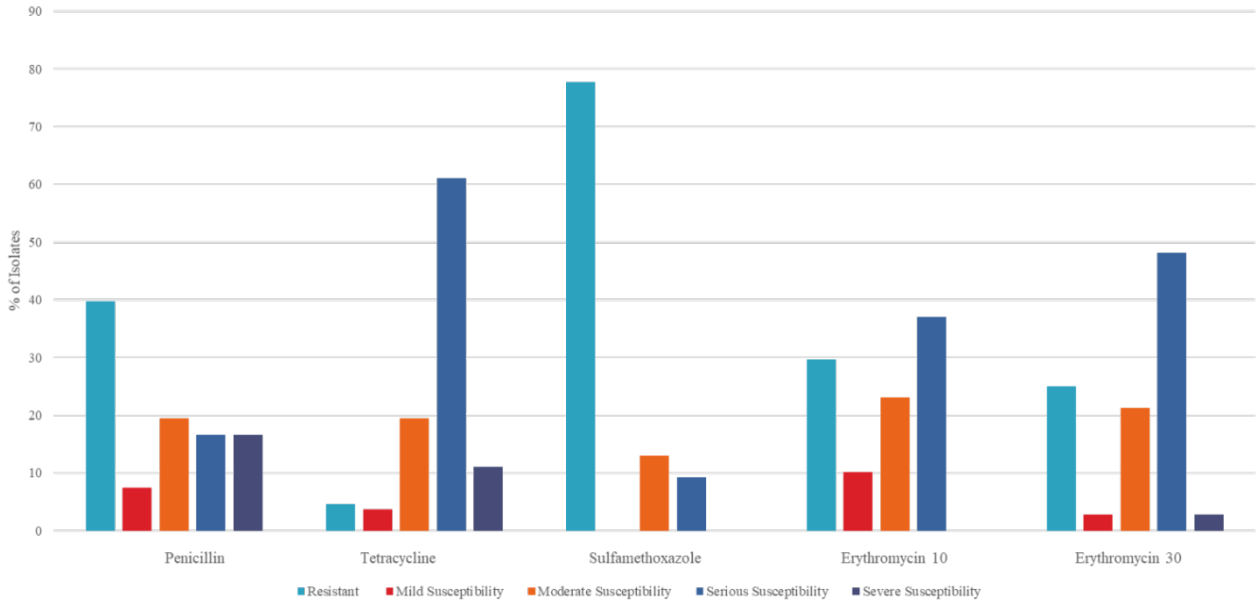


Figure 7.2 Resistance and susceptibility severity (diameter of zone of inhibition, cm) of isolates for antibiotics irrespective of the location of sample collection (First collection)

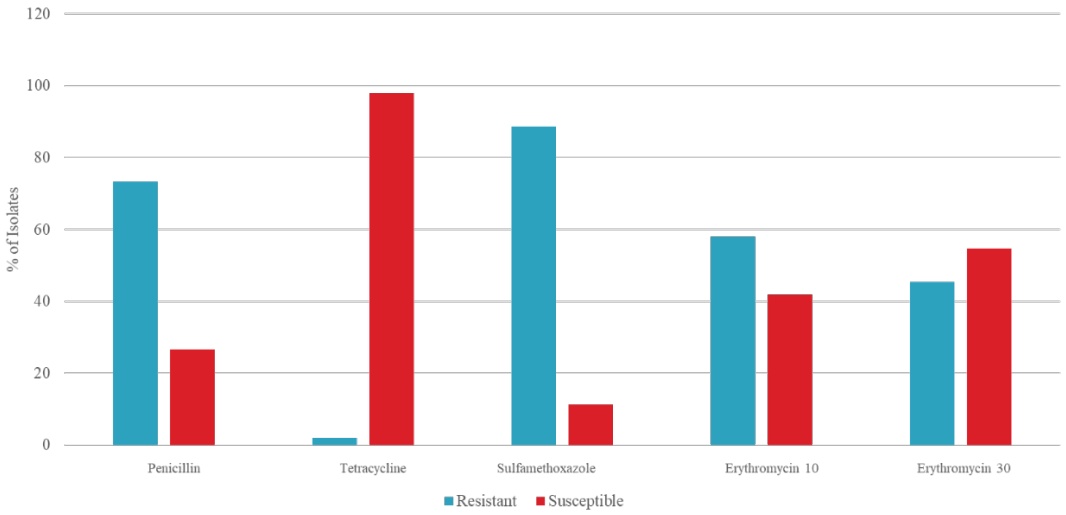


Figure 7.3 Isolates resistant or susceptible to antibiotics tested irrespective of location of sample collection (Second collection)

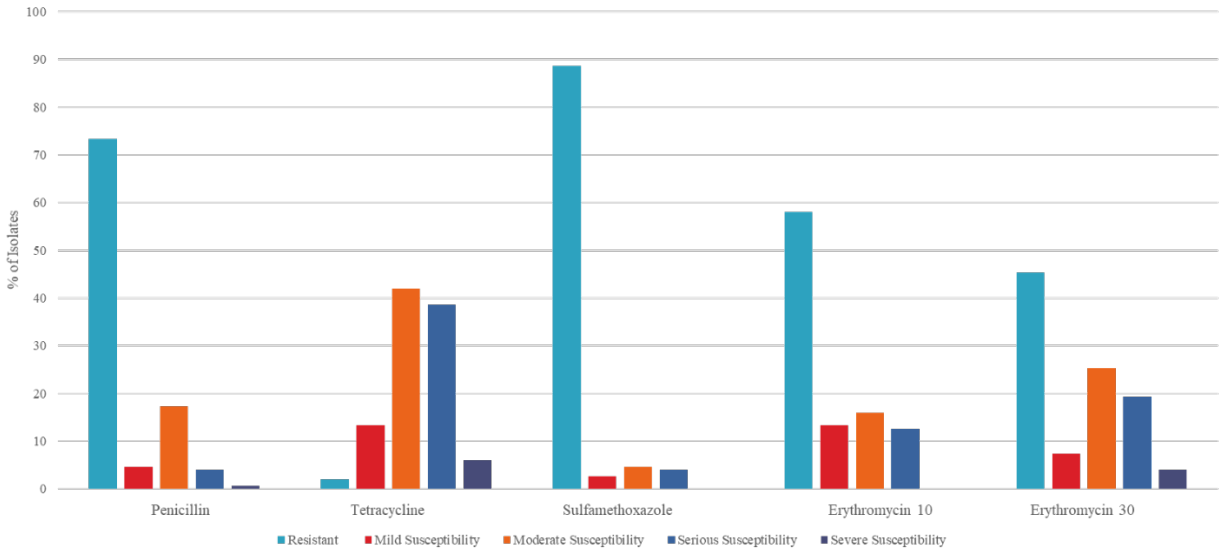


Figure 7.4 Resistance and susceptibility severity (diameter of zone of inhibition, cm) of isolates for antibiotics irrespective of the location of sample collection (Second collection)

Table 7-1 Diameter of Zone Clearance for Susceptibility Classification

		Diameter of zone of clearance (cm)				
Antibiotic	**Disk Conc.**	**Resistant**	**Mild Susceptibility**	**Moderate Susceptibility**	**Serious Susceptibility**	**Severe Susceptibility**
Ampicillin	10mcg	0	> 0 ≤1	> 1 ≤ 2	> 2 ≤ 3	> 3
Tetracycline	30mcg	0	> 0 ≤1	> 1 ≤ 2	> 2 ≤ 3	> 3
Sulphamethoxozole	25mcg	0	> 0 ≤1	> 1 ≤ 2	> 2 ≤ 3	> 3
Erythromycin	10mcg	0	> 0 ≤1	> 1 ≤ 2	> 2 ≤ 3	> 3
Erythromycin	30mcg	0	> 0 ≤1	> 1 ≤ 2	> 2 ≤ 3	> 3

Table 7-2 Pearson correlation between the parameters. 1 indicates a strong or positive correlation, and 0 to -1 shows a negative or weak correlation between variables.

	First Batch					**Second Batch**				
Antibiotics	**PNC**	**ERY10**	**ERY30**	**STZ**	**TTC**	**PNC**	**ERY10**	**ERY30**	**STZ**	**TTC**
PNC	-.174	-.331	-.371	-.111	.047	.106	-.136	-.035	-.128	-.082
TTC	-.032	-.015	-.058	-.041	.089	.060	-.121	-.093	-.154	-.056
ERY	-.106	-.088	-.135	.090	.085	.004	-.208	-.161	-.160	-.050
STZ	-.127	-.071	-.037	.048	.069	.325	.150	.222	-.068	.111

Table 7-3 Significance values of the correlation between the parameters.

	Significance (Chi-square value)	
Antibiotics	**First Batch**	**Second Batch**
PNC	.060	.293
ERY10	.343	.033*
ERY30	.148	.044*
STZ	.748	.692
TTC	.340	.485

*Significance p<.05

7.3 ANTIMICROBIAL-RESISTANT GENES

The results reveal variations in the size of the zone of clearance in response to different antibiotics, indicating the susceptibility or resistance of the isolates. In the first batch, a substantial proportion (78%) of isolates exhibited resistance to Sulphamethoxazole, with only 5% showing resistance to tetracycline. Resistance rates of 40%, 30%, and 20% were observed for ampicillin, erythromycin 10, and erythromycin 30, respectively (Figure 7.1).

In the subsequent batch, the trend shifted, with a significant number (73%, 89%, 58%, and 45%) of isolates demonstrating resistance to ampicillin, Sulphamethoxazole, erythromycin 10, and erythromycin 30, respectively. Conversely, only 2% of isolates were found to be resistant to tetracycline (Figure 7.3). The significance values presented in Tables (7.2 and 7.3) suggest that the emergence of resistance in isolates to antibiotics may be influenced by the source of water discharged into the river.

CHAPTER 8: MODEL DEVELOPMENT

8.1 INTRODUCTION

A mathematical model for the simulation of the antibiotic contaminants in a river is being developed using the concept of first-order reaction kinetics and mass balance of set variables. Reaction kinetics, which has a significant role in the concentration and persistence of antibiotics, is used in the model development. From the reviewed literature (Chee-Sanford et al., 2009; Tong et al., 2009; Chan et al., 2020), the major in-stream processes involved in antibiotic fate and transport are first-order photodegradation (K_p), bio-degradation (K_b), hydrolysis (K_h), and sorption. The reaction kinetics of the instream processes significantly impact the estimation of antibiotic concentration in a river. However, not all antibiotics experience those reactions.

Photodegradation of any substance is the abiotic degradation of compounds from sunlight absorption. Biodegradation is the biological breakdown of pharmaceuticals through human and animal metabolism and breakdown by microorganisms such as bacteria, fungi, microalgae, and protozoa. Microorganisms in the water and sediment-water interface partially or fully decompose degradable antibiotics into stable (no longer affected by biodegradation) solutes. Hydrolysis is the conversion, elimination, and solvation of antibiotics by water as a medium of an agent. Hydrolysis is a critical process in most antibiotics as they can be hydrolysed to form other compounds by bacterial enzymes. Sorption partitions the availability of pharmaceutical compounds in the water and sediment phases. The water-sediment portioning coefficient is used to determine the sorption capacity of sediment. The high concentration of antibiotics in stream beds and suspended matters Is reported in numerous literatures, with sediments considered a significant sink of antibiotics.

8.2 REACTION KINETICS ON SELECTED ANTIBIOTICS

Antibiotic use for human health, animal husbandry, agriculture, preservation and other purposes is a common practice in daily living. However, antibiotic residues from their use and application join the aquatic environment and are transported along rivers or sorbed into sediment and soil (Noutsopoulos et al., 2019; Gothwal and Thatikonda, 2020). As antibiotics travel downstream natural rivers, they undergo various transformations (attenuate, degrade, transform, and decompose). The simulation of antibiotics from a process-based reactive transport model relies on the parameters, especially the critical reaction parameters. Therefore, modelling the fate of antibiotics in a river must represent the possible processes affecting the transport. However, the physical representation of the transport process has dynamic, distinct, and complex properties, making developing a precise quantification mechanism a tough job.

In the water column, the primary attenuation processes of the aqueous concentration of antibiotics are sorption, biodegradation, photodegradation, and hydrolysis. However, the reaction processes of varying antibiotics differ and depend on various factors. For instance, the sorption process is more likely to depend on the river's geological composition and the sediment's organic carbon content. Li and Cui (2020) reported that low amounts of organic carbon content in sediments result in a higher antibiotic sorption rate. Their study showed that the hydrolysis of antibiotics is sensitive to the ion strength and the PH value of the reaction, and the cationic forms of antibiotics are more sensitive to hydrolysis than neutral and anionic forms. The presence of Nitrites in natural water increases the biodegradability of antibiotics, while the density of microbes and the presence of carbon play an increased rate of antibiotic degradation. However, the presence of carbon in the river water is minimal to sediment, sludge, and wastewater, resulting in low biodegradation of antibiotics than in the other water medium (Li and Cui, 2020). Hence, it is essential to understand the effect of specific transport kinetics on a particular antibiotic under consideration. A review of the degradation process for the selected antibiotics is presented in Table 8.1.

Table 8-1 Antibiotic attenuation processes.

Antibiotics Group	Sorption	Biodegradation	Photolysis	Hydrolysis	Citations
Penicillin (B-lactam)	Penicillin has a low rate of degradation due to adsorption by sediment. However, with the help of zinc chloride, a higher rate of biodegradation can be obtained.	β-lactamase enzymes from bacteria degrade penicillin to penicilloic acid by opening the β -lactam ring.		Penicillin is a weak base and hydrophilic. Higher attenuation of penicillin is related to hydrolysis than the other attenuation mechanisms. The unstable structure of β -lactam ring is highly affected by PH and heat, and it can be converted to penicilloic acid, penicilloaldehyde, penicillamine, penicilloic, and isopenillic acid.	(Li et al., 2008) (Yang et al., 2021)
Macrolides (Erythromycin) (ETM)		Erythromycin can be degraded by bacterial degradation (Ochrobactrum sp. Strain) by the transformation of depyranosyloxy	Erythromycin is highly reduced by photodegradation by the mechanism of cladinose ring cleavage.	Erythromycin is used for human and veterinary applications. Hydrophilic (a weak base)	(Yang et al., 2021)
Sulfonamides (Sulfathiazole) (STZ)	It has poor sorption properties and, hence, a low removal rate in WWTP (low sorption to treatment sludges) Has a high concentration in pore water of sediment for more than 50 half-life It is highly affected by photodegradation > 70% and, to a lesser extent, by adsorption. It has a relatively lesser attenuation rate than the other antibiotics.	Sulfathiazole can be biodegraded to N4-acetyl by the mechanism of acetylation.	Sulfathiazol can be degraded with the help of indirect photolysis to form acetylation.	Hydrophilic (Weak acid) Origins from veterinary and human medicine Unlikely to be derived from agriculture Are considered ideal for hydrolysis Sulfonamides do not break down under normal conditions. It requires a higher temperature and higher concentration of strong acids or bases than encountered in the environment.	(Tamtam et al., 2008) (Liu et al., 2019b) (Li and Cui, 2020)
Tetracyclines Tetracycline (TC)	Tetracycline is highly sorbed to the clay and humic substances and clay minerals. Adsorption of tetracycline is influenced by pH and ionic strength. Tetracycline has a higher sorption capacity than any other antibiotic. Tetracycline has a short half-life in the water column as it sorbs into soils and sediment by adsorption due to its hydrophobic properties.		Tetracycline has a moderate rate of direct photodegradation.	Hydrophobic (a weak base). It can be used to treat bacterial infections in humans and animals.	(Gothwal and Shashidhar, 2015) (Yang et al., 2021)

8.3 MODEL FORMULATION

The conceptualized Hybrid Cells in Series (HCIS) model discretises the river into a series of spatial segments, each referred to as a hybrid unit. Each hybrid unit comprises three distinct mixing zones: the plug flow zone, the first thoroughly mixed zone, and the second well-mixed zone. The effluent from the plug flow zone serves as input for the subsequent first mixing zone, and the effluent from the first mixing zone becomes influent for the second mixing zone. This sequential process continues, with the effluent from the second mixing zone of the previous hybrid unit acting as input for the subsequent unit. This pattern persists until the plume reaches the terminal level, as illustrated in Figure 8.1.

The mixing zones in the model consist of time parameters that enable the prediction of the expected concentration range, accounting for variations in dispersion and mixing. The exchange within these zones is characterised by residence times, representing various storage zones. This model structure aims to comprehensively understand antibiotic transport and dispersion dynamics in the river system.

The model incorporates various physical processes, including advection, dispersion, adsorption, degradation, settling, resuspension, and diffusion, to describe the transport of antibiotics, suspended solids, dissolved organic matter, and particulate organic matter. Transformations such as biological degradation, hydrolysis, and photolysis are accounted for in both the adsorbed phase and the aqueous phase. However, this model assumes that all transformations occur solely in the aqueous phase for simplification. The variables are defined based on the total volume and concentrations of contaminants.

The model assumes first-order antibiotic decay and incorporates a point source of contamination. Mass-balance equations are formulated for each variable, encompassing the system's transport processes, inputs, outputs, and degradation processes. This approach allows for a comprehensive analysis of contaminant dynamics within the river system.

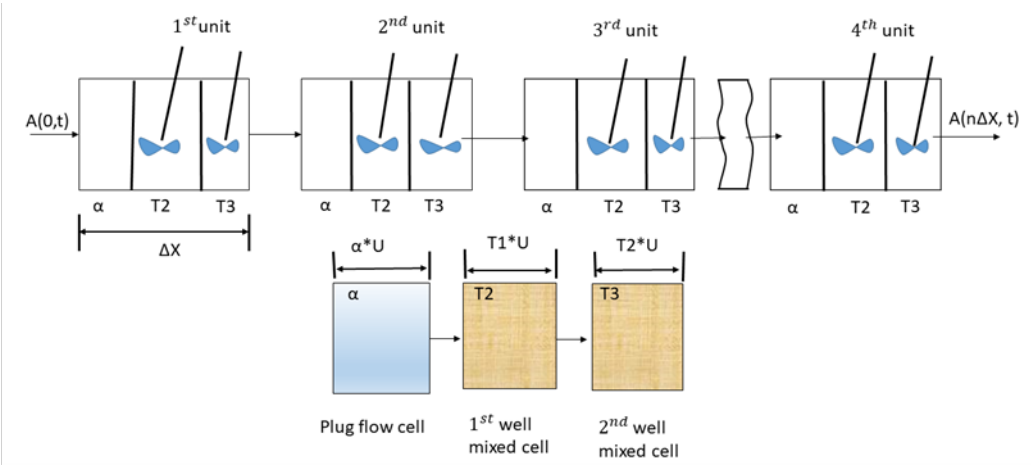


Figure 8.1 Model conceptualisation

8.3.1 Mass balance equation

The partial differential equations are derived by applying mass balance principles to a control volume (V). The expressions for contaminant concentration are formulated based on the rate of change of mass of contaminant within the control volume, equating the mass balance of total mass degraded and the inflow mass. The derivation incorporates considerations for advection, dispersion, settling, diffusion, and instream reactions, comprehensively representing the modelled system's contaminant transport and transformation processes.

8.4 MODELLING OF ANTIBIOTICS

In modelling the presence of antibiotic residues in the riverine system, it is essential to account for various environmental processes influencing their fate and transport. These processes encompass dissolution, dispersion, diffusion, aggregation with solids, settling, and interaction with organic and suspended matter. Hydrolysis, photolysis, biolysis, and sorption, in addition to advection and dispersion, are identified as pivotal processes in the developed model for this study. Using linear partition coefficients, the model incorporates the partitioning between freely dissolved, solids-bound, and DOM-bound phases. Furthermore, the mass balance equation 9 considers the concentration of input from tributaries, expressed using equation 9a-c as follows:

$$\frac{\partial A(x,t)}{\partial t} = -u\frac{dA(x,t)}{dx} - (K_b + K_{\mathrm{h}} + K_p)A(x,t) + \frac{K_r}{\gamma}A_S - \frac{K_d}{\mathrm{h}}\left(A(x,t) - \frac{A_S}{\gamma}\right) - K_{\mathrm{s}}A_p + \frac{\mathrm{q}}{V}Se \quad [9]$$

$$A_{fd} = \frac{A(x,t)}{1+k_{dom}DOM+ k_{solid}TSS} \quad [9a]$$

$$A_d = \frac{A(x,t)\, k_{dom}DOM(x,t)}{1+k_{dom}DOM+ k_{solid}TSS} \quad [9b]$$

$$A_p = \frac{A(x,t)\, k_{solid}TSS(x,t)}{1+k_{dom}DOM+ k_{solid}TSS} \quad [9c]$$

Where A(*x,t*) and A_S are the antibiotic concentration in the water column and sediment, respectively; A_{fd} is the freely dissolved concentration of antibiotics in the water column; A_d is the DOM bounded concentration of antibiotics in the water column, and A_p is the particulate bounded concentration of antibiotics in the water column. u is the advection velocity (m s^{-1}), t is time (day^{-1}), *Se* is the concentration of antibiotics from external sources (mg L^{-1}), h is the depth of river water (m), q is the flow from wastewater treatment plants and tributaries (m^3 s^{-1}) and γ ratio of water depth to a depth of underlying movable sediment layer (h/ψ). The definition of reaction constants, parameters and other terms are provided in Table 8.2.

Dissolved organic matter

Organic matter in stream water is a mixture of various compounds. It is a chemically active substance that plays a vital role in the aquatic ecosystem functioning (carbon cycle, nutrient output, and food web dynamics) and water quality. Dissolved organic matter (DOM) is an abundant substance. It significantly affects the biogeochemical processes that regulate the momentum of mineral colloids and solute transport within the aquatic environment. The presence of DOM facilitates the mechanism for contaminant mobility and transformation in the aqueous phase concentration of antibiotics (Cheng & Saiers, 2015). DOM forms complexes with metals and organic contaminants, affecting their solubility, bioavailability, toxicity, and transport properties that change the reactivity of contaminants in the water column and sediment (Song et al., 2022). In addition, DOM influences the pH level of stream water and affects light penetration. The quantity of availability of DOM in the water quality plays an essential role in eliminating organic contaminants such as antibiotics in the aquatic environment. DOM are active sites for the physical sorption and chemical transformation of antibiotics. Biodegradation of natural organic matter in natural river water produces the main organic fractions, humic and fluvic acids. Humic and fluvic acid transfer light energy to other compounds in the water, which facilitates the excited state of chemical transients and strongly affects the degradation of antibiotics concentration (Makunina et al., 2015).

Consequently, DOM availability is subjected to various biogeochemical processes, and the metabolism of algae (respiration and excretion) breaks down plant residue, producing energy and DOM. The

biogeochemical mechanisms that control the changing rates of supply, mobilization, and retention of soluble organic matter within, above and below-ground reservoirs of the terrestrial environment determine the concentrations of DOM in natural river water (Kim et al., 2003). External load (discharge form point sources) is a major source of DOM. The mass balance of DOM is expressed using equation 10 as:

$$\frac{\partial DOM(x,t)}{\partial t} = -u\frac{\partial DOM(x,t)}{\partial x} - K_s DOM(x,t) + \frac{K_d}{\mathrm{h}} DOM_s - (K_{Pm} + K_p{}^*)DOM(x,t) + K_x \mathrm{POM} + \frac{K_r}{\mathrm{h}} \mathrm{POM}_s + \frac{q}{V} Se \quad [10]$$

DOM and DOMs are the dissolved organic matter concentrations in the water column and sediment.

Particulate organic matter

POM is the fraction of total suspended solids and is a source of dissolved organic matter due to hydrolysis. Algal photosynthesis leads to the transformation of dissolved nutrients into particulate organic matter. Some of this particulate matter may settle and diffuse to the sediment. Many contaminants associate with particulate organic matter. Hydrophobic compounds have a strong affinity to POM and form a complex in the presence of POM. Therefore, the cycle of POM affects their fate and transport (equation 11).

$$\frac{\partial POM(x,t)}{\partial t} = -u\frac{dPOM(x,t)}{dx} - K_s POM(x,t) - (K_{bw} + K_{hw})POM(x,t) + \frac{K_r}{\gamma} POM_s + \frac{q}{V} Se \quad [11]$$

POM and POMs are the particulate organic matter concentrations in the water column and sediment.

Total suspended solids

Total suspended solids (and water turbidity) may influence antibiotic concentration via (1) light availability, (2) water temperature, and (DO) consumption. High TSS increases the light attenuation coefficient and reduces the light available for photosynthesis, leading to less DO production. Suspended particles absorb heat and cause water temperature to increase. The ability of water to hold oxygen is influenced by temperature and salinity. Since warm water holds less DO than cold water, a temperature increase causes a reduction in DO concentrations. Total suspended solids often contain significant organic matter, which attributes active sites to bind antibiotics (equation 12).

$$\frac{\partial TSS(x,t)}{\partial t} = -u\frac{dTSS(x,t)}{dx} - K_s TSS(x,t) + \frac{K_r}{\gamma} TSS_s - Z K_{\mathrm{h}} TSS(x,t) + \frac{\mathrm{q}}{V} Se \quad [12]$$

TSS and TSSs are the total suspended solid concentration in the water column and sediment.

8.4.1 Derivation of concentration through the plug flow zone

In the plug flow zone, a control volume (V) of the water column and the underlying sediment, the concentration of antibiotic contaminant transported downstream with time α to the next control volume (first mixing zone). The plume of antibiotic contaminant entering the plug flow undergoes pure advection transport without changing its concentration. During the translation of antibiotic contaminants in the plug flow zone, a fraction of contaminants might be lost, sources may contribute minimal fractions, and minimal exchanges between the water column and sediment occur. The initial and boundary conditions for the mass balance of antibiotics are given as equations 12 (a-c):

$$A(x, 0) = 0; \quad x > 0 \quad [12a]$$

$A\ (x,\ 0) = 0;\quad t \geq 0$ [12b]

$A\ (au,\ t) = 0;\quad 0 < t > a$ [12c]

The mass balance equation (9) is solved by applying the boundary conditions where the initial boundary concentration of antibiotics in the cell changes from Ci to Cr. Taking the Laplace transform and integration of partial differential equation (9) when A (*0, t*), where t ≥ 0 at x = 0, C_T = Cr +Se and C_T* = (Cr + Se)/s, the inverse Laplace transform; yields the concentration of antibiotics at the end of the plug flow zone to the step input C_T valid for t ≥ 0 (equation 13).

$$A(x,t) = C_T U(t-\alpha)e^{-k\alpha} - \frac{\mu_1}{k}\left(U(t-\alpha)e^{-k\alpha} - 1\right) + \frac{\mu_1}{k}e^{-kt}(U(t-\alpha) - 1) \quad [13]$$

Where, U (t - α) is the step function given as 0 at t < 0 and 1 at t ≥ 0. Similarly, by applying the Laplace transform, boundary conditions, and inverse Laplace transform, the concentration at the end of the plug flow zone is derived for the other variables.

DOM

$$DOM(x,t) = C_T U(t-\alpha)e^{-\mathrm{k}\alpha} + \frac{Ae^{-\mathrm{k}(\mu-\rho)}}{k}\left(\left(1-e^{-\mathrm{k}t}\right) - U(t-\alpha)(e^{-\mathrm{k}\alpha} - e^{-K_m t})\right) + \frac{\omega_1}{k}\left(1-e^{-\mathrm{k}t}\right) - \frac{\omega_1 U(t-\alpha)}{k}\left(e^{-\mathrm{k}\alpha} - e^{-\mathrm{k}t}\right) \quad [13a]$$

POM

$$\mathrm{POM}(x,t) = C_T U(t-\alpha)e^{-\mathrm{k}\alpha} - \frac{\tau_1}{k}\left(U(t-\alpha)e^{-\mathrm{k}\alpha} - 1\right) + \frac{\tau_1 e^{-\mathrm{k}t}}{k}(U(t-\alpha) - 1) \quad [13b]$$

TSS

$$TSS(x,t) = C_T U(t-\alpha)e^{-k\alpha} - \frac{\mu_1}{k}\left(U(t-\alpha)e^{-k\alpha} - 1\right) + \frac{\mu_1}{k}e^{-kt}(U(t-\alpha) - 1) \quad [13c]$$

8.4.2 Derivation of concentration through the first well-mixed zone

Antibiotics

The effluent from the plug flow zone enters the first thoroughly mixed zone. Decaying the pollutants also takes place in this zone. The mass decayed in a duration ∆t equals $k_1V_1CM1\Delta t$, where CM1 is the effluent concentration from the first plug flow zone, which also equals the concentration within the zone. The mass balance is then expressed as:

$$V_1 * \Delta A_1(x,t) = A(x,t) * Q * \Delta t - A_1(x,t) * Q * \Delta t - \mathrm{k}A_1(x,t)V_1\Delta t + \mu_2 V_1 \Delta t \quad [14]$$

Simplifying equation (14), expressing it in differential form, and integrating antibiotic concentration at the end of the first mixing zone derives equation (15). By applying a similar procedure, the concentration of other variables is derived.

$$A_1(x,t) = \frac{C_T U(t-\alpha)e^{-k\alpha}}{1+k_1T_1}a_o - \frac{\mu_1\left(U(t-\alpha)e^{-k\alpha}-1\right)}{(1+kT_1)k}a_1 + \frac{\mu_1(U(t-\alpha)-1)e^{-k\alpha}}{k(1+kT_1)}a_2 + \frac{\mu_2 T_1}{1+k_1T_1}a_o \quad [15]$$

$$\mathrm{DOM}(x,t) = \frac{C_T U(t-\alpha)e^{-\mathrm{k}\alpha}}{1+\mathrm{k}T_1}a_o + \frac{A(U(t-\alpha)-1)e^{-\mathrm{k}\alpha}}{\mathrm{k}(1+(\mu-\rho+\mathrm{k})T_1))}a_3 - \frac{A\left(U(t-\alpha)e^{-\mathrm{k}\alpha}-1\right)e^{-\mathrm{k}\alpha}}{\mathrm{k}(1+(\mu-\rho-k)T_1)}a_4 + \frac{\omega_1(U(t-\alpha)-1)e^{-\mathrm{k}\alpha}}{\mathrm{k}(1+\mathrm{k}T_1)}a_3 - \frac{\omega_1\left(U(t-\alpha)e^{-\mathrm{k}\alpha}-1\right)e^{-\mathrm{k}\alpha}}{\mathrm{k}(1+\mathrm{k}T_1)}a_o + \frac{A_2T_1}{1+\mathrm{k}T_1}a_4 + \frac{\omega_2T_1}{1+\mathrm{k}T_1}a_o \quad [15a]$$

$$\mathrm{POM}_1(x,t) = \frac{C_T U(t-\alpha)e^{-K_p\alpha}}{1+kT_1}a_o - \frac{\tau_1\left(U(t-\alpha)e^{-K_p\alpha}-1\right)}{k_\mathrm{p}(1+k_\mathrm{p}T_1)}a_1 + \frac{\tau_1(U(t-\alpha)-1)e^{-K_p\alpha}}{k_\mathrm{p}(1+k_\mathrm{p}T_1)}a_2 + \frac{\tau_2T_1}{1+k_\mathrm{p}T_1}a_o \quad [15b]$$

$$TSS_1(x,t) = \frac{C_T U(t-\alpha)e^{-k\alpha}}{1+\mathrm{k}T_1}a_0 - \frac{\sigma_1\left(U(t-\alpha)e^{-\mathrm{k}\alpha}-1\right)}{k(1+kT_1)}a_1 + \frac{\sigma_1(U(t-\alpha)-1)e^{-\mathrm{k}\alpha}}{k}a_2 + \frac{\sigma_2T_1}{1+\mathrm{k}T_1}a_0 \quad [15c]$$

Where,

$$a_0 = \left(1 - e^{-(\frac{1}{T_1}+\mathrm{k})(t-\alpha)}\right)$$

$$a_1 = \left(1 + e^{-(\frac{1}{T_1}+\mathrm{k})(t-\alpha)}\right)$$

$$a_2 = \left(e^{-k_\mathrm{T}(t-\alpha)} - e^{-\left(\frac{1}{T_1}+\mathrm{k}\right)(t-\alpha)}\right)$$

$$a_3 = \left(e^{(\mu-\rho-\mathrm{k})t} - e^{-\left(\frac{1}{T_1}+\mathrm{k}\right)(t-\alpha)}\right)$$

$$a_4 = \left(e^{(\mu-\rho)(t-\alpha)} - e^{-\left(\frac{1}{T_1}+\mathrm{k}\right)(t-\alpha)}\right)$$

8.4.3 Derivation of concentration through the second well-mixed zone

Antibiotics

The second well-mixed zone has a retention time of T_2. The outflow from the first well-mixed zone is the inflow to the second well-mixed zone. The mass balance in the second well-mixed zone (equation 16) is formulated considering the sinks and sources. The first term on the right side is the mass inflow from the first mixing zone. The second term represents the mass leaving the zone, and the third and fourth terms represent the mass leaving and entering due to degradation and exchange.

$$V_2 * \Delta A_2(x,t) = A_1(x,t) * Q * \Delta t - A_2(x,t) * Q * \Delta t - kA_2(x,t)V_2\Delta t + \mu_2V_2\Delta t \quad [16]$$

Simplifying and expressing equation 16 in differential form and integration yields equation 17, the step response function for antibiotic concentrations at the end of the first hybrid unit. The step response functions were derived for DOM, POM and TSS by applying the same procedure.

