

**DETECTION AND EFFECTS OF SELECTED PHARMACEUTICAL COMPOUNDS
FROM SELECTED WATER BODIES IN THE EASTERN CAPE PROVINCE,
SOUTH AFRICA**



RHODES UNIVERSITY
Where leaders learn

BY

KOKETSO JOSINAH SETSHEDI

18S5625

DISSERTATION SUBMITTED IN THE FULFILMENT OF THE ACADEMIC
REQUIREMENTS FOR THE AWARD DEGREE OF MASTER OF SCIENCE
PHARMACY IN PHARMACEUTICAL CHEMISTRY

FACULTY OF PHARMACY

RHODES UNIVERSITY

MAKHANDA, 6139

Supervisor: Dr N. Ngqwala

ORCID: <https://orcid.org/0000-0002-8177-378X>

2020

DECLARATION

I, KOKETSO JOSINAH SETSHEDI, proclaim that this thesis is my work. It is due to be submitted for the degree of Master of Science Pharmacy in Pharmaceutical Chemistry at Rhodes University, Makhanda, South Africa. This work has not been submitted before for any award of degree or examination to any other University. This dissertation has no other person's information, diagrams of additional information except explicitly admitted as being cited from other persons'.

Name.....
Signature.....
Date.....

ACKNOWLEDGEMENTS

Allow me to express my sincere gratitude to my supervisor Dr Ngqwala, for her leadership, encouragement, and beneficial critical review of this research piece. I should also like to thank Dr Mutingwende, Dr Gary, Dr Olawole, Dr Teta, and Mr Melamane Siyabonga for their guidance and support by making my progress on the list. I would like to extend my thanks to Mr Adebayo Farounbi, Mr David Gwapedza, Mr Oscar Mogale, Miss Zenande Ngcauzela, and Miss Sesethu Vumazonke for their fieldwork help.

I would also like to extend my thanks to the Lord of Mount Zion for giving me strength in accomplishing this master's degree.

Finally, I would like to express my very most profound appreciation to my parents, siblings, and friends for giving me unwavering support and constant encouragement over the years of the course and through the research process and preparation of the thesis. The completion of this dissertation would have been impossible without them. Special thanks to Mr Moimane Collen for always encouraging me to finish what I have started.

Additionally, I would like to thank the National Research Foundation (NRF) of South Africa for financial support. At this moment, I acknowledge the use of infrastructure provided by the NRF-SAIAB Aquatic Genomics Research Platform and the funding channelled through the NRF-SAIAB Institutional Support System.

Thank you.

DEDICATION

I dedicate this work to my mother, VIOLET MADINGAAN SETSHEDI, father, Dr LEKAU MPHASHA, and siblings LBOGANG, NTHAPE, and ABEL.

ABSTRACT

Water is significantly essential of all-natural assets recognized over the earth. It is necessary to biota, mostly ecosystem, public health, food processing, and economic sustainability. The protection of potable water is crucial for health. Several pollutants that are made up of chemical and microbiological substances affect drinking water. Those pollutants create severe medical challenges, and as a result, the quality of water turns out to be inadequate. Frequently unsatisfactory quality of water induces various health problems in people. Therefore, water quality needs to be tested chemically and microbiologically. This thesis focuses on the prevalence of pharmaceutical products from the rivers (streams) and wastewater treatment plants found along the rivers of Makhanda, Alice, and King William's Town. The physical and chemical parameters were as follows: Temperature ranged from 9.97 to 22.03 °C, pH from 4.32 to 10.60, turbidity from 4.63 to 318 NTU, electrical conductivity from 8.70 to 381.27 mS/m, nitrate from not detected to 100 mg/L, chemical oxygen demand from 5.33 to 296 mg/L, dissolved oxygen from 2.43 to 7.70 mg/L, chloride from not detected to 180.67 mg/L, phosphate from not detected to 0.55 mg/L and sulfate from not detected to 193 mg/L. The results indicate that turbidity, sulfate, and EC adversely affect surface water sources; high concentrations of such parameters verify that further studies are necessary. Hence, some of them do not cause detrimental effects on human health. High levels were principally in rainy seasons, which could have resulted from contaminants' washout from point source pollution into surface water in rains. Other parameters overstepped the acceptable ranges of the Target Water Quality Range, World Health Organisation, and South African National Standard guidelines in some investigation regions. Water samples were freeze-dried to prepare solid-phase extraction and ultra-performance liquid chromatography-tandem mass spectrometer to identify composites. Levonorgestrel and ethinylestradiol were not detected throughout the water samples. Four antiretroviral drugs were detected: Lopinavir ranged from not detected to 1141.8 ng/L, Emtricitabine from not detected to 18757.4 ng/L, Nevirapine from not detected to 10047.2 ng/L, and Efavirenz from 40.6 to 55844.6 ng/L. The bacterial colonies ranged from 1.07E +06 to 9.70E +05 CFU/mL in Mankhanda region, 1.03E +04 to 7.93E +04 CFU/mL in Alice region and 1.08E +05 to 9.33E +05 CFU/mL in King William's Town region. Analytical profile index 20E system identified *Shigella spp* as the most dominant at 81.5% identity. Furthermore, identified *Chromobacterium violaceum*, *Pasteurella pneumotropica*, and *Pneumonia ssp rhinosceromatis* at 97.7 %, 75.1%, and 69% identity. Antibiotic susceptibility testing has shown that Ciprofloxacin was susceptible in all water sample sites except for the lower stream of Tyhume River. Amoxicillin has shown resistance to many parts of the water sample sites. The 16S rRNA (DNA) gene sequencing detected microorganisms in water samples. *Firmicutes* were the most dominant phylum with high abundance in all water samples. Phyla Firmicutes and Bacteroidota have proven that they can survive in wastewater treatment plants. Entirely, this survey reveals that water is potentially unsafe for consumers' health and stresses the need to treat the wastewater treatment plants and support maintainable farming methods for preventing detrimental health damages.

Keywords: Antimicrobial resistance, pharmaceuticals, waterborne diseases, climate change, water quality.

TABLE OF CONTENTS

LIST OF FIGURES.....	ix
LIST OF TABLES.....	xi
LIST OF ACRONYMS AND ABBREVIATIONS.....	xii
UNITS.....	xv
PUBLICATIONS.....	xvi

CHAPTER I

1.1 Background.....	1
1.2 Problem Statement.....	6
1.3 Research Aim.....	7
1.4 Research Objectives.....	7
1.5 Significance of the research.....	7
1.6 Outline of the study.....	8
REFERENCES.....	9

CHAPTER II

ASSESSMENT OF WASTEWATER TREATMENT PLANT AND RIVER WATER QUALITY USING ARTIFICIAL NEURAL NETWORK APPROACH.....	15
Background.....	15
2.1 Natural phenomenon influencing the quality of water.....	15
2.1.1 Water consumption and degradation of water quality.....	16
2.1.2 Water and public health.....	16
2.2.10 Ammonia, Nitrite, and Nitrate.....	21
2.2. The chemical and physical characteristics of water quality.....	17
2.2.1 Chemical Oxygen Demand.....	18
2.2.2 Dissolved Oxygen.....	18
2.2.3 The Electrical Conductivity.....	19
2.2.4 Turbidity.....	19
2.2.5 The pH of the water.....	19
2.2.6 Temperature.....	20
2.2.7 Chloride ions.....	20
2.2.8 Phosphate.....	21
2.2.9 Sulfate.....	21
2.3 Wastewater treatment processes.....	23
2.3.1 Pre-treatment.....	24
2.3.2 Primary treatment.....	24
2.3.3 Secondary treatment.....	24
2.3.4 Tertiary treatment.....	24
2.4 Anthropogenic impacts.....	24
2.4.1 Effects of urbanization activities on water.....	24
2.4.2 Agricultural activities.....	25
2.4.3 Effects of industrial activities on water quality.....	25

2.5 Water is an index of the quality of the river ecosystem.....	25
2.6 Artificial neural network.....	27
2.6.1 Advantages of ANN.....	27
2.6.2 Application area of ANN.....	27
2.7 Essential elements of ANNs.....	27
2.7.1 Input functions.....	28
2.7.2 Output functions.....	28
2.7.2.1 Input layer.....	28
2.7.2.2 Hidden layer.....	28
2.7.2.3 Output layer.....	28
2.7.3 Weights.....	29
2.7.4 Summation function.....	29
2.7.5 Activation function.....	29
2.8 ANN class concerning the structure.....	30
2.8.1 Feedforward network.....	30
2.8.2 Feedback network.....	30
2.9 METHODOLOGY.....	30
2.9.1 THE STUDY REGION.....	30
2.9.2 Approach for data analysis.....	35
2.9.2.1 Sample collection.....	36
2.9.2.2 Physicochemical analysis.....	36
2.9.2.3 ANN.....	37
2.10 RESULTS AND DISCUSSION.....	39
2.10.1 Physicochemical Analyses.....	39
2.10.2 ANN Model structure.....	56
2.11 CONCLUSION.....	63
REFERENCES.....	64

CHAPTER III

EVALUATION OF PHARMACEUTICAL COMPOSITES' (ANTIRETROVIRAL AND CONTRACEPTIVE DRUGS) ECOLOGICAL IMPACT IN RIVER WATER AND WASTEWATER TREATMENT PLANTS.....

3 Background.....	75
3.1 General introduction to pharmaceuticals.....	76
3.2 Class of pharmaceuticals and their occurrence in bodies of water.....	77
3.2.1 Anti-malarial.....	77
3.2.2 Anti-epileptics.....	78
3.2.4 Anti-retroviral.....	78
3.2.5 Antibiotics.....	78
3.2.6 Non-steroidal anti-inflammatory drugs.....	78
3.3 The occurrence of pharmaceuticals.....	79
3.3.1 Personal care products.....	79
3.3.2 Endocrine disruptors.....	79
3.4 Sources of pharmaceuticals.....	79

3.4.1 Source of pharmaceuticals.....	79
3.4.2 Sources of creature pharmaceuticals.....	80
3.4.3 Source of landfills.....	80
3.4.4 Hospitals.....	80
3.4.5 Private houses.....	81
3.4.6 Manufacturers.....	81
3.5 Source control.....	81
3.6 Consequences of pharmaceutical contaminants on the quality of water.....	81
3.7 Impacts of pharmaceutical composites in potable water on the human body.....	82
3.8 The effects of drug combinations.....	82
3.9 Pharmaceutical compounds in the WWTP procedure.....	82
3.10 Antiretroviral metabolites and catch WWTP.....	83
3.11 Removal of pharmaceuticals by the techniques of advanced chemical oxidation.....	83
3.11.1 Ozonation.....	83
3.11.2 Fenton oxidation.....	84
3.11.3 Ultraviolet treatment.....	84
3.11.4 Ionizing irradiation.....	84
3.11.5 Waste management.....	85
3.12 Selected pharmaceutical compounds understudy.....	85
3.13 METHODOLOGY.....	90
3.13.1 Standard preparation.....	90
3.13.2 Extraction of pharmaceuticals.....	90
3.13.3 Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS).....	90
3.13.4 Instruments of analysis and detection facilities.....	91
3.13.5 Statistical analyses.....	92
3.14 RESULTS AND DISCUSSION.....	92
3.14.1 Identification approach.....	94
3.14.2 Qualities of pharmaceuticals.....	95
3.14.3 The existence of ARVs in the upper to downstream.....	96
3.14.4 Concentrations of ARVs in the influent and effluent of WWTPs.....	96
3.14.5 Antiretroviral medications in the streams of water.....	99
3.14.6 Antiretroviral drugs intake.....	99
3.14.7 Remediation of antiretroviral drugs of the environment.....	99
3.14.8. In respect to other surveys.....	100
3.14.9 Health consequences.....	100
3.14.10. The ongoing changes.....	101
3.14.11 Statistical results.....	101
3.15 CONCLUSION.....	103
REFERENCES.....	104