$$A_2(x,t) = \frac{C_T U(t-\alpha)e^{-k\alpha}}{1+k_1T_1}b_1 - \frac{\mu_1\left(U(t-\alpha)e^{-k\alpha}-1\right)}{k(1+kT_1)}b_2 + \frac{\mu_1(U(t-\alpha)-1)e^{-K\alpha}}{k}b_3 + \frac{\mu_2T_1}{1+k_1T_1}b_1 + \frac{\mu_2T_2}{1+kT_2}b_9 \quad [17]$$

$$DOM_2(x,t) = \frac{C_T U(t-\alpha)e^{-\mathrm{k}\alpha}}{1+kT_1}b_1 + \frac{A(U(t-\alpha)-1)e^{-k\alpha}}{\mathrm{k}(1+(\mu-\rho)T_1)}b_4 - \frac{A\left(U(t-\alpha)e^{-k\alpha}-1\right)e^{-\mathrm{k}\alpha}}{\mathrm{k}(1+(\mu-\rho+\mathrm{k})T_1)}b_5 + \frac{\omega_1(U(t-\alpha)-1)e^{-\mathrm{k}\alpha}}{\mathrm{k}}b_6 - \frac{\omega_1\left(U(t-\alpha)e^{-k\alpha}-1\right)}{\mathrm{k}(1+kT_1)}b_1 + \frac{A_2T_1}{1+(\mu-\rho+k)T_1}b_7 + \frac{\omega_2T_1}{1+\mathrm{k}T_1}b_1 + \frac{A_2T_2}{1+\mathrm{k}T_2}b_1 + \frac{\omega_2T_2}{1+\mathrm{k}T_2}b_9 \quad [17a]$$

$$\mathrm{POM}_2(x,t) = \frac{C_T U(t-\alpha)e^{-K_p\alpha}}{1+k_p T_1} b_1 - \frac{\tau_1\left(U(t-\alpha)e^{-k\alpha}-1\right)}{k(1+kT_1)} b_2 + \frac{\tau_1(U(t-\alpha)-1)e^{-K\alpha}}{k} b_3 + \frac{\tau_2 T_1}{1+k_1 T_1} b_1 + \frac{\tau_2 T_2}{1+kT_2} b_9 \quad [17b]$$

$$TSS_2(x,t) = \frac{C_T U(t-\alpha)e^{-\mathrm{k}\alpha}}{1+\mathrm{k}T_1} b_1 - \frac{\sigma_1\left(U(t-\alpha)e^{-k\alpha}-1\right)}{(1+\mathrm{k}T_1)} b_2 + \frac{\sigma_1(U(t-\alpha)-1)e^{-K\alpha}}{\mathrm{k}} b_3 + \frac{\sigma_2 T_1}{1+k_1 T_1} b_1 + \frac{\sigma_2 T_2}{1+\mathrm{k}T_2} b_9 \quad [17c]$$

Where,

$$b_1 = \left[\frac{1-e^{-\left(\frac{1}{T_1}+\mathrm{k}\right)(t-\alpha)}}{1+k_1 T_2} - \frac{T_1}{T_1-T_2}\left(e^{-\left(\frac{1}{T_1}+\mathrm{k}\right)(t-\alpha)} - e^{-\left(\frac{1}{T_2}+\mathrm{k}\right)(t-\alpha)}\right)\right]$$

$$b_2 = \left[\frac{1}{1+k_1 T_2} + \frac{T_1 e^{-\left(\frac{1}{T_1}+\mathrm{k}\right)(t-\alpha)}}{T_1-T_2} + \left(\frac{1}{1+k_1 T_2} + \frac{T_1}{T_1-T_2}\right) e^{-\left(\frac{1}{T_2}+\mathrm{k}\right)(t-\alpha)}\right]$$

$$b_3 = \left[\frac{e^{-K(t-\alpha)}}{(1+\mathrm{k}T_1)} - \frac{T_1 e^{-\left(\frac{1}{T_1}+k\right)(t-\alpha)}}{T_1-T_2} + \frac{(T_1+T_2)e^{-\left(\frac{1}{T_2}+\mathrm{k}\right)(t-\alpha)}}{(T_1-T_2)}\right]$$

$$b_4 = \left[\frac{e^{(\mu-\rho-k)(t-\alpha)} - e^{-\left(\frac{1}{T_2}+\mathrm{k}\right)(t-\alpha)}}{1+(\mu-\rho)T_2} - \frac{T_1}{T_1-T_2}\left(e^{-\left(\frac{1}{T_1}+k_1\right)(t-\alpha)} - e^{-\left(\frac{1}{T_2}+k_1\right)(t-\alpha)}\right)\right]$$

$$b_5 = \left[\frac{e^{(\mu-\rho)(t-\alpha)} + e^{-\left(\frac{1}{T_1}+k_2\right)(t-\alpha)}}{1+(\mu-\rho+k)T_2} + \frac{T_1}{T_1-T_2}\left(e^{-\left(\frac{1}{T_1}+k_1\right)(t-\alpha)} + e^{-\left(\frac{1}{T_2}+k_1\right)(t-\alpha)}\right)\right]$$

$$b_6 = \left[e^{-kt} - 1 - \frac{T_1}{T_1-T_2}\left(e^{-\left(\frac{1}{T_1}+k_1\right)(t-\alpha)} - e^{-\left(\frac{1}{T_2}+k_1\right)(t-\alpha)}\right)\right]$$

$$b_7 = \left[\frac{e^{(\mu-\rho)(t-\alpha)} - e^{-\left(\frac{1}{T_1}+k_2\right)(t-\alpha)}}{1+(\mu-\rho+k)T_2} - \frac{T_1}{T_1-T_2}\left(e^{-\left(\frac{1}{T_1}+k_1\right)(t-\alpha)} - e^{-\left(\frac{1}{T_2}+k_1\right)(t-\alpha)}\right)\right]$$

$$b_8 = \left[e^{(\mu-\rho)(t-\alpha)} - e^{-\left(\frac{1}{T_2}+K_{dom1}\right)(t-\alpha)}\right]$$

$$b_9 = \left[1 - e^{-\left(\frac{1}{T_2}+K_{dom1}\right)(t-\alpha)}\right]$$

Equations 17-17c represent the step response, indicating effluent concentrations, for each variable at the end of the initial hybrid unit within the water column. The step response (K_{VAR}) characterises the system's unit response to a unit step input, applicable for t ≥ α. Moreover, the unit pulse response ($\delta_{VAR}(n,\Delta t)$) to the unit impulse perturbation at the end of the first hybrid unit is determined by differentiating the step response functions of each variable (K_{VAR}) with respect to time 't,' as shown in equation 18.

$$\delta_{VAR}(n,\Delta t) = \frac{K_{VAR}(n\Delta t) - K_{VAR}((n-1)\Delta t))}{\Delta t} \quad [18]$$

To simulate the fate of the pollutant at a downstream location of the river, the convolution technique by the discrete kernel is applied for m ≥ 2. Equation (19) produces the pulse response of a variable at the n^{th} hybrid unit when n ≥ 2 for any number of hybrid units along the river reach.

$$C(m\Delta x, n\Delta t) = \sum_{\gamma=1}^{n} A[(m-1)\Delta x, n\Delta t]\delta_{VAR}[(n-\gamma+1),\Delta t] \quad [19]$$

Table 8-2 Equation Constants

Water Column
Antibiotics
$k = k_a + \frac{K_r}{\gamma} + \frac{K_d}{\mathrm{h}}\left(\frac{1}{1+k_{dom}DOM+ k_{solid}TSS} + \frac{k_{dom}DOM}{1+k_{dom}DOM+ k_{solid}TSS}\right) + K_\mathrm{s}\left(\frac{k_{solid}TSS}{1+k_{dom}DOM+ k_{solid}TSS}\right)$
$\mu_1 = \left(\frac{K_r}{\gamma} + \frac{K_d}{\mathrm{h}}\right)A_S + \frac{Q_1 Se}{\alpha * u * w * \mathrm{h}}$
$\mu_2 = \left(\frac{K_r}{\gamma} + \frac{K_d}{\mathrm{h}}\right)A_S$
DOM
$k = K_s - (K_{Pm} + K_p{}^*) - K_0 Y$
$\omega_1 = \frac{K_d}{\mathrm{h}} DOM_s + K_p \mathrm{POM} + \frac{K_r}{\mathrm{h}}\mathrm{POM}_s + \frac{Q_1}{V} Se$
$\omega_2 = \frac{K_d}{\mathrm{h}} DOM_s + K_p \mathrm{POM} + \frac{K_r}{\mathrm{h}}\mathrm{POM}_s$
POM
$k = K_{bw} + K_{\mathrm{hw}} + K_s + \frac{K_r}{\gamma}$
$\tau_1 = \frac{K_r}{\gamma}\mathrm{POM}_s + \frac{Q_1}{\alpha * u * w * \mathrm{h}}$
$\tau_2 = \frac{K_r}{\gamma}\mathrm{POM}_s$
TSS
$k = K_s + \mathrm{Z}K_\mathrm{h}$
$\sigma_1 = \frac{K_r}{\gamma} TSS_s + \frac{Q_1}{\alpha * u * w * \mathrm{h}}$
$\sigma_2 = \frac{K_r}{\gamma} TSS_s$

8.4.4 Model parameters

The parameters of the proposed model, the residence time of the hybrid unit, are α, T_1 and T_2. The parameters are estimated from the following relations (equations 20a-c) by satisfying the condition of Peclet number, Pe = $\Delta x D_L \geq 4$ (Ghosh et al., 2001; Kumarasamy et al., 2013), where Δx is the size of the unit and D_L Is the dispersion coefficient.

$$\alpha = \frac{0.04\Delta x^2}{D_L} \quad [20a]$$

$$T_1 = \frac{0.05\Delta x^2}{D_L} \quad [20b]$$

$$T_2 = \frac{\Delta x}{u} - \frac{0.04\Delta x^2}{D_L} \quad [20c]$$

8.4.5 Model application

The proposed model has been applied to the Msunduzi River, South Africa. Msunduzi River passes through Pietermaritzburg, the capital of KwaZulu-Natal province, draining two-thirds of the metropolitan region. The watershed is a relatively complex system including numerous discharges, withdrawals and tributaries. The Msunduzi River is densely populated and affected by various anthropogenic activities and developments. It comprises a catchment with several grazing animals, poor sanitation, inadequate wastewater treatment services, industrial pollution, commercial farming sites, and informal settlements. The River is highly affected by anthropogenic activities and is reported to contribute to significant antibiotic pollution in the Umgeni River (Agunbiade & Moodley, 2014, 2016; Matongo et al., 2015). The upstream area is primarily urban and comprises effluents from industries and municipal Wastewater Treatment Plant (WWTP). The downstream area comprises sub-urban areas and agriculture, mostly pasture and confined animal feeding operations.

The river reach from the Hanley Dam before its confluence with the Umgeni River and other water quality sampling points along the river reach were selected for this study. The average inclination of the studied reach of the river is 0.3%. The river's average discharge is 19.09 m^3/s, the average depth is 1 m, with varying river widths along with river length, and the sediment consists predominantly of clay silt. Ten sampling sites along the river and six major tributaries, including Darvile WWTP effluent, were proposed to account for sources from domestic, agricultural, industrial and municipal activities. The WWTP receives 30% from domestic and 70% from commercial, industrial and hospital effluents.

The Fate, transport, and kinetic rate of various antibiotics differ. As a result, frequently detected antibiotics such as tetracycline were selected to test the prediction suitability of the developed model. Appropriate constant values and calibration parameters are chosen from the literature as presented in Table 8.3

Model parameters were calculated using equations 20 (a-c). The dispersion coefficients along the river were determined through tracer taste. The simulation was carried out by dividing the river reach into three. The upstream reach runs through Hanley Dam (HD) to Camps Drift (CD), the middle reach starts at Camps Drift (CD) to Gripthorpe (GRP), and the downstream reach: Gripthorpe (GRP) to Table Mountain (TB). The unit size Δx for the upstream, middle and downstream reaches were 600m, 780m, and 840m, respectively.

The tetracycline residue concentration presented in Table 6-1 was calibrated, as shown in Table 8-3, to match the field data. $K_{d,\ solid}$ was calibrated against water column and sediment bed concentrations. The values are chosen considering silt and fine-grained sediment bed (sand). $K_{d,\ DOM}$ is taken as the average of silt soil and sediment.

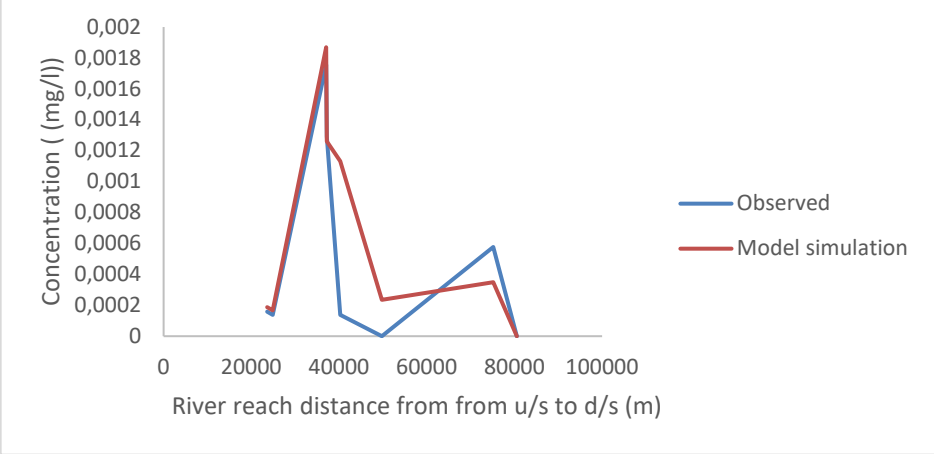


Figure 8.1 Model simulation of tetracycline antibiotics concentration in the water column of the Msunduzi River from upstream (HD) to Downstream (TB).

From Figure 8.1, the developed model successfully captures the spatial distribution of Tetracycline, presenting low concentrations at the catchment heads and higher concentrations at the lower part of the river. Markedly, concentrations increased at the wastewater treatment plants (WWTP) discharge point. However, the downstream section showed lower concentrations. It is essential to acknowledge a discrepancy between the model simulation and the observed curve after the Darville WWTP discharge, potentially arising from the rapid attenuation of tetracycline in observed concentrations due to dilution from the higher river flow rate. The correlation coefficient (R^2) between observed and simulated concentrations is 0.87, indicating a substantial reproduction of the observed spatial pattern of tetracycline in the water column by the model.

The absence of temporal data on antibiotics and organic matters in the studied river has limited the exploration of correlations between antibiotics and other variables. Future studies should investigate the correlation between antibiotics, organic matter, and total suspended solids to understand their interrelationships and dynamics over time.

Table 8-3 Reaction constants and calibration parameters.

Parameter	Term	Unit	Constants	Sources
$\mathbf{K_{photo}}$	Antibiotics photodegradation rate	Day^{-1}	0.063	(Yun et al., 2018)
$\mathbf{K_{bio}}$	Antibiotics biodegradation rate	Day^{-1}	0.0031	
$\mathbf{K_{hy}}$	Antibiotics hydrolysis rate	Day^{-1}	(0.0059) 0.1488(±0.0072)	(Zhong et al., 2022)
k_{dom}	Antibiotics partitioning coefficient with DOM	$L\ kg^{-1}$	199.526	
k_{solid}	Antibiotics partitioning coefficient with TSS	$L\ kg^{-1}$	15848.932	
Kd	Sorption distribution coefficients	$Kg\ L^{-1}$	1147-2370 (silt, clay, sandy loam)	(Sarmah et al., 2006)
K_d	The diffusion constant	m/h	0.000208	
K_r	The resuspension constant	1/h	0.00000239	
Z	constant for hydrolysis (for the suspended solid)		0.0001	(EPA, 1978)
K_{Pm}	Degradation constant for pom production from DOM photolysis-induced transformation	1/hr	0.00471	
K_p^*	Rate constant for dissolved inorganic production from DOM	1/hr	0.00021	
K_{hw}	The rate constant for hydrolysis of POM in the water	1/h	0.00208/h	(Mackay et al., 2006)
K_{bw}	Rate constnat for biotransfromation of POM in the water	1/h		
K_{hs}	The rate constant for the hydrolysis of POM in the sediment	1/h	0.000641	
K_{bs}	Rate constnat for biotransfromation of POM in the sediment	1/h	0.000512	
γ	The ratio of water depth to the depth of the underlying movable sediment layer (h/ψ)			
Mx	Metabolism	1/min	0.00019	
∅	Porosity		0.4	

CHAPTER 9: USER INTERFACE FOR DEVELOPED MODEL

9.1 INTRODUCTION

The mathematical model has been translated into an intuitive user interface tool (Figure 9.1) designed for effortless application. Developed using the Python programming language for both the front and back end, this interface streamlines the modelling process. A user-friendly layout incorporates slots for inputting various parameters essential for driving the backend simulations, with the results presented both graphically and in CSV format (Figure 9.2).

The interface includes a designated slot for entering numerical values representing simulated concentrations, ensuring precision in result interpretation. Simultaneously, the graphical plot visually represents antibiotic variations over time and space along the river stretch. The graphical plot can be conveniently saved and printed for further analysis or documentation. Upon inputting the required parameters, a simple click on the compute button initiates the calculation process, swiftly generating the desired results. This user interface enhances accessibility and facilitates efficient utilization of the mathematical model, catering to a broad spectrum of users.

9.1.1 Tool Operation

The tool features a comprehensive menu structure, including main menus and submenus. Users can seamlessly open, edit, and save text input files within the File menu, enhancing flexibility in managing data. The Help menu provides valuable instructions on the tool's utilization and includes informative content about the developed tool.

A welcome submenu is included for user guidance, offering an operation guideline to ensure a smooth user experience. This well-organized menu system enhances the user interface, providing clear pathways for file management and offering support resources for effective tool navigation. The model's interfaces are visually presented in Figures 9.1 through 9.4, offering a comprehensive view of the diverse facets of the tool's functionality and design. These figures serve as illustrative guides, clearly depicting the interfaces and highlighting the user-friendly features integrated into the model.

Figure 9.1 Front face of Antibiotic Modelling tool

Figure 9.2 Data Input Interface

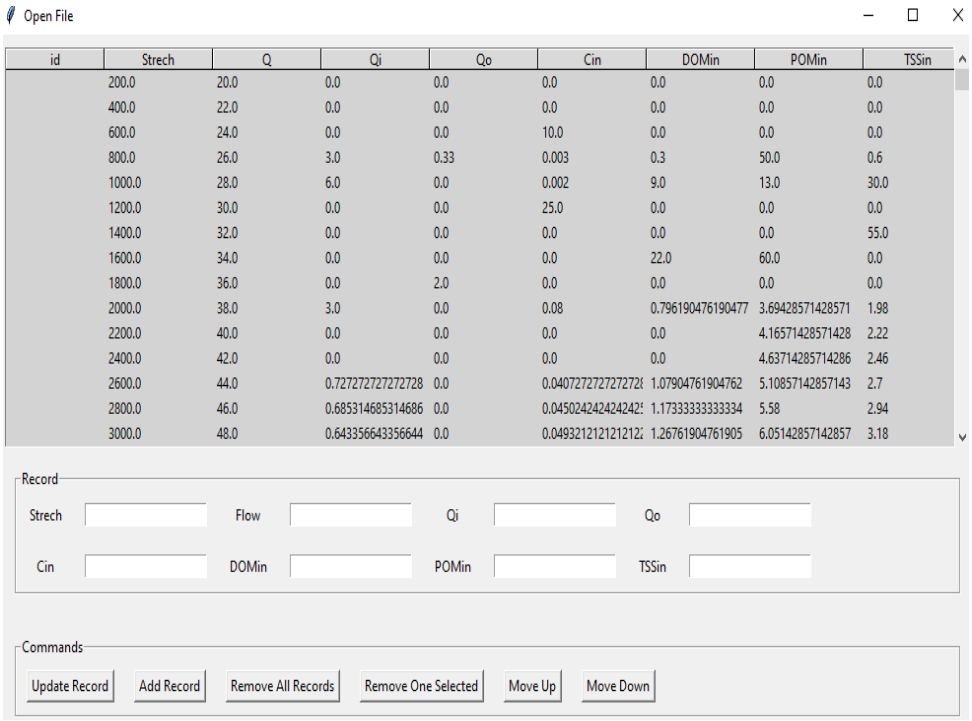


Figure 9.3 Back-End Database Interface

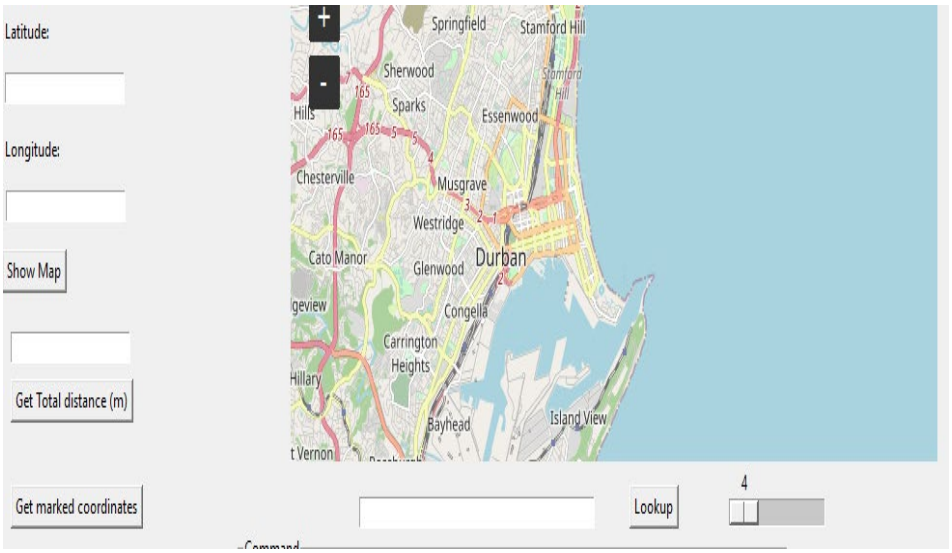


Figure 9.4 GIS integration for Tracing river routes

9.2 SUMMARY

The developed model is a process-based tool requiring minimal data input while maintaining operational simplicity and user-friendliness. Its design prioritizes accessibility without compromising the integrity of its outputs. This tool is essential for water quality managers and all stakeholders involved in providing communal water access. The user-friendly nature of the tool ensures its practical utility in water quality management, emphasizing the need for proactive measures in addressing the presence of antibiotics in our vital river systems.

CHAPTER 10: CONCLUSIONS & RECOMMENDATIONS

10.1 CONCLUSIONS

This study identified antibiotic residues and antimicrobial resistance genes in the Msunduzi River, in wastewater treatment plants and their effluents. The research project has successfully developed a robust and user-friendly mathematical model specifically designed to assess water quality variations in the Msunduzi River, with a particular emphasis on tracking antibiotics as a concerning emerging contaminant. This model represents a significant advancement in water quality management tools, offering an effective solution for understanding the dynamics of antibiotics within river systems. A noteworthy aspect of this model is its reliance on a process-based approach, significantly reducing the amount of data needed for its operation. This ensures the model's practicality in regions where data availability is often limited, a common scenario in many developing countries, including South Africa. Developed using Python, the model boasts an intuitive and straightforward user interface. This interface facilitates the input of parameters and presents results in numerical and graphical formats. The graphical representations of antibiotic variations over time and space along the river reach provide valuable insights for water quality managers, aiding their understanding of contamination patterns and enabling informed decision-making.

The developed model is crucial in managing water quality in the Msunduzi River. Its predictive capabilities regarding antibiotic behaviour empower decision-makers to implement preventive measures to curb antibiotic contamination in water systems. Given the increasing threat posed by antibiotics in river systems, there is an urgent need for proactive and efficient water quality management tools. By targeting antibiotic contamination, this model contributes to environmental sustainability and the preservation of public health.

10.2 LIMITATIONS OF STUDY

This project focused on tracing, monitoring, and modelling emerging contaminants in the Msunduzi River, a vital water resource in the Midlands of KwaZulu-Natal. The river originates from the uMgeni River at Coordinates 29°37′14″S 30°40′36″E and flows through Pietermaritzburg, providing water for domestic, agricultural, and industrial purposes in the Msunduzi Municipality. In response to a prior study revealing elevated concentrations of pharmaceutical residues in KwaZulu-Natal Rivers, the project specifically targeted the four prevalent antibiotics to develop an effective monitoring tool. Standard water quality testing and analysis methods were employed to examine samples collected from various locations along the river. Additional samples from a wastewater treatment plant were analysed to assess the spatial distribution of antibiotic residues within the plant and their contribution to source water. Genomic studies were conducted to evaluate antimicrobial-resistant genes in the water system. However, the project faced challenges, including COVID-related restrictions and delays caused by floods and riots in KwaZulu-Natal. The extraction of antibiotic residues and Genomic studies proved time-consuming and very expensive, impacting the depth and number of analyses required; therefore, the planned metagenomic sequencing could not be completed as intended. Also, obtaining approval for sample collection from the wastewater treatment plant added further delays, limiting sampling and testing.

While the developed model achieved its intended objectives, and the current focus on simulating water quality along the river reach provides valuable insights, it is essential to recognise that its applicability may be limited in scenarios requiring broader catchment-scale analysis. Although Geographic information system (GIS) was used in generating the land use map, delineating spatio-temporal

variations of water quality parameters and water quality mapping along the Msunduzi River in this project, future research could explore integrating GIS in the model at a two-dimensional catchment scale to understand variations within catchments better.

10.3 RECOMMENDATIONS

This project has made significant strides in understanding the dynamics of water quality variations in the Msunduzi River, particularly concerning antibiotics; there are, however, avenues for further studies that could enhance the depth of knowledge in this area. Further recommendations for future research include:

Model Validation: Further validation of the model using additional data sets and under different environmental conditions would enhance its reliability. Continuous refinement and calibration of the model based on new data will contribute to its accuracy in predicting water quality variations.

Efficiency of Wastewater Treatment Technologies: Investigating the efficiency of different wastewater treatment technologies in mitigating antibiotic residues before discharge into water bodies could provide insights into sustainable treatment practices. Comparing the performance of various treatment methods can guide improvements in wastewater treatment infrastructure.

Multi Temporal Studies: Conducting long-term temporal studies to capture seasonal variations and trends in antibiotic concentrations could provide a more comprehensive understanding of the dynamic nature of emerging contaminants. Monitoring the river over an extended period will contribute valuable data on fluctuations and potential factors influencing antibiotic levels.

Genomic Analysis: Expanding the investigation to include a more in-depth genomic analysis of microbial communities in the river could offer insights into the genetic basis of antibiotic resistance. This could involve studying the metagenomic profile of bacterial communities to identify specific resistance genes and their prevalence.

Additional Emerging Contaminants: Broadening the focus to include other emerging contaminants beyond antibiotics, such as Antiretrovirals, personal care products, and endocrine-disrupting compounds, would provide a holistic view of water quality. Understanding multiple contaminants' interactions and combined effects is essential for comprehensive water management.

Human Health Impact Assessment: Incorporating a human health impact assessment to evaluate the potential risks posed by antibiotic residues in the water to the surrounding communities would be beneficial. This could involve assessing exposure pathways' potential health outcomes and establishing guidelines for safe water consumption.

Community Engagement and Education: Integrating community engagement and education initiatives into future studies can foster a better understanding of water quality issues among the local population. This may include awareness campaigns, training programs, and collaborative efforts to promote responsible water use.

Policy and Regulatory Assessment: Assessing the existing policies and regulations related to water quality, particularly concerning emerging contaminants and

- Establishing benchmarking thresholds for antibiotics and other emerging contaminants in water bodies based on scientific evidence and risk assessment.
- Enhancing monitoring programs to detect the presence of emerging contaminants more effectively.
- Strengthening regulations to control the release of antibiotics from various sources, such as pharmaceutical manufacturing, agriculture, and healthcare facilities.

- Implementing pollution prevention measures and promoting sustainable practices to reduce the introduction of antibiotics into the environment.
- Improving public awareness and education on the impacts of emerging contaminants on water quality and the importance of proper disposal and use of pharmaceuticals.

By addressing these recommendations, future studies can contribute to the ongoing efforts to manage emerging contaminants in water bodies, ensuring sustainable and safe water resources for both human and environmental well-being.

REFERENCES

ABATIH EN, ALBAN L, ERSBØLL AK, LO FO and WONG DM. (2009) Impact of antimicrobial usage on the transmission dynamics of antimicrobial resistant bacteria among pigs. Journal of theoretical biology **256** 561-73.

ABDELMALIK KW (2016) Role of statistical remote sensing for Inland water quality parameters prediction. The Egyptian Journal of Remote Sensing and Space Science **21 (2)** 193-200.

ABED BS, DAHAM MH and ISMAIL AH (2021) Water quality modelling and management of Diyala river and its impact on Tigris River. Journal of Engineering Science and Technology **16 (1)** 122-135.

ACHARYA TD and YANG I (2015) Exploring Landsat 8. International Journal of IT, Engineering and Applied Sciences Research **4 (4**) 4-10.

ACHARYA TD, LEE DH, YANG IT and LEE JK (2016) Identification of water bodies in a Landsat 8 OLI image using a J48 decision tree. Sensors **16 (7**) 1075.

ADU JT (2021) Analytical solution of hyporheic exchange in natural rivers with non-point source pollution loads. Journal of Hydrology **593** 125860.

ADU JT and KUMARASAMY MV (2020a) Development of non-point source hybrid cells in series model for reactive pollutant transport in natural rivers. Polish Journal of Environmental Studies **29 (5)** 3031-3039.

ADU JT and KUMARASAMY MV (2020b) Mathematical model development for non-point source in-stream pollutant transport. Archives of Environmental Protection **46 (2)** 91-99.

AGUNBIADE FO and MOODLEY B (2014) Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa. Environmental Monitoring and Assessment **186 (11)** 7273-7291.

AGUNBIADE FO and MOODLEY B (2016) Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, KwaZulu-Natal, South Africa. Environmental toxicology and chemistry **35 (1)** 36-46.

AHMAD A, ZACHARIASEN C, CHRISTIANSEN LE, GRÆSBØLL K, TOFT N, MATTHEWS L, NIELSEN SS and OLSEN JE (2016) Modeling the growth dynamics of multiple Escherichia coli strains in the pig intestine following intramuscular ampicillin treatment. BMC Microbiology **16** 205.

AHMED MB, ZHOU JL, NGO HH and GUO W (2015) Adsorptive removal of antibiotics from water and wastewater: progress and challenges. Science of the Total Environment **532** 112-126.

ALABI ME and ESSACK SY (2022) Antibiotic prescribing amongst South African general practitioners in private practice: an analysis of a health insurance database. JAC-Antimicrobial Resistance ***4*** **(5)**.

ALDEKOA J, MARCÉ R and FRANCÉS F (2016) Fate and Degradation of Emerging Contaminants in Rivers: Review of Existing Models. Emerging Contaminants in River Ecosystems: Occurrence and Effects Under Multiple Stress Conditions 159-193.

ALLAM M, YAWAR ALI KHAN M and MENG Q (2020) Retrieval of Turbidity on a Spatio-Temporal Scale Using Landsat 8 SR: A Case Study of the Ramganga River in the Ganges Basin, India. Applied Sciences **10 (11)** 3702.

ALLEN, HK, DONATO J, WANG HH, CLOUD-HANSEN KA, DAVIES JE and HANDELSMAN J (2010) Call of the wild: Antibiotic resistance genes in natural environments. Nature Reviews. Microbiology, **8** 251-259.

ALPARSLAN E, AYDÖNER C, TUFEKCI V and TÜFEKCI H (2007) Water quality assessment at Ömerli Dam using remote sensing techniques. Environmental Monitoring and Assessment **135 (1)** 391-398.

APALATA T, BAMFORD C, BENJAMIN D, BOTHA M, BRINK A, CROWTHER-GIBSON P, DEVENISH L, DU PLESSIS M, DUSE AG, EAGER H and ESSACK SY (2011) Global Antibiotic Resistance Partnership—Situation Analysis: Antibiotic use and resistance in South Africa. South African Medical Journal **101** 549-596.

ARCHANA G, DHODAPKAR R and KUMAR A (2016) Offline solid-phase extraction for preconcentration of pharmaceuticals and personal care products in environmental water and their simultaneous determination using the reversed phase high-performance liquid chromatography method. Environmental Monitoring and Assessment **188 (9)** 1-10.

ARLOS MJ, BRAGG LM, SERVOS MR and PARKER WJ (2014) Simulation of the fate of selected pharmaceuticals and personal care products in a highly impacted reach of a Canadian watershed. Science of the Total Environment **485** 193-204.

ARNOT JA, MACKAY D, PARKERTON T F, ZALESKI RT and WARREN CS (2010) Multimedia modeling of human exposure to chemical substances: the roles of food web biomagnification and biotransformation. Environmental Toxicology and Chemistry ***29*****(1)** 45-55.

AUSTIN T, BREGOLI F, HÖHNE D, HENDRIKS AJ and RAGAS AM (2022). Ibuprofen exposure in Europe; ePiE as an alternative to costly environmental monitoring. Environmental Research **209** 112777.

AYDIN E and TALINLI I (2013) Analysis, occurrence and fate of commonly used pharmaceuticals and hormones in the Buyukcekmece Watershed, Turkey Chemosphere **90 (6)** 2004-2012.

BABIĆ S, AŠPERGER D, MUTAVDŽIĆ D, HORVAT AJM and KAŠTELAN-MACAN M (2006) Solid phase extraction and HPLC determination of veterinary pharmaceuticals in wastewater. Talanta **70 (4)** 732-738.

BAI J, ZHAO J, ZHANG Z and TIAN Z (2022) Assessment and a review of research on surface water quality modeling. Ecological Modelling **466** 109888.

BAI Y, ZHOU Y, CHE X, LI C, CUI Z, SU R and QU K (2021) Indirect photodegradation of sulfadiazine in the presence of DOM: Effects of DOM components and main seawater constituents. Environmental Pollution **268**.

BAKER M, HOBMAN JL, DODD CER, RAMSDEN SJ and STEKEL DJ (2016) Mathematical modelling of antimicrobial resistance in agricultural waste highlights importance of gene transfer rate FEMS Microbiology. Ecology **92** 1-10.

BANDE P, ADAM E, ELBASIT MAA and ADELABU S (2018) Comparing Landsat 8 and sentinel-2 in mapping water quality at Vaal dam. In: *Proceedings of the International Geoscience and Remote Sensing Symposium,* July 2018*,* 22-27. Valencia, Spain.

BANKS RB (1974) A mixing cell model for longitudinal dispersion in open channels. Water Resources Research **10** 357-358.

BARCELÓ D and PETROVIC M (2007) Pharmaceuticals and Personal Care Products EDCs in the Environment. Analytical and Bioanalytical Chemistry **387** 1141-1142.

BEAR J (1972) *Dynamics of Fluids in porous media*, American Elsevier Publishing Company, Inc., New York, 764.

BECK MB (1987) Water quality modeling: a review of the analysis of uncertainty. Water resources research **23 (8)** 1393-1442.

BEER T and YOUNG PC (1983) Longitudinal dispersion in natural streams. Journal of environmental engineering. Div., ASCE **109 (5)** 1049-1067.