CHAPTER V

THE PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN AQUATIC ENVIRONMENTS (WASTEWATER TREATMENT PLANTS AND RIVER WATER)

4.1 Background.....	113
4.2 General view of Antibiotics.....	117
4.3 The effect of Antibiotic Resistance on public health.....	118
4.4 Components contributing to the occurrence of antibiotic resistance.....	119
4.5 The surrounding and dissemination of resistance.....	119
4.6 The emergence of resistance in species of bacteria usually cause human infections.....	120
4.7 The spread of antibiotic resistance in potable water distribution channels.....	120
4.7.1 Suspended solids.....	120
4.7.2 Loose deposits.....	121
4.7.3 Biofilms.....	121
4.8 Antibiotic mechanisms and the prior existence of antimicrobial resistance genes.....	122
4.9 Antimicrobial resistance co-selection by heavy metals.....	122
4.10 Antimicrobial co-selection by biocidal products.....	123
4.11 Measurement of the concentration of water antibiotic.....	124
4.12 Current Wastewater purification failure.....	124
4.13 Class of antibiotics under the study.....	124
4.13.1 Macrolide antibiotics and mechanism resistance.....	124
4.13.1.1 Erythromycin.....	125
4.13.2 Fluoroquinolone antibiotics and mechanism resistance.....	125
4.13.2.1 Ciprofloxacin.....	126
4.13.3 Beta-lactam antibiotics and mechanism resistance.....	126
4.13.3.1 Amoxicillin.....	126
4.13.4 Tetracycline antibiotics and mechanism resistance.....	126
4.13.4.1 Doxycycline.....	127
4.14 Threats to health and hazard assessment.....	129
4.15 METHODOLOGY.....	129
4.15.1 Preparation of nutrient agar.....	129
4.15.1.1 The procedure of pour plate and spread plate techniques.....	129
4.15.1.2 Colony count technique.....	130
4.15.2 Identification of bacterial isolates.....	130
4.15.2.1 Materials and reagents.....	130
4.15.2.2 Preparation of bacterial suspension in saline tube.....	130
4.15.2.3 Set up of API 20E Biochemical Test Strip.....	130
4.15.3 Antibiotic susceptibility testing using the disk diffusion technique.....	130
4.15.3.1 Mueller-Hinton agar (MHA).....	131
4.15.3.2 Preparation of antibiotic stock solution and antibiotic disks.....	131
4.15.4 Polymerase Chain Reaction (PCR) and gene sequencing.....	131
4.15.4.1 PCR procedural steps.....	131
4.15.4.2 The extraction of DNA, amplification of PCR and 16S rDNA gene	

sequencing.....	132
4.16 RESULTS AND DISCUSSION.....	133
4.16.1 Microbial analysis.....	133
4.16.2 Counting of bacterial colonies.....	134
4.16.3 Identification of bacterial isolates.....	137
4.16.4 Antibiotic susceptibility testing.....	139
4.16.5 Sequencing analysis.....	142
4.17 CONCLUSION.....	152
REFERENCES.....	154
CHAPTER V	
GENERAL CONCLUSION AND RECOMMENDATION.....	167
Appendix A.....	170

LIST OF FIGURES

Figure 2.1: Indication of treatment steps inside a wastewater treatment plant (modified from UNEP, 2012).....	23
Figure 2.2: Diagrammatic representation of an artificial neuron (Oliveira et al., 2017).....	28
Figure 2.3: ANN structure with its layers (Aydin et al., 2014).....	29
Figure 2.4: The map of the Bloukrans River Catchment demonstrating the study region with sampling sites.....	32
Figure 2.5: The map of the Tyhume River Catchment demonstrating the study region with sampling sites.....	33
Figure 2.6: The map of the Buffalo River Catchment demonstrating the study region with sampling sites.....	34
Figure 2.7: Water temperature representing all seasons of sampling.....	45
Figure 2.8: Water samples of pH representing all seasons of sampling.....	46
Figure 2.9: Turbidity of water samples during seasons of sampling.....	48
Figure 2.10: Electrical conductivity of water samples representing all seasons of sampling.....	49
Figure 2.11: Nitrate in water samples representing all seasons of sampling.....	50
Figure 2.12: Sulfate content in water samples representing all seasons of sampling.....	51
Figure 2.13: Phosphate content in water samples representing all seasons of sampling.....	52
Figure 2.14: Chloride content in water samples representing all sites of sampling.....	53
Figure 2.15: Dissolved oxygen in water samples representing all seasons of sampling.....	55
Figure 2.16: Chemical oxygen demand in water samples representing all seasons of sampling.....	56
Figure 3.1: Pharmaceutical compounds in potable water (Mutiyar & Mittal, 2013).....	77
Figure 3.2: The concentration of LPV, FTC, NVP, and EFV in the WWTPs of Alice, Makhanda, and King William's Town regions.....	94
Figure 3.2: The concentration of LPV, FTC, NVP, and EFV in the WWTPs of Alice, Makhanda, and King William's Town regions.....	94
Figure 4.1: Components associated with the occurrence and dissemination of antibiotic resistance. The identification of four broad areas related to the growth of antibiotic resistance: i) Human medicine in society and hospitals, ii) livestock breeding, iii) and agriculture, and iv) the environment's compartments.....	119
Figure 4.2: Transport of antibiotic-resistant bacteria and antibiotic resistance genes in the potable water distribution system (Sanganyado & Gwenzi, 2019).....	122
Figure 4.3: Co-occurrence mechanisms of heavy metal and antibiotic resistance (Barancheshme & Munir, 2018).....	123
Figure 4.4: Bacterial colony count representing all sampling sites of Tyhume, Bloukrans, and Buffalo Rivers and WWTPs found along the riverbanks.....	135

Figure 4.5: Conceptual graphic of the effect of chemistry or water and geographical extent on the community of bacteria streams from the upper stream to downstream (Wang et al., 2019).....	136
Figure 4.6: Abundance of OTUs found in all water samples.....	146
Figure 4.7: NMDS plots of water samples. The NMDS plots are based on bacterial OTUs in the water samples.....	147
Figure 4.8: Bacterial community composition at the phylum level.....	148
Figure 4.9: Bacterial community composition at class level.....	149
Figure 4.10: Bacterial community composition at the family level.....	150
Figure 4.11: Bacterial community composition at the genus level.....	151

LIST OF TABLES

Table 2.1: Processes influencing the quality of water (Fate et al., n.d.).....	15
Table 2.2: Types of parameters of water quality.....	17
Table 2.3: Geographic database of seasonally sampling points of the streams and the full description site.....	31
Table 2.4: Parameters of water quality and analytical technique for the source of water assessment.....	35
Table 2.5: Overall average values of water physical and chemical properties from Tyhume, Buffalo, and Bloukrans Rivers (means \pm standard deviations).....	40
Table 2.6: The average water physicochemical parameters of the Bloukrans River and WWTP in autumn, winter, and spring seasons.....	41
Table 2.7: The average water physicochemical parameters of Buffalo River and WWTP in autumn, winter, and spring seasons.....	42
Table 3.1: Selected pharmaceutical compounds and their CAS numbers, molar masses, molecular structures, pKa, and Log K _{ow} qualities (Kim et al., 2019).....	89
Table 3.2: The instrumental conditions of LC-MS/MS.....	91
Table 3.3: Gradient elution for the aimed analyte of the mobile phase.....	91
Table 3.4: Physicochemical characteristics of individual drugs.....	92
Table 3.5: Pharmaceutical concentrations (ng/L) at different sites determined by Solid Phase Extraction and analysis by UPLC-ESI-MS/MS.....	93
Table 3.6: Optimized MRM parameters of ARVs by UPLC-ESI-MS/MS. Retention time, precursor ion, product-ion, R ² in different matrices (rivers and sewage works). The calibration curve linearity for both analytes was possible with a regression coefficient of both analytes.....	93
Table 3.7: Univariate statistics for the treatment plant of Grahamstown, Alice, and King William's Town regions.....	97
Table 3.8: Univariate statistics for streams of Bloukrans, Buffalo, and Tyhume Rivers.....	97
Table 3.9: Correlation coefficient of ARVDs for the treatment plant of Makhanda, Alice and King William's town.....	102
Table 3.10: Correlation coefficient of ARVDs for streams of Bloukrans, Buffalo, and Tyhume Rivers.....	102
Table 4.1: Antibiotic grouping by their mode of action.....	117
Table 4.2: Selected antibiotic drugs and their properties (Kim et al., 2019).....	128
Table 4.3: Thermal Cycling Parameters for amplification of 16S rDNA.....	132

ACRONYMS AND ABBREVIATIONS

ACN	Acetonitrile
ADH	Arginine/Antidiuretic hormone
AIDS	Acquired Immunodeficiency Syndrome
AMR	Antimicrobial Resistance
ANN	Artificial Neural Network
API	Analytical Profile Index
ARB	Antibiotic-Resistant Bacteria
ARG	Antibiotic-Resistant Genes
AR	Antibiotic Resistance
ARVD	Antiretroviral drugs
AST	Antimicrobial Susceptibility Testing
BEH	Ethylene Bridged Hybrid
BFGS	Broyden-Fletcher-Goldfarb-Shanno
BOD	Biological Oxygen Demand
CAS	Chemical Abstract Service/
CDC	Centre for Disease Control and Prevention
CETP	Common Effluent Treatment Plant
CFU	Colony Forming Unit
CH₃CN	Acetonitrile
CLSI	Clinical and Laboratory Standard Institute
COD	Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity
<i>E. coli</i>	Escherichia coli
EDC	Endocrine Disrupting Chemicals
ECDC	European Centre for Disease Prevention and Control
EDTA	Ethylenediaminetetraacetic Acid
EE	Ethinylestradiol
EFV	Efavirenz
F	Forward
FAO	Food and Agriculture Organization
FeCl₃	Iron Trichloride
FTC	Emtricitabine
GPS	Global Positioning System
HCOOH	Formic acid
H₂O	Water
H₂O₂	Hydrogen Peroxide
H₂S	Na Thiosulphate
HGT	Horizontal Gene Transfer
HIV	Human Immunodeficiency Virus

<i>HLB</i>	Hydrophilic-Lipophilic-Balanced
<i>ICU</i>	Intensive Care Unit
<i>IDSA</i>	Infectious Diseases Society of America
<i>IS</i>	Internal Standard
<i>LC-MS/MS</i>	Liquid Chromatography-Tandem Mass Spectroscopy
<i>LDC</i>	Lysine
<i>LNG</i>	Levonorgestrel
<i>LOD</i>	Limit of Detection
<i>LPV</i>	Lopinavir
<i>LOQ</i>	Limit of Quantification
<i>MeOH</i>	Methanol
<i>MHA</i>	Mueller-Hinton Agar
<i>MIC</i>	Minimum Inhibitory Concentration
<i>MLP</i>	Multi-Layer Perception
<i>MSE</i>	Mean Square Error
<i>MRM</i>	Multiple-Reaction Monitoring
<i>NCCLS</i>	National Committee for Clinical Laboratory Standards
<i>NGS</i>	Next-Generation Sequencing
<i>NMDS</i>	Non-metric Multidimensional Scaling
<i>NNRTI</i>	Non-Nuclear Reverse Transcriptase Inhibitors
<i>NRTI</i>	Nuclear Reverse Transcriptase Inhibitors
<i>NTU</i>	Nephelometric Turbidity Unit
<i>NVP</i>	Nevirapine
<i>NSAID</i>	Non-Steroidal Anti-Inflammatory Drugs
<i>ODC</i>	Ornithine
<i>OTP</i>	Operational Taxonomic Unit
<i>PCoA</i>	Principal Co-ordinate Analysis
<i>PCR</i>	Polymerase Chain Reaction
<i>PK-PD</i>	Pharmacokinetic-Pharmacodynamics
<i>PPCP</i>	Pharmaceutical and Personal Care Products
<i>pH</i>	Potential of Hydrogen
<i>R²</i>	Regression coefficient
<i>R</i>	Reverse
<i>r</i>	Ribosome
<i>RMSE</i>	Root Mean Square Error
<i>RNA</i>	Ribonucleic Acid
<i>SA</i>	South Africa
<i>SAIAB</i>	South African Institute for Aquatic Biodiversity
<i>SANS</i>	South African National Standard
<i>SOP</i>	Standard Operating Procedure
<i>SOS</i>	Self-organization System
<i>SRM</i>	Selected Reaction Monitoring

<i>SPE</i>	Solid Phase Extraction
<i>STP</i>	Sewage Treatment Plant
<i>TAE</i>	Tris-acetate-EDTA
<i>TSS</i>	Total Suspended Solids
<i>USA</i>	United States of America
<i>USEPA</i>	United States Environmental Protection Agency
<i>TWQR</i>	Target Water Quality Range
<i>UNEP</i>	United Nations Environment Programme
<i>UNESCO</i>	United Nations Educational, Scientific and Cultural Organisation
<i>UNICEF</i>	United Nations International Children's Emergency Fund
<i>URE</i>	Urea
<i>UPLC-ESI-MS/MS</i>	Ultra Performance Liquid Chromatography-Electrospray Ionisation-Tandem Mass Spectrometry
<i>UV</i>	Ultraviolet
<i>VBNC</i>	Viable But Non-Cultural
<i>WHO</i>	World Health Organization
<i>WISA</i>	Water Institute of South Africa
<i>WQI</i>	Water Quality Indices
<i>WWTPs</i>	Wastewater Treatment Plants

UNITS

<i>cm</i>	Centimetre
<i>mm</i>	Millimetre
<i>g</i>	Gram
<i>kg</i>	Kilogram
<i>km</i>	Kilometre
<i>ng</i>	Nanogram
<i>L</i>	Litre
<i>Mm³</i>	Million cubic metre
<i>mg</i>	Milligram
<i>Atm</i>	Standard Atmosphere
<i>μg</i>	Microgram
<i>K</i>	Kelvin
<i>S</i>	Svedberg
<i>Mi</i>	Mile
<i>m/z</i>	Mass-to-charge ratio
<i>pKa</i>	Acid Dissociation Constant
<i>K_{ow}</i>	Octanol/ water partition coefficient
<i>mS/m</i>	Millisiemens per metre
<i>μmol</i>	Micromole
<i>μm</i>	Micron/micrometre
<i>kGy</i>	Gray
<i>°C</i>	Degrees Centigrade/ Celsius
<i>°F</i>	Fahrenheit
<i>°</i>	Degree
<i>"</i>	Minutes
<i>'</i>	Seconds

PUBLICATIONS

Paper 1

Submitted paper

1. The use of an Artificial Neural network to predict physicochemical characteristics of water quality in three district municipalities, Eastern Cape Province, South Africa

Authors:

Koketso J Setshedi, Nhamo Mutingwende, and Nosiphiwe P Ngqwala

Journal:

International Journal of Environmental Research and Public Health

Paper 2

To be submitted

2. Evaluation and ecological impact of pharmaceutical residues in river water and wastewater treatment plants. A case study of the Eastern Cape Province, South Africa

Journal name:

Applied Science Journal

CHAPTER I

1.1 BACKGROUND

South Africa (SA) is regarded as a water-scarce nation (Statistics South Africa, 2010a); because of climate and rainfall variations, SA is ranked the 35th, based on its dryness in the world (DWAF, 2010). SA being semi-arid due to limited resources, prevailing high rates of evaporation, and the median rainfall of 450 mm each year (Department of Water Affairs and Forestry, 2004a), in comparison to the earth's median rainfall of 860 mm, the median rainfall of South Africa is low. Furthermore, South Africa shares water with neighbouring countries such as Namibia, Lesotho, Botswana, Mozambique, Zimbabwe, and Swaziland (DWAF, 2004a).

There are specific parameters that can be utilized to examine wastewater treatment plants (WWTPs; Hamada et al., 2018). These parameters are as follows: chemical oxygen demand (COD), total suspended solids (TSS), and biological oxygen demand (BOD) (Türkmenler & Pala, 2017). Up to now, most studies for modelling WWTPs have utilized these variables. The artificial neural network-based models give satisfactory results (Tümer & Edebali, 2015). The BOD, TSS, and COD parameters design and work on the treatment system, inspecting whether the discharge limit of wastewater is satisfactory for the receiving environment are essential parameters utilized to examine the execution of the treatment systems (Türkmenler & Pala, 2017).

Physicochemical properties are parameters that do not recognize specific chemical species; however, they had long been utilized to indicate how water quality could affect water uses (Tadesse et al., 2018). For instance, variations of pH prove specific effluents such as metals, whereas differences in turbidity prove to dredge in the region (Girardi et al., 2016). Color, temperature, and total dissolved solids are supplementary physical characteristics of a river. Changes in water physical chemistry implicate eutrophication, conductivity, and temperature (Rydin et al., 2017).

WWTP is a facility that was planned to separate solids, dissolved organic matter, and nutrients from the wastewater. Wastewater treatment is a crucial innovation that must be taken seriously to improve the community's livelihood and the coming times (Vijayan & Mohan, 2016). Wastewater treatments are classified as physical, chemical, and biological. The Sewage Treatment Plant (STP) and the Common Effluent Treatment Plant (CETP) are some of the main types of processes of wastewater treatments (Vijayan & Mohan, 2016). The sewage works' primary drive is to enable humanity and commercial refuse to be discarded other than posing risks to humans' well-being or unsuitable dangers to the environment (Gutberlet & Uddin, 2017; Mjalli et al., 2007). For that reason, the environmental regulatory bodies set restrictions on effluents to be abided by whichever WWTP (Mjalli et al., 2007).

There is anxiety about poisonous composites' results on wastewater biological treatment

(Rajasulochana & Preethy, 2016; Ries et al., 2007). However, as after the oxidative stress in surgery inducing chemical changes, the toxicity assessment is needed because the oxidation can create more poisonous by-products than their parent composites (Wigh et al., 2018).

The discharge of treated effluents having toxic substances into water bodies can damage aquatic organisms' health and, eventually, the ecological equilibrium (Wigh et al., 2018). Ecotoxicological assessment through the correct bioassays is required to ensure that the released effluents are not toxic (Norberg-King et al., 2018).

In previous years, divergent artificial neural network (ANN) procedures have been put in the application of numerous research on forecasting water resource crises (Oyebode et al., 2019). Kaman et al. (2017) developed artificial neural network models to estimate the sulfate and nitrate in water sources; their results displayed the ANN models' excellent precision. Another study investigated the ANN models' suitability in forecasting water pollution sources in work mentions Ghahavand, Iran (Alizamir & Sobhanardakani, 2017). It has been found that this model can produce satisfactory results. Alizamir and Sobhanardakani (2017) put in an application of artificial neural networks to forecast the concentration of Arsenic, Zinc, and Lead in the underground water resources of Asadabad Plain. The results displayed an artificial neural network's practicality in modelling the heavy metals concentration (Alizamir & Sobhanardakani, 2017). Furthermore, Alizamir and Sobhanardakani (2017) applied two ANN models for calculating the level of heavy metals in the Asadabad plain, and their results have shown better results.

Artificial Neural Network (ANN) is a method of artificial intelligence for the transference of information (data) and processing (Abiodun et al., 2018; He et al., 2018). This adaptive and nonlinear network merges bioscience and computer science (He et al., 2018). In the previous two decades, artificial neural networks have been applied to different areas of environmental matters such as WWTPs (Tümer & Edebali, 2015). The process of wastewater treatment is multiplex (Tümer & Edebali, 2015). Artificial Neural Network is a mathematical modelling tool that helps predict and forecast in multiplex settings, and its primary purpose is to calculate the output from input values through several internal estimations (Türkmenler & Pala, 2017). The ANN model can be utilized for data collection and variable prediction problems. For data classification problems, the artificial neural network uses a specific algorithm to examine information cases for resemblances and isolate them into a pre-defined number of types such as feedback neural network, radial basis function neural network, Kohonen self-organizing neural network, convolutional neural network, and modular neural network (Vyas et al., 2011). For variable prediction problems, the ANN learns precisely to predict the output variable value given sufficient input variable information (Vyas et al., 2011). The increased anxiety about the environment's matters has influenced the experts to focus on legitimate operation and manage the WWTPs (Mjalli et al., 2007). The features of influent to the wastewater treatment plants differ based on living in the communities. Consequently, whichever WWTP's operation relies on a process engineer's local experience who recognizes the plant's specific conditions (Hong et al., 2003; Kehrein et al., 2020).

Human beings and veterinary medicines mainly utilize pharmaceutical compounds to prevent

or cure sicknesses (Mehdi et al., 2018). Medications can be converted in the human body into more polar molecules and soluble forms as conjugates of glucuronic and sulphuric acid or metabolites (Mehdi et al., 2018). Pharmaceuticals arrive at WWTPs via excreta and discarding unemployed or expired medications (Martín et al., 2012). Wastewater from domestic, industrial, and agricultural practices has been reported to cause acute pressure on wastewater treatment to eradicate various organic micro-pollutants from the surface water system. A survey carried out in the USA revealed that these drugs eventually reach the site of the solid waste landfill or WWTPs (Kotchen et al., 2009).

Pharmaceuticals are not eliminated throughout the WWTPs; consequently, they are released into the receiving streams (Martín et al., 2012). The mass balance between the effluent and influent wastewaters (Martín et al., 2012; Zorita et al., 2009) assesses pharmaceutical medicines' eradication in the WWTPs. Biodegradation and sorption to sludge are accountable for eliminating pharmaceutical compounds in WWTPs (Martín et al., 2012). Ozonation has been found to minimize the number of drugs leaving WWTP (Schoeman et al., 2017).

These chemicals' outcomes on aquatic living organisms are not known, mostly when multiplex mixtures are regarded. The verification of pharmaceuticals' influence on the aquatic environment originated from the ingredients utilized in the birth control pill, the synthetic estrogen Ethinylestradiol (EE) (Runnalls et al., 2015). Estrogen Ethinylestradiol is used in birth control pill formulations and hormone emergency treatment (Barr, 2010). Along with estrogen Ethinylestradiol, synthetic progestin such as Levonorgestrel is used as contraceptives, both on their own or in the amalgamation of estrogen Ethinylestradiol (Shapiro et al., 2000). Progestins are mainly accountable for the contraceptive effect in human beings; on the other hand, the estrogens take action on the endometrium. In fish, the hypothalamic-pituitary-gonadal axis disturbance is the initial root of reproductive failure (Li et al., 2011). The estrogen Ethinylestradiol is only taken away, partly, from sewage works; thus, it can be identified in several nations' surface waters, though in low concentration. Levonorgestrel is one of the most used synthetic progestin since its first use and has shown the negative consequences of fathead minnow replica at a low level (Frankel et al., 2017). Levonorgestrel has long ago presented to masculinize female fish at moderately higher concentrations because of its androgenic possessions (Overturf et al., 2014; Runnalls et al., 2015). Levonorgestrel has so far been discovered to exist in the surroundings in the low-level nanogram per cubic decimeter scope due to its slight metabolism and incomplete removal in the course of sewage treatment (Eckert et al., 2012). In France, Levonorgestrel has been identified in wastewater at concentrations of 30 kg/m³, whereas in the surface water at 11 ng/L (Vulliet et al., 2007).