BELL KY, BANDY J, FINNEGAN BJ, KEEN O, MAUTER MS, PARKER AM, SIMA LC and STRETZ HA (2013) Emerging Pollutants – Part II: Treatment. Water Environment Research **85** 2022-2071.

BELLOTINDOS LM, CHANG AT and LU MC (2015) Degradation of acetaminophen by different Fenton processes. Desalination and Water Treatment **56** 1372-1378.

BELTAOS, S (1980) Longitudinal dispersion in rivers. Journal of Hydrology. Div., ASCE **106 (1)** 151-171.

BEN Y, FU C, HU M, LIU L, WONG MH and ZHENG C (2019) Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environmental research **169** 483-493.

BENCALA KE and WALTERS RA (1983) Simulation of solute transport in a mountain pool-and-riffle stream with a kinetic mass transfer model for sorption, Water Resources Research **19 (3)** 732-738.

BENCALA KE, MCKNIGHT DM and ZELLWEGER GW (1990) Characterization of transport in an acidic and metal-rich mountain stream based on a lithium tracer injection and simulations of transient storage, Water Resources Research **26 (5)** 989-1000.

BENEDICT KB, MCFALL AS and ANASTASIO C (2017) Quantum Yield of Nitrite from the Photolysis of Aqueous Nitrate above 300 nm. Environmental Science and Technology **51 (8)** 4387-4395.

BENEDINI M and TSAKIRIS G (2013) *Water quality modelling for rivers and streams*. Springer Science & Business Media.

BENOTTI MJ, TRENHOLM RA, VANDERFORD BJ, HOLADY JC, STANFORD BD and SNYDER SA (2009) Pharmaceuticals and endocrine disrupting compounds in US drinking water. Environmental Science and technology **43 (3)** 597-603.

BERDING V and MATTHIES M (2002) European scenarios for EUSES regional distribution model. Environmental Science and Pollution Research **9** 193-198.

BERDING V, SCHWARTZ S and MATTHIES M (1999) Visualisation of the Complexity of EUSES. Environmental Science and Pollution Research **6** 37-43.

BERHANE TM, LANE CR, WU Q, AUTREY BC, ANENKHONOV OA, CHEPINOGA VV and LIU H (2018) Decision-tree, rule-based, and random forest classification of high-resolution multispectral imagery for wetland mapping and inventory. Remote sensing **10 (4)** 580.

BEVEN KJ and YOUNG PC (1988) An aggregated mixing zone model of solute transport through porous media. Journal of contaminant hydrology **3 (2-4)** 129-143.

BHAGUNDE PR, NIKOLAOU M and TAM VH (2015) Modeling heterogeneous bacterial populations exposed to antibiotics: the logistic-dynamics case. AICHE Journal **61** 2385-93.

BIRKEGARD AC, HALASA T, TOFT N, FOLKESSON A and GRAESBOLL K (2018) Send more data: a systematic review of mathematical models of antimicrobial resistance. Antimicrobial Resistance & Infection Control. **2018 7** 117.

BÖCKELMANN U, DÖRRIES HH, AYUSO-GABELLA MN, DE MARÇAY MS, TANDOI V, LEVANTESI C, MASCIOPINTO C, VAN HOUTTE E, SZEWZYK U, WINTGENS T and GROHMANN E (2009) Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Applied and environmental microbiology **75 (1)** 154-163.

BORAH DK and BERA M (2003) Watershed-scale hydrologic and nonpoint-source pollution models: Review of mathematical bases. Transactions of the ASAE **46 (6)** 1553.

BORAH DK and BERA M (2004) Watershed-scale hydrologic and nonpoint-source pollution models: Review of applications. Transactions of the ASAE **47 (3)** 789.

BOUKI C, VENIERI D and DIAMADOPOULOS E (2013) Review: Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. Ecotoxicology and Environmental Safety **91** 1–9.

BRANDO VE and DEKKER AG (2003) Satellite hyperspectral remote sensing for estimating estuarine and coastal water quality. IEEE transactions on geoscience and remote sensing **41 (6)** 1378-1387.

BROWN KD, KULIS J, THOMSON B, CHAPMAN TH and MAWHINNEY DB (2006) Occurrence of antibiotics in hospital, residential, and dairy effluent, municipal wastewater, and the Rio Grande in New Mexico. Science of the Total Environment **366 (2-3)** 772-783

BRUCE GM, PLEUS RC and SNYDER SA (2010) Toxicological relevance of pharmaceuticals in drinking water. Environmental science and technology, **44 (14)** 5619-5626.

BURGMANN H, FRIGON D, GAZE W, MANAIA CM, PRUDEN A, SINGER AC, SMETS BF and ZHANG T (2018) Water and sanitation: An essential battlefront in the war on antimicrobial resistance. FEMS Microbiology **94 (9)** 1–14.

CALDERON-PRECIADO D, JIMENEZ-CARTAGENA C, MATAMOROS V and BAYONA JM (2011) Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. Water Research **45 (1)** 221-231.

CAMERON DR and KLUTE A (1977) Convective dispersive solute transport with combined equilibrium and kinetic adsorption model. Water Resources Research **13 (1)** 183-188.

CAMPBELL JB and WYNNE RH (2011) Introduction to remote sensing. 5th ed., Guilford Press. New York, USA.

CAPDEVIELLE M, VAN EGMOND R, WHELAN M, VERSTEEG D, HOFMANN-KAMENSKY M, INAUEN J, CUNNINGHAM V and WOLTERING D (2008) Consideration of exposure and species sensitivity of triclosan in the freshwater environment. Integrated environmental assessment and management **4 (1)** pp.15-23.

CENTER FOR DISEASE CONTROL AND PREVENTION (2019) AR threats report. Biggest Threats and Data.

CHANG PH, JIANG WT, LI Z, JEAN JS and KUO CY (2015) Antibiotic tetracycline in the environments – A review. Research and Reviews: Journal of Pharmaceutical Analysis **4 (3)** 86-111.

CHATWIN PC (1980) Presentation of longitudinal dispersion data. Journal of the Hydraulics Division **106 (1)** 71-83.

CHATWIN PC and ALLEN CM (1985) Mathematical models of dispersion in rivers and estuaries. Annual Review of Fluid Mech **17 (1)** 119-149.

CHEN H, JING L, TENG Y and WANG, J. (2018) Multimedia fate modeling and risk assessment of antibiotics in a water-scarce megacity. Journal of hazardous materials **348** 75-83.

CHEN J, ZHU W, TIAN YQ, YU Q, ZHENG Y and HUANG L (2017) Remote estimation of colored dissolved organic matter and chlorophyll-a in Lake Huron using Sentinel-2 measurements. Journal of Applied Remote Sensing **11 (3)** 036007.

CHEN Y, HU C, DENG D, LI Y and LUO L (2020) Factors affecting sorption behaviors of tetracycline to soils: Importance of soil organic carbon, pH and Cd contamination. Ecotoxicology and Environmental Safety **197**.

CHEN Z, CHEN S, WEN J, CHEN J, HE X and ZHU Y (2020) Occurrence and distribution of antibiotics in the surface sediments of Poyang Lake, the largest freshwater lake in China. Environmental Science and Pollution Research **27 (12)** 13656-13666.

CHENG T and SAIERS JE (2015) Effects of dissolved organic matter on the co-transport of mineral colloids and sorptive contaminants. Journal of Contaminant Hydrology **177** 148-157.

CHOLLOM M, RATHILAL S, SWALAHA F and BAKARE B (2018) Fate, transport, and toxicity of veterinary antimicrobials with an insight on Africa: A review. Ecology, Environment and Conservation **24** 1201-1220.

CHUNHUI Z, LIANGLIANG W, XIANGYU G and XUDAN H (2016) Antibiotics in WWTP discharge into the Chaobai River, Beijing. Archives of Environmental Protection **42** 48-57.

CONTE D, PALMEIRO JK, DA SILVA NOGUEIRA K, DE LIMA TMR, CARDOSO MA, PONTAROLO R, PONTES FLD and DALLA-COSTA LM (2017) Characterization of CTX-M enzymes, quinolone resistance determinants, and antimicrobial residues from hospital sewage, wastewater treatment plant, and river water. Ecotoxicology and environmental safety **136** 62-69.

COONEY CM (2009) Study detects trace levels of pharmaceuticals in US drinking water. Environmental Science and Technology **43** 551.

CRUZEIRO C, ROCHA E, PARDAL MÂ and ROCHA MJ (2016) Seasonal-spatial survey of pesticides in the most significant estuary of the Iberian Peninsula-the Tagus River estuary. Journal of Cleaner Production **126** 419-427.

CZERNUSZENKO W and ROWINSKI PM (1997) Properties of the dead zone model of longitudinal dispersion in rivers. Journal of Hydraulic Research **35 (4)** 491-504.

DA SILVA AK, AMADOR J, CHERCHI C, MILLER SM, MORSE AN, PELLEGRIN ML and WELLS MJM (2013) Emerging Pollutants – Part I: Occurrence, Fate and Transport. Water Environment Research **85** 1978-2021.

DAHANE S, GIL GARCIA MD, MARTINEZ BUENO MJ, UCLES MORENO A, MARTINEZ GALERA M and DERDOUR A (2013) Determination of drugs in river and wastewaters using solid-phase extraction by packed multi-walled carbon nanotubes and liquid chromatography-quadrupole-linear ion trap-mass spectrometry. Journal of Chromatography. *A* **1297** 17-28.

DANNER MC, ROBERTSON A, BEHRENDS V and REISS J (2019) Antibiotic pollution in surface fresh waters: occurrence and effects. Science of the Total Environment, **664** 793-804.

DAVIES J and DAVIES D (2010) Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews **74** 417-433.

DAY TJ and WOOD IR (1976) Similarity of the mean motion of fluid particles dispersing in natural channels. Water Resources Research **12 (4)** 655-666.

DE SMEDT F, BREVIS W and DEBELS P (2005) Analytical solution for solute transport resulting from instantaneous injection in streams with transient storage Journal of hydrology (Amsterdam) **315 (1-4)** 25-39.

DEBLONDE T, COSSU-LEGUILLE C AND HARTEMANN P (2011) Emerging pollutants in wastewater: a review of the literature. International journal of hygiene and environmental health **214 (6)** 442-448.

DEMETRACOPOULOS AC (1981) Cells in series simulation of riverine transport Journal of the Hydraulics Division **107 (6)** 675-697.

DÍAZ-CRUZ MS and BARCELÓ D (2006) Determination of antimicrobial residues and metabolites in the aquatic environment by liquid chromatography tandem mass spectrometry. Analytical and Bioanalytical Chemistry, **386 (4)** 973-985.

DUNCA AM (2018) Water pollution and water quality assessment of major transboundary rivers from Banat (Romania). Journal of Chemistry **2018**.

ECDPC. (2018) 33000 people die every year due to infections with antibiotic-resistant bacteria. Available from: https://www.ecdc.europa.eu/en/news-events/33000-people-die-every-year-due-infections-antibiotic-resistant-bacteria.

EPA (1978) *Rates, Constants, and a Kinetics Formulations in Surface Water Quality Modeling* (p. 338).

EPA. (1978). *Rates, Constants, and a Kinetics Formulations in Surface Water Quality Modeling* (p. 338).

ESSACK SY (2006) Strategies for the Prevention and Containment of Antibiotic Resistance. South African family practice **48 (1)** 17-17e.

ESSACK SY, CONNOLLY C and STURM AW (2005) Antibiotic use and resistance in public-sector hospitals in KwaZulu-Natal. South African Medical Journal, **95 (11)** 865-870.

ESSACK SY, SCHELLACK N, POPLE T, VAN DER MERWE L, SULEMAN F, MEYER JC and BENJAMIN D (2011) Part III. Antibiotic supply chain and management in human health. SAMJ: South African Medical Journal, **101 (8)** 562-566.

FAIRBAIRN DJ, KARPUZCU ME, ARNOLD WA, BARBER BL, KAUFENBERG EF, KOSKINEN WC, NOVAK PJ, RICE PJ and SWACKHAMER DL (2016) Sources and transport of contaminants of emerging concern: A two-year study of occurrence and spatiotemporal variation in a mixed land use watershed. Science of the Total Environment **551** 605-613.

FALEYE AC, ADEGOKE AA, RAMLUCKAN K, BUX F and STENSTRÖM TA (2018) Antibiotic Residue in the Aquatic Environment: Status in Africa. Open Chemistry **16** 890-903.

FALEYE AC, ADEGOKE AA, RAMLUCKAN K, FICK J, BUX F and STENSTRÖM TA (2019) Concentration and reduction of antibiotic residues in selected wastewater treatment plants and receiving waterbodies in Durban, South Africa. Science of The Total Environment **678** 10-20.

FATAEI E (2011) Assessment of surface water quality using Principle Component Analysis and Factor Analysis. World Journal of Fish and Marine Sciences **3 (2)** 159-166.

FEKADU S, ALEMAYEHU E, DEWIL R and VAN DER BRUGGEN B (2019) Pharmaceuticals in freshwater aquatic environments: A comparison of the African and European challenge. Science of the Total Environment, **654** 324-337.

FENT K, WESTON AA and CAMINADA D (2006) Ecotoxicology of human pharmaceuticals. Aquatic toxicology **76 (2)** 122-159.

FERGUSON PJ, BERNOT MJ, DOLL JC and LAUER TE (2013) Detection of pharmaceuticals and personal care products (PPCPs) in near-shore habitats of southern Lake Michigan. Science of the Total Environment **458** 187-196.

FERNÁNDEZ-CALVIÑO D, BERMÚDEZ-COUSO A, ARIAS-ESTÉVEZ M, NÓVOA-MUÑOZ JC, FERNÁNDEZ-SANJURJO MJ, ÁLVAREZ-RODRÍGUEZ E and NÚÑEZ-DELGADO A (2014) Kinetics of tetracycline, oxytetracycline, and chlortetracycline adsorption and desorption on two acid soils. Environmental Science and Pollution Research **22 (1)** 425-433.

FERNÁNDEZ-CALVIÑO D, BERMÚDEZ-COUSO A, ARIAS-ESTÉVEZ M, NÓVOA-MUÑOZ JC, FERNÁNDEZ-SANJURJO MJ, ÁLVAREZ-RODRÍGUEZ E and NÚÑEZ-DELGADO A (2015) Competitive adsorption/desorption of tetracycline, oxytetracycline and chlortetracycline on two acid soils: Stirred flow chamber experiments. Chemosphere **134** 361-366.

FERNÁNDEZ-CALVIÑO D, RODRÍGUEZ-GIL JL, CID L and ARIAS-ESTÉVEZ, M (2014) The environmental fate of antibiotics in water and sediments. Analytical and Bioanalytical Chemistry **406 (22)** 5787-5798.

FERNÁNDEZ-CALVIÑO D, RODRÍGUEZ-GIL JL, SIMAL-GÁNDARA J and ARIAS-ESTÉVEZ M (2015) Impact of Veterinary Antibiotics on the Environment: Source, Fate and Effects. In F. T. de Oliveira & M. Simeonov (Eds.), Veterinary Infection Biology: Molecular Diagnostics and High-Throughput Strategies (pp. 397-422). Springer International Publishing.

FERRER DL and DELEO PC (2017) Development of an in-stream environmental exposure model for assessing down-the-drain chemicals in Southern Ontario. Water Quality Research Journal **52 (4)** 258-269.

FICK J, LINDBERG RH, FÅNG J, MAGNÉR J, KAJ L and BRORSTRÖM-LUNDÉN E (2015) *Screening 2014: Analysis of pharmaceuticals and hormones in samples from WWTPs and receiving waters*. IVL-report; C 135, Swedish Environmental Research Institute, Stockholm, Sweden.

FIGUEIRA V, VAZ-MOREIRA I, SILVA M and MANAIA CM (2011) Diversity and antibiotic resistance of Aeromonas spp. in drinking and waste water treatment plants. Water research **45 (17)** 5599-5611.

FISCHER HB (1967) The mechanics of dispersion in natural streams. Journal of the Hydraulics division **93 (6)** 187-216.

FISCHER HB (1968) Dispersion predictions in natural streams. Journal of the Sanitary Engineering Division **94** 927-943.

FISCHER HB, IMBERGER J, LIST EJ, KOH RCY and BROOKS NH (1979) *Mixing in inland and coastal waters*, Academic, New York.

FONT C, BREGOLI F, ACUÑA V, SABATER S and MARCÉ R (2019) GLOBAL-FATE (version 1.0. 0): A geographical information system (GIS)-based model for assessing contaminants fate in the global river network. *Geoscientific Model Development* **12(12)** 5213-5228.

FOUHSE JM, YANG K, MORE BAYONA J, GAO Y, GORUK S, PLASTOW G, FIELD C, BARREDA DR and Willing BP (2019) Neonatal exposure to amoxicillin alters long-term immune response despite transient effects on gut-microbiota in piglets. Frontiers in immunology **10** 2059.

GARCIA-GALAN MJ, SILVIA DIAZ-CRUZ M and BARCELO D (2008) Identification and determination of metabolites and degradation products of sulfonamide antibiotics. Trac-Trends in Analytical Chemistry, **27** 1008-1022.

GAVRILESCU M, DEMNEROVA K, AAMAND J, AGATHOS S and FAVA F (2015) Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. New Biotechnology **32 (1)** 147-156.

GEHRING R, SCHUMM P, YOUSSEF M, SCOGLIO C (2010) A network-based approach for resistance transmission in bacterial populations Journal of theoretical biology **262** 97-106.

GHOLIZADEH MH, MELESSE AM AND REDDI L (2016) A comprehensive review on water quality parameters estimation using remote sensing techniques. Sensors, **16 (8)** 1298.

GHOSH NC (2001) Study of solute transport in a river. Roorkee, India: Dept. of Civil Eng., IIT, PhD Thesis.

GHOSH NC, MISHRA GC and KUMARASAMY MV (2008) Hybrid-cells-in-series model for solute transport in streams and relation of its parameters with bulk flow characteristics, Journal of Hydraulic Engineering, **134 (4)** 497-503.

GHOSH NC, MISHRA GC and OJHA CSP (2004) Hybrid-cells-in-series Model for Solute Transport in a River, Journal of Environmental Engineering, **130** 1198.

GIBS J, HECKATHORN HA, MEYER MT, KLAPINSKI FR, ALEBUS M, and LIPPINCOTT RL (2013) Occurrence and partitioning of antibiotic compounds found in the water column and bottom sediments from a stream receiving two wastewater treatment plant effluents in Northern New Jersey, 2008. Science of the total environment **458** 107-116.

GILMORE S, SALEEM A and DEWAN A (2015) Effectiveness of DOS (Dark-Object Subtraction) method and water index techniques to map wetlands in a rapidly urbanising megacity with Landsat 8 data. Research@ Locate'15, 100-108.

GITELSON AA, GAO BC, LI RR, BERDNIKOV S and SAPRYGIN V (2011) Estimation of chlorophyll-a concentration in productive turbid waters using a Hyperspectral Imager for the Coastal Ocean—the Azov Sea case study. Environmental Research Letters **6 (2)** 024023.

GORELICK N, HANCHER M, DIXON M, ILYUSHCHENKO S, THAU D and MOORE R (2017) Google Earth Engine: Planetary-scale geospatial analysis for everyone. Remote sensing of Environment **202** 18-27.

GOTHWAL R and SHASHIDHAR T (2015) Antibiotic pollution in the environment: a review. Clean-Soil, Air, Water **43** 479-489.

GOTHWAL R and THATIKONDA S (2020) Modeling transport of antibiotic resistant bacteria in aquatic environment using stochastic differential equations. Scientific Reports **10 (1)** 15081

GRACIA-LOR E, SANCHO JV, SERRANO R and HERNANDEZ F (2012) Occurrence and removal of pharmaceuticals in wastewater treatment plants at the Spanish Mediterranean area of Valencia. Chemosphere **87 (5)** 453-462.

GROS M, RODRIGUEZ-MOZAZ S and BARCELO D (2012) Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. Journal of Chromatography A **1248** 104-121.

GUMBI BP, MOODLEY B, BIRUNGI G and NDUNGU PG (2017) Detection and quantification of acidic drug residues in South African surface water using gas chromatography-mass spectrometry. Chemosphere **168** 1042-1050.

GUO H, HUANG JJ, CHEN B, GUO X and SINGH VP (2021) A machine learning-based strategy for estimating non-optically active water quality parameters using Sentinel-2 imagery. International Journal of Remote Sensing **42 (5)** 1841-1866.

HADJIMITSIS DG and CLAYTON C (2011) Field spectroscopy for assisting water quality monitoring and assessment in water treatment reservoirs using atmospheric corrected satellite remotely sensed imagery. Remote Sensing **3 (2)** 362-377.

HAKVOORT H, DE HAAN J, JORDANS R, VOS R, PETERS S and RIJKEBOER M (2002) Towards airborne remote sensing of water quality in The Netherlands—validation and error analysis. ISPRS Journal of Photogrammetry and Remote Sensing **57 (3)** 171-183.

HALME E, PELLIKKA P, and MÕTTUS M (2019) Utility of hyperspectral compared to multispectral remote sensing data in estimating forest biomass and structure variables in Finnish boreal forest. International Journal of Applied Earth Observation and Geoinformation **83** 101942.

HAN Y, DU X, FARJAD B, GOSS G, GUPTA, A and FARAMARZI M (2022) A numerical modeling framework for simulating the key in-stream fate processes of PAH decay in Muskeg River Watershed, Alberta, Canada. *Science of The Total Environment* **848** 157246.

HANAMOTO S, NAKADA N, JÜRGENS MD, JOHNSON AC, YAMASHITA N and TANAKA H (2018) The different fate of antibiotics in the Thames River, UK, and the Katsura River, Japan. Environmental Science and Pollution Research **25 (2)** 1903-1913.

HANNA N, SUN P, SUN Q, LI X, YANG X, JI X, ZOU H, OTTOSON J, NILSSON LE, BERGLUND B and DYAR OJ (2018) Presence of antibiotic residues in various environmental compartments of Shandong province in eastern China: its potential for resistance development and ecological and human risk. Environment international **114** 131-142.

HAO C, ZHAO X and YANG P (2007) GC-MS and HPLC-MS analysis of bioactive pharmaceuticals and personal-care products in environmental matrices. Trends in Analytical Chemistry **26 (6)** 569-580.

HAO H, SANDER P, IQBAL Z, WANG Y, CHENG G, YUAN Z. (2016) The risk of some veterinary antimicrobial agents on public health associated with antimicrobial resistance and their molecular basis. Front Microbiology **7** 1626.

HARRIS S, CORMICAN M and CUMMINS E (2012) The effect of conventional wastewater treatment on the levels of antimicrobial-resistant bacteria in effluent: a meta-analysis of current studies. Environmental geochemistry and health **34 (6)** 749-762.

HAYS JR, KRENKEL PA and SCHNELLE KB (1966) Mass transport mechanisms in open channel flow. Tech. Rep. No. 8, Vanderbilt Univ., Nashville, TN.

HELLWEGER FL (2013) Simple model of tetracycline antibiotic resistance in aquatic environment: accounting for metal Co-selection Journal of Environmental Engineering **139** 913-21.

HERNÁNDEZ F, IBÁÑEZ M, BADE R, BIJLSMA L and SANCHO JV (2014) Investigation of pharmaceuticals and illicit drugs in waters by liquid chromatography-high-resolution mass spectrometry TrAC Trends in Analytical Chemistry **63** 140-157.

HIRSCH R, TERNES TA, HABERER K, MEHLICH A, BALLWANZ F and KRATZ KL (1998) Determination of antibiotics in different water compartments via liquid chromatography-electrospray tandem mass spectrometry. Journal of chromatography A, **815 (2)** 213-223.

HORNING N (2004) Selecting the appropriate band combination for an RGB image using Landsat imagery Version 1.0. American Museum of Natural History, Center for Biodiversity and Conservation. Available from http://biodiversityinformatics.amnh.org (Accessed 8th June 2021).

HUGO R and SHARON P (2019) An Integrated Water Resources Decision Support System for the Olifants Catchment Facilitating real-time monitoring, early warning & systemic decision-making for water resources [INWARDS].

IBARGÜEN-MONDRAGÓN E, MOSQUERA S, CERÓN M, BURBANO-ROSERO EM, HIDALGO-BONILLA SP, ESTEVA L, et al. (2014) Mathematical modeling on bacterial resistance to multiple antibiotics caused by spontaneous mutations. Biosystems. **117** 60-7.

JELIC A, GROS M, GINEBREDA A, CESPEDES-SANCHEZ R, VENTURA F, PETROVIC M and BARCELO D (2011) Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Research **45 (3)** 1165-1176.

K'OREJE KO, DEMEESTERE K, DE WISPELAERE P, VERGEYNST L, DEWULF J and VAN LANGEHOVE H (2012) From multi-residue screening to target analysis of pharmaceuticals in water: Development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya. Science of the Total Environment **437** 153-164.

KANAMA KM, DASO AP, MPENYANA-MONYATSI L and COETZEE MA (2018) Assessment of pharmaceuticals, personal care products, and hormones in wastewater treatment plants receiving inflows from health facilities in Northwest Province, South Africa. Journal of toxicology *2018*.

KAPO KE, DELEO PC, VAMSHI R, HOLMES CM, FERRER D, DYER SD, WANG X and WHITE-HULL C (2016) iSTREEM®: An approach for broad-scale in-stream exposure assessment of "down-the-drain" chemicals. *Integrated Environmental Assessment and Management* **12(4)** pp.782-792.

KARTHIKEYAN KG and MEYER MT (2006) Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. Science of the Total Environment **361(1-3)** 196-207.

KATENDE-KYENDA NL, LUBBE MS, SERFONTEIN JHP and TRUTER I (2006) Usage of antimicrobial agents in a private primary healthcare setting in South Africa. International journal of pharmacy practice **14 (4)** 283-287.

KAWAMOTO K, MACLEOD M and MACKAY D (2001) Evaluation and comparison of multimedia mass balance models of chemical fate: application of EUSES and ChemCAN to 68 chemicals in Japan. *Chemosphere **44*(4)** 599-612.

KHORRAM S (1981) Water quality mapping from Landsat digital data. International Journal of Remote Sensing **2 (2)** 145-153.

KHORRAM S, CHESHIRE H, GERACI AL and ROSA GL (1991) Water quality mapping of Augusta Bay, Italy from Landsat-TM data. International Journal of Remote Sensing **12 (4)** 803-808.

KIM SB, YAVUZ CORAPCIOGLU M and KIM DJ (2003) Effect of dissolved organic matter and bacteria on contaminant transport in riverbank filtration. Journal of Contaminant Hydrology **66 (1-2)** 1-23.

KIM SC and CARLSON K (2007) Quantification of human and veterinary antibiotics in water and sediment using SPE/LC/MS/MS. Analytical and bioanalytical chemistry **387 (4)** 1301-1315.

KIM W, LEE Y and KIM SD (2017) Developing and applying a site-specific multimedia fate model to address ecological risk of oxytetracycline discharged with aquaculture effluent in coastal waters off Jangheung, Korea. *Ecotoxicology and environmental safety* **145** 221-226.

KINSELLA B, O'MAHONY J, MALONE E, MOLONEY M, CANTWELL H, FUREY A and DANAHER M (2009) Current trends in sample preparation for growth promoter and veterinary drug residue analysis. Journal of Chromatography A, **1216 (46)** 7977-8015.

KIRCHELLE C (2018) Pharming animals: a global history of antibiotics in food production (1935-2017). Palgrave Communications **4 (96)** 1-13.

KIRCHELLE C (2019) *Pyrrhic progress. Antibiotics in Anglo-American food production 1935-2013*. Newark: Rutgers University Press.

KLEIN EY, VAN BROECKEL TP, MATINEZ EM, PANT S, GANDRE S, LEVIN S, GOOSSENS H and LAXMINARAYAN R (2017) Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. In: *Proceedings of the National Academy of Sciences,* **115**, E3463-E3470.

KOLPIN DW, FURLONG ET, MEYER MT, THURMAN EM, ZAUGG SD, BARBER LB and BUXTON HT (2002) Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, a national reconnaissance. Environmental Science and Technology **36 (6)** 1202-1211.

K'OREJE KO, VERGEYNST L, OMBAKA D, DE WISPELAERE P, OKOTH M, VAN LANGENHOVE H and DEMEESTERE K (2016) Occurrence patterns of pharmaceutical residues in wastewater, surface water and groundwater of Nairobi and Kisumu city, Kenya. Chemosphere **149** 238-244.

KUMARASAMY M, GHOSH NC, MISHRA GC and KANSAL ML (2013) Hybrid model development for the decaying pollutant transport in streams. International Journal of Environment and Waste Management **12 (2)** 130-145.

KUMARASAMY M, MISHRA GC, GHOSH NC and KANSAL ML (2011) Semi analytical solution for non-equilibrium sorption of pollutant transport in streams. Journal of Environmental Engineering **137 (11)** 1066-1074.

KUMARASAMY MV (2015) De-oxygenation and Reaeration Coupled hybrid mixing cells Based Pollutant Transport Model to Assess Water Quality Status of a River. International Journal of Environmental Research **9 (1)** 341-350.

KÜMMERER K (2009a) Antibiotics in the aquatic environment – A review – Part I. *Chemosphere* **75 (4)** 417-434.

KUMMERER K (2009b) The presence of pharmaceuticals in the environment due to human use-present knowledge and future challenges. Journal of Environmental Management **90 (8)** 2354-2366.

LÄMMCHEN V, NIEBAUM G, BERLEKAMP J and KLASMEIER J (2021) Geo-referenced simulation of pharmaceuticals in whole watersheds: application of GREAT-ER 4.1 in Germany. *Environmental Science and Pollution Research* **28 (17)** 21926-21935.

LANDGREBE DA (1999) Some fundamentals and methods for hyperspectral image data analysis. In Systems and technologies for clinical diagnostics and drug discovery II. International Society for Optics and Photonics **3603** 104-113.

LAPWORTH DJ, BARAN N, STUART ME and WARD RS (2012) Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. Environmental Pollution **163** 287-303.

LARSSON DGJ, DE PEDRO C and PAXEUS N (2007) Effluent from drug manufactures contains extremely high levels of pharmaceuticals. Journal of hazardous materials **148** 751-755.

LAUNAY MA, DITTMER U and STEINMETZ H (2016) Organic micropollutants discharged by combined sewer overflows – Characterisation of pollutant sources and stormwater-related processes. Water Research **104** 82-92.

LAXMINARAYAN R, DUSE A, WATTA C, ZAIDI AK, WERTHEIM HF, SUMPRADIT N, VLIEGHE E, HARA GL, GOULD IM, GOOSSENS H and GREKO C (2013) Antibiotic resistance: The need for global solutions. The Lancet infectious diseases **13 (12)** 1057-1098.

LAXMINARAYAN R, MATSOSO P, PANT S, BROWER C, RØTTINGEN JA, KLUGMAN K and DAVIES S (2016) Access to effective antimicrobials: a worldwide challenge. The Lancet **387 (10014)** 168-175.

LE T, STACH ND, DUC HT, ABBT-BRAUN DM, GIERÉ S, and WELLINGTON EMH (2014) The Application of Polyketide Synthase (PKS) and Nonribosomal Peptide Synth

LEIJ FJ and DANE JH (1990) Analytical solutions of the one-dimensional advection equation and two- or three-dimensional dispersion equation. Water Resources Research, **26 (7)** 1475-1482

LEIJ FJ and TORIDE N (1998) Analytical solutions for non-equilibrium transport models, in physical non-equilibrium in soils modelling and application, H. M. Selim and L. Ma, eds., Ann Arbor Press, Chelsea, MI.

LEOPOLD SJ, VAN LETH F, TAREKEGN H and SCHULTSZ C (2014) Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: a systematic review. Journal of Antimicrobial Chemotherapy **69 (9)** 2337-2353.

LEVIN BR, BAQUERO F and JOHNSEN PJ (2014) A model-guided analysis and perspective on the evolution and epidemiology of antibiotic resistance and its future. Current opinion in microbiology **19** 83-9.

LEVIN BR, STEWART FM and RICE VA (1979) The kinetics of conjugative plasmid transmission: fit of a simple mass action model. Plasmid **2** 247-260.

LI C, WANG J, WANG L, HU L and GONG P (2014) Comparison of classification algorithms and training sample sizes in urban land classification with Landsat thematic mapper imagery. Remote sensing **6 (2)** 964-983.

LI D, YANG M, HU J, REN L, ZHANG Y and LI K (2008) Determination and fate of oxytetracycline and related compounds in oxytetracycline production wastewater and the receiving river. Environmental Toxicology and Chemistry: An International Journal **27 (1)** 80-86.

LI D, YANG M, HU J, ZHANG Y, CHANG H, JIN F and GE W (2008) Determination of Penicillin-G and its degradation products in a penicillin production wastewater treatment plant and the receiving river. Environmental Science and Technology **42 (2)** 487-492.

LI J, HU C, SHEN Q, BARNES BB, MURCH B, FENG L, ZHANG M and ZHANG B (2017) Recovering low quality MODIS-Terra data over highly turbid waters through noise reduction and regional vicarious calibration adjustment: A case study in Taihu Lake. Remote Sensing of Environment **197** 72-84.

LI J, LI W, LIU K, GUO Y, DING C, HAN J and LI P (2022) Global review of macrolide antibiotics in the aquatic environment: Sources, occurrence, fate, ecotoxicity, and risk assessment. In Journal of Hazardous Materials 439.

LI J, XIE L, CHEN Y, WANG Q, XIAO Z, LUO Z, LI Y, WANG M, CHEN Y, GUO L, LI Y, LIU J, WAN Y, ZANG Y, WU H, and CHANG H (2022) Degradation of erythromycin by a new Ochrobactrum sp. strain ECR001 from activated sludge: The effect of salinity and Cu 2+ on degradation. Journal of Environmental Management **307** 114407.

LI S, ZHUANG Y, ZHANG L, DU Y and LIU H (2014) Worldwide performance and trends in non-point source pollution modeling research from 1994 to 2013: A review based on bibliometrics. Journal of soil and water conservation **69 (4)** 121A-126A.

LI T, WANG C, XU Z and CHAKRABORTY A (2020) A coupled method of on-line solid phase extraction with the UHPLC–MS/MS for detection of sulfonamides antibiotics residues in aquaculture. *Chemosphere* 254.

LI Y, LI Q, ZHOU K, SUN XL, ZHAO LR and ZHANG YB (2016) Occurrence and distribution of the environmental pollutant antibiotics in Gaoqiao mangrove area, China. Chemosphere **147** 25-35.

LIEN LTQ, HOA NQ, CHUC NTK, THOA NTM, PHUC HD, DIWAN V, DAT NT, TAMHANKAR AJ and LUNDBORG CS (2016) Antibiotics in wastewater of a rural and an urban hospital before and after wastewater treatment, and the relationship with antibiotic use a one year study from Vietnam. International Journal of Environmental Research and Public Health **13 (6)** 588.

LIM C, TAKAHASHI E, HONGSUWAN M, WUTHIEKANUN V, THAMLIKITKUL V, HINJOY S. DAY, NP, PEACOCK SJ and LIMMATHUROTSAKUL D (2016) Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. eLife **5** 18082.

LIM J and CHOI M (2015) Assessment of water quality based on Landsat 8 operational land imager associated with human activities in Korea. Environmental monitoring and assessment **187 (6)** 1-17.

LINDBERG RH, BJÖRKLUND K, RENDAHL P, JOHANSSON MI, TYSKLIND M and ANDERSSON BA (2007) Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. Water Research **41 (3)** 613-619.

LIU F, LIU X, ZHAO S, WANG J, QIAN X, CUI B and BAI J (2019) Photochemical transformations of tetracycline antibiotics influenced by natural colloidal particles: Kinetics, factor effects and mechanisms. *Chemosphere* **235** 867-875.

LIU F, YING G-G, TAO R, ZHAO J-L AND YANG J-F (2019) Sorption behavior of antibiotics in sediments from two contrasting freshwater systems. Chemosphere **224** 334-341.

LIU H, ZHOU X, HUANG H and ZHANG J (2019) Prevalence of antibiotic resistance genes and their association with antibiotics in a wastewater treatment plant: process distribution and analysis. Water, **11 (12)** 2495.

LIU X, LV K, DEN C, YU Z, SHI J and JOHNSON AC (2019) Persistence and migration of tetracycline, sulfonamide, fluoroquinolone, and macrolide antibiotics in streams using a simulated hydrodynamic system. *Environmental Pollution* **252** 1532-1538.

LIU X, ZHANG L and HONG S (2011) Global biodiversity research during 1900-2009: a bibliometric analysis. Biodiversity and Conservation **20(4)** 807-826.

LIU Y, LI C, ANDERSON B, ZHANG S, SHI X and ZHAO S (2017) A modified QWASI model for fate and transport modelling of mercury between the water-ice-sediment in Lake Ulansuhai. *Chemosphere* **176** 117-124.

LÖFFLER D and TERNES TA (2003). Determination of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using liquid chromatography-tandem mass spectrometry. Journal of chromatography A, **1021 (1-2)** 133-144.

LUNETTA RS, KNIGHT JF, PAERL HW, STREICHER JJ, PEIERLS BL, GALLO T, LYON JG, MACE TH and BUZZELLI CP (2009) Measurement of water colour using AVIRIS imagery to assess the potential for an operational monitoring capability in the Pamlico Sound Estuary, USA. International Journal of Remote Sensing **30 (13)** 3291-3314.

MACKAY D, SHIU W Y, MA KC and LEE SC (2006) Handbook of physical-chemical properties and environmental fate for organic chemicals: Volume I: Introduction and hydrocarbons. In *Handbook of Physical-Chemical Properties and Environmental Fate For Organic Chemicals: Volume I: Introduction and Hydrocarbons: Vol. III*.

MADIKIZELA LM, TAVENGWA NT and CHIMUKA L (2017) Status of pharmaceuticals in African water bodies: occurrence, removal and analytical methods. Journal of environmental management **193** 211-220.

MAGWIRA CA, ANECK-HAHN N and TAYLOR MB (2019) Fate, occurrence and potential adverse effects of antimicrobials used for treatment of tuberculosis in the aquatic environment in South Africa. Environmental Pollution **254** 1-10.

MAHLOCH JL (1974) Comparative analysis of modeling techniques for coliform organisms in streams. Applied microbiology **27 (2)** 340-5.

MAKUNINA MP, POZDNYAKOV IP, CHEN Y, GRIVIN VP, BAZHIN NM and PLYUSNIN VF (2015) Mechanistic study of fulvic acid assisted propranolol photodegradation in aqueous solution. *Chemosphere* **119** 1406-1410.

MAMUN AA and SALLEH MN (2014) Challenges in non-point source pollution-sampling and testing. 5th Brunei International Conference on Engineering and Technology (BICET 2014) November 2014, 1-6, Bandar Seri Begawan, Brunei.

MANZETTI S and GHISI R (2014) The environmental release and fate of antibiotics. Marine pollution bulletin **79 (1-2)** 7-15.

MARTÍNEZ-CARBALLO E, GONZÁLEZ-BARREIRO C, SCHARF S and GANS O (2007) Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environmental Pollution **148 (2)** 570-579.

MARUYA KA, DODDER NG, SENGUPTA A, SMITH DJ, LYONS JM, HEIL AT and DREWES JE (2016) Multimedia screening of contaminants of emerging concern (CECS) in coastal urban watersheds in southern California (USA). Environmental Toxicology and Chemistry **35 (8)** 1986-1994.

MASSÉ DI, SAADY NMC and GILBERT Y (2014) Potential of biological processes to eliminate antibiotics in livestock manure: an overview. Animals **4 (2)** 146-163.

MATONGO S, BIRUNGI G, MOODLEY B and NDUNGU P (2015a) Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa. Environmental Science and Pollution Research **22 (13)** 10298-10308.

MATONGO S, BIRUNGI G, MOODLEY B and NDUNGU P (2015b) Pharmaceutical residues in water and sediment of Msunduzi River, KwaZulu-Natal, South Africa. Chemosphere **134** 133-140.

MBUH M (2019) Use of Hyperspectral Remote Sensing to Estimate Water Quality: In Processing and Analysis of Hyperspectral Data. Geography & Geographic Information Science Faculty Publications, **13**.

MBUH MJ, HOUSER PR and HEIDARI A (2016) Water quality estimation using combined water chemistry and field spectroscopy in the Shenandoah River, Virginia. International Journal of Applied Geospatial Research **7 (2)** 14-37.

MEADOR JP, YEH A, YOUNG G and GALLAGHER EP (2016) Contaminants of emerging concern in a large temperate estuary. Environmental Pollution **213** 254-267.

MELLOR A, HAYWOOD A, JONES S and WILKES P (2012). Forest classification using random forests with multisource remote sensing and ancillary GIS data. In: *Proceedings of 16th Australian Remote Sensing and Photogrammetry Conference,* Melbourne, VIC, Australia, August 2012, 2728.

MIKULIK K, JIRBIIOVB A, JANDA I and WEISER J (1983) *Susceptibility of ribosomes of the tetracycline-producing strain of Streptomyces aureofaciens to tetracyclines*.

MIKULIK K, PALECKOVA J, DEMNEROVA A, and CORPE MR (1983) Transformation of Penicillin-G by Streptomyces aureofaciens. Z. Allg. Mikrobiol. **23** 237-242.

MILIĆ N, MILANOVIĆ M, LETIĆ NG, SEKULIĆ MT, RADONIĆ J, MIHAJLOVIĆ I and MILORADOV MV (2013a) Occurrence of antibiotics as emerging contaminant substances in aquatic environment. In International Journal of Environmental Health Research **23 (4)** 296-310.

MILIĆ N, MILANOVIĆ M, LETIĆ N, SEKULIĆ M T, RADONIĆ J, AGBABA J and MILORADOV MV (2013b) Determination of antibiotics in surface and wastewater samples in Serbia using SPE-UHPLC-MS/MS method. Environmental Science and Pollution Research **20 (11)** 7916-7925.

MIRZAEI R, YUNESIAN M, NASSERI S, GHOLAMI M, JALILZADEH E, SHOEIBI S and MESDAGHINIA A (2018) Occurrence and fate of most prescribed antibiotics in different water environments of Tehran, Iran. Science of the Total Environment **619** 446-459

MONAHAN C, NAG R, MORRIS D and CUMMINS E (2021) Antibiotic residues in the aquatic environment-current perspective and risk considerations. Journal of Environmental Science and Health, Part A, **56 (7)** 733-751.

MOODLEY B, BIRUNGI G and NDUNGU P (2016) *Detection and quantification of emerging organic pollutants in the Umgeni and Msunduzi Rivers.* Research report no. 2215/1/16, Water Research Commission, Pretoria, South Africa.

MOSLAH B, HAPESHI E, JRAD A., FATTA-KASSINOS D and HEDHILI A (2018) Pharmaceuticals and illicit drugs in wastewater samples in north-eastern Tunisia. Environmental Science and Pollution Research **25 (19)** 18226-18241.

MOTSOANE N, MAPUTSOE X, RICHARDS H, TUTU H, KOTZE I, CUKROWSKA E and CHIMUKA L (2021) *Development and Application of Passive Samplers for Determining the Fate of Toxic Metals in Wetlands Polluted by Mining Activities Report to the Water Research Commission* (2551/1/21). Water Resources Commission. www.wrc.org.za

MTHOMBENI TC, BURGER JR, LUBBE MS, JULYAN M and MTHOMBENI T (2014) Southern African Journal of Infectious Diseases. S Afr J Infect Dis **37 (1)** 462

MURATA A, TAKADA H, MUTOH K, HOSODA H, HARADA A and NAKADA N (2011) Nationwide monitoring of selected antibiotics: distribution and sources of sulfonamides, trimethoprim, and macrolides in Japanese rivers. Science of the Total Environment **409 (24)** 5305-5312.

MURRAY KE, THOMAS SM and BODOUR AA (2010) Prioritising research for trace pollutants and emerging contaminants in the freshwater environment. Environmental pollution **158 (12)** 3462-3471

MUSHTAQ F and NEE LALA MG (2016) Remote estimation of water quality parameters of Himalayan Lake (Kashmir) using Landsat 8 OLI imagery. Geocarto international **32 (3)** 274-285.

NA G, FANG X, CAI Y, GE L, ZONG H, YUAN X, YAO Z and ZHANG Z (2013) Occurrence, distribution, and bioaccumulation of antibiotics in coastal environment of Dalian, China. *Marine Pollution Bulletin* **69 (1-2)** 233-237.

NAS B, EKERCIN S, KARABÖRK H, BERKTAY A, and MULLA DJ (2010) An application of Landsat-5TM image data for water quality mapping in Lake Beysehir, Turkey. Water, Air, & Soil Pollution **212 (1)** 183-197.

NASA (2018) Accuracy assessment of a land cover classification. Remote sensing training program. Advanced webinar: (unpublished)

NDIHOKUBWAYO JB, YAHAYA AA, DESTA AT, KI-ZERBO G, ODEI EA, KEITA B, PANA AP and NKHOMA W (2013) Antimicrobial resistance in the African Region: Issues, challenges and actions proposed. *African* Health Monitor, **16** 27-30.

NGUMBA E, GACHANJA A and TUHKANEN T (2016) Occurrence of selected antibiotics and antiretroviral drugs in Nairobi River Basin, Kenya. Science of the Total Environment **539** 206-213.

NGUYEN TT, GUEDJ J, CHACHATY E, DE GUNZBURG J, ANDREMONT A and MENTRÉ F (2014) Mathematical modeling of bacterial kinetics to predict the impact of antibiotic colonic exposure and treatment duration on the amount of resistant Enterobacteria excreted. PLoS computational biology **10 (9)** 1-10.

NIETO-JUÁREZ JI, TORRES-PALMA RA, BOTERO-COY AM and HERNÁNDEZ F (2021) Pharmaceuticals and environmental risk assessment in municipal wastewater treatment plants and rivers from Peru. *Environment international* **155** 106674.

NIKOLAOU M and TAM VH (2006) A new modeling approach to the effect of antimicrobial agents on heterogeneous microbial populations. Journal of mathematical biology **52 (2)** 154-82.

NÖDLER K, LICHA T, BARBIERI M and PEREZ S (2012) Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification. Water Research **46 (7)** 2131-2139.

NORDIN CF and TROUTMAN BM (1980) Longitudinal dispersion in rivers: The persistence of skewness in observed data. Water resources research **16 (1)** 123-128.

NOUTSOPOULOS C, KOUMAKI E, SARANTOPOULOS V and MAMAIS D (2019) Analytical and mathematical assessment of emerging pollutants fate in a river system. Journal of hazardous materials **364** 48-58.

OGATA A and BANKS RB (1961) A solution of the deferential equation of longitudinal dispersion in porous media. Professional paper No. 411-A, USGS, Reston, VA.

OHARISI IO, AGHOGHOVWIA EA and EKERE NR (2023) Occurrence and Distribution of Selected Antibiotics in Wastewater and Surface Water: A Case Study of a Tertiary Institution in Nigeria. Journal of Water Process Engineering **45** 102383.

OKIN SG, ROBERTS AD, MURRAY B and OKIN JW (2001). Practical limits on hyperspectral vegetation discrimination in arid and semiarid environments. Remote Sensing of Environment **77 (2)** 212-225.

OKOH A, ODJADJARE EE, IGBINOSA EO and OSODE AE (2007) Wastewater treatment plants as a source of microbial pathogens in receiving watersheds. African Journal of Biotechnology **6 (25)** 2932–2944

OLARINMOYE O, BAKARE A, UGWUMBA O and HEIN A (2016) Quantification of pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria. Journal of Environmental Chemistry and Ecotoxicology **8 (3)** 14-24.

OLDENKAMP R, HOEKS S, ČENGIĆ M, BARBAROSSA V, BURNS EE, BOXALL AB and RAGAS A M (2018) A high-resolution spatial model to predict exposure to pharmaceuticals in European surface waters: EPiE. *Environmental science & technology* **52(21)** 12494-12503.

OLOWE KO and KUMARASAMY M (2017) Development of the hybrid cells in series model to simulate ammonia nutrient pollutant transport along the Umgeni River. Environmental Science and Pollution Research **24 (29)** 22967-22979.

OLOWE KO AND KUMARASAMY MV (2020) Analytical solution of a mathematical model to simulate Nitrite pollutant transport along river. Journal of sustainability Science and Management. **15 (7)** 83-101.

O'NEILL J (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Review on Antimicrobial Resistance. December 2014. 1-20. Wellcome Trust and the UK Government, United Kingdom.

ONGLEY ED, XIAOLAN Z AND TAO Y (2010) Current status of agricultural and rural non-point source pollution assessment in China. Environmental pollution **158 (5)** 1159-1168.

OPATOWSKI L, GUILLEMOT D, BOËLLE P-Y and TEMIME L. (2011) Contribution of mathematical modeling to the fight against bacterial antibiotic resistance. Current opinion in infectious diseases **24 (3)** 279-87.

ÖSTMAN M, LINDBERG RH, FICK J, BJÖRN E and TYSKLIND M (2017) Screening of biocides, metals and antibiotics in Swedish sewage sludge and wastewater. Water Research **115** 318-328.

PAL A, HE Y, JEKEL M, REINHARD M and GIN KY-H (2014) Emerging contaminants of public health significance as water quality indicator compounds in the urban water cycle. Environment International **71** 46-62.

PALA-OZKOK I, SPONZA M and SPONZA A (2019a) Assessment of selective antimicrobial resistance effects of different antibiotics on three denitrifying bacterial strains. Water Res. **156**:244-255.

PALA-OZKOK I, UBAY-COKGOR E, JONAS D and ORHON D (2019b) Kinetic and microbial response of activated sludge community to acute and chronic exposure to tetracycline. Journal of Hazardous Materials **367** 418-426.

PARROTT JL and BENNIE DT (2009) Life-cycle exposure of fathead minnows to a mixture of six common pharmaceuticals and triclosan. Journal of Toxicology and Environmental Health, Part A, **72 (10)** 633-641.

PATEL M, KUMAR R, KISHOR K, MLSNA T, PITTMAN CU and MOHAN D (2019) Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. In *Chemical Reviews* (**Vol. 119, Issue 6** pp. 3510-3673). American Chemical Society.

PEREZ S and BARCELO D (2008) Advances in the Analysis of Pharmaceuticals in the Aquatic Environment. In: Aga DS (ed) *Fate of Pharmaceuticals in the Environment and in Water Treatment Systems.* New York, United States of America: CRC Press, 53-76.

PHILLIPS PJ, SMITH SG, KOLPIN DW, ZAUGG SD, BUXTON HT, FURLONG ET, ESPOSITO K and STINSON B (2010) Pharmaceutical formulation facilities as sources of opioids and other pharmaceuticals to wastewater treatment plant effluents. Environmental science and technology **44 (13)** 4910-4916.

PIRT SJ (1990) Penicillin Hydrolysis Rate: Its Effect on the Kinetics of Penicillin Fermentation. In JOURNAL OF FERMENTATION AND BIOENGINEERING **(Vol. 69, Issue 4)**.

PIRT SJ (1990). Biodegradation of Penicillin in Sewage Plants. Journal of Applied Bacteriology **69(1)** 39-52.

POLIANCIUC SI, GURZĂU, AE, KISS B, ŞTEFAN MG and LOGHIN F (2020) Antibiotics in the environment: causes and consequences. Medicine and Pharmacy Reports **93 (3)** 231.

PRUDEN A, LARSSON DJ, AMÉZQUITA A, COLLIGNON P, BRANDT KK, GRAHAM DW, LAZORCHAK JM, SUZUKI S, SILLEY P, SNAPE JR and TOPP E (2013) Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. Environmental health perspectives **121 (8)** 878-885.

RAMASWAMY BR, SHANMUGAM G, VELU G, RENGARAJAN B and LARSSON DJ (2011) GC-MS analysis and ecotoxicological risk assessment of triclosan, carbamazepine and parabens in Indian rivers. Journal of hazardous materials **186 (2-3)** 1586-1593.

REICHERT P (1994) AQUASIM-A tool for simulation and data analysis of aquatic systems. *Water Science and Technology* **30(2)** 21.

REYNOLDS J, WESSON K, DESBIEZ AL, OCHOA-QUINTERO JM and LEIMGRUBER P (2016) Using remote sensing and random forest to assess the conservation status of critical Cerrado habitats in Mato Grosso do Sul, Brazil. Land **5 (2**) 12.

RIBEIRO AR, NUNES OC, PEREIRA MFR and SILVA AMT (2015) An overview on the advanced oxidation processes applied for the treatment of water pollutants defined in the recently launched Directive 2013/39/E.U. Environment International **75** 33-51.

RICHARDS JA and JIA X (2006) Feature reduction. *Remote sensing digital image analysis: an introduction*, 267-294. Springer, Heidelberg, Germany.

RICHARDSON SD and TERNES TA (2014) Water analysis: emerging contaminants and current issues. Analytical Chemistry, **86 (6)** 2813-2848.

RIZZO L, MANAIA C, MERLIN C, SCHWARTZ T, DAGOT C, PLOY MC, MICHAEL I and FATTA-KASSINOS D (2013) Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Science of the total environment **447** 345-360.

RODRIGUEZ-GALIANO VF, GHIMIRE B, ROGAN J, CHICA-OLMO M and RIGOL-SANCHEZ JP (2012) An assessment of the effectiveness of a random forest classifier for land-cover classification. Remote Sensing **67** 93-104.

RODRIGUEZ-MOZAZ S, CHAMORRO S, MARTI E, HUERTA B, GROS M, SÀNCHEZ-MELSIÓ A, BORREGO CM, BARCELÓ D, BALCÁZAR JL (2015) Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. Water Research **69** 234-242.

ROY DP, KOVALSKYY V, ZHANG HK, VERMOTE EF, YAN L, KUMAR SS and EGOROV A (2016) Characterization of Landsat-7 to Landsat-8 reflective wavelength and normalized difference vegetation index continuity. Remote sensing of Environment **185** 57-70.

RUNKEL RL (1998) One dimensional transport with inflow and storage (OTIS): A solute transport model for streams and rivers. Water Resources Investigations Rep. No. 98-4018., USGS, Denver.

RUNKEL RL and BROSHEARS RE (1991) One-dimensional transport with inflow and storage (OTIS): A solute transport model for small streams. Technical Rep. 91-01, Center for Advanced Decision Support for Water and Environmental System, Department of Civil Engineering, University of Colorado.

RUNKEL RL and CHAPRA SC (1993) An efficient numerical solution of the transient storage equations for solute transport in small streams. Water Resources Research **29 (1)** 211-215.

RUTHERFORD JC (1994) River mixing, Wiley, Chichester, UK.

SARMAH AK, MEYER MT and BOXALL ABA (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. In *Chemosphere* **65 (5)** 725-759.

SAUNDERS LJ, MAZUMDER A and LOWE CJ (2016) Pharmaceutical concentrations in screened municipal wastewaters in Victoria, British Columbia: A comparison with prescription rates and predicted concentrations. Environmental Toxicology and Chemistry **35 (4)** 919-929.

SCHEURER M, STORCK FR, GRAF C, BRAUCH HJ, RUCK W, LEV O and LANGE FT (2011) Correlation of six anthropogenic markers in wastewater, surface water, bank filtrate, and soil aquifer treatment. Journal of Environmental Monitoring **13 (4)** 966-973.

SCHOLZ M (2016) Activated Sludge Processes Wetlands for Water Pollution Control (Second Edition) 91-105

SCHOUPS G, VAN DE GIESEN NC and SAVENIJE HHG (2008) Model complexity control for hydrologic prediction. Water Resources Research, **44(12)**.

SCHULMAN LS (2017) Bacterial resistance to antibodies: a model evolutionary study. Journal of theoretical biology **417** 61-7.

SEIFRTOVÁ M, NOVÁKOVÁ L, LINO C, PENA A and SOLICH P (2009) An overview of analytical methodologies for the determination of antibiotics in environmental waters. Analytica Chimica Acta, **649 (2)** 158-179.

SENF C, HOSTERT P and VAN DER LINDEN S (2012). Using MODIS time series and random forests classification for mapping land use in South-East Asia. In: *2012 IEEE International Geoscience and Remote Sensing Symposium*, Munich, Germany, 22-27 July 2012, 6733-6736.

SHAFIQUE NA, AUTREY BC, FULK F, CORMIER SM and ENVIRONMENTAL S (2001). The selection of narrow wavebands for optimizing water quality monitoring on the Great Miami River, Ohio using hyperspectral remote sensor data. Journal of Spatial Hydrology **1 (1)** 1-22.

SHAFIQUE NA, FULK F, CORMIER SM, and AUTREY BC (2002) Coupling hyperspectral remote sensing with field spectrometer to monitor inland water quality parameters. In: *AVIRIS workshop proceedings*. US Environmental agency,

SHARMA VK, JOHNSON N, CIZMAS L, MCDONALD TJ and KIM H (2016) A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. Chemosphere **150** 702-714.

SHEFFIELD J, WOOD EF, PAN M, BECK H, COCCIA G, SERRAT-CAPDEVILA A and VERBIST K (2018) Satellite remote sensing for water resources management: Potential for supporting sustainable development in data-poor regions. Water Resources Research **54 (12)** 9724-9758.

SHIPPERT P (2003) Introduction to Hyperspectral Image Analysis, Online Journal of Space Communication **2 (3)**. Available at: https://ohioopen.library.ohio.edu/spacejournal/vol2/iss3/8

SILBERGELD EK, GRAHAM J and PRICE LB (2008) Industrial food animal production, antimicrobial resistance, and human health. Annual Review of Public Health **29** 151-69.

ŠIMATOVIĆ A and UDIKOVIĆ-KOLIĆ N (2019) Antibiotic resistance in pharmaceutical industry effluents and effluent-impacted environments. In: *Antibiotic Resistance in the Environment*, 101, Springer, Cham.

SINGH R, SINGH AP, KUMAR S, GIRI BS and KIM KH (2019) Antibiotic resistance in major rivers in the world: a systematic review on occurrence, emergence, and management strategies. Journal of Cleaner Production **234** 1484-1505.

SIVANPILLAI R, and MILLER SN (2010) Improvements in mapping water bodies using ASTER data. Ecological Informatics **5 (1)** 73-78.

SLAUGHTER AR and MANTEL SK (2018) Water quality modelling of an impacted semi-arid catchment using flow data from the WEAP model. Proceedings of the International Association of Hydrological Sciences, April 2018, 377, 25-33. IAHS & Agrocampus Ouest, France.

SLAUGHTER AR, HUGHES DA and MANTEL SK (2012) The development of a Water Quality Systems Assessment Model (WQSAM) and its application to the Buffalo River catchment, Eastern Cape, South Africa. In: Proceedings of the 6th International Environmental Modelling and Software Society (iEMSs) Biennial Meeting, July 2012, Leipzig, Germany.

SMITH RB (2012) Introduction to remote sensing of environment (RSE), MicroImages, Inc., Nebraska, U.S.A.

SONG HX, PENG YY and ZHU ZF (2006) Competition between plasmid-bearing and plasmid-free organisms in the host: population dynamics and antibiotic resistance. Medical Principles and Practice 15 436-42.

SONG X, ZHAO Y, ZHANG L, XIE X, WU J, WEI Z, YANG H, ZHANG S, SONG C and JIA L (2022) Photodegradation, bacterial metabolism, and photosynthesis drive the dissolved organic matter cycle in the Heilongjiang River. *Chemosphere* **295**.

SOOKY AA (1969) Longitudinal dispersion in open channels. Journal of the Hydraulics Division **95(4)** 1327-1346

SOUSA JC, RIBEIRO AR, BARBOSA MO, RIBEIRO C, TIRITAN ME, PEREIRA MFR and SILVA AM (2019) Monitoring of the 17 EU Watch List contaminants of emerging concern in the Ave and the Sousa Rivers. Science of the Total Environment **649** 1083-1095.

SPANIOL O, BERGHEIM M, DAWICK J, KÖTTER D, MCDONOUGH K, SCHOWANEK D, STANTON K, WHEELER J and WILLING A (2021) Comparing the European Union System for the Evaluation of Substances (EUSES) environmental exposure calculations with monitoring data for alkyl sulphate surfactants. *Environmental Sciences Europe* **33** 1-9.

Statistics South Africa. Mid-year population estimates (2019). Statistical release P0302. http://www.statssa.gov.za/publications/P0302/P03022019.pdf. (Accessed 7 July 2020).

SUTHAR N, ROY S, CALL DR, BESSER TE and DAVIS MA (2014) An individual-based model of transmission of resistant bacteria in a veterinary teaching hospital. PLoS One 9.

SVERDRUP HU, FROLOVA LL and SVERDRUP AE (2020) Using a system dynamics model for investigating potential levels of antibiotics pollution in the Volga River. Water, Air, & Soil Pollution, **231 (4)**, 1-32.

TAHRANI AA, BARNETT AH and BAILEY CJ (2016) Pharmacology and therapeutic implications of current drugs for type 2 diabetes mellitus. Nature Reviews Endocrinology **12 (10)** 566.

TAM VH, SCHILLING AN, POOLE K and NIKOLAOU M (2007) Mathematical modelling response of Pseudomonas aeruginosa to meropenem Journal of antimicrobial chemotherapy **60** 1302-9.

TAMTAM F, MERCIER F, LE BOT B, EURIN J, TUC DINH Q, CLÉMENT M and CHEVREUIL M (2008) Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Science of the Total Environment **393 (1)** 84-95.

TANG J, SHI T, WU X, CAO H, LI X, HUA R, TANG F and YUE Y (2015) The occurrence and distribution of antibiotics in Lake Chaohu, China: seasonal variation, potential source and risk assessment. Chemosphere **122** 154-161.

TANG X, CUI Z, BAI Y and SU R (2021) Indirect photodegradation of sulfathiazole and sulfamerazine: Influence of the CDOM components and seawater factors (salinity, pH, nitrate and bicarbonate). Science of the Total Environment **750**.

TAYLOR GI (1953) Dispersion of soluble matter in solvent flowing slowly through a tube. In: *Proceedings of the Royal Society of London. Series A. Mathematical and Physical Sciences* **219 (1137)** 186-203.

TAYLOR GI (1954) The dispersion of matter in turbulent flow through a pipe. In: *Proceedings of the Royal Society of London. Series A. Mathematical and Physical Sciences* **223 (1155)** 446-468.

THACKSON EL and KRENKEL PA (1967) Longitudinal mixing in natural streams Journal of the Sanitary Engineering Division ASCE **93 (5)** 67-90.

TONG J and WEI Y (2012) State-of-the-art removal of antibiotic resistance bacteria (ARB) and antibiotic resistance gens (ARG) in wastewater treatment plants (WWTPs). Huanjing Kexue Xuebao/Acta Scientiae Circumstantiae **32 (11)** 2650-2659.

TRUTER I (2015) Antimicrobial prescribing in South Africa using a large pharmacy database: a drug utilisation study: original research-autopsies at an academic hospital. Southern African Journal of Infectious Diseases **30 (2)** 52-56.

United States Geological Survey (USGS) (2018). What is the Landsat satellite program and why is it important? https://landsat.gsfc.nasa.gov/landsat-8/landsat-8-overview (Accessed 15th July 2021)

USGS (2013) Using the USGS Landsat 8 Product. U.S. Department of the Interior, U.S. Geological Survey. http://landsat.usgs.gov/Landsat8_Using_Product.php (Accessed 8th June 2021).

UYAK V, SOYLU S, TOPAL T, KARAPINAR N, OZDEMIR K, OZAYDIN S and AVSAR E (2014) Spatial and seasonal variations of disinfection by products (DBPs) in drinking water distribution systems of Istanbul City, Turkey. Environmental Forensics **15 (2)** 190-205

VAN BOECKEL TP, BROWER C, GILBERT M, GRENFELL BT, LEVIN SA, ROBINSON TP, TEILLANT A and LAXMINARAYAN R (2015) Global trends in antimicrobial use in food animals. In: *Proceedings of the National Academy of Sciences* **112 (18)** 5649-5654.

VAN BOECKEL TP, GANDRE S, ASHOK A, CAUDON Q, GRENFELL B, LEVIN SA and LAXMINARAYAN R (2014) Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data Lancet **14** 742-751.

VAN BUNNIK BAD and WOOLHOUSE MEJ (2017) Modelling the impact of curtailing antibiotic usage in food animals on antibiotic resistance in humans. Royal Society Open Science*,* **4** 161067.

VAN GENUCHTEN MT and JURY WA (1987) Progress in unsaturated flow and transport modelling. Reviews of Geophysics **25 (2)** 135-140.

VAN OMMEN HC (1985) The mixing cell concept applied to transport of non-reactive and reactive components in soils and groundwater Journal of Hydrology **78** 201-213.

VERMEIRE TG, JAGER DT, BUSSIAN B, DEVILLERS J, DEN HAAN K, HANSEN B, LUNDBERG I, NIESSEN H, ROBERTSON S, TYLE H and VAN DER ZANDT PT (1997) European union system for the evaluation of substances (EUSES). Principles and structure. *Chemosphere* **34 (8)** 1823-1836.

VILANOVA R, ROJAS JD and ALFARO VM (2011) Digital control of a waste water treatment plant. International Journal of Computers Communications & Control **6 (2)** 367-374

VOROVENCII I and MUNTEAN DM (2012). Evaluation of supervised classification algorithms for Landsat 5 TM images. RevCAD Journal of Geodesy and Cadastre **11 (1)** 229-238.

WALLACE JS and AGA, D. S. (2016). Enhancing Extraction and Detection of Veterinary Antibiotics in Solid and Liquid Fractions of Manure. Journal of Environmental Quality **45 (2)** 471-479.

WANG GT and CHEN S (1996) A new model describing convective-dispersive phenomena derived using the mixing-cell concept Applied mathematical modelling **20 (4)** 309-320.

WANG X and YANG W (2019) Water quality monitoring and evaluation using remote-sensing techniques in China: A systematic review. Ecosystem Health and Sustainability **5 (1)** 47-56.

WANG X, LIU S and GUO H (2017) A nosocomial-pathogens-infections model with impulsive antibiotics treatment on multiple bacteria Applied Mathematics and Computation **296** 64-87.

WANG Y, KHAN SJ, FAN L and RODDICK F (2020) Application of a QWASI model to produce validated insights into the fate and transport of six emerging contaminants in a wastewater lagoon system. Science of the Total Environment **721** 1-11.

WANG Z, DU Y, YANG C, LIU X, ZHANG J, LI E, ZHANG Q and WANG X (2017) Occurrence and ecological hazard assessment of selected antibiotics in the surface waters in and around Lake Honghu, China. Science of the total environment **609** 1423-1432.

WEBB GF, D'AGATA EMC, MAGAL P and RUAN S (2005) A model of antibiotic-resistant bacterial epidemics in hospitals. In: *Proceedings of the National Academy of Sciences* USA **102** 13343-13348.

WEI CJ, LI XY, XIE YF and WANG XM (2019) Direct photo transformation of tetracycline and sulfanomide group antibiotics in surface water: Kinetics, toxicity and site modeling. *Science of the Total Environment*, *686*, 1-9.

WEI M, ZHANG N, ZHANG Y and ZHENG B (2009) Integrated assessment of river health based on water quality, aquatic life and physical habitat. Journal of Environmental Sciences **21 (8)** 1017-1027.

WEI Z, GUO J, WANG Y and HUA R (2019) Occurrence, distribution, and risk assessment of antibiotics in an important water system of northeast China. Environmental Geochemistry and Health **41(4)** 1713-1723.

WILKINSON JL, SWINDEN J, HOODA PS, BARKER J and BARTON S (2016) Markers of anthropogenic contamination: A validated method for quantification of pharmaceuticals, illicit drug metabolites, perfluorinated compounds, and plasticisers in sewage treatment effluent and rain runoff. Chemosphere **159** 638-646.

WINTGENS T, SALEHI F, HOCHSTRAT R and MELIN T (2008) Emerging contaminants and treatment options in water recycling for indirect potable use. *Water Science and Technology: A Journal of the International* Association on Water Pollution Research **57 (1)** 99-107.

WOOD DE and SALZBERG SL (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome biology **15 (3)** 1-12.

WOOL T, AMBROSE RB, MARTIN JL and COMER A (2020) WASP 8: The next generation in the 50-year evolution of USEPA's water quality model. Wate*r* **12 (5)** 1398.

WOOLHOUSE M, WARD M, VAN BUNNIK B and FARRAR J (2015) Antimicrobial resistance in humans, livestock and the wider environment. Philosophical Transactions of the Royal Society B, **370 (20)** 140, 083.

WORLD HEALTH ORGANIZATION (WHO) (2006) Emerging issues in water and infectious disease. 2003. WHO report Geneva, Switzerland. http://www.who.int/water_sanitation_health/wwdrchap6p4_7.pdf (Accessed 10th June 2020).