During this recent and increasing group of water micro-pollutants, antiretroviral drugs in surface water and wastewater have been the current study's focal point (Ngumba et al., 2016; Peng et al., 2014; Russo et al., 2018; Wood et al., 2015). Since their establishment in the 90s, antiretroviral drugs have been widespread because of their success in HIV treatment (Russo et al., 2018). Zidovudine and Nevirapine are the most applied as an amalgamation treatment to increase their success in preventing the replication of HIV (Kumari & Singh, 2012). The latest estimates show that seven million people are HIV infected, and of those, half are on medication

(Schoeman et al., 2017). The excretion of drugs differs depending on the compounds, as Nevirapine is excreted at 2.7% via urine (Schoeman et al., 2017) and Tipranavir at 80%. Assuming an average of 30% secretion to sewage through urine and faeces, it is calculated that nearly 380 tons of the ARVD could get into the aquatic system of SA annually (Swanepoel et al., 2015). Nevirapine is likely discovered in WWTP influents. The procedures of activated sludge are utilized in WWTPs because they fabricate effluents that meet the quality standard at sensible costs (Jelic et al., 2011; Schoeman et al., 2017), yet were not planned to separate pharmaceuticals and do not remove them successfully (Gros et al., 2010; Schoeman et al., 2017; WHO, 2011).

In the later years, scientists have noticed that hospital wastewater may be dangerous to people and the environment because of pathogens, laboratories' products, pharmaceutical substances, and research activities (Amouei et al., 2015; Wang et al., 2020). Many of these substances are held in the faeces and urine of sick people and are discharged as non-metabolized remedies in the sewer system (Verlicchi et al., 2012). Several studies on hospital wastewater (HWW) investigated the limited number of pharmaceutical compounds, their destination in the cycle of management of freshwater resources, also upon the surroundings (Carraro et al., 2016; Kalyva, 2017; Le Corre et al., 2012). One or two nations have reference standards and particular treatment processes to control the wastewater (Edokpayi et al., 2017). Nonetheless, there are specific reference standards for manufacturing effluents and the treatment method at regional or community levels by skilled experts regarding the immediate release, the reuse after appropriate treatment, and discharge in a community sewage works (Carraro et al., 2016).

The surface waters in densely populated nations have become the reservoir of antimicrobial-resistant pathogenic microorganisms because of non-selective usage of antimicrobials in both human beings and veterinary medicinal products and the inclusion of faecal pollution via point-and non-point sources as well as storm drain infrastructure (Kraemer, 2019; Meirelles-Pereira et al., 2002). The inclination of species distribution and the commonness of the background level of antimicrobial-resistance is affected by a diversity of living and non-living components, plus communal area and population analysis (Davies & Davies, 2010). In general, the river and its side streams supply 40% of water requirements for different aims, including daily irrigation use and drinking (Loucks & van Beek, 2017). Roughly 0.00000246 Mm³ each day of household sewage refuse and about 4570 million litres every day of raw sewage find a route into the river through its side streams.

In the content of international regulations, the pollution of reservoirs by natural micro-pollutants is the theme of persistent interest and is regularly under inspection (Jung et al., 2014). However, at the origin of many epidemics of gastrointestinal diseases and public health anxiety, the microbes' pollution is infrequently considered (Hlavsa et al., 2011). However, several studies have displayed a constant and vital connection between massive rainfall events and the outbreaks of waterborne disease and climate change outcomes (Jung et al., 2014; Levy et al., 2016).

The microbial pollution of water has been frequently in human beings' faecal nature, such as combined sewage overflow, sewage treatment plants, non-collective sewage systems, or wildlife (Newton & McClary, 2019). The primary sources of the microbial pollution of natural aquatic resources are excreted treatment facilities, cleansing stations, health centres, and industries are regarded as point sources (Kraemer et al., 2019). The plentiful and significance of pathogenic organisms in the water relies on aspects such as the degree of pollution, pathogens' perseverance in reservoirs, physical storages, and the pathogens' capacity to be transported (Harmel et al., 2010).

La-Para et al. (2011) elucidated that antibiotic-resistant bacteria are the main difficulty in medicine today- they are plentiful in sewage that infiltrates municipal wastewater treatment plants. The purpose of the treatment plant is to destroy the microorganisms, and it detaches most of the genes of the bacteria that result in antibiotic resistance (Peterson & Kaur, 2018; Principi et al., 2019). However, the genes may be allowed to move in the wastewater from the plant. In a vigorous attempt to regulate the significance of community sewage works as a source of the genes' antimicrobial resistance, the researchers examined the liberation of that gene (Singer et al., 2016). La-Para and the team stated, "*Even the most high-tech sewage treatment plants may be significant sources of antibiotic resistance genes in waterways*" (La-Para et al., 2011).

The effluents have pollutants from the WWTPs influence the surface water bodies, stormwater flow, and drainage from non-point sources such as arable or agricultural land and the urban city's surfaces (Edokpayi et al., 2017). Notwithstanding the public health significance, antibiotic resistance genes (ARGs), and their appearance in aquatic microbiomes, a small concentration has been given to the research are in SA (Kraemer et al., 2019). The other report from other areas of the world discussed the outcome of ARGs and antibiotic-resistant bacteria in the drainage basin on other water-related applications in African continent nations and few other countries (Anthony et al., 2018).

Over the past years, studies from Canada displayed antibiotics and antimicrobial-resistant bacteria in the water supplies and soil samples (Fernando et al., 2016). That is disturbing since a variety of antimicrobial microorganisms in societies makes the therapy of community-acquired infections difficult. A study by Gwimbi et al. (2016) showed that water samples from communities with no access to drinking water have many bacteria. They do have a water treatment plant; however, the community is provided with piped tap water. Many households do not possess piped tap water from the sewage treatment work; thus, the truck water is used to fill up the sewage treatment work (Luby et al., 2020). Whereas a high bacterial count at the time of water delivery itself creates a danger to health, the existence of antibiotic-resistant bacteria causes the threat to be dangerous (Aslam et al., 2018). Having access to water that is not pure and safe is an issue that is connected with developing countries (Hunter et al., 2010).

The worldwide usage of agents that kill microorganisms in stocks and the food cycle makes up the crucial origin of antimicrobial resistance (AMR; Founou et al., 2016). However, the effect of such use on the health of humans stays contentious (Roca et al., 2015). The enormous

amount of antibiotics was utilized as growth promoters for prevention and the therapy of illnesses among livestock and aquaculture. It is escalating the selective pressure on commensal bacteria and pathogens that can proliferate to human beings via direct connection or through food chains or indirect from the environmental contamination of farm effluents (Liebana et al., 2012). Antimicrobial resistance within the society has been growing throughout the last ten years, predominately considering resistance to quinolones, carbapenems, and third-generation cephalosporin (Munita & Arias, 2016; Roca et al., 2015). Surveillance studies of antibiotic resistance and the utilization of a drug used to treat bacterial infections have focused on this occurrence.

The coincidence of high antibiotic utilization in seriously sick sufferers and a consistent influx of pathogenic species inside the hospital settings promotes resistance growth. It supplies the perfect scenario for the distribution of resistant microscopic organisms and horizontal transmission of resistance genes (Aslam et al., 2018). Antimicrobial utilization displays significant inter-hospital dissimilarities that could be partly elucidated by case-mix and by the divergences in the flux of sick people, conveying resistance microorganisms transported from one hospital facility to another (Berger et al., 2014; Blommaert et al., 2013). The environment is exposed to a broad diversity of antimicrobials via agricultural runoff, wastewater treatment plant effluents, and animal-related and anthropogenic activities (Taruna et al., 2016). Antimicrobials are utilized in animals such as poultry for treatment and disease control and growth promoters (Economou & Gousia, 2015). In environmental settings, interactions between the environment and resistant bacteria supply perfect selective and ecological conditions for the appearance of resistant strains, in the end, contributing to the expansion of ARGs (Fletcher, 2015). The β -lactams are extensively used in the prevention and treatment of bacterial infections caused by susceptible organisms. Numerous β -lactamase enzymes have been found, and their corresponding resistance encoding genes had long been described (Taruna et al., 2016).

The pollution of the environment with the unabated and extensive use of antibiotics through sewage, water, and soil causes a severe threat to patients or diseased animals (Manyi-Loh et al., 2018). Particularly, aquatic environments have been regarded to be a potential reservoir for ARGs because they permit sewage-derived ARGs to continue and proliferate in the background (Taruna et al., 2016). The pollution of such pristine components of the ecosystem with ARGs may build a pleasant environment for their transmission to native microorganisms that may contribute to their human food chain (Chu & Karr, 2017).

1.2 Problem Statement

The water that flows over the ground surface may collect contaminants from the wildlife and soils (Pandey et al., 2014) and often occurs after floods. The disposal of inappropriate products may pollute the groundwater; this problem can result from imperfect septic tanks. The elderly who ingest such water for a long time may develop kidney problems or high blood pressure. Quick expansions of industrialization and urbanization have contributed to the surroundings' direct influence (Siddiquee et al., 2015). Popuri et al. (2008) have highlighted that all

technologies except for adsorption and ion exchange are unproductive and high-priced. Pharmaceuticals are used throughout the entire world, such as antibiotics, contraceptives, and antiretroviral drugs, to mention a few. After intake of pharmaceutical compounds are subjected to metabolism processes and discharged in urine and faeces, succeeding in sewage works. The considerable anxiety is that some other pharmaceutical compounds cannot be eradicated in the treatment course. Consequently, they are noticed in surface water (Castiglioni et al., 2004; Celle-Jeanton et al., 2014; Gašo-Sokač et al., 2017), effluents of sewage treatment plant (Kanama et al., 2018; Gebhardt & Schröder, 2007), rarely in groundwater (Ebele et al., 2017), and potable water in every part of the world.

Fish change their way of living or normal behaviour once exposed to pharmaceutical compounds present in waterways. Residing in environments affected by people may change how a species of fish respond to stress. Contraceptive pills harm sexual development on masculine fish at low concentrations, and intersex fish have been observed at the WWTPs ground level in streams in the entire world (Svensson, 2016).

1.3 Research Aim

The aim of the current study is:

To determine the prevalence and effects of pharmaceutical compounds (Zidovudine, Efavirenz, Levonorgestrel, and Ethinylestradiol) in the rivers (streams) of Makhanda, Alice, and King William's Town and WWTPs.

1.4 Research Objectives

The above aim was accomplished by addressing the following purposes:

1. To determine the physical, chemical, and biological characteristics of the related rivers (streams) and WWTPs found along the riverbanks of Makhanda, Alice, and King William's Town and compare the obtained data to TWQR, SANS, and WHO guidelines.
2. To forecast the physicochemical parameters of the rivers and WWTPs of the municipalities stated in (1) based on the ANN model.
3. To screen the target and non-target pharmaceutical compounds in the WWTPs and related streams using Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS).
4. To determine the spread and effects of antimicrobial resistance in bodies of water.
5. To identify bacterial species isolated from the water samples by using 16S rDNA gene sequencing.

1.5 Significance of the research

Different poisonous gases, particle material, and liquid waste enter the environment day by day. The percentage of literate people is inadequate, and most of the populace is under minimum subsistence income, and they do not have the idea of pollution and its implications. Countless illnesses, which are water-based, arise in some regions of these investigation regions.

As to get the state of water quality of diverse backgrounds, methodological research was carried out in municipalities mentioned above. These three municipalities are major towns; public, commercial, and culture-related activities of some areas depend upon it. River water and wastewater were sampled from specific sites. Hence, the regions under study can examine the state of water body pollution. Water samples were collected in rural, urban, and industrial areas in autumn, winter, and spring. After completing the examination, the parameters were compared with TWQR, WHO, and SANS guidelines. The method of ANN has been employed to predict and forecast data of water quality parameters. Pharmaceutical composites were also analyzed in water samples by employing SPE and LC-MS/MS techniques. ARVs and contraceptives are most used with alarming toxicity levels and prevalence in different environmental areas and have worsened the biosphere. The spread of antibiotic-resistant bacteria and its detrimental impacts have also been incorporated in this study. This research was carried out to inform the inhabitants of the three District Municipalities to be conscious of the WWTPs, and river water's physicochemical parameter status as that would enable them to manage the dangers of their exposure to dangerous chemicals in the water. It would also be beneficial to the municipal management to sensitize society against the utilization of contaminated water and increase the understanding of people on safer use of river water since the number of contaminants overstepped the limits. That is to strengthen reliable water coverage in the region by the municipal management.