WORLD HEALTH ORGANIZATION (WHO) (2011) Pharmaceuticals in drinking water. WHO/HSE/WSH/11.05 49 pages. World Health Organization (WHO) (2015a). Antibiotic resistance http://www.who.int/mediacentre/factsheets/antibiotic-resistance/en/.(Accessed 10th June 2020).

WORLD HEALTH ORGANIZATION (WHO) (2015) Global action plan on antimicrobial resistance. http://apps. who. int/iris/bitstream/10665/193736/1/9789241509763_eng.pdf. (Accessed 10th June 2020).

WORLD HEALTH ORGANIZATION (WHO) (2017) The world is running out of antibiotics, WHO report confirms. News release. https://www.who.int/news-room/detail/20-09-2017-the-world-is-running-out-of-antibiotics-who-report-confirms. (Accessed 10th June 2020).

WORMAN A (1998) Analytical solution and time scale for transport of reacting solutes in rivers and streams. Water Resource Research **34 (10)** 2703-2716.

WORMAN A, PACKMAN AI, JOHANSSON H and JONSSON K (2002) Effect of flow-induced exchange in hyporheic zones on longitudinal transport of solutes in streams and rivers. Water Resource Research **38 (1)** 1001.

WU S, HUA P, GUI D, ZHANG J, YING G and KREBS P (2022) Occurrences, Transport Drivers, and Risk Assessments of Antibiotics in Typical Oasis Surface and Groundwater. Water Research 119138.

YAMAGUCHI Y and NAITO C (2003) Spectral indices for lithologic discrimination and mapping by using the ASTER SWIR bands. International Journal of Remote Sensing **24 (22)** 4311-4323.

YANG JH, BHARGAVA P, MCCLOSKEY D, MAO N, PALSSON BO and COLLINS JJ (2017) Antibiotic-induced changes to the host metabolic environment inhibit drug efficacy and alter immune function. Cell host and microbe **22 (6)** 757-765.

YANG Q, GAO Y, KE J, SHOW PL, GE Y, LIU Y, GUO R and CHEN J (2021) Antibiotics: An overview on the environmental occurrence, toxicity, degradation, and removal methods. Bioengineered **12(1)** 7376-7416.

YANG Y, SONG W, LIN H and HUANG H (2021) Occurrence, Distribution, and Ecotoxicological Impacts of Antibiotics in Surface Water and Sediments. Environmental Science & Technology **55(5)** 2819-2829.

YAO L, WANG Y, TONG L, DENG Y, LI Y, GAN Y, GUO W, DONG C, DUAN Y and ZHAO K (2017) Occurrence and risk assessment of antibiotics in surface water and groundwater from different depths of aquifers: a case study at Jianghan Plain, central China. Ecotoxicology and Environmental Safety **135** 236-242.

YIGIT AZ, KAPLAN G, GONCU S and AVDAN U (2019) Monitoring the water quality of small water bodies using high-resolution remote sensing data. ISPRS International Journal of Geo-Information **8 (12)** 553.

YOUNG PC and WALLIS SG (1993) Solute transport and dispersion in channels. Channel network hydrology, K. Beven and M. J. Kirkby, eds., Wiley, New York, 129-171.

YUN SH, JHO EH, JEONG S, CHOI S, KAL Y and CHA S (2018) Photodegradation of tetracycline and sulfathiazole individually and in mixtures. Food and Chemical Toxicology **116** 108-113.

YUNUS AP, MASAGO Y, and HIJIOKA Y (2020). COVID-19 and surface water quality: Improved Lake water quality during the lockdown. Science of the Total Environment **731** 139012.

ZAFIRIOU OC (1979) NITRATE PHOTOLYSIS IN SEAWATER BY SUNLIGHT* (Vol. 8).

ZHANG R, TANG J, LI J, CHENG Z, CHAEMFA C, LIU D, ZHENG Q, SONG M, LUO C and ZHANG G (2013) Occurrence and risks of antibiotics in the coastal aquatic environment of the Yellow Sea, North China. Science of the Total Environment **450** 197-204.

ZHANG R, ZHANG G, ZHENG Q, TANG J, CHEN Y, XU W, ZOU Y and CHEN X (2012) Occurrence and risks of antibiotics in the Laizhou Bay, China: impacts of river discharge. Ecotoxicology and Environmental Safety **80** 208-215.

ZHANG XX, ZHANG T and FANG HH (2009) Antibiotic resistance genes in water environment. Applied microbiology and Biotechnology **82** 397-414.

ZHANG Y, ZHAO YG, HU Y, GAO M, GUO L and JI J (2022) Insight in degradation of tetracycline in mariculture wastewater by ultraviolet/persulfate advanced oxidation process. Environmental Research, 212.

ZHAO L, JI Y, YAO J, LONG S, LI D and YANG Y (2017) Quantifying the fate and risk assessment of different antibiotics during wastewater treatment using a Monte Carlo simulation. Journal of Cleaner Production **168** 626-631.

ZHAO S, LIU X, CHENG D, LIU G, LIANG B, CUI B and BAI J (2016) Temporal-spatial variation and partitioning prediction of antibiotics in surface water and sediments from the intertidal zones of the Yellow River Delta, China. Science of the Total Environment **569** 1350-1358.

ZHI H, WEBB DT, SCHNOOR JL, KOLPIN DW, KLAPER RD, IWANOWICZ, LR and LEFEVRE GH (2022) Modeling risk dynamics of contaminants of emerging concern in a temperate-region wastewater effluent-dominated stream. Environmental Science: Water Research & Technology **8 (7)** 1408-1422.

ZHONG SF, YANG B, XIONG Q, CAI W, LAN ZG and YING GG (2022) Hydrolytic transformation mechanism of tetracycline antibiotics: Reaction kinetics, products identification and determination in WWTPs. Ecotoxicology and Environmental Safety 229.

ZHOU JL, ZHANG ZL, BANKS E, GROVER D and JIANG J (2009) Pharmaceutical residues in wastewater treatment works effluents and their impact on receiving river water. Journal of Hazardous Materials **166 (2-3)** 655-661.

ZIEMIŃSKA-STOLARSKA A and SKRZYPSKI J (2012) Review of mathematical models of water quality. Ecological Chemistry and Engineering S ***19*** **(2)** 197-211.

ZOU L, MILLER SN and SCHMIDTMANN ET (2006) Mosquito larval habitat mapping using remote sensing and GIS: implications of coalbed methane development and West Nile virus. Journal of medical entomology **43 (5)** 1034-1041.

ZUR WIESCH PS, ENGELSTÄDTER J and BONHOEFFER S (2010) Compensation of fitness costs and reversibility of antibiotic resistance mutations. Antimicrobial agents and chemotherapy. **54** 2085-95.

APPENDIX A: REMOTE SENSING AND LANDUSE

A1: Water Quality Parameters

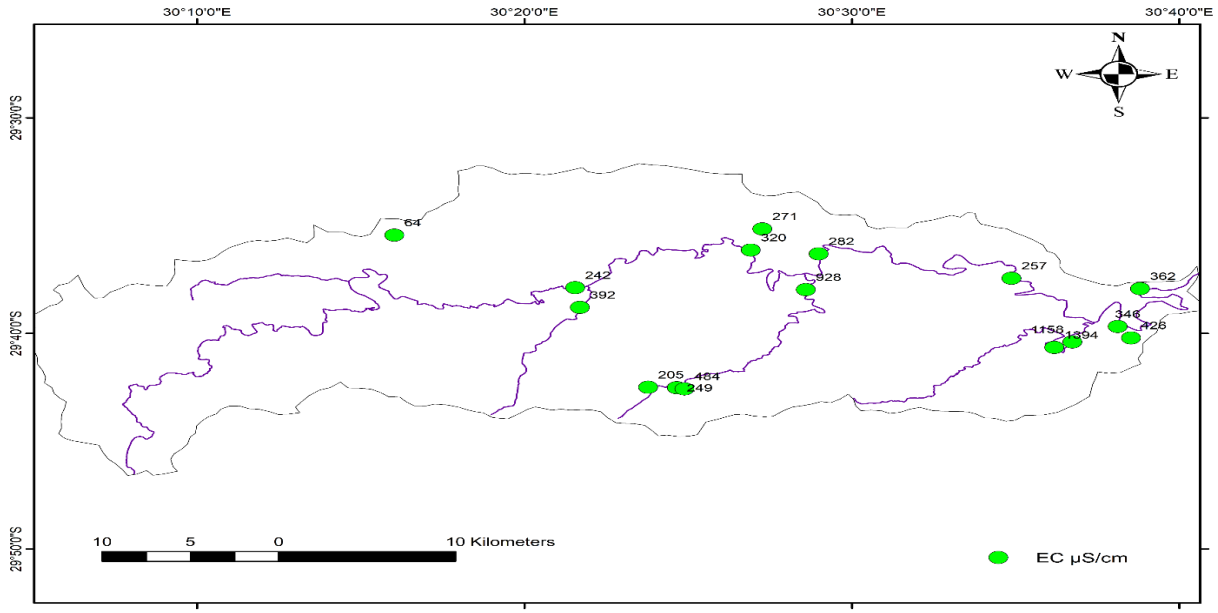
Table A1 Descriptive statistics of Water quality parameters

	18-21 October 2021			26-28 May 2022		
	Min.	Max.	SD	Min.	Max.	SD
Temp (oC)	16,60	24,71	2,53	14,33	21,20	1,93
pH	7,00	8,56	0,40	6,15	8,16	0,55
ORP (mV)	92,50	257,30	41,01	87,40	248,50	50,43
EC (µS/cm)	64,00	1394,00	369,65	77,00	1136,67	262,56
TDS (ppm)	37,00	754,00	199,39	39,00	568,33	131,27
DO %	2,20	57,00	12,68	87,23	102,43	3,92
DO (ppm)	1,23	5,15	0,94	7,43	9,42	0,49
NO3	0,06	19,22	6,90	1,43	3,87	0,71
NO2 (mg/l)	0,46	3,39	1,07	0,01	1,98	0,48
PO4	0,15	0,52	0,14	0,09	1,53	0,34
SO4 (mg/l)	10,95	107,93	25,81	0,00	70,00	17,85
Turbidity				8,46	149,67	33,34
Mg (mg/l)	7,42	39,32	11,16			
K (mg/l)	0,25	24,61	6,41			
Ca (mg/l)	13,08	52,57	13,85			
Cl (mg/l)	24,08	177,43	50,78			
NH4+ (mg/l)	0,02	2,85	1,18			
Na (mg/l)	22,15	156,30	39,01			
F (mg/l)	0,07	1,59	0,43			
Fe (ug/l)	4,38	331,64	90,88			
Al (ug/l)	3,51	500,27	140,04			
Mn (ug/l)	0,54	103,76	29,00			

A2: Spatio-Temporal Variation of Water Quality Parameters

- Spatial variability water quality parameters are mainly influenced by land use activities.
  - Runoff from cultivated areas
  - Darvill Wastewater Treatment plant
  - Industrial areas
  - Informal settlements
  - Urban runoff

SPRING 2021



AUTUMN 2021

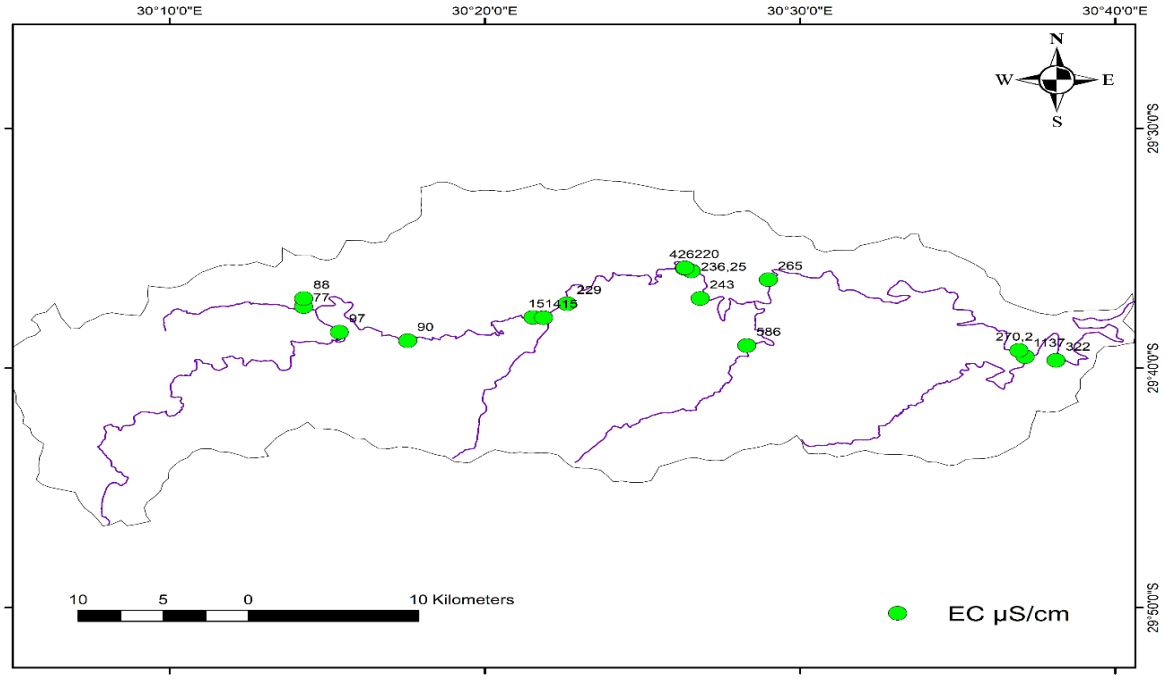
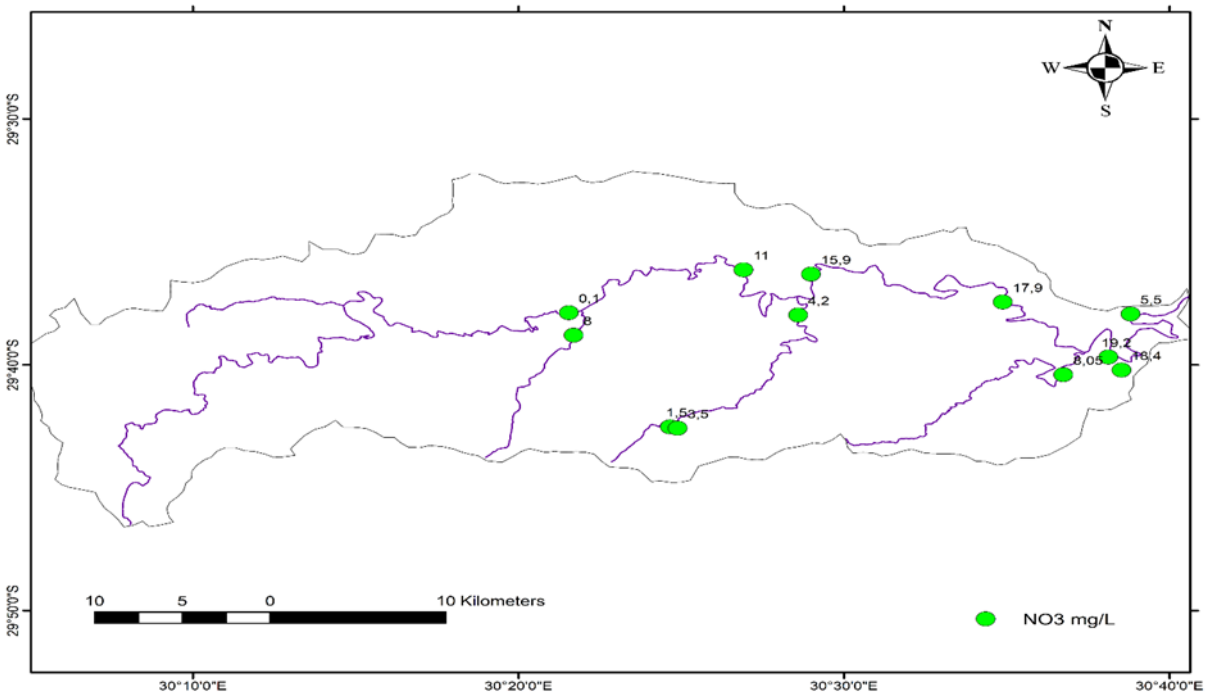


Figure A1 EC Variations along Msunduzi River

SPRING 2021



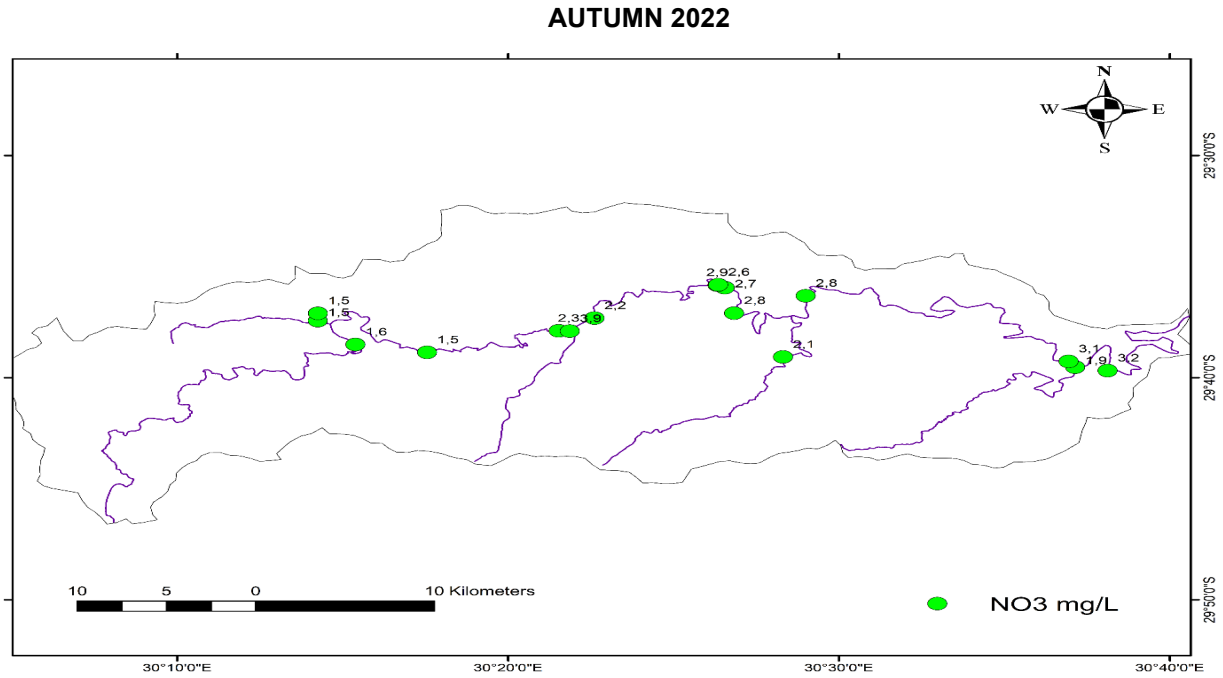


Figure 10 EC Variations along Msunduzi River

A3: Water quality mapping along the Msunduzi River using Remote sensing and GIS

Linear regression between sentinel-2B reflectance values and measured water quality parameters was undertaken. The resulting regression equation for Turbidity and Dissolved Oxygen is given by equations A1 and A2, respectively and later mapped along the Msunduzi River for various times using GIS.

$$\text{Turbidity} = -62.403 + 0.167\text{xBand } 12 - 0.117\text{xBand } 11 + 0.019\text{xBand } 8a \quad [A1]$$

$$\text{Dissolved Oxygen} = 1.673 + 0.001\text{xBand } 11 + 0.003\text{xBand } 5 - 0.000485\text{xBand } 7 \quad [A2]$$

Where the bands refer to the Sentinel-2B image bands.

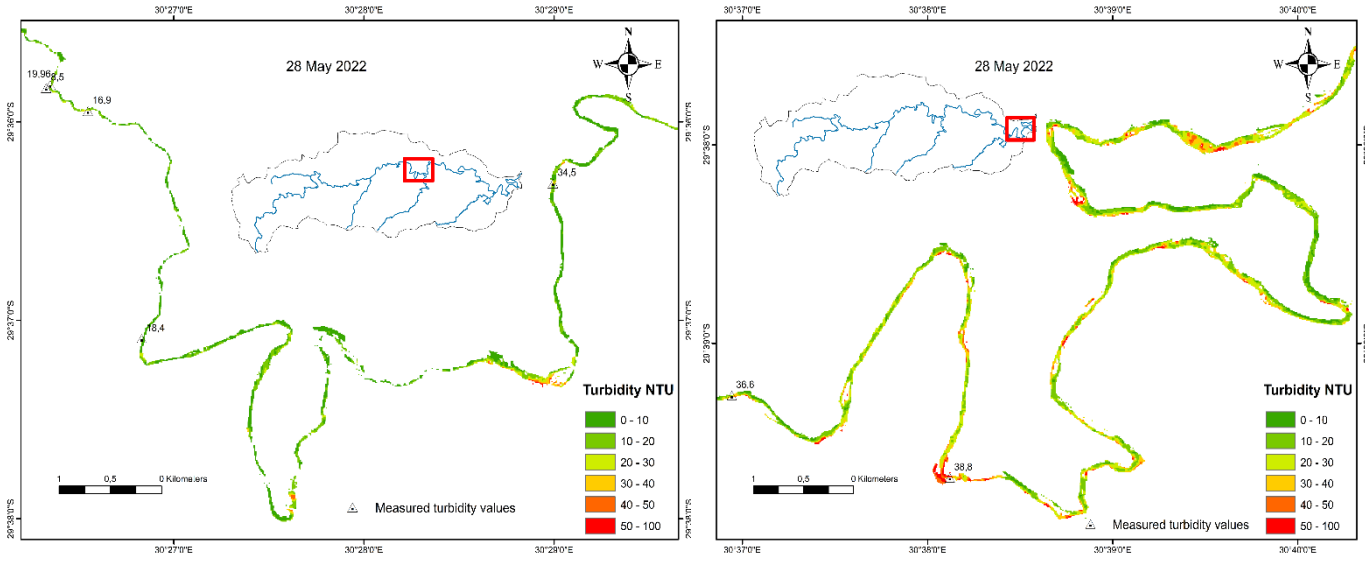


Figure A3 Turbidity map of the Msunduzi River on 28 May 2022 derived from Sentinel-2B satellite imagery interpretation

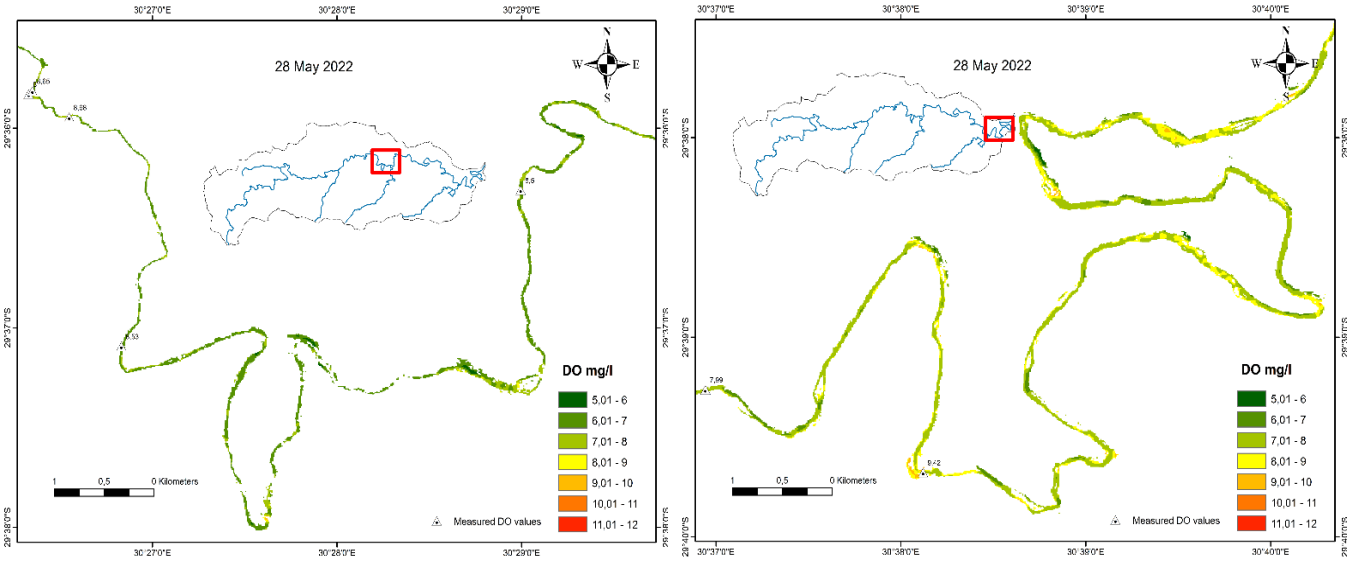


Figure A4 Dissolved Oxygen map of the Msunduzi River on 28 May 2022 derived from Sentinel-2B satellite imagery interpretation

APPENDIX B: FIELD AND LABORATORY WATER QUALITY RESULTS

B1: Continuous Data Collection in Situ for two days

Sample data was continuously collected on-site for a field trip campaign over two days using a handheld Hanna Probe.

Table B1 Select On-Site Water Quality Parameters

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.59689	30.43909	2023/03/25	10:39:52	16,27	7,13	-7,6	245,7	1	1	999999	0	0,00	13,752	27,6	2,53	311
29.64165	30.25633	2023/03/25	11:00:09	19,69	8,51	-87,5	192,2	70	63	14290	35	3,20E-02	13,270	2,6	0,22	1000
29.64157	30.25636	2023/03/25	11:00:16	19,56	8,34	-77,9	201,6	70	63	14290	35	3,20E-02	13,269	1,4	0,12	1000
29.62394	30.23745	2023/03/25	13:00:55	23,14	8,14	-66,9	222,7	89	86	11240	45	4,09E-02	13,266	0,6	0,05	5,8
29.61844	30.23751	2023/03/25	13:24:10	22,73	8,23	-72,0	223,1	96	92	10420	48	0,04	13,236	1,2	0,10	28,6
29.62337	30.25236	2023/03/25	14:19:11	22,88	7,63	-36,9	252,2	105	101	9520	53	4,87E-02	13,310	2,2	0,17	49,3
29.64761	30.29238	2023/03/25	15:15:59	24,30	7,47	-27,9	268,4	117	116	8550	59	5,42E-02	13,592	1,6	0,13	52,5
29.64761	30.29238	2023/03/25	15:15:59	24,30	7,47	-27,9	268,4	117	116	8550	59	5,42E-02	13,592	1,6	0,13	52,5
29.63131	30.35909	2023/03/25	15:51:07	24,72	7,59	-35,1	66,7	213	212	4695	106	9,99E-02	13,702	1,2	0,09	110
29.62227	30.37643	2023/03/25	16:29:56	25,86	7,63	-37,2	185,4	247	251	4049	123	0,12	13,698	1,1	0,08	323
29.63150	30.36436	2023/03/25	17:07:09	27,76	7,48	-28,8	202,6	413	434	2421	206	0,20	13,688	1,5	0,11	30,7
29.63155	30.36439	2023/03/25	17:09:11	26,73	7,57	-34,2	203,0	2	2	500000	1	0,00	13,688	1,5	0,11	0,0
29.63154	30.36436	2023/03/25	17:09:31	27,73	7,52	-30,9	201,2	413	435	2421	207	0,20	13,689	1,5	0,11	18,7
29.63157	30.36434	2023/03/25	17:10:39	27,72	7,57	-33,8	196,5	413	434	2421	206	0,20	13,690	1,4	0,10	34,1
29.59714	30.43886	2023/03/26	10:07:57	25,09	7,96	-56,9	171,1	547	548	1828	274	0,26	13,751	3,3	0,26	0,0
29.59690	30.43912	2023/03/26	10:15:49	23,21	7,82	-48,5	182,1	229	221	4367	115	0,11	13,760	3,1	0,25	76,5
29.59693	30.43911	2023/03/26	10:16:07	23,22	7,75	-43,9	183,1	229	221	4367	115	0,11	13,760	3,2	0,26	78,7
29.59691	30.43909	2023/03/26	10:16:18	23,22	7,76	-44,6	180,3	229	222	4367	115	0,11	13,759	3,2	0,25	77,2
29.59330	30.43359	2023/03/26	11:02:37	23,42	7,92	-53,9	183,4	229	222	4367	114	0,11	13,762	3,3	0,26	70,5
29.59331	30.43359	2023/03/26	11:02:45	23,39	7,67	-39,2	197,6	229	222	4367	115	0,11	13,759	3,3	0,26	69,3
29.59329	30.43360	2023/03/26	11:03:02	23,39	7,55	-32,6	202,2	230	223	4348	115	0,11	13,762	3,3	0,26	71,0
29.59931	30.44318	2023/03/26	11:27:29	23,80	7,70	-41,5	188,5	269	263	3717	135	0,13	13,766	3,1	0,24	158
29.59931	30.44319	2023/03/26	11:27:44	23,81	7,71	-42,1	187,8	269	263	3717	134	0,13	13,770	3,1	0,24	162
29.61821	30.44713	2023/03/26	12:12:59	23,51	7,59	-34,8	182,8	253	245	3953	126	0,12	13,776	3,2	0,26	93,3

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.61820	30.44713	2023/03/26	12:13:07	23,50	7,58	-34,4	181,4	253	246	3953	126	0,12	13,778	3,2	0,26	82,6
29.61821	30.44714	2023/03/26	12:13:24	23,50	7,59	-34,7	178,8	253	246	3953	126	0,12	13,775	3,2	0,25	94,8
29.60505	30.48316	2023/03/26	12:49:15	23,52	7,72	-42,4	187,7	252	245	3968	126	0,12	13,903	3,3	0,27	139
29.60503	30.48314	2023/03/26	12:49:25	23,52	7,72	-42,5	187,8	252	245	3968	126	0,12	13,905	3,3	0,27	142
29.60503	30.48319	2023/03/26	12:49:36	23,52	7,70	-41,4	188,1	253	246	3953	126	0,12	13,906	3,3	0,26	134
29.65115	30.47167	2023/03/26	13:29:50	23,81	7,89	-52,7	194,9	669	654	1495	335	0,32	13,767	3,3	0,26	11,2
29.65113	30.47167	2023/03/26	13:29:59	23,81	7,89	-52,3	195,7	669	653	1495	334	0,32	13,767	3,3	0,26	10,4
29.65112	30.47167	2023/03/26	13:30:08	23,81	7,90	-52,8	195,6	669	654	1495	335	0,33	13,766	3,3	0,26	11,5
29.65113	30.47169	2023/03/26	14:04:53	23,59	8,01	-59,4	205,1	669	651	1495	334	0,32	13,753	3,0	0,24	12,2
29.65114	30.47169	2023/03/26	14:04:58	23,59	8,00	-59,1	205,3	669	651	1495	334	0,32	13,750	3,1	0,24	22,3
29.65114	30.47171	2023/03/26	14:05:03	23,59	8,00	-58,9	205,0	669	651	1495	334	0,32	13,751	3,1	0,24	11,6
29.65114	30.47171	2023/03/26	14:05:08	23,59	8,01	-59,4	204,2	669	651	1495	334	0,32	13,753	3,1	0,24	12,6
29.65116	30.47170	2023/03/26	14:05:13	23,59	8,01	-59,6	203,8	669	651	1495	334	0,32	13,755	3,1	0,25	11,5
29.65116	30.47170	2023/03/26	14:05:18	23,59	8,04	-61,0	202,1	669	651	1495	335	0,33	13,753	3,1	0,25	16,7
29.65116	30.47170	2023/03/26	14:05:23	23,59	8,05	-62,0	200,9	669	651	1495	335	0,33	13,753	3,1	0,25	11,4
29.65116	30.47169	2023/03/26	14:05:28	23,59	8,04	-61,4	201,2	669	651	1495	335	0,33	13,753	3,1	0,25	11,0
29.65116	30.47169	2023/03/26	14:05:33	23,59	8,04	-61,4	200,8	669	651	1495	335	0,33	13,756	3,1	0,25	12,8
29.65116	30.47169	2023/03/26	14:05:38	23,59	8,07	-62,8	199,4	669	651	1495	335	0,33	13,757	3,1	0,25	14,3
29.65116	30.47169	2023/03/26	14:05:43	23,58	8,07	-63,2	199,0	669	652	1495	335	0,33	13,756	3,1	0,25	11,4
29.65116	30.47169	2023/03/26	14:05:48	23,59	8,07	-63,0	199,1	669	651	1495	335	0,33	13,757	3,1	0,25	12,8
29.65115	30.47167	2023/03/26	14:05:53	23,58	8,07	-62,9	198,8	669	651	1495	335	0,33	13,757	3,1	0,25	11,3
29.65114	30.47167	2023/03/26	14:05:58	23,58	8,06	-62,4	199,3	670	652	1493	335	0,33	13,756	3,1	0,25	10,6
29.65114	30.47167	2023/03/26	14:06:03	23,59	8,07	-63,2	198,4	674	656	1484	337	0,33	13,754	3,1	0,25	10,9
29.65114	30.47167	2023/03/26	14:06:08	23,59	8,08	-63,4	198,0	723	703	1383	361	0,35	13,753	3,2	0,25	10,5
29.65114	30.47167	2023/03/26	14:06:13	23,58	8,08	-63,7	196,8	898	874	1114	449	0,44	13,753	3,2	0,25	11,9
29.65116	30.47167	2023/03/26	14:06:18	23,58	8,06	-62,7	196,6	1420	1382	704	710	0,71	13,753	3,2	0,25	12,1
29.65116	30.47167	2023/03/26	14:06:23	23,58	8,09	-64,1	195,5	1542	1501	649	771	0,78	13,752	3,2	0,25	12,6
29.65116	30.47167	2023/03/26	14:06:28	23,58	8,10	-64,9	195,4	1366	1329	732	683	0,68	13,755	3,2	0,25	13,0
29.65116	30.47167	2023/03/26	14:06:33	23,58	8,12	-65,8	195,3	1120	1090	893	560	0,56	13,755	3,2	0,25	12,4
29.65115	30.47167	2023/03/26	14:06:38	23,58	8,11	-65,3	195,3	1085	1055	922	542	0,54	13,755	3,2	0,25	13,0
29.65114	30.47167	2023/03/26	14:06:43	23,58	8,12	-66,0	195,1	942	917	1062	471	0,46	13,753	3,2	0,25	12,7
29.65115	30.47167	2023/03/26	14:06:48	23,58	8,12	-65,9	195,5	834	812	1199	417	0,41	13,756	3,2	0,25	12,5
29.65114	30.47167	2023/03/26	14:06:53	23,58	8,13	-66,4	194,9	784	763	1276	392	0,38	13,755	3,2	0,25	11,9
29.65114	30.47167	2023/03/26	14:06:58	23,58	8,11	-65,3	195,8	735	716	1361	368	0,36	13,754	3,2	0,25	11,0

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.65115	30.47167	2023/03/26	14:07:03	23,58	8,12	-66,2	194,9	729	710	1372	365	0,36	13,756	3,2	0,25	12,4
29.65115	30.47167	2023/03/26	14:07:08	23,58	8,12	-66,1	194,6	709	690	1410	355	0,35	13,754	3,2	0,25	11,9
29.65115	30.47167	2023/03/26	14:07:13	23,58	8,11	-65,2	195,4	695	676	1439	348	0,34	13,755	3,2	0,25	11,9
29.65115	30.47167	2023/03/26	14:07:18	23,58	8,10	-65,0	196,0	685	666	1460	342	0,33	13,753	3,2	0,25	11,9
29.65115	30.47167	2023/03/26	14:07:23	23,58	8,11	-65,3	195,4	681	663	1468	341	0,33	13,754	3,2	0,25	11,9
29.65115	30.47167	2023/03/26	14:07:28	23,58	8,10	-65,0	195,8	677	659	1477	339	0,33	13,755	3,2	0,25	11,6
29.65115	30.47167	2023/03/26	14:07:33	23,58	8,12	-65,8	194,9	678	659	1475	339	0,33	13,752	3,2	0,25	11,7
29.65115	30.47166	2023/03/26	14:07:38	23,58	8,11	-65,5	195,1	677	659	1477	339	0,33	13,752	3,2	0,25	11,2
29.65115	30.47166	2023/03/26	14:07:43	23,58	8,13	-66,6	194,4	677	658	1477	338	0,33	13,753	3,2	0,25	11,3
29.65115	30.47165	2023/03/26	14:07:48	23,58	8,13	-66,3	194,1	674	656	1484	337	0,33	13,754	3,2	0,25	11,0
29.65115	30.47165	2023/03/26	14:07:53	23,58	8,14	-67,0	193,8	676	658	1479	338	0,33	13,752	3,2	0,25	10,9
29.65115	30.47167	2023/03/26	14:07:58	23,58	8,11	-65,4	195,1	672	654	1488	336	0,33	13,753	3,2	0,25	11,3
29.65115	30.47167	2023/03/26	14:08:03	23,58	8,12	-66,2	194,5	673	655	1486	336	0,33	13,754	3,2	0,25	11,0
29.65115	30.47167	2023/03/26	14:08:08	23,58	8,12	-66,1	194,2	672	654	1488	336	0,33	13,752	3,2	0,25	11,5
29.65116	30.47167	2023/03/26	14:08:13	23,58	8,13	-66,7	193,8	672	654	1488	336	0,33	13,752	3,2	0,26	11,8
29.65116	30.47167	2023/03/26	14:08:18	23,58	8,15	-68,0	192,9	672	654	1488	336	0,33	13,754	3,2	0,25	16,8
29.65116	30.47167	2023/03/26	14:08:23	23,58	8,16	-68,5	191,9	672	654	1488	336	0,33	13,750	3,2	0,25	12,2
29.65116	30.47167	2023/03/26	14:08:28	23,58	8,16	-68,4	192,2	672	654	1488	336	0,33	13,751	3,3	0,26	21,1
29.65116	30.47169	2023/03/26	14:08:33	23,58	8,16	-68,0	192,5	673	655	1486	336	0,33	13,752	3,2	0,25	12,2
29.65116	30.47167	2023/03/26	14:08:38	23,58	8,18	-69,2	191,5	674	655	1484	337	0,33	13,753	3,2	0,25	18,0
29.65115	30.47167	2023/03/26	14:08:43	23,58	8,17	-68,6	191,9	673	655	1486	337	0,33	13,751	3,2	0,25	12,4
29.65115	30.47167	2023/03/26	14:08:48	23,58	8,16	-68,2	192,3	673	655	1486	336	0,33	13,751	3,1	0,25	17,7
29.65115	30.47167	2023/03/26	14:08:53	23,58	8,16	-68,4	192,7	672	654	1488	336	0,33	13,752	3,2	0,25	16,6
29.65115	30.47167	2023/03/26	14:08:58	23,58	8,16	-68,1	192,5	672	654	1488	336	0,33	13,753	3,1	0,25	17,5
29.65115	30.47167	2023/03/26	14:09:03	23,58	8,14	-67,4	193,3	672	653	1488	336	0,33	13,752	3,2	0,25	12,3
29.65114	30.47167	2023/03/26	14:09:08	23,57	8,15	-67,9	192,8	671	653	1490	336	0,33	13,753	3,2	0,25	12,3
29.65115	30.47167	2023/03/26	14:09:13	23,57	8,15	-67,4	193,3	671	653	1490	336	0,33	13,755	3,2	0,25	12,5
29.65114	30.47169	2023/03/26	14:09:18	23,57	8,15	-67,7	192,9	671	653	1490	336	0,33	13,757	3,2	0,25	14,4
29.65114	30.47169	2023/03/26	14:09:23	23,57	8,15	-67,7	193,0	671	653	1490	336	0,33	13,754	3,2	0,25	14,6
29.65115	30.47169	2023/03/26	14:09:28	23,57	8,15	-68,0	192,7	671	653	1490	336	0,33	13,752	3,2	0,25	12,8
29.65115	30.47169	2023/03/26	14:09:33	23,57	8,15	-67,8	192,7	671	652	1490	335	0,33	13,755	3,2	0,25	11,1
29.65114	30.47169	2023/03/26	14:09:38	23,57	8,15	-67,6	192,9	671	652	1490	335	0,33	13,754	3,2	0,25	10,4
29.65114	30.47169	2023/03/26	14:09:43	23,57	8,15	-67,9	192,8	670	652	1493	335	0,33	13,755	3,2	0,25	11,4
29.65114	30.47169	2023/03/26	14:09:48	23,57	8,15	-67,5	193,3	671	653	1490	335	0,33	13,755	3,2	0,25	11,3

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.65113	30.47169	2023/03/26	14:09:53	23,57	8,15	-67,9	192,7	671	652	1490	335	0,33	13,757	3,2	0,25	11,4
29.65113	30.47169	2023/03/26	14:09:58	23,57	8,16	-68,3	192,5	670	652	1493	335	0,33	13,757	3,3	0,26	11,7
29.65113	30.47169	2023/03/26	14:10:03	23,57	8,15	-67,8	193,0	670	652	1493	335	0,33	13,757	3,2	0,26	11,3
29.65113	30.47169	2023/03/26	14:10:08	23,57	8,15	-67,7	193,0	670	652	1493	335	0,33	13,756	3,2	0,25	11,1
29.65113	30.47169	2023/03/26	14:10:13	23,56	8,14	-67,4	193,4	670	652	1493	335	0,33	13,756	3,2	0,25	11,7
29.65439	30.61578	2023/03/26	15:17:57	22,99	8,00	-58,7	199,8	233	224	4292	117	0,11	14,117	3,1	0,26	835
29.65439	30.61578	2023/03/26	15:18:05	23,00	7,98	-57,3	201,0	233	225	4292	117	0,11	14,117	3,1	0,26	825
29.65437	30.61577	2023/03/26	15:18:13	22,99	8,00	-58,8	199,2	233	225	4292	117	0,11	14,113	3,2	0,26	832
29.65878	30.61884	2023/03/26	15:40:55	22,30	7,83	-48,5	214,3	871	826	1148	435	0,43	14,118	3,2	0,27	22,5
29.65878	30.61884	2023/03/26	15:41:04	22,32	7,84	-49,5	212,4	1056	1002	947	528	0,52	14,119	3,2	0,27	22,9
29.65878	30.61884	2023/03/26	15:41:13	22,76	7,86	-50,2	211,7	1694	1622	590	847	0,86	14,117	3,2	0,26	24,1
29.66129	30.63539	2023/03/26	16:13:25	22,97	8,11	-65,1	198,1	255	245	3922	127	0,12	14,153	3,1	0,26	1000
29.66131	30.63540	2023/03/26	16:13:33	22,97	8,01	-59,6	203,7	255	245	3922	127	0,12	14,152	3,1	0,26	1000
29.64162	30.25646	2023/05/24	11:19:12	12,19	6,04	54,4	301,0	119	90	8400	60	5,63E-02	13,265	34,3	3,31	46,2
29.64134	30.25626	2023/05/24	11:25:53	11,99	7,29	-16,1	313,9	113	85	8850	56	5,30E-02	13,280	29,8	2,89	47,1
29.64135	30.25625	2023/05/24	11:26:05	11,85	7,10	-5,9	327,1	113	85	8850	56	5,32E-02	13,279	30,0	2,92	36,8
29.62577	30.24697	2023/05/24	11:51:09	16,08	7,16	-8,9	344,7	110	92	9090	55	5,16E-02	13,304	31,8	2,83	63,5
29.62577	30.24697	2023/05/24	11:51:16	16,03	7,18	-10,3	342,8	111	92	9010	56	5,20E-02	13,300	31,4	2,80	62,9
29.62577	30.24697	2023/05/24	11:51:24	16,04	7,18	-10,3	343,3	110	92	9090	55	5,17E-02	13,297	30,9	2,75	62,9
29.62345	30.25253	2023/05/24	12:12:33	15,52	7,01	-0,5	327,9	111	91	9010	55	5,19E-02	13,323	32,0	2,89	29,3
29.62343	30.25253	2023/05/24	12:12:42	15,24	7,07	-4,0	327,8	111	90	9010	55	5,20E-02	13,326	31,3	2,84	27,2
29.62343	30.25253	2023/05/24	12:13:00	15,16	7,13	-7,4	329,9	110	90	9090	55	5,18E-02	13,323	31,3	2,84	53,5
29.62387	30.23752	2023/05/24	12:56:12	14,28	7,21	-12,2	293,7	88	70	11360	44	4,08E-02	13,256	31,3	2,89	211
29.62387	30.23753	2023/05/24	12:56:18	14,15	7,07	-3,7	304,0	82	65	12200	41	0,04	13,256	30,5	2,82	26,3
29.62387	30.23751	2023/05/24	12:56:33	14,14	6,98	1,0	310,3	81	64	12350	41	3,76E-02	13,260	30,2	2,80	1000
29.62390	30.23750	2023/05/24	13:10:21	14,26	6,85	8,3	346,3	23	18	43500	11	9,38E-03	13,273	30,4	2,81	369
29.62436	30.23822	2023/05/24	13:23:50	14,45	6,93	4,2	346,6	90	72	11110	45	4,18E-02	13,265	30,3	2,78	6,0
29.62436	30.23822	2023/05/24	13:23:55	14,45	6,95	3,1	347,4	90	72	11110	45	4,18E-02	13,266	30,3	2,78	6,2
29.62436	30.23823	2023/05/24	13:24:00	14,45	6,94	3,6	345,8	90	72	11110	45	4,18E-02	13,269	30,3	2,78	5,4
29.62436	30.23824	2023/05/24	13:24:05	14,46	6,94	3,5	347,0	90	72	11110	45	4,18E-02	13,269	30,3	2,78	6,2
29.62436	30.23824	2023/05/24	13:24:10	14,46	6,97	1,7	345,3	90	72	11110	45	4,19E-02	13,269	30,2	2,78	7,5
29.62436	30.23825	2023/05/24	13:24:15	14,46	6,95	2,6	346,9	90	72	11110	45	4,18E-02	13,269	30,2	2,78	7,5
29.62436	30.23825	2023/05/24	13:24:20	14,46	6,95	3,1	347,7	90	72	11110	45	4,18E-02	13,269	30,2	2,78	6,8
29.62436	30.23824	2023/05/24	13:24:25	14,46	7,00	-0,1	342,7	90	72	11110	45	4,18E-02	13,269	30,2	2,78	6,8

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62436	30.23824	2023/05/24	13:24:30	14,46	6,97	1,6	344,6	90	72	11110	45	4,19E-02	13,269	30,2	2,77	7,1
29.62436	30.23824	2023/05/24	13:24:35	14,46	6,98	1,2	343,8	90	72	11110	45	4,18E-02	13,267	30,2	2,77	7,6
29.62436	30.23822	2023/05/24	13:24:40	14,46	6,95	2,6	347,2	90	72	11110	45	4,18E-02	13,267	30,2	2,77	7,0
29.62436	30.23822	2023/05/24	13:24:45	14,46	7,02	-1,0	342,7	90	72	11110	45	4,18E-02	13,267	30,1	2,77	8,1
29.62436	30.23821	2023/05/24	13:24:50	14,46	6,99	0,5	343,7	90	72	11110	45	4,18E-02	13,267	30,1	2,76	8,1
29.62436	30.23821	2023/05/24	13:24:55	14,46	7,00	0,2	346,0	90	72	11110	45	4,18E-02	13,265	30,1	2,76	6,9
29.62434	30.23822	2023/05/24	13:25:00	14,46	7,01	-0,5	344,7	90	72	11110	45	4,18E-02	13,269	30,1	2,77	8,3
29.62434	30.23822	2023/05/24	13:25:05	14,47	7,00	0,2	344,5	90	72	11110	45	4,18E-02	13,267	30,0	2,76	7,0
29.62434	30.23822	2023/05/24	13:25:10	14,47	7,01	-0,3	346,9	90	72	11110	45	4,18E-02	13,265	30,0	2,76	7,3
29.62433	30.23823	2023/05/24	13:25:15	14,46	7,02	-1,1	344,4	90	72	11110	45	0,04	13,265	30,0	2,76	7,1
29.62433	30.23824	2023/05/24	13:25:20	14,47	7,03	-1,5	343,6	90	72	11110	45	4,18E-02	13,265	30,0	2,76	8,0
29.62433	30.23824	2023/05/24	13:25:25	14,47	7,01	-0,3	344,9	90	72	11110	45	4,18E-02	13,270	30,0	2,76	7,2
29.62431	30.23824	2023/05/24	13:25:30	14,47	7,01	-0,7	346,2	90	72	11110	45	4,18E-02	13,269	30,0	2,76	7,5
29.62431	30.23825	2023/05/24	13:25:35	14,47	7,00	0,1	347,8	90	72	11110	45	4,18E-02	13,271	30,0	2,76	6,2
29.62431	30.23825	2023/05/24	13:25:40	14,46	6,98	1,0	346,0	90	72	11110	45	4,18E-02	13,272	30,1	2,76	6,9
29.62431	30.23825	2023/05/24	13:25:45	14,46	7,01	-0,7	344,2	90	72	11110	45	4,19E-02	13,270	30,1	2,77	7,6
29.62431	30.23824	2023/05/24	13:25:50	14,47	7,03	-2,0	342,9	90	72	11110	45	4,19E-02	13,271	30,0	2,76	6,9
29.62433	30.23823	2023/05/24	13:25:55	14,47	6,95	2,9	349,2	90	72	11110	45	4,20E-02	13,270	30,0	2,76	7,9
29.62433	30.23823	2023/05/24	13:26:00	14,47	6,95	2,6	348,7	90	72	11110	45	4,18E-02	13,270	30,0	2,76	7,3
29.62433	30.23823	2023/05/24	13:26:05	14,47	6,97	1,4	348,0	90	72	11110	45	4,17E-02	13,272	29,9	2,75	7,2
29.62433	30.23823	2023/05/24	13:26:10	14,47	7,00	0,0	346,6	90	72	11110	45	0,04	13,270	30,0	2,75	7,0
29.62433	30.23823	2023/05/24	13:26:15	14,47	7,01	-0,6	346,6	90	72	11110	45	4,18E-02	13,270	29,9	2,75	7,9
29.62434	30.23822	2023/05/24	13:26:20	14,47	7,05	-3,1	343,4	90	72	11110	45	4,18E-02	13,272	29,9	2,75	7,9
29.62434	30.23822	2023/05/24	13:26:25	14,48	7,01	-0,7	346,0	90	72	11110	45	4,18E-02	13,273	29,9	2,75	7,3
29.62434	30.23822	2023/05/24	13:26:30	14,47	7,07	-3,9	344,5	90	72	11110	45	0,04	13,275	29,9	2,75	8,1
29.62434	30.23822	2023/05/24	13:26:35	14,47	7,08	-4,3	344,5	90	72	11110	45	4,17E-02	13,271	30,0	2,75	7,6
29.62434	30.23822	2023/05/24	13:26:40	14,47	7,07	-4,2	343,3	90	72	11110	45	0,04	13,270	30,0	2,75	7,5
29.62434	30.23821	2023/05/24	13:26:45	14,47	7,04	-2,2	344,7	90	72	11110	45	0,04	13,268	30,0	2,75	6,5
29.62434	30.23821	2023/05/24	13:26:50	14,47	7,08	-4,4	342,2	90	72	11110	45	0,04	13,271	29,9	2,75	6,4
29.62434	30.23821	2023/05/24	13:26:55	14,47	7,06	-3,2	344,5	90	72	11110	45	4,17E-02	13,271	29,9	2,75	7,2
29.62434	30.23821	2023/05/24	13:27:00	14,47	7,04	-2,4	345,0	90	72	11110	45	0,04	13,271	29,9	2,75	7,1
29.62434	30.23822	2023/05/24	13:27:05	14,47	7,09	-5,0	341,7	90	72	11110	45	4,17E-02	13,269	29,9	2,75	7,0
29.62434	30.23822	2023/05/24	13:27:10	14,47	7,03	-1,9	345,3	90	72	11110	45	4,17E-02	13,271	29,9	2,75	6,8
29.62434	30.23822	2023/05/24	13:27:15	14,48	7,08	-4,8	343,0	90	72	11110	45	4,17E-02	13,272	29,9	2,75	6,4

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62434	30.23822	2023/05/24	13:27:20	14,47	7,10	-5,5	342,3	90	72	11110	45	4,17E-02	13,274	29,8	2,74	8,1
29.62433	30.23822	2023/05/24	13:27:25	14,47	7,08	-4,4	344,0	90	72	11110	45	4,17E-02	13,272	29,8	2,74	8,4
29.62433	30.23823	2023/05/24	13:27:30	14,48	7,08	-4,8	342,2	90	72	11110	45	4,17E-02	13,275	29,8	2,74	8,4
29.62433	30.23823	2023/05/24	13:27:35	14,48	7,07	-4,3	343,6	90	72	11110	45	4,17E-02	13,275	29,9	2,74	7,4
29.62433	30.23823	2023/05/24	13:27:40	14,48	7,09	-5,1	342,5	90	72	11110	45	4,17E-02	13,273	29,9	2,75	6,5
29.62433	30.23823	2023/05/24	13:27:45	14,48	7,07	-3,8	344,0	90	72	11110	45	4,17E-02	13,274	29,9	2,75	6,6
29.62433	30.23823	2023/05/24	13:27:50	14,48	7,10	-5,8	342,4	90	72	11110	45	4,17E-02	13,275	29,9	2,75	6,6
29.62433	30.23822	2023/05/24	13:27:55	14,48	7,12	-7,0	341,9	90	72	11110	45	4,17E-02	13,274	29,9	2,75	6,8
29.62433	30.23822	2023/05/24	13:28:00	14,48	7,06	-3,2	344,2	90	72	11110	45	4,17E-02	13,271	29,9	2,75	7,2
29.62433	30.23822	2023/05/24	13:28:05	14,48	7,09	-5,4	342,6	90	72	11110	45	4,17E-02	13,271	29,9	2,75	6,9
29.62433	30.23821	2023/05/24	13:28:10	14,48	7,03	-2,0	345,4	90	72	11110	45	4,17E-02	13,270	29,9	2,75	6,6
29.62433	30.23821	2023/05/24	13:28:15	14,49	7,11	-6,3	341,5	90	72	11110	45	4,17E-02	13,273	29,8	2,74	7,0
29.62433	30.23821	2023/05/24	13:28:20	14,48	7,05	-2,8	345,2	90	72	11110	45	4,17E-02	13,273	29,9	2,74	7,0
29.62433	30.23821	2023/05/24	13:28:25	14,48	7,04	-2,5	347,7	90	72	11110	45	4,17E-02	13,274	30,0	2,76	6,9
29.62433	30.23821	2023/05/24	13:28:30	14,48	7,07	-3,8	345,5	90	72	11110	45	4,17E-02	13,274	30,0	2,76	7,0
29.62434	30.23820	2023/05/24	13:28:35	14,48	7,06	-3,3	345,8	90	72	11110	45	4,17E-02	13,270	30,1	2,76	8,6
29.62434	30.23819	2023/05/24	13:28:40	14,49	7,07	-4,2	345,3	90	72	11110	45	4,17E-02	13,271	30,0	2,76	6,7
29.62434	30.23819	2023/05/24	13:28:45	14,49	7,05	-2,9	346,5	90	72	11110	45	4,17E-02	13,272	30,0	2,76	7,6
29.62433	30.23820	2023/05/24	13:28:50	14,49	7,08	-4,3	345,0	90	72	11110	45	4,17E-02	13,271	30,0	2,76	7,4
29.62433	30.23820	2023/05/24	13:28:55	14,49	7,08	-4,4	344,4	90	72	11110	45	4,17E-02	13,268	30,0	2,76	5,8
29.62434	30.23820	2023/05/24	13:29:00	14,49	7,09	-4,9	343,8	89	72	11240	45	4,17E-02	13,269	30,0	2,76	5,8
29.62434	30.23820	2023/05/24	13:29:05	14,49	7,08	-4,6	344,0	90	72	11110	45	4,17E-02	13,268	30,0	2,76	5,8
29.62434	30.23820	2023/05/24	13:29:10	14,49	7,09	-5,4	343,1	90	72	11110	45	4,17E-02	13,268	30,0	2,76	7,0
29.62434	30.23820	2023/05/24	13:29:15	14,49	7,09	-5,0	343,3	90	72	11110	45	4,17E-02	13,272	30,1	2,76	8,5
29.62434	30.23820	2023/05/24	13:29:20	14,49	7,10	-5,5	342,8	90	72	11110	45	4,17E-02	13,272	30,1	2,76	7,6
29.62434	30.23820	2023/05/24	13:29:25	14,49	7,09	-5,4	342,4	89	72	11240	45	4,17E-02	13,272	30,1	2,76	6,8
29.62434	30.23819	2023/05/24	13:29:30	14,49	7,10	-5,6	343,1	89	72	11240	45	4,17E-02	13,271	30,1	2,76	5,7
29.62434	30.23819	2023/05/24	13:29:35	14,49	7,10	-5,6	342,7	90	72	11110	45	4,17E-02	13,272	30,1	2,76	6,0
29.62434	30.23819	2023/05/24	13:29:40	14,49	7,23	-13,4	334,7	90	72	11110	45	4,17E-02	13,271	30,0	2,76	5,9
29.62434	30.23820	2023/05/24	13:29:45	14,49	7,23	-13,1	335,8	90	72	11110	45	4,17E-02	13,272	30,0	2,75	6,0
29.62434	30.23820	2023/05/24	13:29:50	14,49	7,21	-12,1	336,0	89	72	11240	45	0,04	13,271	29,9	2,75	5,8
29.62434	30.23820	2023/05/24	13:29:55	14,49	7,20	-11,4	337,4	90	72	11110	45	4,17E-02	13,272	29,8	2,74	6,6
29.62434	30.23821	2023/05/24	13:30:00	14,49	7,28	-16,1	332,4	89	72	11240	45	4,17E-02	13,273	29,8	2,74	5,6
29.62434	30.23821	2023/05/24	13:30:05	14,49	7,22	-12,5	335,8	89	72	11240	45	4,17E-02	13,274	29,8	2,73	6,6

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62434	30.23821	2023/05/24	13:30:10	14,49	7,22	-12,7	337,0	90	72	11110	45	4,17E-02	13,272	29,8	2,74	5,4
29.62434	30.23821	2023/05/24	13:30:15	14,49	7,14	-7,8	341,3	89	72	11240	45	4,17E-02	13,271	29,8	2,74	6,6
29.62434	30.23821	2023/05/24	13:30:20	14,49	7,19	-10,9	336,9	89	72	11240	45	4,17E-02	13,272	29,8	2,74	6,9
29.62434	30.23821	2023/05/24	13:30:25	14,49	7,07	-4,2	347,2	89	72	11240	45	4,17E-02	13,271	29,8	2,74	7,4
29.62434	30.23821	2023/05/24	13:30:30	14,49	7,09	-5,3	342,2	89	72	11240	45	4,17E-02	13,272	30,0	2,76	5,7
29.62434	30.23821	2023/05/24	13:30:35	14,50	7,03	-1,9	345,4	89	72	11240	45	4,17E-02	13,274	30,1	2,76	6,8
29.62436	30.23820	2023/05/24	13:30:40	14,49	7,09	-5,3	344,6	89	72	11240	45	4,17E-02	13,276	30,1	2,77	5,8
29.62436	30.23820	2023/05/24	13:30:45	14,50	7,18	-10,3	337,2	89	72	11240	45	4,17E-02	13,274	30,1	2,77	3,8
29.62436	30.23820	2023/05/24	13:30:50	14,50	7,09	-5,2	343,5	89	72	11240	45	4,17E-02	13,274	30,1	2,77	6,5
29.62436	30.23820	2023/05/24	13:30:55	14,50	7,11	-6,4	341,7	89	72	11240	45	4,16E-02	13,272	30,1	2,77	6,6
29.62436	30.23820	2023/05/24	13:31:00	14,50	7,20	-11,5	341,6	89	72	11240	45	0,04	13,271	30,1	2,77	3,5
29.62436	30.23820	2023/05/24	13:31:05	14,51	7,12	-6,7	341,7	89	72	11240	45	0,04	13,271	30,1	2,76	5,4
29.62436	30.23820	2023/05/24	13:31:10	14,51	7,10	-5,7	342,0	89	72	11240	45	0,04	13,269	30,1	2,76	4,7
29.62436	30.23820	2023/05/24	13:31:15	14,50	7,26	-14,9	332,7	89	72	11240	45	0,04	13,269	30,1	2,76	5,9
29.62436	30.23820	2023/05/24	13:31:20	14,49	7,26	-14,9	332,9	90	72	11110	45	4,17E-02	13,270	29,8	2,74	5,8
29.62436	30.23820	2023/05/24	13:31:25	14,50	7,24	-13,9	334,5	90	72	11110	45	4,17E-02	13,271	29,8	2,73	6,4
29.62436	30.23822	2023/05/24	13:31:30	14,51	7,08	-4,6	342,9	89	72	11240	45	0,04	13,270	29,9	2,74	5,0
29.62436	30.23822	2023/05/24	13:31:35	14,51	7,18	-10,3	336,4	90	72	11110	45	4,17E-02	13,272	30,0	2,76	5,6
29.62436	30.23822	2023/05/24	13:31:40	14,51	7,17	-9,6	336,5	90	72	11110	45	4,17E-02	13,268	30,1	2,76	5,3
29.62436	30.23822	2023/05/24	13:31:45	14,51	7,27	-15,3	331,5	90	72	11110	45	4,17E-02	13,273	30,1	2,76	5,8
29.62436	30.23822	2023/05/24	13:31:50	14,51	7,20	-11,6	336,2	90	72	11110	45	4,18E-02	13,274	30,1	2,76	5,2
29.62436	30.23822	2023/05/24	13:31:55	14,50	7,15	-8,6	338,8	90	72	11110	45	4,18E-02	13,271	29,9	2,75	6,8
29.62436	30.23822	2023/05/24	13:32:00	14,50	7,16	-9,2	337,8	90	72	11110	45	4,18E-02	13,268	30,0	2,76	6,3
29.62436	30.23822	2023/05/24	13:32:05	14,50	7,13	-7,3	338,6	90	72	11110	45	4,20E-02	13,266	30,1	2,76	6,7
29.62436	30.23822	2023/05/24	13:32:10	14,49	7,23	-13,2	334,1	90	72	11110	45	4,20E-02	13,266	30,0	2,76	5,3
29.62436	30.23822	2023/05/24	13:32:15	14,50	7,20	-11,4	335,6	90	72	11110	45	4,21E-02	13,268	30,0	2,75	5,5
29.62436	30.23822	2023/05/24	13:32:20	14,50	7,15	-8,7	337,8	91	73	10990	45	0,04	13,269	30,1	2,76	5,1
29.62436	30.23822	2023/05/24	13:32:25	14,51	7,15	-8,7	337,9	91	73	10990	45	4,24E-02	13,268	30,1	2,77	6,5
29.62436	30.23822	2023/05/24	13:32:30	14,51	7,13	-7,4	338,3	91	73	10990	46	4,26E-02	13,267	30,2	2,77	5,6
29.62436	30.23821	2023/05/24	13:32:35	14,50	7,15	-8,4	337,2	91	73	10990	46	0,04	13,265	30,2	2,77	5,4
29.62436	30.23821	2023/05/24	13:32:40	14,51	7,14	-7,8	338,3	92	74	10870	46	4,28E-02	13,265	30,2	2,77	5,7
29.62436	30.23822	2023/05/24	13:32:45	14,51	7,19	-11,1	336,7	92	74	10870	46	4,30E-02	13,266	30,2	2,77	5,1
29.62436	30.23822	2023/05/24	13:32:50	14,51	7,17	-9,5	336,5	93	74	10750	46	4,33E-02	13,264	30,2	2,77	5,6
29.62436	30.23822	2023/05/24	13:32:55	14,51	7,09	-4,9	341,4	93	75	10750	47	4,35E-02	13,265	30,2	2,77	5,7

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62436	30.23822	2023/05/24	13:33:00	14,51	7,09	-5,3	339,7	95	76	10530	47	4,42E-02	13,264	30,2	2,77	6,1
29.62434	30.23822	2023/05/24	13:33:05	14,51	7,11	-6,2	340,0	95	76	10530	48	4,44E-02	13,267	30,2	2,77	5,4
29.62434	30.23822	2023/05/24	13:33:10	14,51	7,18	-10,3	335,4	96	77	10420	48	4,47E-02	13,266	30,2	2,77	5,4
29.62434	30.23822	2023/05/24	13:33:15	14,51	7,18	-10,5	335,1	97	77	10310	48	4,51E-02	13,267	30,2	2,78	5,4
29.62434	30.23822	2023/05/24	13:33:20	14,51	7,18	-10,1	334,7	98	78	10200	49	4,57E-02	13,267	30,2	2,77	4,9
29.62433	30.23822	2023/05/24	13:33:25	14,51	7,18	-10,5	335,9	98	79	10200	49	4,59E-02	13,268	30,2	2,78	4,9
29.62433	30.23822	2023/05/24	13:33:30	14,51	7,18	-10,1	335,3	100	80	10000	50	4,68E-02	13,270	30,2	2,78	5,5
29.62433	30.23822	2023/05/24	13:33:35	14,51	7,18	-10,4	334,2	101	81	9900	50	4,70E-02	13,271	30,2	2,78	6,1
29.62433	30.23823	2023/05/24	13:33:40	14,51	7,23	-13,2	332,0	102	82	9800	51	4,77E-02	13,270	30,2	2,78	6,5
29.62433	30.23823	2023/05/24	13:33:45	14,51	7,18	-10,2	333,9	102	82	9800	51	4,78E-02	13,268	30,2	2,77	5,6
29.62434	30.23822	2023/05/24	13:33:50	14,51	7,19	-10,7	334,6	103	82	9710	51	4,82E-02	13,268	30,1	2,77	5,7
29.62434	30.23823	2023/05/24	13:33:55	14,52	7,18	-10,2	334,6	105	84	9520	52	0,05	13,268	30,1	2,76	6,0
29.62434	30.23823	2023/05/24	13:34:00	14,52	7,19	-11,1	333,3	106	85	9430	53	4,95E-02	13,270	30,1	2,76	5,0
29.62434	30.23823	2023/05/24	13:34:05	14,52	7,20	-11,1	333,8	106	85	9430	53	4,94E-02	13,269	30,1	2,76	5,7
29.62434	30.23823	2023/05/24	13:34:10	14,52	7,19	-10,9	333,8	108	86	9260	54	5,06E-02	13,270	30,1	2,76	5,2
29.62436	30.23823	2023/05/24	13:34:15	14,52	7,21	-12,1	332,8	108	86	9260	54	5,05E-02	13,273	30,1	2,76	5,2
29.62436	30.23822	2023/05/24	13:34:20	14,52	7,17	-9,8	334,3	109	88	9170	55	5,13E-02	13,272	30,1	2,76	5,4
29.62436	30.23822	2023/05/24	13:34:25	14,52	7,19	-10,6	334,5	109	88	9170	55	5,13E-02	13,268	30,0	2,76	5,5
29.62436	30.23822	2023/05/24	13:34:30	14,52	7,19	-10,8	335,3	111	89	9010	56	5,21E-02	13,268	30,0	2,76	5,3
29.62436	30.23822	2023/05/24	13:34:35	14,52	7,19	-10,7	333,6	110	88	9090	55	5,15E-02	13,268	30,1	2,77	5,2
29.62436	30.23822	2023/05/24	13:34:40	14,53	7,08	-4,8	339,5	110	88	9090	55	5,14E-02	13,266	30,2	2,77	5,0
29.62436	30.23822	2023/05/24	13:34:45	14,52	7,10	-5,6	338,5	111	89	9010	56	5,23E-02	13,268	30,2	2,77	4,8
29.62436	30.23822	2023/05/24	13:34:50	14,53	7,15	-8,4	336,5	113	91	8850	57	5,32E-02	13,271	30,2	2,77	5,6
29.62434	30.23822	2023/05/24	13:34:55	14,53	7,15	-8,3	336,5	114	91	8770	57	5,36E-02	13,270	30,2	2,77	6,7
29.62436	30.23823	2023/05/24	13:35:00	14,53	7,18	-10,4	333,8	115	92	8700	57	5,39E-02	13,269	30,2	2,77	5,5
29.62436	30.23824	2023/05/24	13:35:05	14,53	7,20	-11,1	333,6	116	93	8620	58	5,43E-02	13,269	30,1	2,77	6,5
29.62436	30.23824	2023/05/24	13:35:10	14,52	7,17	-9,7	334,7	117	93	8550	58	5,48E-02	13,270	30,1	2,76	5,8
29.62436	30.23824	2023/05/24	13:35:15	14,53	7,19	-11,1	333,2	118	94	8470	59	5,53E-02	13,267	30,1	2,76	5,1
29.62436	30.23825	2023/05/24	13:35:20	14,53	7,17	-9,8	335,2	118	94	8470	59	0,06	13,267	30,1	2,76	5,5
29.62434	30.23826	2023/05/24	13:35:25	14,53	7,21	-12,1	332,3	118	95	8470	59	5,55E-02	13,268	30,1	2,76	5,1
29.62434	30.23826	2023/05/24	13:35:30	14,53	7,19	-10,6	334,3	119	96	8400	60	5,61E-02	13,269	30,1	2,76	5,5
29.62434	30.23827	2023/05/24	13:35:35	14,53	7,24	-13,4	331,0	118	95	8470	59	5,57E-02	13,268	30,1	2,76	5,8
29.62434	30.23827	2023/05/24	13:35:40	14,53	7,27	-15,3	329,1	119	95	8400	59	5,58E-02	13,267	30,0	2,75	5,6
29.62434	30.23827	2023/05/24	13:35:45	14,53	7,26	-14,7	329,8	119	95	8400	59	5,59E-02	13,267	29,9	2,74	5,9