1.6 Outline of the study

This thesis is composed of the following chapters:

- Chapter I: provides a general introduction, problem statement, aim, objectives, and significance of the study.
- Chapter II: the assessment of physicochemical characteristics and seasonal variations of surface water quality of Makhanda, Alice, and King William's town municipalities in the Eastern Cape, South Africa.
- Chapter III: the evaluation of pharmaceutical composites' ecological consequences (antiretroviral and contraceptives) from water bodies (river water and WWTP).
- Chapter IV: the occurrence of antibiotic-resistant bacteria in wastewater treatment plants and river water
- Chapter V: general conclusion and recommendation.
- Each chapter is structured with a brief mini introduction, literature review, methodology, results and discussion, and conclusion.

REFERENCES

- Abiodun, O. I., Jantan, A., Omolara, A. E., Dada, K. V., Mohamed, N. A., & Arshad, H. (2018). State-of-the-art in artificial neural network applications: A survey. *Heliyon*, *4*(11), e00938.
- Alizamir, M., & Sobhanardakani, S. (2017). Predicting arsenic and heavy metals contamination in groundwater resources of Ghahavand plain based on an artificial neural network optimized by the imperialist competitive algorithm. *Environmental Health Engineering and Management Journal*, *4*(4), 225-231.
- Amouei, A., Asgharnia, H., Fallah, H., Faraji, H., Barari, R., & Naghipour, D. (2015). Characteristics of effluent wastewater in hospitals of Babol University of Medical Sciences, Babol, Iran. *Health Scope*, *4*(2).
- Anthony, A. A., Adekunle C, F., & Thor A, S. (2018). Residual antibiotics, antibiotic-resistant superbugs and antibiotic resistance genes in surface water catchments: public health impact. *Physics and Chemistry of the Earth*, *105*, 177-183.
- Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M., & Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and drug resistance*, *11*, 1645–1658.
- Barr, N. G. (2010). Managing adverse effects of hormonal contraceptives. *American Family Physician*, *82*(12), 1499-1506.
- Berger, A., Edelsberg, J., Oster, G., Huang, X., & Weber, D. (2014). Patterns of Initial Antibiotic Therapy for Community-Acquired Pneumonia in US Hospitals, 2000 to 2009. *The American Journal of the Medical Sciences*, *347*(5), 347-356.
- Blommaert, A., Marais, C., Hens, N., Coenen, S., Muller, A., Goossens, H., & Beutels, P. (2013). Determinants of between-country differences in ambulatory antibiotic use and antibiotic resistance in Europe: a longitudinal observational study. *Journal of Antimicrobial Chemotherapy*, *69*(2), 535-547.
- Bruyndonckx, R., Hens, N., Aerts, M., Goossens, H., Molenberghs, G., & Coenen, S. (2014). Measuring trends of outpatient antibiotic use in Europe: jointly modelling longitudinal data in defined daily doses and packages. *Journal of Antimicrobial Chemotherapy*, *69*(7), 1981-1986.
- Carraro, E., Bonetta, S., Bertino, C., Lorenzi, E., Bonetta, S., & Gilli, G. (2016). Hospital effluents management: Chemical, physical, microbiological risks, and legislation in different countries. *Journal of Environmental Management*, *168*, 185-199.
- Castiglioni, S., Fanelli, R., Calamari, D., Bagnati, R., & Zuccato, E. (2004). Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy. *Regulatory Toxicology and Pharmacology*, *39*(1), 25-32.
- Celle-Jeanton, H., Schemberg, D., Mohammed, N., Huneau, F., Bertrand, G., Lavastre, V., & Le Coustumer, P. (2014). Evaluation of pharmaceuticals in surface water: Reliability of PECs compared to MECs. *Environment international*, *73*, 10-21.
- Chu, E. W., & Karr, J. R. (2017). Environmental Impact: Concept, Consequences, Measurement. *Reference Module in Life Sciences*, B978-0-12-809633-8.02380-3.

- Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews: MMBR*, 74(3), 417–433.
- DWAF: Department of Water Affairs and Forestry. 2004a. National Water Resource Strategy. Chapter 2. Pretoria, South Africa: Government Printer.
- Ebele, A. J., Abdallah, M. A. E., & Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants*, 3(1), 1-16.
- Eckert, V., Bensmann, H., Zegenhagen, F., Weckenmann, J., & Sörensen, M. (2012). Elimination of hormones in pharmaceutical wastewater. *Pharm. Ind. Pharmind*, 74, 487-492.
- Economou, V., & Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and drug resistance*, 8, 49.
- Edokpayi, J. N., Odiyo, J. O., & Durowoju, O. S. (2017). Impact of wastewater on surface water quality in developing countries: a case study of South Africa. *Water quality*, 401-416.
- Founou, L. L., Founou, R. C., & Essack, S., Y. (2016). Antibiotic Resistance in the Food Chain: a Developing Country-Perspective. *Frontiers in microbiology*, 7, 1881.
- Frankel, T., Yonkos, L., & Frankel, J. (2017). Exposure effects of Levonorgestrel on oogenesis in the fathead minnow (*Pimephales promelas*). *Environmental toxicology and chemistry*, 36(12), 3299-3304.
- Fernando, D., Tun, H., Poole, J., Patidar, R., Li, R., & Mi, R. et al. (2016). Detection of Antibiotic Resistance Genes in Source and Drinking Water Samples from a First Nations Community in Canada. *Applied and Environmental Microbiology*, 82(15), 4767-4775.
- Fletcher, S. (2015). Understanding the contribution of environmental factors in the spread of antimicrobial resistance. *Environmental health and preventive medicine*, 20(4), 243–252.
- Gašo-Sokač, D., Habuda-Stanić, M., Bušić, V., & Zobundžija, D. (2017). The occurrence of pharmaceuticals in surface water. *Croatian journal of food science and technology*, 9(2), 204-210.
- Gebhardt, W., & Schröder, H. (2007). Liquid chromatography–(tandem) mass spectrometry for the follow-up of the elimination of persistent pharmaceuticals during wastewater treatment applying biological wastewater treatment and advanced oxidation. *Journal of Chromatography A*, 1160(1-2), 34-43.
- Girardi, R., Pinheiro, A., Garbossa, L. H. P., & Torres, É. (2016). Water quality change of rivers during rain events in a watershed with different land uses in Southern Brazil. *RBRH*, 21(3), 514-524.
- Gros, M., Petrović, M., Ginebreda, A., & Barceló, D. (2010). Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. *Environment International*, 36(1), 15-26.
- Gutberlet, J., & Uddin, S. M. N. (2017). Household waste and health risks affecting waste pickers and the environment in low-and middle-income countries. *International Journal of occupational and environmental health*, 23(4), 299-310.
- Gwimbi, P., George, M. & Ramphalile, M. (2019). Bacterial contamination of drinking water

- sources in rural villages of Mohale Basin, Lesotho: exposures through neighbourhood sanitation and hygiene practices. *Environmental Health and Preventive Medicine*, 24, 33.
- Hamada, M., Adel Zaqoot, H., & Abu Jreiban, A. (2018). Application of artificial neural networks for the prediction of the Gaza wastewater treatment plant performance-Gaza strip. *Journal of Applied Research in Water and Wastewater*, 5(1), 399-406.
- He, F., Wang, J., & Chen, W. (2018). Cleaner Production Assessment for Wastewater Treatment Plants Based on Backpropagation Artificial Neural Network. *NeuroQuantology*, 16(6).
- Hong, Y. S. T., Rosen, M. R., & Bhamidimarri, R. (2003). Analysis of a municipal wastewater treatment plant using neural network-based pattern analysis. *Water Research*, 37(7), 1608-1618.
- Hunter, P. R., MacDonald, A. M., & Carter, R. C. (2010). Water supply and health. *PLoS medicine*, 7(11), e1000361.
- Jelic, A., Gros, M., Ginebreda, A., Cespedes-Sánchez, R., Ventura, F., Petrovic, M. & Barcelo, D. (2011). occurrence, partition, and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. *Water Research*, 45(3), 1165-1176.
- Kalyva, M. (2017). The fate of pharmaceuticals in the environment-A review.
- Kaman, k. k., Faramarzi, M., Ibrahim, S. & Yunus, M. A. M. (2017). Artificial Neural Network for Non-Intrusive Electrical Energy Monitoring System. *Indonesian Journal of Electrical Engineering and Computer Science*, 6(1), 124 – 131.
- Kanama, K. M., Daso, A. P., Mpenyana-Monyatsi, L., & Coetzee, M. A. (2018). Assessment of pharmaceuticals, personal care products, and hormones in wastewater treatment plants receiving inflows from health facilities in North West province, South Africa. *Journal of toxicology*, 2018.
- Kehrein, P., van Loosdrecht, M., Osseweijer, P., Garfi, M., Dewulf, J., & Posada, J. (2020). A critical review of resource recovery from municipal wastewater treatment plants–market supply potentials, technologies, and bottlenecks. *Environmental Science: Water Research & Technology*, 6(4), 877-910.
- Kotchen, M., Kallaos, J., Wheeler, K., Wong, C., & Zahller, M. (2009). Pharmaceuticals in wastewater: behaviour, preferences, and willingness to pay for a disposal program. *Journal of Environmental Management*, 90(3), 1476-1482.
- Kraemer, S. A., Ramachandran, A., & Perron, G. G. (2019). Antibiotic Pollution in the Environment: From Microbial Ecology to Public Policy. *Microorganisms*, 7(6), 180.
- Kumari, G., & Singh, R. K. (2012). Highly active antiretroviral therapy for the treatment of HIV/AIDS patients: Current status and prospects and the Indian scenario. *HIV & AIDS Review*, 11(1), 5-14.
- LaPara, T. M., Burch, T. R., McNamara, P. J., Tan, D. T., Yan, M., & Eichmiller, J. J. (2011). Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbour. *Environmental science & technology*, 45(22), 9543-9549.
- Le Corre, K., Katouli, M., Stratton, H., Ort, C., & Keller, J. (2012). Hospital Wastewater. Urban Water Security Research Alliance Technical Report No. 76.
- Levy, K., Woster, A. P., Goldstein, R. S., & Carlton, E. J. (2016). Untangling the impacts of

- climate change on waterborne diseases: a systematic review of relationships between diarrheal conditions and temperature, rainfall, flooding, and drought. *Environmental science & technology*, 50(10), 4905-4922.
- Li, Z., Kroll, K. J., Jensen, K. M., Villeneuve, D. L., Ankley, G. T., Brian, J. V. ... & Armstrong, B. (2011). A computational model of the hypothalamic-pituitary-gonadal axis in female fathead minnows (*Pimephales promelas*) exposed to 17 α -ethynylestradiol and 17 β -trenbolone. *BMC systems biology*, 5(1), 63.
- Liebana, E., Carattoli, A., Coque, T., Hasman, H., Magiorakos, A., & Mevius, D. et al. (2012). Public Health Risks of Enterobacterial Isolates Producing Extended-Spectrum -Lactamases or AmpC -Lactamases in Food and Food-Producing Animals: An EU's Perspective of Epidemiology, Analytical Methods, Risk Factors, and Control Options. *Clinical Infectious Diseases*, 56(7), 1030-1037.
- Loucks, D. P., & van Beek, E. (2017). Water resources planning and management: An overview. In *Water Resource Systems Planning and Management* (pp. 1-49). Springer, Cham.
- Luby, S., Davis, J., Brown, R., Gorelick, S., & Wong, T. (2020). Broad approaches to cholera control in Asia: Water, sanitation, and handwashing. *Vaccine*, 38, A110-A117.
- Martín, J., Camacho-Muñoz, D., Santos, J., Aparicio, I., & Alonso, E. (2012). The occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: Removal and Eco-toxicological impact of wastewater discharges and sludge disposal. *Journal of Hazardous Materials*, 239-240, 40-47.
- Mehdi, Y., Létourneau-Montminy, M., Gaucher, M., Chorfi, Y., Suresh, G., & Rouissi, T. et al. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition*, 4(2), 170-178.
- Meirelles-Pereira, F., Pereira, A., Silva, M., Gonçalves, V., Brum, P., & Castro, A. et al. (2002). Ecological aspects of the antimicrobial resistance in bacteria of importance to human infections. *Brazilian Journal of Microbiology*, 33(4).
- Mjalli, F., Al-Asheh, S., & Alfadala, H. (2007). Use of artificial neural network black-box modelling for the prediction of wastewater treatment plants' performance. *Journal of Environmental Management*, 83(3), 329-338.
- Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiology Spectrum*, 4(2).
- Newton, R. J., & McClary, J. S. (2019). The flux and impact of wastewater infrastructure microorganisms on human and ecosystem health. *Current opinion in biotechnology*, 57, 145-150.
- Ngumba, E., Gachanja, A., & Tuhkanen, T. (2016). The occurrence of selected antibiotics and antiretroviral drugs in the Nairobi River Basin, Kenya. *Science of the Total Environment*, 539, 206-213.
- Norberg-King, T. J., Embry, M. R., Belanger, S. E., Braunbeck, T., Butler, J. D., Dorn, P. B., ... & Journal, R. (2018). An international perspective on the tools and concepts for effluent toxicity assessments in the context of animal alternatives: reduction in vertebrate use. *Environmental toxicology and chemistry*, 37(11), 2745-2757.
- Overturf, M. D., Overturf, C. L., Carty, D. R., Hala, D., & Huggett, D. B. (2014). Levonorgestrel exposure to fathead minnows (*Pimephales promelas*) alters survival,

- growth, steroidogenic gene expression, and hormone production. *Aquatic Toxicology*, 148, 152-161.
- Oyebode, O., Babatunde, D. E., Monyei, C. G., & Babatunde, O. M. (2019). Water demand modelling using evolutionary computation techniques: integrating water equity and justice to realize sustainable development goals. *Heliyon*, 5(11), e02796.
- Peterson, E., & Kaur, P. (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Frontiers in microbiology*, 9, 2928.
- Principi, N., Silvestri, E., & Esposito, S. (2019). Advantages and Limitations of Bacteriophages for the Treatment of Bacterial Infections. *Frontiers in pharmacology*, 10, 513.
- Rajasulochana, P., & Preethy, V. (2016). Comparison of the efficiency of various techniques in treating waste and sewage water—A comprehensive review. *Resource-Efficient Technologies*, 2(4), 175-184.
- Roca, I., Akova, M., Baquero, F., Carlet, J., Cavalieri, M., & Coenen, S. et al. (2015). The global threat of antimicrobial resistance: science for intervention. *New Microbes and New Infections*, 6, 22-29.
- Runnalls, T., Beresford, N., Kugathas, S., Margiotta-Casaluci, L., Scholze, M., Scott, A & Sumpter, J. (2015). From single chemicals to mixtures—Reproductive effects of Levonorgestrel and Ethinylestradiol on the fathead minnow. *Aquatic Toxicology*, 169, 152-16.
- Russo, D., Siciliano, A., Guida, M., Andreozzi, R., Reis, N. M., Puma, G. L., & Marotta, R. (2018). Removal of antiretroviral drugs stavudine and zidovudine in water under UV254 and UV254/H₂O₂ processes: Quantum yields, kinetics, and ecotoxicology assessment. *Journal of hazardous materials*, 349, 195-204.
- Rydin, E., Kumblad, L., Wulff, F., & Larsson, P. (2017). Remediation of a eutrophic bay in the Baltic Sea. *Environmental Science & Technology*, 51(8), 4559-4566.
- Schoeman, C., Dlamini, M., & Okonkwo, O. J. (2017). The impact of wastewater treatment works in Southern Gauteng, South Africa, on Efavirenz and Nevirapine discharges into the aquatic environment. *Emerging Contaminants*, 3(2), 95-106.
- Shapiro, S., Rosenberg, L., Hoffman, M., Truter, H., Cooper, D., Rao, S. ... & Bailie, R. (2000). Risk of breast cancer in relation to the use of injectable progestogen contraceptives and combined estrogen/progestogen contraceptives. *American Journal of Epidemiology*, 151(4), 396-403.
- Siddiquee, S., Rovina, K., & Azad, S. (2015). Heavy Metal Contaminants Removal from Wastewater Using the Potential Filamentous Fungi Biomass: A Review. *Journal of Microbial & Biochemical Technology*, 07(06), 384-393.
- Stats SA: Statistics in South Africa. (2010a). Environmental-economic accounts, water management areas in South Africa. *Statistics release D0405.8*. Pretoria, South Africa.
- Svensson, J. (2016). *Progestagenic Aquatic Contaminants Act as Potent Androgens in Fish: Experimental Studies in Three-spined Stickleback and Zebrafish* (Doctoral dissertation, Acta Universitatis Upsaliensis).
- Swanepoel, C., Bouwman, H., Peters, R. & Bezuidenhoudt, C. (2015). Presence, Levels and Potential Implications of HIV Antiretrovirals in Drinking Treated and Natural Waters. *Water Research Commission Report*.

- Tadesse, M., Tsegaye, D., & Girma, G. (2018). Assessment of the level of some physicochemical parameters and heavy metals of the Rebu River in the Oromia region, Ethiopia. *MOJ Biol Med*, 3(4), 99-118.
- Tümer, A.B. & Edebali, S. (2015). An Artificial Neural Network Model for Wastewater Treatment Plant of Konya. *International Journal of Intelligent Systems and Applications in Engineering*, 3(4), 131-135.
- Türkmenler, H., & Pala, M. (2017). Performance Assessment of Advanced Biological Wastewater Treatment Plants Using Artificial Neural Networks.
- Vijayan, A., & Mohan, G. (2016). Prediction of Effluent Treatment Plant Performance in a Dairy Industry Using Artificial Neural Network Technique. *Journal of Civil & Environmental Engineering*, 06(06).
- Verlicchi, P., Al Aukidy, M., Galletti, A., Petrovic, M., & Barceló, D. (2012). Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Science of the Total Environment*, 430, 109-118.
- Vulliet, E., Baugros, J. B., Flament-Waton, M. M., & Grenier-Loustalot, M. F. (2007). Analytical methods for the determination of selected steroid sex hormones and corticosteroids in wastewater. *Analytical and bioanalytical chemistry*, 387(6), 2143.
- Vyas, M., Modherab, B. & Sharma, A., K. (2011). ARTIFICIAL NEURAL NETWORK BASED MODEL IN EFFLUENT TREATMENT PROCESS. *International Journal of Advanced Engineering Technology*, 2(2), 271-275.
- Wang, J., Shen, J., Ye, D., Yan, X., Zhang, Y., Yang, W. ... & Pan, L. (2020). Disinfection technology of hospital wastes and wastewater: Suggestions for disinfection strategy during coronavirus Disease 2019 (COVID-19) pandemic in China. *Environmental Pollution*, 114665.
- Wigh, A., Aït-Aïssa, S., Creusot, N., Terrisse, H., Delignette-Muller, M. L., Bergé, A... & Devaux, A. (2018). Assessment of Ozone or Not-Treated Wastewater Ecotoxicity Using Mechanism-Based and Zebrafish Embryo Bioassays. *Journal of Environmental Protection* 09(04), 325-346
- World Health Organization. (2011). *Guidelines for drinking water quality fourth edition*. World Health Organization.
- World Health Organization Report. (2011). *Pharmaceuticals in drinking water*. World Health Organization.
- Wood, T. P., Basson, A. E., Duvenage, C., & Rohwer, E. R. (2016). The chlorination behaviour and environmental fate of the antiretroviral drug nevirapine in South African surface water. *Water Research*, 104, 349-360.
- Zorita, S., Mårtensson, L., & Mathiasson, L. (2009). Occurrence and removal of pharmaceuticals in a municipal sewage treatment system in the south of Sweden. *Science of the total environment*, 407(8), 2760-2770.

CHAPTER II

ASSESSMENT OF WASTEWATER TREATMENT PLANT AND RIVER WATER QUALITY USING ARTIFICIAL NEURAL NETWORK APPROACH

2. BACKGROUND

2.1 Natural phenomenon influencing the quality of water

Though water quality deterioration is continuously the consequence of human-made activities, specific natural processes may cause water quality to fall under the requirement for particular uses. A naturally occurring phenomenon like heavy rainfall and storms causes immoderate erosion and mudflows because of increasing the content of suspended substances in affected streams and lakes. The seasonal overthrow of water in certain rivers may result in water with almost no dissolved oxygen to the surface (Van Loon, 2015). These natural phenomena can sometimes be standard. Persistent natural circumstances in certain parts can make the water unsuitable for drinking or other purposes. Groundwaters are rich in carbonates, hence requiring treatment before administration for specific industrial uses. Groundwaters in some areas have certain ions and harmful elements in abundance that are health hazards, whereas others have composites that lead to other potential problems (Sharma & Bhattacharya, 2017). The nature and level of chemical agents and composites in freshwater are subject to variations in natural phenomena such as physical, chemical, hydrological, and biological processes. The processes influencing the water quality are mentioned in Table 2.1. Under the effect of these main environmental conditions, the levels of most chemicals in the streams are likely to change seasonally (Hamid et al., 2020). Chemical agents have a high-affinity for particles, and the particles may be separated from the material in solution by filtering water samples (0.45 μm or 0.50 μm filters; Viret & Grand, 2019). However, it is a known fact that fine colloids permeate the filter. The utility of more excellent filters is expensive and demands lengthy filtration times.

Table 2.1: Processes influencing the quality of water (Fate et al., n.d.).

<i>Type of Process</i>	<i>Process in bodies of water</i>	<i>Bodies of water</i>
<i>Physical</i>	Evaporation of dissolved samples	Streams and lakes
	Diffusion	
	Adsorption	All waterways
<i>Chemical</i>	Photo-degradation	
	Reactions of acid-base	All waterways
	Particle dissolution	All waterways
<i>Biological</i>	Primary production	Surface waters
	Microbial fade and expansion	All bodies of water
	Deterioration of organics	Streams and lakes
	Accumulation of substances	Streams and lakes
<i>Hydrological</i>	Dilution	All waterways
	Vaporization	Surface waters
	Percolation and leaching	Groundwaters
	Suspension and settling	Surface waters

2.1.1 Water consumption and degradation of water quality

The development of a society makes a society shift in water usage pattern, usually from agricultural to industrial. The water demand follows the order of drinking and hygiene kits, fishery, livestock water use, irrigation, hydroelectricity, manufacturing, industrial cooling, recreation, and wildlife conservation. Water consumption with the most demanding requirements for quantity frequently has low demands for quality.

Potable water, conversely, demands a high level of quality water, however, relatively small amounts. From old times, water in streams, ponds, and sea have been introduced as favourable wastes receivers (Edokpayi et al., 2017).

The usage of water influences the quality of the aquatic environment. The unsustainable use of water gives rise to water quality degradation, which then restricts its usage. Human activities are the root cause of suspended matter, dissolved, and volatile compounds in water (Manisalidis et al., 2020). Dissolved matter and particulate substances are released into water bodies when particulate substances and volatiles contaminate the environment are collected by rain and deposited in water.

2.1.2 Water and public health

Despite the imperative need for water for life, it could be the transporter of most sicknesses. The unsatisfactory quality of drinking water may seriously affect the consumer's health. Water-based contagious diseases occur when pathogens are present in water and swallowed. Many of the causative agents of diseases are derived from human excreta. Other primary faecal-oral illnesses are diarrhoea, cholera, hepatitis A, and many others.

Other communicable diseases such as typhoid fever, gastroenteritis, and renal disorders spread via contaminated water (Haseena et al., 2017). People may be exposed to various infections such as Dracunculiasis when they ingest contaminated water with microscopic crustaceans (Hlavsa et al., 2014), though it is not a faecal-oral illness.