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62434	30.23828	2023/05/24	13:35:50	14,53	7,23	-13,3	330,8	120	96	8330	60	0,06	13,265	29,9	2,74	5,6
29.62434	30.23828	2023/05/24	13:35:55	14,53	7,25	-14,2	330,9	119	96	8400	60	5,61E-02	13,267	29,9	2,74	5,5
29.62434	30.23829	2023/05/24	13:36:00	14,53	7,26	-14,9	329,4	120	96	8330	60	5,62E-02	13,269	29,8	2,74	5,1
29.62436	30.23829	2023/05/24	13:36:05	14,53	7,26	-15,1	329,8	119	96	8400	60	5,61E-02	13,270	29,8	2,74	5,1
29.62436	30.23829	2023/05/24	13:36:10	14,53	7,25	-14,1	329,9	119	96	8400	60	5,61E-02	13,270	29,8	2,74	6,3
29.62436	30.23828	2023/05/24	13:36:15	14,53	7,26	-14,6	330,4	119	95	8400	60	0,06	13,271	29,8	2,73	5,4
29.62436	30.23828	2023/05/24	13:36:20	14,53	7,21	-12,1	332,3	119	95	8400	59	5,59E-02	13,269	29,8	2,73	5,3
29.62436	30.23828	2023/05/24	13:36:25	14,53	7,20	-11,7	332,2	119	95	8400	59	5,58E-02	13,271	29,8	2,73	6,0
29.62436	30.23827	2023/05/24	13:36:30	14,53	7,23	-13,2	331,6	119	95	8400	59	5,58E-02	13,270	29,8	2,73	5,7
29.62436	30.23827	2023/05/24	13:36:35	14,53	7,23	-12,9	331,7	118	95	8470	59	5,57E-02	13,271	29,8	2,74	4,9
29.62437	30.23827	2023/05/24	13:36:40	14,53	7,23	-12,9	330,9	118	95	8470	59	5,56E-02	13,268	29,8	2,73	5,2
29.62436	30.23826	2023/05/24	13:36:45	14,53	7,23	-13,0	332,3	118	94	8470	59	5,53E-02	13,268	29,8	2,74	5,4
29.62436	30.23826	2023/05/24	13:36:50	14,53	7,22	-12,5	332,0	117	94	8550	59	5,51E-02	13,265	29,8	2,74	5,9
29.62436	30.23825	2023/05/24	13:36:55	14,54	7,23	-13,0	332,0	117	94	8550	58	5,50E-02	13,267	29,8	2,74	6,1
29.62434	30.23824	2023/05/24	13:37:00	14,54	7,22	-12,6	332,2	117	94	8550	58	5,48E-02	13,269	29,8	2,73	5,4
29.62434	30.23823	2023/05/24	13:37:05	14,54	7,23	-13,4	331,3	116	93	8620	58	5,47E-02	13,271	29,8	2,73	4,2
29.62434	30.23822	2023/05/24	13:37:10	14,54	7,25	-14,4	329,4	116	93	8620	58	5,44E-02	13,270	29,8	2,74	4,9
29.62434	30.23821	2023/05/24	13:37:15	14,54	7,21	-11,8	332,1	116	93	8620	58	5,43E-02	13,270	29,8	2,74	5,8
29.62434	30.23821	2023/05/24	13:37:20	14,54	7,22	-12,5	331,1	115	92	8700	57	5,39E-02	13,270	29,8	2,74	6,0
29.62434	30.23821	2023/05/24	13:37:25	14,54	7,23	-13,4	330,7	114	92	8770	57	5,37E-02	13,273	29,8	2,73	5,5
29.62436	30.23821	2023/05/24	13:37:30	14,54	7,21	-12,1	332,4	114	91	8770	57	5,35E-02	13,270	29,8	2,73	5,1
29.62436	30.23822	2023/05/24	13:37:35	14,54	7,23	-13,3	330,1	113	91	8850	57	5,32E-02	13,270	29,8	2,73	5,1
29.62436	30.23822	2023/05/24	13:37:40	14,54	7,23	-12,8	331,3	113	91	8850	57	5,31E-02	13,271	29,8	2,73	5,3
29.62436	30.23822	2023/05/24	13:37:45	14,54	7,24	-13,5	331,1	113	90	8850	56	5,29E-02	13,270	29,8	2,73	5,6
29.62436	30.23823	2023/05/24	13:37:50	14,54	7,22	-12,4	331,1	112	90	8930	56	5,27E-02	13,271	29,8	2,73	5,7
29.62436	30.23822	2023/05/24	13:37:55	14,54	7,22	-12,8	330,6	111	89	9010	56	0,05	13,272	29,8	2,73	4,5
29.62436	30.23823	2023/05/24	13:38:00	14,54	7,20	-11,4	331,9	111	89	9010	55	5,19E-02	13,269	29,8	2,73	5,4
29.62436	30.23823	2023/05/24	13:38:05	14,54	7,23	-13,2	329,9	110	89	9090	55	5,18E-02	13,268	29,8	2,73	5,5
29.62436	30.23824	2023/05/24	13:38:10	14,54	7,22	-12,5	330,9	110	88	9090	55	5,17E-02	13,268	29,8	2,73	5,2
29.62438	30.23824	2023/05/24	13:38:15	14,54	7,21	-12,1	331,9	110	88	9090	55	5,15E-02	13,269	29,8	2,74	5,5
29.62438	30.23824	2023/05/24	13:38:20	14,54	7,21	-12,2	331,8	110	88	9090	55	5,15E-02	13,270	29,9	2,75	5,3
29.62438	30.23824	2023/05/24	13:38:25	14,54	7,19	-10,9	332,5	109	87	9170	54	5,10E-02	13,269	29,9	2,75	6,0
29.62438	30.23823	2023/05/24	13:38:30	14,54	7,20	-11,4	332,2	109	87	9170	54	5,10E-02	13,268	29,9	2,75	5,0
29.62438	30.23822	2023/05/24	13:38:35	14,54	7,21	-11,8	331,8	108	87	9260	54	5,08E-02	13,269	30,0	2,75	5,6

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62438	30.23822	2023/05/24	13:38:40	14,54	7,19	-10,8	332,9	108	86	9260	54	5,05E-02	13,266	30,0	2,75	4,6
29.62438	30.23822	2023/05/24	13:38:45	14,54	7,16	-9,2	333,3	107	86	9350	54	5,04E-02	13,267	29,9	2,75	6,5
29.62438	30.23822	2023/05/24	13:38:50	14,55	7,19	-10,7	333,1	107	86	9350	54	0,05	13,266	29,9	2,75	5,3
29.62438	30.23822	2023/05/24	13:38:55	14,55	7,23	-13,4	330,4	107	85	9350	53	0,05	13,267	29,9	2,75	5,8
29.62438	30.23822	2023/05/24	13:39:00	14,55	7,15	-8,5	334,9	106	85	9430	53	4,98E-02	13,268	29,9	2,75	5,4
29.62437	30.23822	2023/05/24	13:39:05	14,55	7,19	-10,7	332,4	106	85	9430	53	4,99E-02	13,266	29,9	2,74	5,1
29.62436	30.23821	2023/05/24	13:39:10	14,55	7,18	-10,2	333,0	106	85	9430	53	4,99E-02	13,266	30,0	2,75	6,6
29.62436	30.23822	2023/05/24	13:39:15	14,56	7,21	-11,9	331,2	106	85	9430	53	4,96E-02	13,265	30,0	2,75	4,8
29.62436	30.23822	2023/05/24	13:39:20	14,56	7,18	-10,3	332,8	106	85	9430	53	4,94E-02	13,265	29,9	2,74	6,6
29.62434	30.23822	2023/05/24	13:39:25	14,56	7,18	-10,5	332,1	105	84	9520	53	4,92E-02	13,266	29,9	2,75	5,8
29.62433	30.23822	2023/05/24	13:39:30	14,56	7,22	-12,6	331,0	105	84	9520	52	4,91E-02	13,266	30,0	2,75	5,3
29.62433	30.23822	2023/05/24	13:39:35	14,56	7,21	-12,1	330,9	105	84	9520	52	0,05	13,268	29,9	2,75	4,9
29.62433	30.23824	2023/05/24	13:39:40	14,56	7,19	-10,6	332,4	104	83	9620	52	4,85E-02	13,267	30,0	2,75	5,1
29.62433	30.23824	2023/05/24	13:39:45	14,56	7,20	-11,4	331,9	103	83	9710	52	4,84E-02	13,269	30,0	2,75	5,3
29.62430	30.23825	2023/05/24	13:39:50	14,56	7,13	-7,5	336,4	103	83	9710	52	4,83E-02	13,268	30,0	2,75	4,6
29.62430	30.23825	2023/05/24	13:39:55	14,56	7,16	-9,0	334,7	103	83	9710	52	4,82E-02	13,267	30,0	2,76	6,0
29.62430	30.23825	2023/05/24	13:40:00	14,56	7,14	-8,2	334,8	102	82	9800	51	4,79E-02	13,267	30,1	2,76	6,1
29.62430	30.23824	2023/05/24	13:40:05	14,56	7,16	-9,1	334,7	103	82	9710	51	4,81E-02	13,265	30,0	2,75	6,3
29.62430	30.23824	2023/05/24	13:40:10	14,56	7,17	-9,6	333,7	101	81	9900	51	4,74E-02	13,265	30,1	2,76	5,0
29.62430	30.23824	2023/05/24	13:40:15	14,56	7,13	-7,6	334,7	102	82	9800	51	4,76E-02	13,266	30,0	2,75	4,8
29.62430	30.23824	2023/05/24	13:40:20	14,56	7,15	-8,7	333,8	100	81	10000	50	4,70E-02	13,264	30,1	2,76	5,4
29.62430	30.23824	2023/05/24	13:40:25	14,56	7,17	-9,6	332,6	100	80	10000	50	4,68E-02	13,264	30,1	2,76	5,3
29.62430	30.23824	2023/05/24	13:40:30	14,56	7,16	-9,1	332,8	100	80	10000	50	4,68E-02	13,266	30,1	2,76	5,9
29.62430	30.23824	2023/05/24	13:40:35	14,56	7,15	-8,8	333,4	100	80	10000	50	4,68E-02	13,266	30,1	2,76	5,4
29.62434	30.23823	2023/05/24	13:40:40	14,56	7,17	-9,7	331,9	100	80	10000	50	4,69E-02	13,265	30,1	2,76	6,0
29.62434	30.23823	2023/05/24	13:40:45	14,56	7,16	-9,4	331,4	99	79	10100	49	4,62E-02	13,266	30,1	2,76	5,6
29.62434	30.23823	2023/05/24	13:40:50	14,56	7,16	-9,1	331,2	99	80	10100	50	4,64E-02	13,266	30,1	2,76	5,1
29.62434	30.23823	2023/05/24	13:40:55	14,56	7,19	-11,1	328,9	99	79	10100	49	4,61E-02	13,266	30,1	2,76	5,3
29.62434	30.23823	2023/05/24	13:41:00	14,56	7,21	-11,7	328,9	98	79	10200	49	4,59E-02	13,264	30,0	2,75	5,6
29.62434	30.23824	2023/05/24	13:41:05	14,56	7,20	-11,4	328,8	99	79	10100	49	4,62E-02	13,263	30,0	2,75	5,9
29.62434	30.23824	2023/05/24	13:41:10	14,56	7,19	-10,8	329,4	98	79	10200	49	4,60E-02	13,262	30,0	2,75	5,5
29.62436	30.23824	2023/05/24	13:41:15	14,57	7,19	-10,6	330,1	99	79	10100	49	0,05	13,263	30,0	2,75	5,0
29.62436	30.23823	2023/05/24	13:41:20	14,57	7,20	-11,6	329,2	98	79	10200	49	4,59E-02	13,264	30,0	2,75	5,9
29.62436	30.23823	2023/05/24	13:41:25	14,57	7,20	-11,5	328,7	98	79	10200	49	4,57E-02	13,264	30,0	2,75	5,8

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62436	30.23822	2023/05/24	13:41:30	14,57	7,21	-12,1	328,4	97	78	10310	49	4,55E-02	13,265	30,0	2,75	5,7
29.62436	30.23822	2023/05/24	13:41:35	14,57	7,20	-11,7	327,9	98	78	10200	49	4,56E-02	13,264	30,0	2,75	4,6
29.62436	30.23822	2023/05/24	13:41:40	14,57	7,21	-12,1	327,8	97	78	10310	49	4,55E-02	13,265	30,0	2,75	5,7
29.62436	30.23822	2023/05/24	13:41:45	14,57	7,20	-11,2	328,7	97	78	10310	49	4,54E-02	13,267	30,0	2,75	5,2
29.62436	30.23822	2023/05/24	13:41:50	14,57	7,20	-11,6	328,5	97	78	10310	48	0,05	13,265	30,0	2,75	5,4
29.62436	30.23822	2023/05/24	13:41:55	14,57	7,21	-12,0	328,1	96	77	10420	48	4,49E-02	13,264	30,0	2,75	5,0
29.62436	30.23822	2023/05/24	13:42:00	14,57	7,18	-10,4	329,6	96	77	10420	48	4,49E-02	13,264	30,0	2,75	4,9
29.62436	30.23822	2023/05/24	13:42:05	14,57	7,23	-13,3	326,7	96	77	10420	48	4,48E-02	13,263	30,0	2,75	5,2
29.62434	30.23823	2023/05/24	13:42:10	14,57	7,20	-11,5	328,1	97	77	10310	48	4,51E-02	13,263	30,0	2,75	4,7
29.62434	30.23823	2023/05/24	13:42:15	14,57	7,19	-11,0	329,6	96	77	10420	48	4,47E-02	13,261	30,0	2,75	5,1
29.62434	30.23823	2023/05/24	13:42:20	14,57	7,18	-10,4	329,5	96	77	10420	48	4,47E-02	13,263	30,0	2,75	5,9
29.62434	30.23823	2023/05/24	13:42:25	14,57	7,17	-9,8	329,6	96	77	10420	48	4,48E-02	13,261	30,0	2,75	6,5
29.62434	30.23823	2023/05/24	13:42:30	14,57	7,15	-8,7	332,4	96	77	10420	48	4,46E-02	13,262	30,1	2,76	5,6
29.62434	30.23823	2023/05/24	13:42:35	14,57	7,24	-13,5	326,0	95	76	10530	48	4,44E-02	13,263	30,0	2,75	5,9
29.62434	30.23823	2023/05/24	13:42:40	14,57	7,16	-8,9	329,9	95	76	10530	48	4,44E-02	13,264	30,0	2,75	5,0
29.62434	30.23823	2023/05/24	13:42:45	14,57	7,20	-11,4	327,8	95	76	10530	48	4,44E-02	13,263	30,0	2,75	5,9
29.62434	30.23823	2023/05/24	13:42:50	14,57	7,21	-11,7	327,2	95	76	10530	47	4,42E-02	13,262	30,0	2,75	5,5
29.62434	30.23823	2023/05/24	13:42:55	14,57	7,20	-11,4	327,7	95	76	10530	47	4,42E-02	13,264	30,0	2,75	5,0
29.62434	30.23823	2023/05/24	13:43:00	14,57	7,20	-11,1	328,5	95	76	10530	47	4,42E-02	13,263	30,0	2,75	4,5
29.62434	30.23823	2023/05/24	13:43:05	14,57	7,18	-10,4	328,9	95	76	10530	47	0,04	13,260	30,0	2,75	5,3
29.62434	30.23823	2023/05/24	13:43:10	14,57	7,20	-11,5	327,6	94	76	10640	47	4,40E-02	13,261	30,0	2,75	5,3
29.62434	30.23824	2023/05/24	13:43:15	14,58	7,19	-10,6	328,3	95	76	10530	47	4,41E-02	13,261	29,9	2,74	5,2
29.62434	30.23823	2023/05/24	13:43:20	14,58	7,22	-12,7	325,9	94	75	10640	47	4,39E-02	13,265	30,0	2,75	7,8
29.62434	30.23823	2023/05/24	13:43:25	14,58	7,20	-11,3	327,9	94	76	10640	47	4,40E-02	13,265	30,0	2,75	5,6
29.62434	30.23824	2023/05/24	13:43:30	14,58	7,18	-10,1	329,4	94	75	10640	47	0,04	13,263	30,0	2,75	5,4
29.62434	30.23824	2023/05/24	13:43:35	14,58	7,21	-12,0	327,4	94	75	10640	47	4,37E-02	13,263	30,0	2,75	6,2
29.62434	30.23824	2023/05/24	13:43:40	14,58	7,21	-11,9	327,1	94	75	10640	47	4,39E-02	13,262	30,0	2,75	5,1
29.62434	30.23824	2023/05/24	13:43:45	14,58	7,21	-12,1	327,9	94	75	10640	47	4,37E-02	13,261	30,0	2,75	5,1
29.62436	30.23825	2023/05/24	13:43:50	14,58	7,20	-11,3	327,7	94	75	10640	47	4,37E-02	13,260	30,0	2,75	5,2
29.62436	30.23826	2023/05/24	13:43:55	14,58	7,21	-12,0	327,4	94	75	10640	47	4,36E-02	13,261	30,0	2,75	5,2
29.62436	30.23826	2023/05/24	13:44:00	14,58	7,20	-11,4	328,6	93	75	10750	47	4,35E-02	13,261	30,0	2,75	4,4
29.62436	30.23826	2023/05/24	13:44:05	14,58	7,18	-10,3	328,9	93	75	10750	47	4,36E-02	13,260	30,0	2,75	5,2
29.62436	30.23826	2023/05/24	13:44:10	14,58	7,23	-13,1	325,8	93	75	10750	47	4,36E-02	13,261	30,0	2,75	5,1
29.62436	30.23826	2023/05/24	13:44:15	14,58	7,21	-12,1	326,6	93	75	10750	47	4,35E-02	13,263	30,0	2,75	5,2

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.62434	30.23826	2023/05/24	13:44:20	14,58	7,18	-10,5	328,5	93	75	10750	47	0,04	13,264	30,0	2,75	6,9
29.62434	30.23826	2023/05/24	13:44:25	14,58	7,20	-11,3	327,9	93	75	10750	47	4,34E-02	13,262	30,0	2,75	5,3
29.62434	30.23826	2023/05/24	13:44:30	14,58	7,22	-12,5	326,4	93	75	10750	47	4,34E-02	13,259	30,0	2,74	5,5
29.62434	30.23826	2023/05/24	13:44:35	14,58	7,22	-12,5	327,0	93	75	10750	46	4,33E-02	13,259	30,0	2,74	6,0
29.62434	30.23826	2023/05/24	13:44:40	14,58	7,20	-11,6	326,7	93	74	10750	46	4,33E-02	13,260	30,0	2,75	5,2
29.62436	30.23826	2023/05/24	13:44:45	14,58	7,19	-10,9	328,9	93	75	10750	46	4,33E-02	13,262	30,0	2,75	5,7
29.62436	30.23827	2023/05/24	13:44:50	14,58	7,17	-10,0	329,5	93	74	10750	46	0,04	13,261	30,0	2,75	5,4
29.62436	30.23827	2023/05/24	13:44:55	14,58	7,20	-11,4	326,8	93	75	10750	46	4,33E-02	13,260	30,0	2,75	5,5
29.62436	30.23826	2023/05/24	13:45:00	14,58	7,19	-11,0	327,9	93	74	10750	46	4,32E-02	13,258	30,0	2,75	5,1
29.62436	30.23827	2023/05/24	13:45:05	14,58	7,17	-9,5	328,7	93	74	10750	46	4,32E-02	13,259	30,0	2,75	5,7
29.62436	30.23827	2023/05/24	13:45:10	14,58	7,14	-8,0	331,4	92	74	10870	46	4,31E-02	13,260	30,0	2,75	5,4
29.62436	30.23827	2023/05/24	13:45:15	14,59	7,15	-8,5	330,0	92	74	10870	46	4,31E-02	13,261	30,0	2,75	4,9
29.62436	30.23827	2023/05/24	13:45:20	14,59	7,15	-8,7	330,1	92	74	10870	46	4,30E-02	13,261	30,0	2,75	5,3
29.61847	30.23751	2023/05/24	13:57:02	16,08	6,96	2,4	324,0	98	81	10200	49	4,56E-02	13,227	30,6	2,71	134
29.61847	30.23752	2023/05/24	13:57:08	15,40	6,88	7,0	332,2	98	80	10200	49	4,58E-02	13,228	30,8	2,76	148
29.61847	30.23753	2023/05/24	13:57:18	15,38	6,82	10,2	338,8	98	80	10200	49	4,58E-02	13,228	30,6	2,75	126
29.64752	30.29236	2023/05/24	14:26:00	15,48	6,92	4,7	305,5	39	32	25600	20	1,72E-02	13,614	32,1	2,97	1000
29.64758	30.29239	2023/05/24	14:27:05	15,17	7,04	-2,1	322,3	46	37	21740	23	2,04E-02	13,613	31,6	2,93	1000
29.64754	30.29250	2023/05/24	14:28:12	15,22	7,11	-6,4	312,6	122	100	8200	61	5,75E-02	13,613	31,5	2,93	24,6
29.64752	30.29249	2023/05/24	14:28:28	15,22	7,14	-8,3	311,9	122	100	8200	61	0,06	13,615	31,5	2,93	24,3
29.64754	30.29245	2023/05/24	14:28:42	15,22	7,18	-10,6	309,8	123	100	8130	61	5,76E-02	13,613	31,4	2,92	25,0
29.63130	30.35910	2023/05/24	15:00:46	15,46	7,16	-9,4	280,4	229	188	4367	115	0,11	13,722	31,5	2,93	30,4
29.63124	30.35913	2023/05/24	15:00:53	15,09	7,00	0,1	288,4	232	188	4310	116	0,11	13,718	29,8	2,80	25,3
29.63119	30.35911	2023/05/24	15:01:03	15,07	7,03	-2,0	286,0	232	188	4310	116	0,11	13,719	28,7	2,69	29,6
29.62199	30.37663	2023/05/24	15:24:07	16,74	7,60	-34,5	246,6	240	203	4167	120	0,11	13,718	30,3	2,74	12,0
29.62202	30.37662	2023/05/24	15:24:24	16,86	7,39	-22,6	259,4	240	203	4167	120	0,11	13,718	28,3	2,55	10,8
29.62202	30.37663	2023/05/24	15:24:34	16,81	7,42	-24,2	258,7	240	203	4167	120	0,11	13,722	27,6	2,50	10,7
29.63156	30.36436	2023/05/24	15:39:34	19,64	7,39	-22,5	262,7	388	348	2577	194	0,19	13,716	29,7	2,53	17,0
29.63154	30.36438	2023/05/24	15:39:42	19,57	7,21	-12,4	276,0	390	350	2564	195	0,19	13,715	25,2	2,15	9,8
29.63154	30.36441	2023/05/24	15:39:50	19,56	7,18	-10,7	276,7	390	349	2564	195	0,19	13,716	24,1	2,06	12,1
29.59730	30.43887	2023/05/25	10:18:53	21,80	6,59	23,8	214,8	0	0	999900	0	4,88E-10	13,724	31,9	2,61	0,0
29.59729	30.43888	2023/05/25	10:24:04	23,31	6,36	37,7	219,4	0	0	999900	0	4,75E-10	13,736	31,3	2,49	0,0
29.59690	30.43911	2023/05/25	10:32:02	14,56	7,07	-3,8	226,3	234	188	4274	117	0,11	13,752	30,9	2,94	11,6
29.59687	30.43916	2023/05/25	10:36:35	14,53	7,15	-8,6	242,6	234	188	4274	117	0,11	13,756	29,0	2,76	10,8

GPS Lat. (S)	GPS Long. (E)	Date	Time	Temp. [°C]	pH	mV [pH]	ORP [mV]	EC [µS/cm]	EC Abs. [µS/cm]	RES [Ohm-cm]	TDS [ppm]	Sal. [psu]	Press. [psi]	D.O .[%]	D.O. [ppm]	Turb. FNU
29.59689	30.43911	2023/05/25	10:38:35	16,10	7,15	-8,9	243,7	2	1	500000	1	0,00	13,756	26,6	2,45	141
29.59689	30.43911	2023/05/25	10:38:49	16,19	7,15	-8,4	244,2	1	1	999999	1	0,00	13,757	26,4	2,42	150
29.59689	30.43907	2023/05/25	10:40:13	16,36	7,14	-8,0	246,3	1	1	999999	0	0,00	13,758	27,4	2,51	393
29.59716	30.43885	2023/05/25	10:42:35	18,00	6,96	2,2	245,8	7	6	143000	3	1,82E-03	13,750	30,6	2,70	204
29.59718	30.43883	2023/05/25	10:44:21	18,48	7,11	-6,4	243,8	11	9	91000	5	3,63E-03	13,750	29,7	2,60	54,4
29.59932	30.44309	2023/05/25	11:19:58	28,92	6,70	18,0	263,8	----	----	----	----	----	13,746	27,4	1,97	0,0
29.59931	30.44309	2023/05/25	11:27:24	16,13	7,55	-31,7	241,3	295	245	3390	148	0,14	13,754	28,9	2,66	60,5
29.61823	30.44687	2023/05/25	11:56:30	21,02	7,03	-1,5	274,2	0	0	999999	0	0,00	13,746	33,6	2,80	0,0
29.61825	30.44697	2023/05/25	11:59:45	26,51	6,56	26,2	271,4	0	0	999900	0	6,44E-10	13,744	29,4	2,21	0,0
29.61823	30.44726	2023/05/25	12:01:26	15,34	7,09	-4,9	273,2	273	223	3663	136	0,13	13,755	29,5	2,76	15,4
29.60516	30.48351	2023/05/25	12:34:49	27,34	6,88	7,2	268,8	0	0	999999	0	0,00	13,866	29,3	2,19	112
29.60509	30.48354	2023/05/25	12:35:35	27,95	6,90	6,0	267,4	0	0	999999	0	0,00	13,867	29,1	2,15	104
29.60516	30.48331	2023/05/25	12:39:20	15,42	6,98	1,0	254,9	274	224	3650	137	0,13	13,872	33,4	3,14	9,4
29.60516	30.48333	2023/05/25	12:39:54	15,42	6,97	1,6	266,3	274	225	3650	137	0,13	13,870	32,7	3,08	9,6
29.65116	30.47164	2023/05/25	13:11:14	22,28	6,79	12,2	263,5	0	0	999999	0	0,00	13,747	33,0	2,68	0,0
29.65110	30.47174	2023/05/25	13:14:58	15,87	7,09	-5,3	244,2	125	104	8000	63	5,90E-02	13,732	38,0	3,51	0,0
29.65111	30.47169	2023/05/25	13:16:07	15,95	7,31	-17,9	256,4	10	8	100000	5	3,30E-03	13,733	35,1	3,24	0,0
29.65114	30.47169	2023/05/25	13:17:35	18,24	7,21	-12,1	252,6	6	5	167000	3	1,52E-03	13,731	32,1	2,82	0,0
29.65114	30.47165	2023/05/25	13:34:13	21,04	6,94	3,3	237,6	4	4	250000	2	9,26E-04	13,717	29,8	2,47	0,0
29.65401	30.61563	2023/05/25	14:33:07	30,82	6,64	22,0	243,9	1	1	999999	0	0,00	14,026	29,6	2,10	0,0
29.65437	30.61587	2023/05/25	14:37:02	17,74	7,17	-9,6	242,4	2	1	500000	1	0,00	14,044	34,9	3,18	15,6
29.65907	30.61928	2023/05/25	14:50:47	26,41	7,10	-5,7	243,8	1	1	999999	0	0,00	14,040	30,3	2,33	0,0
29.65907	30.61926	2023/05/25	14:51:53	25,51	6,80	11,7	242,9	1	1	999999	0	0,00	14,040	31,3	2,44	0,0
29.66131	30.63543	2023/05/25	15:24:16	19,33	7,23	-13,1	242,4	1	1	999999	1	0,00	14,076	34,4	3,03	31,7
29.66130	30.63542	2023/05/25	15:25:05	18,27	7,63	-36,4	237,4	374	327	2674	187	0,18	14,077	35,5	3,20	27,4

B2: Laboratory Results for Select Water Quality Parameters (2023 CAMPAIGN)

Table B2 Select Water Quality Parameters

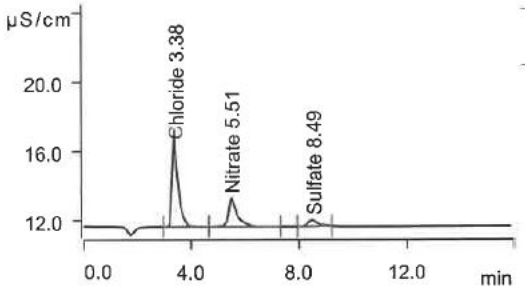
Coordinates			Concentrations in ppm								
Site No	GPS Lat. (S)	GPS Long. (E)	Fluoride	Chloride	Nitrate	Sulfate	Phosphate	Calcium	Iron	Magnesium	Sodium
1	29.64169	30.25631		12,364	7,341	2,341		9,690	9,783	5,806	5,088
2	29.64169	30.23749		11,066	6,032	2,381		4,817	0,031	3,560	7,124
3	29.61837	30.23751		12,959	7,341	5,954		5,060	0,028	3,766	8,035
4	29.64755	30.27233		14,533	6,884	3,349		7,551	0,269	4,773	8,968
5	29.63135	30.35887		35,119	7,946	15,352		12,351	0,682	6,705	19,207
6	29.6226	30.37500		31,324	10,878	11,917		15,213	1,486	8,355	19,614
7	29.631363	30.36443		48,482	16,411	24,07		26,272	0,368	14,717	35,469
8	29.59909	30.44254		30,735	12,822	12,452		14,186	0,127	7,564	19,082
9	29.59725	30.43886		88,096	16,181	40,927	3,093	43,101	6,831	-	57,548
10	29.3549	30.2625		41	15,288	17,491		17,142	0,720	7,624	23,286
11	29.61822	30.44724		37,952	12,094	17,982		15,956	0,418	7,550	22,033
12	29.60502	30.48338		40,28	11,914	17,117		16,374	-	7,763	22,322
13	29.65112	30.47177	0,054	176,862	2,654	31,443		41,875	-	27,139	60,601
14	29.6613	30.63542		69,266	13,472	25,176		16,359	2,157	8,607	23,500
15	29.6588	30.61899	1,152	493,521	22,745	37,442		83,931	0,151	59,284	190,401
16	29.65444	30.61583		45,755	12,255	20,438		15,369	1,605	7,467	20,046
17	29.62342	30.2524		12,317	6,417	2,497		6,913	0,273	4,435	7,520
18	29.62568	30.24701		12,437	6,152	2,67					

B3: SUMMARY OF LABORATORY RESULTS (2022 SAMPLING CAMPAIGN)

Sample data

Ident . . . . . Location 2
Sample type . . . . . Sample
Determination start 2022-06-17 12:18:42

Anions

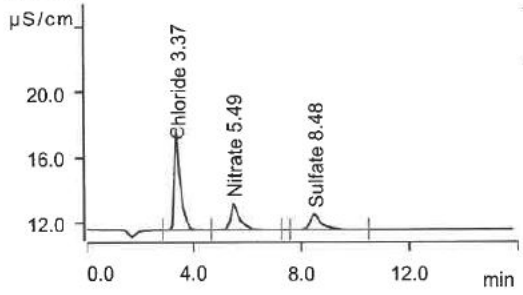


Retention Time	Concentration ppm	Component name
3.38	10.770	Chloride
5.51	12.499	Nitrate
8.49	2.420	Sulfate

Sample data

Ident . . . . . Location 3
Sample type . . . . . Sample
Determination start 2022-06-17 12:37:10

Anions



Retention Time	Concentration ppm	Component name
3.37	12.733	Chloride
5.49	12.772	Nitrate
8.48	6.840	Sulfate

MagIC Net

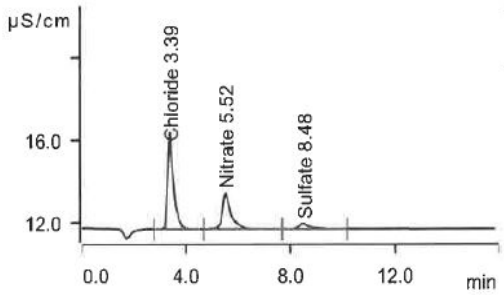
Summary Report

2022-06-22 05:17:52

Sample data

Ident . . . . . Location 1
Sample type . . . . . Sample
Determination start 2022-06-17 12:00:15

Anions



Retention Time	Concentration ppm	Component name
3.39	9.664	Chloride
5.52	13.500	Nitrate
8.48	2.199	Sulfate

APPENDIX C: ANTIBIOTIC RESIDUES IN MSUNDUZI RIVER

C1: Antibiotics Residues in Surface and Wastewater

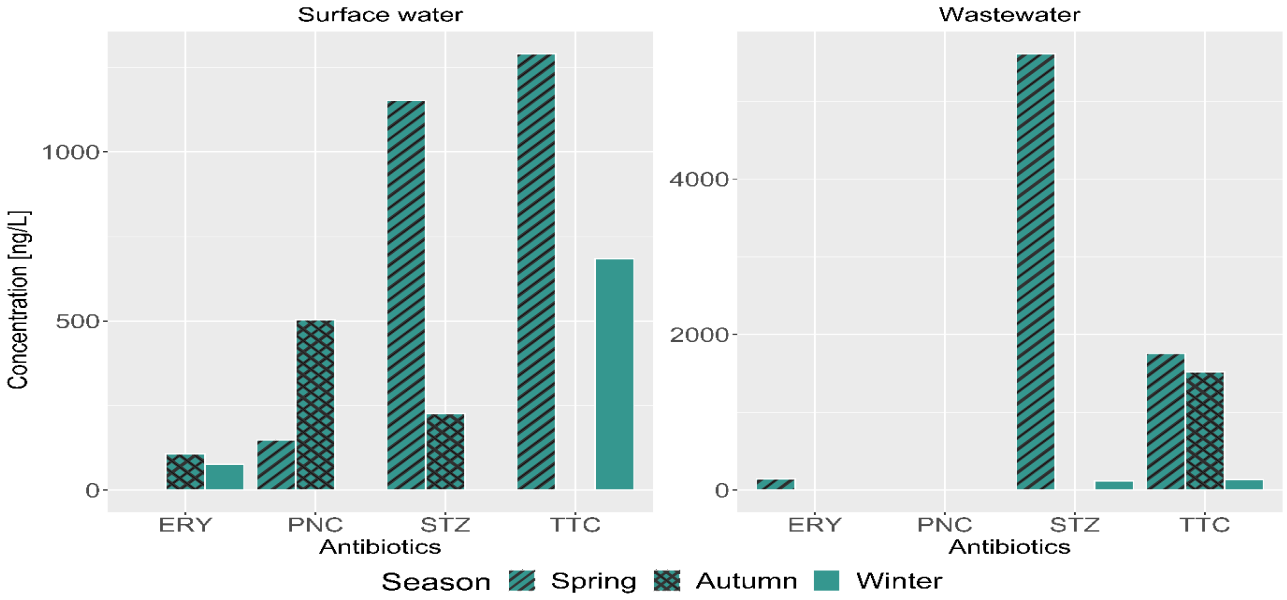


Figure C1 Comparison of antibiotic detection during spring, autumn and winter seasons for wastewater effluent and surface water samples along the Msunduzi River.