Water should be made accessible in sufficient amounts to allow bathing, washing, cooking, cleaning, and so forth. The quantities needed for such uses is significantly higher than drinking. The effects of contaminated water on human health show up when a person uses water that contains detrimental amounts of toxicants. The unregulated use of nitrate fertilizers may raise its concentration in the adjacent water bodies. Increased nitrates in water may result in blue baby syndrome and cancer (Bryan et al., 2012; Majumdar, 2003). The fatality rate of cancer is higher in rural than urban communities since rural inhabitants have no treated water facilities but consume raw water (Omarova et al., 2019).

The prolonged period of water utilization containing excessive fluorides brings about fluorosis. WHO (2018) reported that greater than or equal to 2 billion of the world population consumed water contaminated with faeces (WHO, 2018). Understanding the factors that affect the quality of drinking water is a decisive step to take correct decisions on the protection and management of drinking water quality. The water source influences the quality of drinking water, for instance, treatment in WWTP before distribution, water supply networks, and water storage containers (Li & Wu, 2019). Substandard water quality damages agricultural production and contaminates foods; this is harmful to human and aquatic lives (Edokpayi et al., 2017).

Nonetheless, potable water is usually pumped straight from the river or wells in rural communities without being adequately treated. Hence, the source of water contributes to determining the quality of drinking water. Damage of plants and animal foods directly influence public health. Water contaminant kills aquatic algae, molluscs, sea birds, crustaceans, and fishes that give humans food.

2.2. The chemical and physical characteristics of water quality

The quality of water is evaluated by measuring its physical and chemical parameters. Various water quality standards have been established to determine water pollution (Gholizadeh et al., 2016). Water is a chemical compound and has some physicochemical characteristics, including colour, taste, dissolved chemicals, and hydrogen ion concentration (pH). Water is a natural resource; its accessibility makes such a difference to ecological sustainability (UNESCO, 2012). Table 2.2 below shows the types of parameters of water quality.

Table 2.2: Types of parameters of water quality.

<i>Parameters</i>		
<i>Physical parameters</i>	<i>Chemical parameters</i>	<i>Biological parameters</i>
<i>Turbidity</i>	Chloride	Bacteria
<i>Temperature</i>	Sulfate	Algae
<i>Electrical conductivity (EC)</i>	Nitrate	Viruses
<i>Colour</i>	Phosphate	Protozoa
<i>Taste and odour</i>	Dissolved oxygen	
<i>Solids</i>	Chemical oxygen demand (COD)	
	Biochemical oxygen demand (BOD)	
	Toxic inorganic and organic substances	
	Iron and manganese	
	Copper and zinc	
	Acidity and alkalinity	
	pH	

Note: Data for types of parameters of water quality from Omer (2019)

Water quality measurement is grouped into biological, physical, and chemical parameters (Ami' & Tadi', 2018). Physicochemical parameters are an essential component that determines potable water quality. Biological parameters matter more than physical and chemical parameters concerning the direct impact on people's health (Rahmanian et al., 2015). Microorganisms are factors that affect the quality of potable water (Prest et al., 2016). Physical parameters of water are determined by tactile sense, vision, odour, and taste, for instance, temperature, colour, and turbidity (Shah et al., 2017). Chemical parameters of water, for example, nitrate, chemical oxygen demand (COD), dissolved oxygen (DO), phosphate, temperature, turbidity, chloride, Electrical Conductivity (EC), Total Dissolved Solids (TSS), and pH (Popa et al., 2012) are also considered in determining the quality of water. For this study, the following parameters were investigated: COD, DO, sulfate, nitrate, phosphate, temperature, turbidity, chloride, pH, and electrical conductivity.

2.2.1 Chemical Oxygen Demand

Chemical Oxygen Demand is the quantity of oxygen equal to the natural material content of a sample susceptible to oxidation by a potent chemical oxidant (DWAF, 1996c; Shen et al., 2018). The COD is also the amount of oxidation of reduced chemicals in the water; it can be linked empirically to the biochemical oxygen demand (BOD) or organic material. The COD measures the quality of water and wastewater. The COD test is used to monitor and regulate water treatment plants' efficiency (Weiner & Matthews, 2003; Shen et al., 2018). The COD test indicates the amount of dissolved oxygen consumed during the oxidation of organic compounds. The higher the COD value, the greater the presence of organic contaminants in the sample, and *vice versa* (Yao et al., 2014). The high COD concentration indicates many oxidizable organics that usually lower the water's dissolved oxygen level (Aniyikaiye et al., 2019). Hence, a decrease in DO may result in anaerobic states that are detrimental to aquatic life forms. It is expressed in the mass of oxygen consumed over the volume of solution using SI unit mg/L. The COD test is commonly used as an alternative to BOD because of the short length of the test period.

2.2.2 Dissolved Oxygen

Dissolve Oxygen (DO) quantifies the amount of oxygen dissolved in water (Radhakrishnan et al., 2016). DO is essential to water pollution, and its amount is dependent on the pressure, temperature, and water salinity (Schmidt et al., 2018). Therefore, water becomes unpalatable when there is very little or no oxygen. Oxygen solubility and gases decrease when the water temperature rises. Cold rivers hold DO better than warm waters; hence, the higher the DO, the better the water quality (Woodward et al., 2010). Generally, oxygen gas forms approximately 21% of the atmosphere and can easily dissolve in water (Neira et al., 2015). The temperature and salinity have some influence on the ability of water to absorb oxygen. Oxygen tends to diffuse from the atmosphere into the water below the saturation concentration (Neira et al., 2015).

When there is no photosynthesis, particularly between evening and next morning hours, respiration is active in the streams, causing the separation of oxygen (Kemker, 2015). External loading of nutrients and eutrophication can decrease DO (Wang et al., 2014). For instance, some fish and aquatic creatures adjust to the low level of oxygen, while most fish species are affected if DO concentration drops below 3 to 4 mg/L (Radhakrishnan et al., 2016). Therefore, larvae and small fishes are less tolerant of low levels of DO. Devangee et al. (2013) confirmed that when free oxygen is below 2 mg/L in the river, aquatic organisms in such an environment might die. DO indicates the changes transpire in biological parameters because of the aerobic or anaerobic phenomenon and denotes the state of streams with the aim of human and aquatic life (Botheju & Bakke, 2011). In treatment plant processes, DO is added to the aeration lagoon to improve oxidation by giving oxygen to aerobes to convert organic waste materials to inorganic by-products (Malovanyy et al., 2016).

2.2.3 The Electrical Conductivity

Electrical Conductivity (EC) is the measure of a sample of water to transport the electric current (Uwidia & Ukulu, 2013). The Electrical Conductivity unit is $\mu\text{S}/\text{cm}$, and it estimates the level

of dissolved salts in water and soil. Ions in solution carry EC; therefore, the more the ions present in water, the higher the conductivity it becomes in water. In contrast, the fewer ions present in water, water's less conductivity (McCleskey et al., 2012). Conductivity is temperature-dependent; it increases when the water temperature rises. The EC is a fast and accurate method of determining natural and wastewater salinity, and the values are expressed at 25 °C (Uwidia & Ukulu, 2013). A survey has shown that the increased range of pH results from ions (Manyatshe et al., 2016). Amongst the different elements that influence the EC of water, temperature is the primary element. The increase in water temperature will increase the EC since temperature influences salts' solubility in water. A rise of 1 °C may raise EC by 2-3% (Bathiany et al., 2018). It is vital to measure the EC of water since it is directly proportional to the dissolved salts. However, even small temperature changes could lead to significant EC variations. Another element that dictates how EC varies is natural and human impacts (Khatri & Tyagi, 2015). Evaporation and rain are physical factors that influence water EC, whereas agricultural runoff, gritting, and septic leachate may increase the water EC. Discharges to rivers could make changes to the conductivity of water, depending on their structure. A septic system failure would increase the conductivity due to the presence of phosphate, nitrate, chloride, and oil spillage would reduce the conductivity (Bhateria & Jain, 2016).

2.2.4 Turbidity

Turbidity is an indirect measurement of substances in suspension in a sample of water or wastewater. Turbidity measures the light-transmitting properties of water and is a sign of the water's cloudiness or clarity. An increase in turbidity leads to a disturbance of light penetration. Thus, this harms aquatic life and degrades surface water quality (Kjellang et al., 2015; Gupta et al., 2017). Substances in suspension such as clay, organic and inorganic material, and microorganisms cause turbidity in wastewater. Turbidity is still the most pollution indicator used only in waterworks to observe their performance (Cawood & Vos, 2016). Suspended particulate materials can be detrimental to fish gills, lowering their growth rate and affecting their eggs. Water temperature increases when there is high turbidity; consequently, dissolved oxygen concentration can be reduced due to water holding less dissolved oxygen than cold water (Ling et al., 2017). The separation of turbidity by any treatment process is essential for upcoming treatment processes (Haarhoff et al., 2013). A study carried out by Barakat et al. (2016) has shown that the Oum Er Rbia river water had high turbidity of 61107 NTU, which indicates that there was a high degree of pollution (Barakat et al., 2016). The sharp rise of turbidity in surface water reduces the filter runs that provoke disease causing-organisms to become most dangerous to human lives.

2.2.5 The pH of the water

The extreme or extremely low pH can adversely affect water use, and the aquatic organism will die (Kulthanan et al., 2013). Extreme pH makes the water taste bitter and reduces chlorine disinfection efficiency, leading to extra chlorine demand. Water pH influences the solubility and toxicity of heavy metals and chemical substances (Tchounwou et al., 2012). Water pH is also temperature-dependent; as the temperature rises or falls, pH shifts the value. If the temperature increases, the equation moves to the left to reach the equilibrium.

In poorly buffered streams and rivers, the high density of aquatic vegetation may affect the carbonate buffering system attributed to biological processes (Arnold et al., 2017) and cause

high day-to-day variability in pH. The uptake of CO₂ by plants in photosynthesis separates the carbonic acid from the water, giving rise to pH variation (Poschenrieder et al., 2018).

In contrast, the pH level may be lower at night because of the absence of photosynthesis and can lead to a successive release of CO₂ by plants during respiration (Arnold et al., 2017). The fish membrane denatures when the pH higher than nine, debris will not undergo decomposition, and fish eggs will not hatch under this condition. Generally, the best pH range for surface water is six (DWAF, 1996; Kulthanan et al., 2013). The pH of <6.5 stops the production of vitamin and mineral substances in the human system.

In contrast to pH >8.5, water becomes salty and results in red eyes (Kulthanan et al., 2013), and pH >11 causes skin disease (Proksch et al., 2018). The determination of pH has an essential role in the processes of sewage works. Excessive concentrations, the presence of suspended materials, and the accumulation of poisonous substances are commonly encountered in wastewater. Untreated wastewater has a pH of almost seven; however, it may vary between six & eight.

2.2.6 Temperature

Temperature influences palatability, viscosity, solubility, odour, and chemical reactions (Barham et al., 2010). Furthermore, the temperature affects the biological and chemical characteristics of surface water. It influences the DO concentration in water, photosynthesis of water plants, aquatic organisms' metabolism, and sensibility of such microorganisms to pollution. Wastewater temperature ranges from 10 to 20 °C, which is higher than that of the water supply (van den Brand et al., 2015). It occurs because of the addition of warm water and heating in the plumbing system at home (Dallas & Ross-Gillespie, 2015). Effluent water with higher temperatures can affect aquatic life (Bashir et al., 2020). It can lead to a mutation of different fish species in the habitat (Dallas & Ross-Gillespie, 2015; Hamilton et al., 2017). An increased surface temperature could lead to stratification or layering of water bodies. The processes of sedimentation and chlorination depend on temperature. It also influences the process of biosorption of heavy metals.

2.2.7 Chloride ions

Chloride is an anion and is a functional and dependable chemical indicator of the river water (Ali et al., 2014). It also acts as the authoritative disinfectant of wastewater (Kim et al., 2018; WISA, 2002) and water desalination (Al-Abrih et al., 2019). Water regulating agencies use chloride to check waterways' pollution levels and potable water sources (Ali et al., 2014; Omer, 2019). Chloride is always present in sewage and clean water (Bond et al., 2013). Chloride ions increase the EC of water, therefore, increases corrosiveness. In metallic pipes, chloride ions react with metal ions to create soluble salts. As a result, they are increasing the concentration of metals in potable water. Chloride ions may damage metals and can influence the taste of foodstuffs.