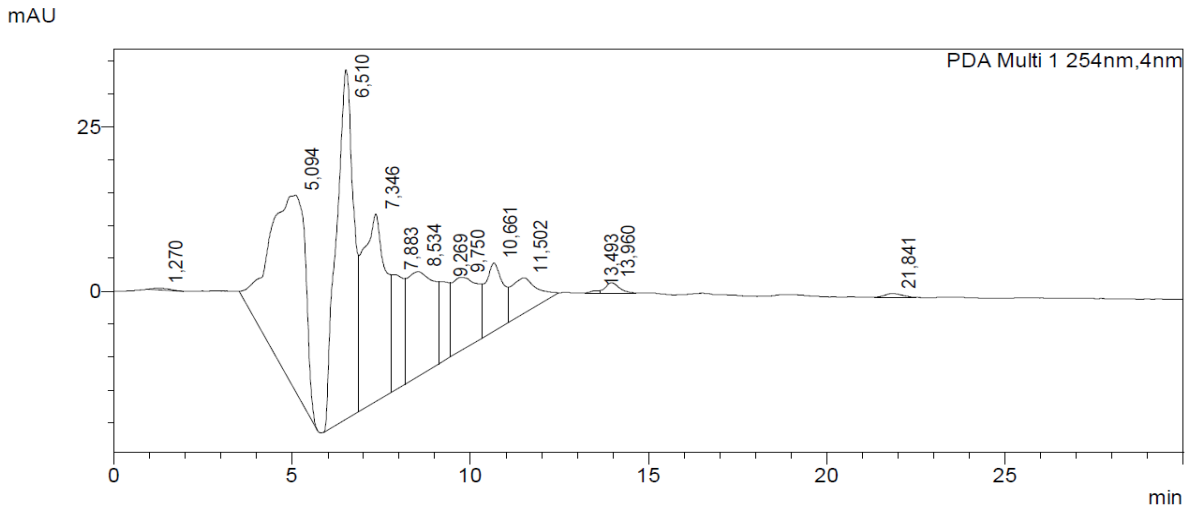


Figure C2 Chromatogram for Sample collected at the inlet of the WWTP in Spring

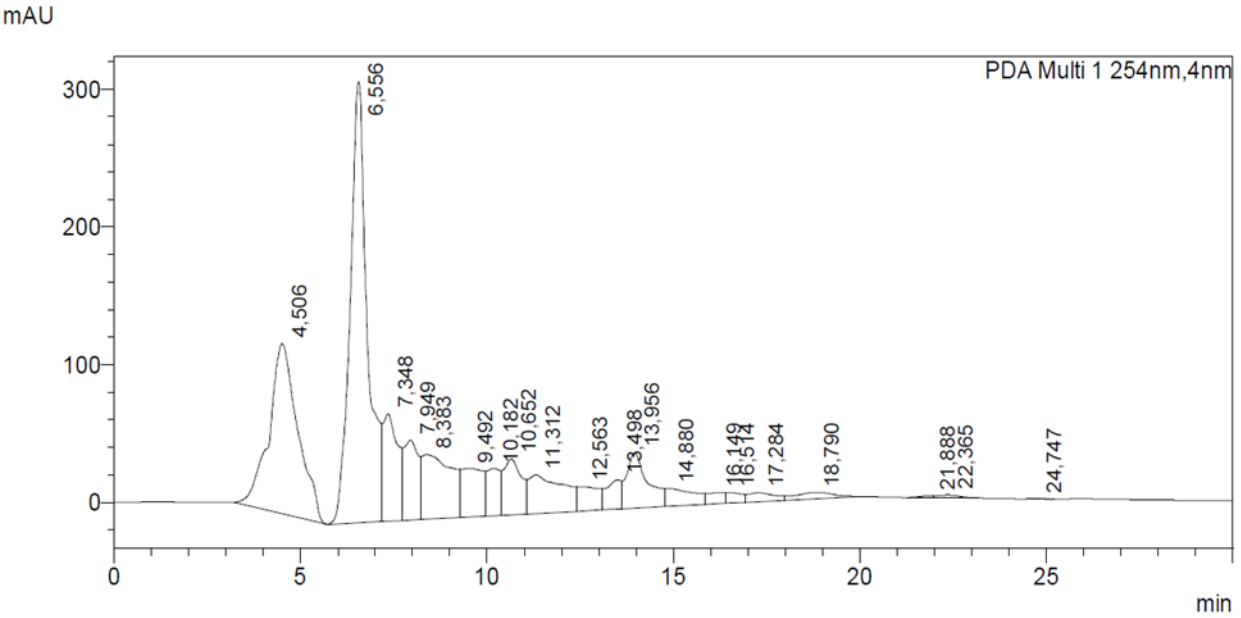


Figure C3 Chromatogram for Sample collected at the Outlet to Primary Settling Tank and Inlet to Aeration Basin of the WWTP in Winter

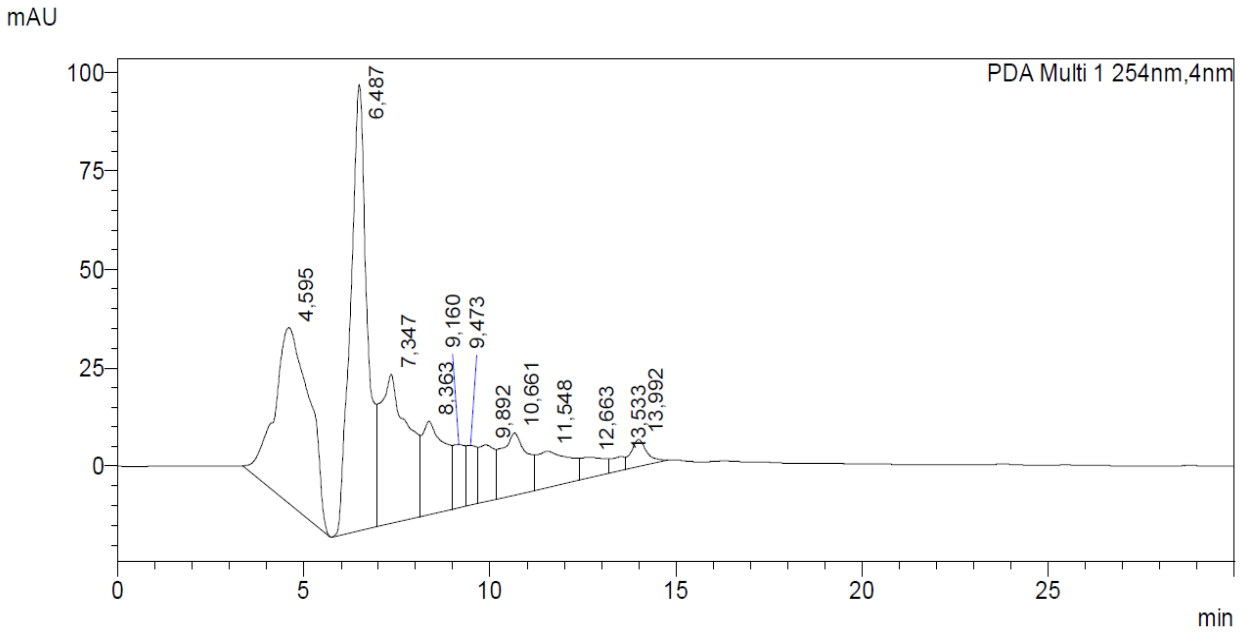


Figure C4 Chromatogram for Sample collected at the Outlet to Primary Settling Tank and Inlet to Aeration Basin of the WWTP in Spring

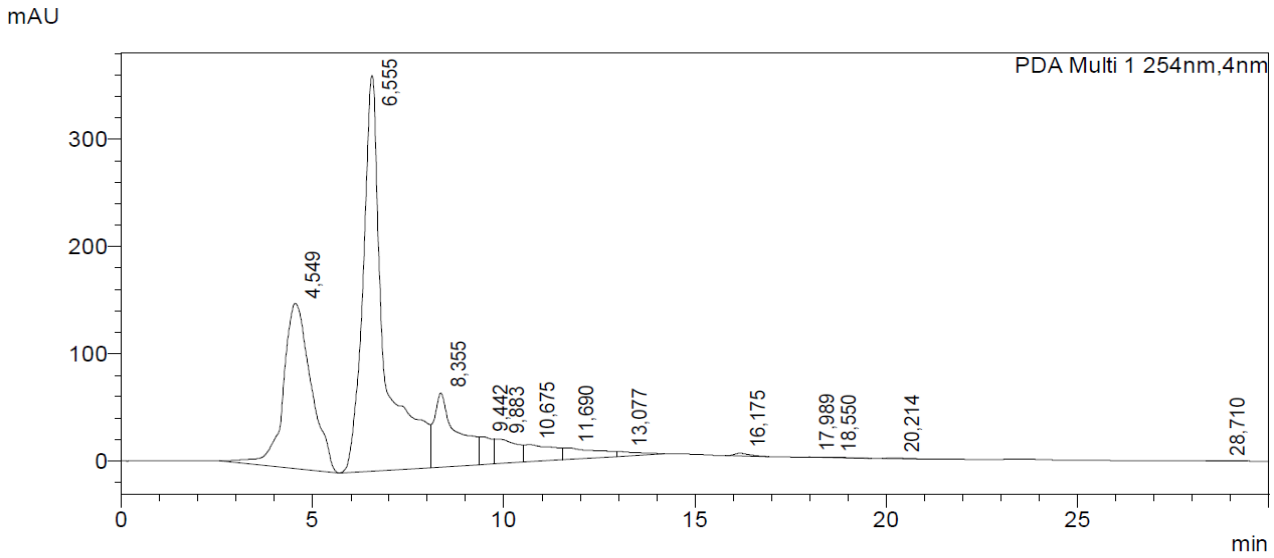


Figure C5 Chromatogram for Sample collected at the WWTP Effluent Point in Winter

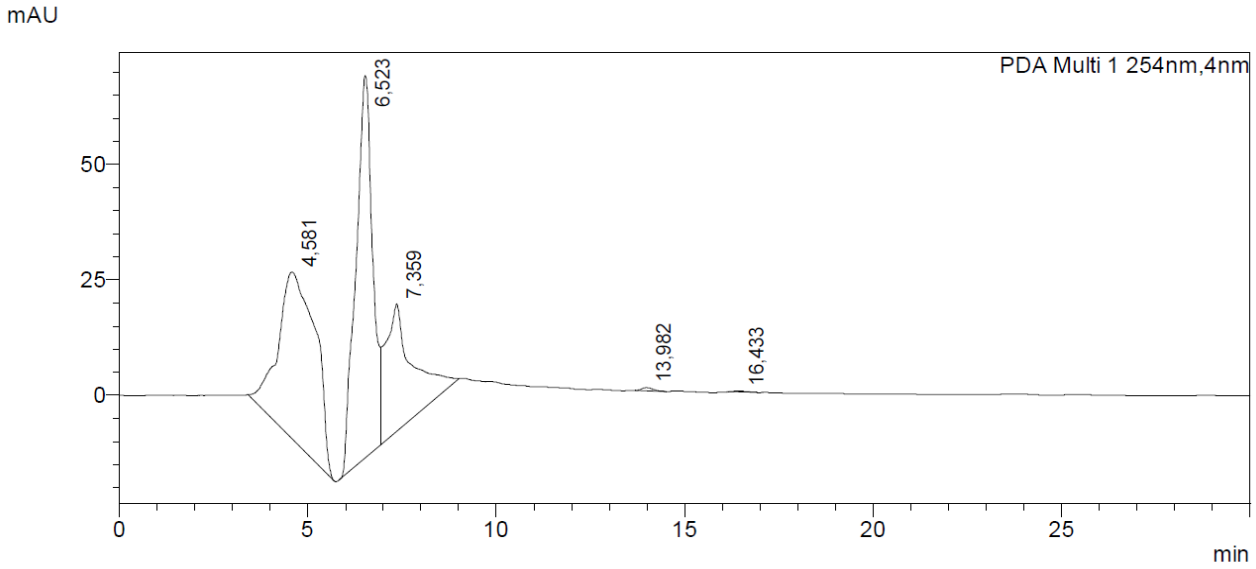


Figure C6 Chromatogram for Sample collected WWTP Effluent Point in Spring.

APPENDIX D: ESTROGENIC ACTIVITY IN MSUNDUZI RIVER

Table D1 AST results for subtypes.

Location	AMP	E10	E30	SU	TE
1-01 A	0	0	0	0	0
1-01 B	0	0	0	0	0,8
1-01 C	3,3	3	3,2	0	2,8
1-01 D	0	0	0	0	0
1-01 AA	1,1	0	0	0	1,4
1-01 AA (2)	0	0	0	0	0,9
1-01 AB	0	0	0	0	0
1-01 AC	0	0	0	0	0
1-01 AD	2	0	0	0	1
1-01 E	3,6	1,5	1,8	2	3,1
2-01 A	3,6	2,6	3	1,8	3,3
2-01 B	2,2	2	2,3	1,3	2,7
2-01 C	0	1,1	1,5	0	3,1
2-01 DD	0	2,7	2,8	0	1,7
2-01 E	2,6	1,9	2,5	1,5	2,6
2-01 EE	1,9	2,1	2,2	0	2,8
5-01 A	0	0	0	0	2,1
5-01 B	1,9	0	0	0	2,3
5-01 D	2,2	0,8	1,1	0	2,8
5-01 E	0	0	0	0	2,8
3-01 A	0	1,3	1,6	0	3
3-01 B	0	0	2,2	0	2,8
3-01 C	1,4	0	0	0	1
3-01 D	0	0	0	0	0
3-01 F	0	2	2,3	1,5	2,6
3-01 H	3,4	1,2	1,5	2,6	3,2

Antibiotics	Resistant	Intermediate	Susceptible
Ampicillin	59	1	48
Tetracycline	11	16	81
Sulphamethoxozole	84	3	21
Erythromycin (10)	48	25	35
Erythromycin (30)	37	25	46

Location	AMP	E10	E30	SU	TE
01-01	0	0	0	0	0
02-01	5,4	3,5	3,9	2,6	4
03-01	2,3	1,8	2,1	1,6	2,6
04-01A	0	0	0	0	0
04-01B	0	1,1	1,7	0	1,5
05-01A	1,6	0	1,8	0	2
05-01B	0	0	0	0	2,1
06-01	0	0	1,3	0	2,4
07-01	0,7	2,5	2,7	0	2,4
08-01	1,1	2,1	2,5	0	2,6
09-01	1,5	2,5	2,5	0	3
10-01	0	2,5	2,9	0	2,3
11-01	3,2	2,3	2,4	1,5	3
12-01	1	2,6	2,9	0	3
13-01	1	2,4	2,8	0	2,9
14-01	0	0	1	0	2,3
15-01	0,8	2,5	0	0	1,6
16-01	1,1	1,7	2,6	0	3
17-01	0	2,1	2,5	0	1,5

Location	AMP	E10	E30	SU	TE
4-01 A	0	0	0	0	2,1
4-01 B	0	0	0	0	2,5
4-01 D	0	0	0	0	2,9
6-01 A	1,6	0	1,1	0	2,4
6-01 B	0	0	1,4	0	2,6
7-01 A	0	0	0	0	2,7
7-01 B	1,1	0	0	0	2,6
8-01 A	3,4	0,8	1,1	0	1,5
8-01 B	3,4	0,8	1,1	0	1,5
8-01 BB	3,1	0,9	1,1	0	1,5
8-01 C	0,7	2,5	2,8	0	2,8
8-01 CC	0,9	2,5	2,8	0	2,8
8-01 F	2,4	2,5	2,5	2,1	3
9-01 A	2,4	2,1	2,4	1,9	3
9-01 B	1,2	2,4	2,6	0	3
9-01 C	0	1,9	2,2	1,7	2,7
9-01 D	1,4	2,5	3	0	3
9-01 E	0	2	2,2	1,7	2,8
10-01 A	0	2,2	2,5	0	2
10-01 B	3,3	1,8	2,2	0	3,5
10-01 BB	3,1	2	2,5	0	3,3
10-01 C	2,9	2,7	3	0	3
10-01 D	3,3	2	2,5	0	3,2
10-01 E	3	1,4	2	0	3
11-01 A	3,3	2	2,6	0	3,1
11-01 B	3,1	2	2,5	0	3
11-01 C	0	0	0	0	2,4
11-01 D	0	0	0	0	2,5
12-01 A	0	0,9	1,1	0	2,5
12-01 B	0	0	0	0	1,5
12-01 C	0	0	0	0	1,6
13-01 A	2,5	2,5	2,7	2,1	3

Antibiotics	**Resistant**	**Intermediate**	**Susceptible**

	Resistant	Intermediate	Susceptible
Ampicillin	14	0	5
Tetracycline	2	3	14
Sulphamethoxozole	16	1	2
Erythromycin (10)	7	4	8
Erythromycin (30)	6	3	8

Location	AMP	E10	E30	SU	TE
13-01 B	2,5	1,9	2,2	1,7	3
13-01 C	0	1,4	1,7	0	3
14-01 A	0	0	1,4	0	2,6
14-01 B	0,8	2,5	2,7	0	2,2
15-01 A	2,5	2,5	2,6	2,1	3
15-01 B	1,1	2,5	2,7	0	1,9
15-01 C	0	1,4	2	0	3,2
16-01 A	1,5	0	0	0	2,3
16-01 B	0	2,1	2,6	0	2,5
17-01 A	3,7	2,8	3,1	0	1,8
17-01 B	1,1	2,5	2,7	0	3
8-01 B	3	0,8	1	0	1,3
13-01 C-B	0	1	1	0	3
13-01 C-C	2,5	1,8	2,1	1,8	2,7
14-01 A-A	2	1,1	1,7	2,8	3,1
14-01 A-B	1	2,5	2,8	0	2,6
15-01 B-B	0,9	2,3	2,5	0	1,6
15-01 B-C	0	1	1,5	0	2,6
15-01 B-X	2,6	2,5	2,8	2,3	3
16-01 C-B	1,6	2,3	2,6	2	2,9
16-01 C-C	3,1	2,7	3	0	1,6
17-01 A-A	1,1	2,3	2,5	0	3
17-01 A-B	3,4	2,8	3	0	1,7
11-01 D	0	0	0	0	2,5
12-01 A	0	0,9	1,1	0	2,5
12-01 B	0	0	0	0	1,5
12-01 C	0	0	0	0	1,6
13-01 A	2,5	2,5	2,7	2,1	3
13-01 B	2,5	1,9	2,2	1,7	3
13-01 C	0	1,4	1,7	0	3
13-01 C-B	0	1	1	0	3
13-01 C-C	2,5	1,8	2,1	1,8	2,7

Antibiotics	R

Location	AMP	E10	E30	SU	TE
14-01 A	0	0	1,4	0	2,6
14-01 A-A	2	1,1	1,7	2,8	3,1
14-01 A-B	1	2,5	2,8	0	2,6
14-01 B	0,8	2,5	2,7	0	2,2
15-01 A	2,5	2,5	2,6	2,1	3
15-01 B	1,1	2,5	2,7	0	1,9
15-01 B-B	0,9	2,3	2,5	0	1,6
15-01 B-C	0	1	1,5	0	2,6
15-01 B-X	2,6	2,5	2,8	2,3	3
15-01 C	0	1,4	2	0	3,2
16-01 A	1,5	0	0	0	2,3
16-01 B	0	2,1	2,6	0	2,5
16-01 C-B	1,6	2,3	2,6	2	2,9
16-01 C-C	3,1	2,7	3	0	1,6
17-01 A	3,7	2,8	3,1	0	1,8
17-01 A-A	1,1	2,3	2,5	0	3
17-01 A-B	3,4	2,8	3	0	1,7
17-01 B	1,1	2,5	2,7	0	3

Antibiotics	R

Table D2 Observed the antibiotic susceptibility test (AST) results

Diameter in Centimetres (LL, IR and TI)					
Location	**AMP**	**E10**	**E30**	**RL**	**TE**
1-01 A	0	0	0	0	0
1-01 B	0.9 LL	0	0	0	0,8
1-01 C	3,3	3	3,2	0	2,8
1-01 D	2,4 LL	0	1,5 LL	1,4 LL	0
1-01 AA	1,1	0	0,9 LL	0	1.4
1-01 AA (2)	1 LL	0	0,8 LL	2,2 LL	0,9
1-01 AB	0	0	0	0	1,8 LL+R
1-01 AC	2,5 LL	0	2 LL	0	4 LL
1-01 AD	2	0	0	0	1
1-01 E	3,6	1,5	1,8	2	3,1
2-01 A	3,6	2,6	3	1,8	3,3
2-01 B	2,2	2	2,3	1,3	2,7
2-01 C	0	1,1	1,5	1,0 LL	3,1
2-01 DD	3,3 IR	2,7	2,8	1,5 LL	1,7
2-01 E	2,6	1,9	2,5	1,5	2,6
2-01 EE	1,9	2,1	2,2	2,0 LL	2,8
5-01 A	0	0	0	0	2,1
5-01 B	1,9	0	0,8 LL	0	2,3
5-01 D	2,2	0,8	1,1	0	2,8
5-01 E	2,0 LL	0	0	1,2 LL	2,8
3-01 A	0	1,3	1,6	1,4 LL	3
3-01 B	2,4 IR	1,9 IR	2,2	1,6 IR	2,8
3-01 C	1,4	0	0	0	1
3-01 D	3,0 IR	2,8 IR	3,0 IR	1,0 LL	1,8 IR
3-01 F	2,4 IR	2	2,3	1,5	2,6
3-01 H	3,4	1,2	1,5	2,6	3,2
4-01 A	0	0	0	0	2,1
4-01 B	1,6 IR	0	1,0 LL	1,4 LL	2,5
4-01 D	0	1,3 IR	1,6 IR	0	2,9
6-01 A	1,6	0	1,1	3,0 IR	2,4

Diameter of zone of inhibition (ZOI)				
Antibiotic	**Disk Conc.**	**Resistant**	**Intermediate**	**Susceptible**
Ampicillin	10mcg	≤11	12-13	≥14
Tetracycline	30mcg	≤14	15-18	≥19
Sulphamethoxozole	25mcg	≤10	11-15	≥16
Erythromycin	10mcg			
Erythromycin	30mcg			
Erythromycin	15mcg	≤13	14-22	≥23

(LL=lawn lightening; IR=inhibition and regrowth; TI=total inhibition)

LL means that the area surrounding the antibiotic disc reduced the growth density of the bacteria but did not inhibit growth.

IR means that bacteria around the disc were inhibited by the antibiotic but grew back overtime.

TI means the bacteria growth was fully inhibited

6-01 B	1,0 LL	0,8 LL	1,4	0	2,6
7-01 A	0,8 LL	2,0 LL	2,5 LL	0	2,7
7-01 B	1,1	2,0 IR	2,2 IR	1,5 LL	2,6
7-01 C	NO BACTERIAL GROWTH				
8-01 A	3,4	0,8	1,1	0	1,5
8-01 B	3,4	0,8	1,1	1,0 LL	1,5
8-01 BB	3,1	0,9	1,1	0	1,5
8-01 C	0,7	2,5	2,8	0	2,8
8-01 CC	0,9	2,5	2,8	0	2,8
8-01 F	2,4	2,5	2,5	2,1	3
9-01 A	2,4	2,1	2,4	1,9	3
9-01 B	1,2	2,4	2,6	0	3
9-01 C	2,3 IR	1,9	2,2	1,7	2,7
9-01 D	1,4	2,5	3	0	3
9-01 E	2,2 IR	2	2,2	1,7	2,8
10-01 A	0	2,2	2,5	0	2
10-01 B	3,3	1,8	2,2	1,2 IR	3,5
10-01 BB	3,1	2	2,5	0	3,3
10-01 C	2,9	2,7	3	0	3
10-01 D	3,3	2	2,5	1,4 IR	3,2
10-01 E	3	1,4	2	0	3
11-01 A	3,3	2	2,6	1,0 IR	3,1
11-01 B	3,1	2	2,5	0	3
11-01 C	0	0	0	0	2,4
11-01 D	1,5 IR	0	0,9 LL	0	2,5
12-01 A	0	0,9	1,1	0	2,5
12-01 B	0	0	1,5 LL	0	1,5
12-01 C	0	0	0	1,5 IR	1,6
13-01 A	2,5	2,5	2,7	2,1	3
13-01 B	2,5	1,9	2,2	1,7	3
13-01 C	0	1,4	1,7	0,9 LL	3
14-01 A	1,9 IR	0	1,4	0	2,6
14-01 B	0,8	2,5	2,7	0	2,2

15-01 A	2,5	2,5	2,6	2,1	3
15-01 B	1,1	2,5	2,7	0	1,9
15-01 C	0	1,4	2	0	3,2
16-01 A	1,5	0	0,9 LL	0	2,3
16-01 B	2,5 LL	2,1	2,6	0	2,5
17-01 A	3,7	2,8	3,1	0	1,8
17-01 B	1,1	2,5	2,7	1,6 LL	3
13-01 C-B	0	1	1	0	3
13-01 C-C	2,5	1,8	2,1	1,8	2,7
14-01 A-A	2	1,1	1,7	2,8	3,1
14-01 A-B	1	2,5	2,8	0	2,6
15-01 B-B	0,9	2,3	2,5	0	1,6
15-01 B-C	0	1	1,5	0	2,6
15-01 B-X	2,6	2,5	2,8	2,3	3
16-01 C-B	1,6	2,3	2,6	2	2,9
16-01 C-C	3,1	2,7	3	0	1,6
17-01 A-A	1,1	2,3	2,5	0	3
17-01 A-B	3,4	2,8	3	0	1,7

APPENDIX E: MODELLING TOOL

E1- Expected Model Output Configurations

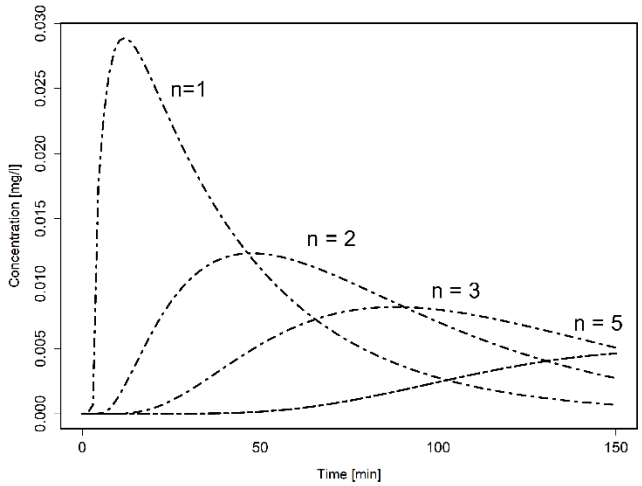


Figure 10 Concentration of antibiotics in the water column in response to unit impulses of developed model simulated at different hybrid units; first (n=1), second (n=2), third (n=3) and fifth (n=5); at x equals 200, 400, 600, and 1000 m respectively from the source.

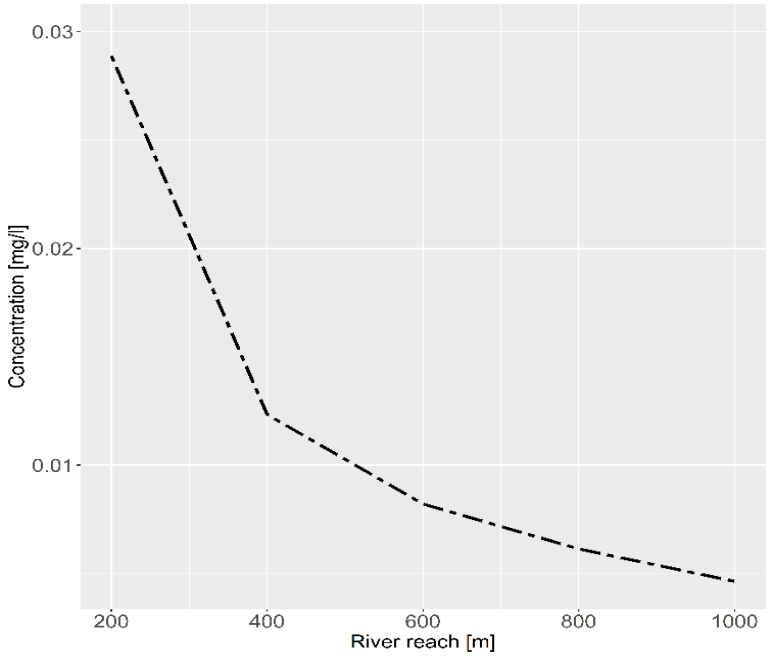


Figure E2 Simulated peak antibiotic concentration along the reach of the stream at distances of 200, 400, 600, 800 and 1000 m from the source.

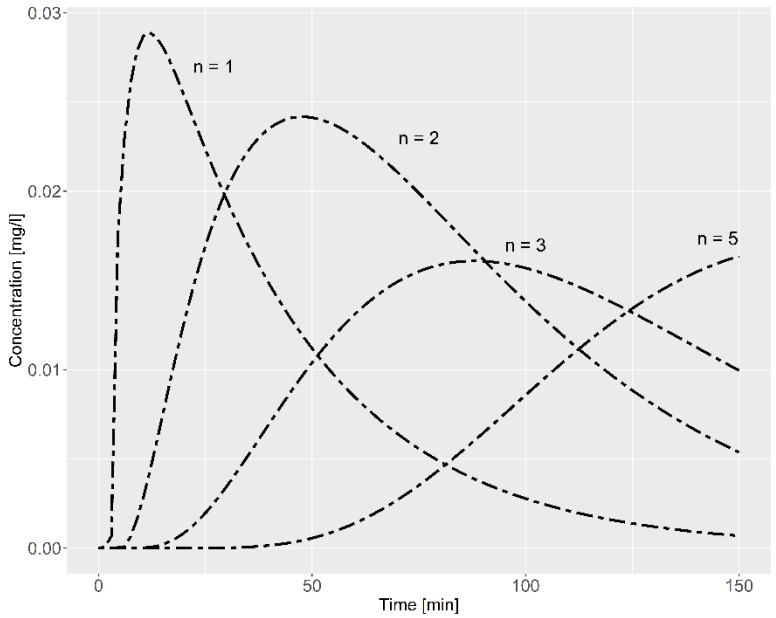


Figure E3 Unit impulse response of the antibiotic model in the water column for multiple external sources (Se = 0.02 mg/l) where external sources join the stream at x = 400m and x = 800m.

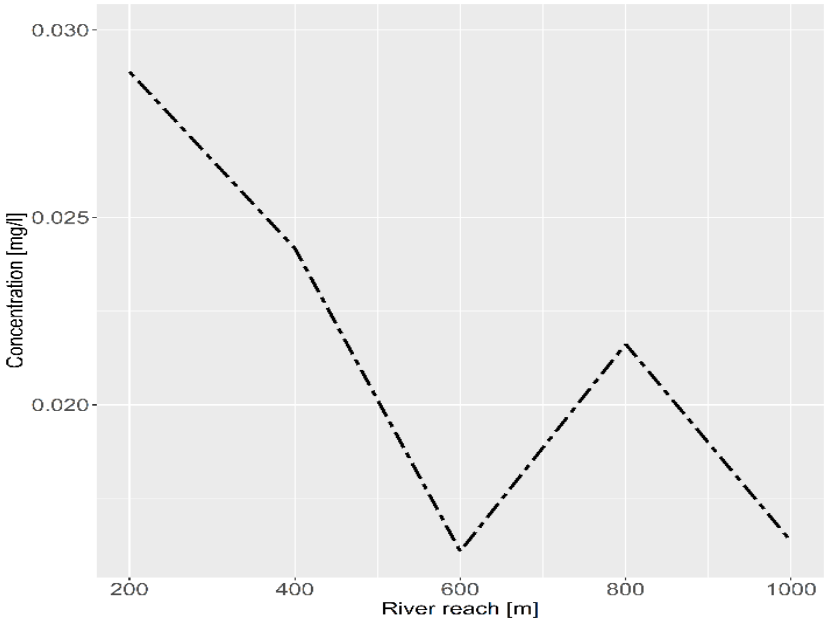


Figure E4 The unit impulse response of the developed model to simulate peak concentrations due to the introduction of multiple external sources along the reach of the stream where external sources join the stream at the second (x = 400m) and fourth (x = 800m) hybrid units.