Furthermore, it can contaminate streams and lakes. Fish cannot live in water that contains chloride ions above 250 mg/L (Shukla et al., 2018). Chloride ions are not detrimental to human

health; nonetheless, sodium chloride has been associated with renal impairment and cardiovascular disease (Oppelaar & Vog, 2019). Chloride ions occur in nature in lakes, rivers, and streams and enter surface waters via agricultural runoff and wastewater (Strifling, 2018). When more chloride ions are present in water, the higher the conductivity becomes. The presence of inorganic dissolved solids such as sulfate, nitrate, and phosphate ions influences chloride.

2.2.8 Phosphate

Phosphate is a chemical that contains phosphorus (P). P, which is a required nutrient for humans, plants, and animals. It is the primary nutrient for determining the alga bloom phenomenon (Kasan et al., 2016). Human activities aid the unlimited loading of phosphorus in freshwater. The quality of water can be low when bacteria eat up dead algae and use up dissolved oxygen (*e.g.*, asphyxiating fish) (Rebich et al., 2011). The non-point sources of phosphorus are urban runoff, non-agricultural rural runoff, and seepage from wastewater systems (Rebich et al., 2011; Wang et al., 2016). Phosphate is a limiting reagent required for aquatic plants' growth, causing eutrophication (Oberholster & Ashton, 2008). It influences surface water systems such as lakes, rivers, and ponds. The increased phosphate concentration occurs in water that receives sewage, wastes from the animal, and runoff from cultivated lands (Dallas & Day, 2004). Phosphate is significant in determining water quality, as its concentration level serves as a tool to identify and understand the source of phosphate contamination in surface waters (Vadde et al., 2018). Phosphate is a constraint of eutrophication because microorganisms and algae can fix atmospheric nitrogen and change it to oxidizable nitrate and nitrite states (Ngatia et al., 2019). In water, phosphate feeds algae, which damages the way of living and yields toxic substances, and it affects the water quality by causing excessive growth of algae (Weigelhofer et al., 2018). Elevated phosphate concentration of above 0.5 mg/L in drinking water induces muscle strains, respiratory problems, and renal impairment (Nyamangara et al., 2013). The rise in phosphate levels gives rise to eutrophication and reduced DO levels (Schindler et al., 2016). The manageable level of phosphate is 0.1 mg/L.

2.2.9 Sulfate

Sulfate is a salt resulting from the reaction of the formation of sulphuric acid and other chemicals. Sulfate occurs naturally in freshwater, including surface water and groundwater (Khatri & Tyagi, 2015). When sulfate ions concentration in water is high, it can lead to short-term or severe reactions in humans and animals, including diarrhoea and dehydration (Darbi et al., 2003). However, young farm animals and human beings will tolerate sulfate with time, and the symptoms will no longer be visible. The United States Environmental Protection Agency (EPA) recommended a limit of no more than 500 mg/L of sulfate in potable water. The maximum secondary concentration is 250 mg/L (Darbi et al., 2003; Meride & Ayenew, 2016). Generally, the level of sulfate in the surface water is approximately 5 mg/L. A higher concentration of greater than 100 mg/L may be recorded when sulfate-rich effluents from acid mine drainage are discharged into water bodies (Khatri & Tyagi, 2015). For instance, sulfate concentration in seawater is above 900 mg/L (Ayers et al., 2016; DWAF, 1996). Excessive sulfate levels in water result from the leaching of natural deposits of sodium sulfate or

magnesium sulfate. The extreme concentration of sulfate can have a laxative effect on humans and young stockbreeding (Sengupta, 2013).

2.2.10 Ammonia, Nitrite, and Nitrate

Ammonia salts such as nitrite, nitrate, and nitrogen gas are the most common form of nitrogen. Nitrates are compounds that contain nitrogen and oxygen, while nitrites are salt of nitrous acid. However, both are the oxyanions of nitrogen. Under oxidizing conditions, ammonia is converted to nitrite and nitrite to nitrate by nitrifying bacteria. Vapour is a conventional pollutant and is one of the nutrients that cause eutrophication (DWAF, 1996; Gheorghe & Ion, 2011). Nitrates are present in aquatic environments and the soil and tend to increase groundwater concentration, mainly agricultural and urban runoff. Various farming activities enhance nitrate levels in surface and groundwater (Gupta et al., 2017). The rise in nitrate-nitrogen levels in surface water poses challenges; for instance, the water's reduced oxygen levels will adversely affect aquatic life, plants, and algae. Infant methemoglobinemia in humans arises because of the reaction of nitrite and iron in red blood cells. Consequently, it produces methemoglobin that hinders the level of oxygen. Infants suffer the most in cases of ingestion of nitrate-contaminated water (Fewtrell, 2004; Sharma & Bhattacharya, 2017).

2.3 Wastewater treatment processes

The WWTP is essential for eradicating environmental effects (Baharvand & Mansourie, 2019). Physical, chemical, and biological processes may be utilized for treating wastewater. Wastewater may be recycled and reused for different water activities such as farming, combating fires, flushing, fishing, and suchlike (Rashidi & Pandit, 2019; Yang & Abbaspour, 2007). Wastewater harms the ecological system in various forms; for instance, wastewater can have higher nitrates and phosphate nourishments (Saad et al., 2017). When water bodies receive surplus masses of nourishment, it can induce extreme cultivation of plants, releasing toxicants into waterways resulting in loss of oxygen, causing de-oxygenated dead regions (Saad et al., 2017). Water is a fundamental prerequisite for the survival of humanity and oil for economic growth and is essential for supportable economics (Voulvoulis, 2018). Figure 2.1 demonstrates the treatment steps inside a wastewater treatment plant (UNEP, 2012).

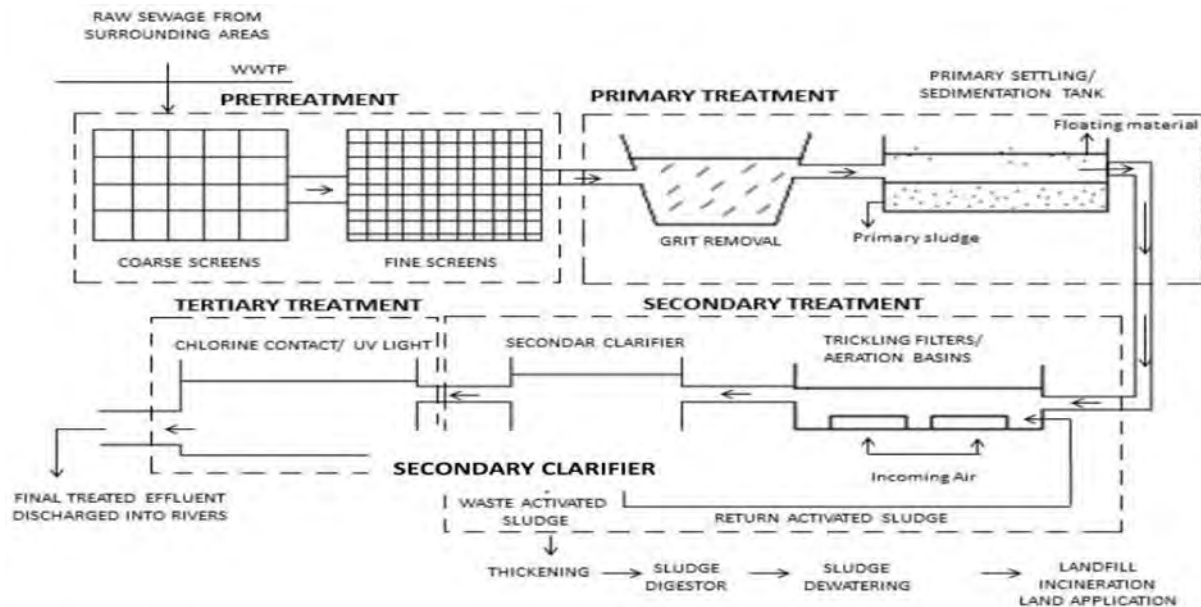


Figure 2.1: Indication of treatment steps inside a wastewater treatment plant (modified from UNEP, 2012).

2.3.1 Pre-treatment

The first step of treatment includes screening to separate the considerable debris that may harm downstream plant apparatus, followed by the separation of grit and silt (Naidoo & Olaniran, 2013). Additionally, the screened constituents are frequently dangerous, and they need to be discarded to ward off the dangerous downstream impacts on the public and the environment (Naidoo & Olaniran, 2013). The incineration of solids before burial is frequently preferred (DWAF, 2011). Excess grit can cause a severe operational problem, affecting a range of succeeding treatment stages, eventually producing a severe pump blockage (Naidoo & Olaniran, 2013). The separation of grit is crucial to keep the mechanical apparatus and pumps safe from abrasion and minimize obstruction (Naidoo & Olaniran, 2013).

2.3.2 Primary treatment

Primary treatment's central objective is to reduce settleable solids, oils, and grit in the wastewater through settling and sedimentation (Naidoo & Olaniran, 2013). All the complicated stages in this primary treatment are entirely mechanical and in the form of sedimentation and filtration (Sonune & Ghate, 2004; Zaharia, 2017). Afterward, the first screening of separating the massive debris, wastewater holds dissolved organic and inorganic materials and suspended solids that are separated through the procedure of primary settling, deposit, chemical coagulation, or percolation (Crini & Lichtfouse, 2019). It enables removing the liquid and solid phases of the sewage by separating those settled natural solid substances and drifting constituents (Naidoo & Olaniran, 2013). Wastewater infiltrates a sedimentation cistern, permitting the sewage to settle in settling tanks designated to carry sewage for some hours (Gill et al., 2018; Naidoo & Olaniran, 2013). Some heavy metals come to the lowermost of the cistern making primary sludge throughout the time, thus minimizing the sewage's suspended matter content (Tytila, 2019).

2.3.3 Secondary treatment

Successive primary treatment, sewage runs within the following step by which the residual suspended solids are rotten, and the microbes' burden are minimized (Naidoo & Olaniran, 2013). Wastewater stabilization ponds are either built exceptionally or comparable with plenty of lakes growing as the capacity of unwanted being processed by the plant rises (Naidoo & Olaniran, 2013). This treatment implicates the biological treatment processes that dissolved organic material from the primary effluent. About 90% of this separation is reached through microorganisms taking in the organic matter in the wastewater as their source of food, mostly utilizing aerobic biological treatment processes (Doorn et al., 2006; Sonune & Ghate, 2004). Secondary treatment occurs in fixed or suspended growth reactors operating variants of activated sludge, rotating biological contactors, and constructed wetland processes (Samer, 2015; US EPA, 1997).

2.3.4 Tertiary treatment

The tertiary treatment step involves separating nutrients and carbon adsorption to separate chemicals (Samer, 2015). This treatment includes the advanced separation of dissolved and suspended solids and the separation of nutrients (Englande Jr et al., 2015). Utilizing processes such as polishing ponds, biological processes, improved filtration, carbon adsorption, and ion exchange (Doorn et al., 2006; Samer, 2015) can reach the treatment.

2.4 Anthropogenic impacts

Agriculture, urbanization, and industrialization are human activities that resulted in eutrophication (Varioli et al., 2005). The household activities also lead to synthetic organic chemicals to excrete wastewater, run-off from the agricultural field, run-off from urban, leachate from polluted soils, and such organic pollutants implicate pesticides and solvents compounds formerly in use (Ritter, 2010).

2.4.1 Effects of urbanization activities on water

Urbanization involves constructing buildings, roadways, and walkways, which leads to an increase of impermeable surfaces that speed up the run-off and the pollution of receiving reservoirs (Yang & Zhang, 2011). Just as urbanization occurs, soils are enwrapped by increasing the number of impervious surfaces like car parks, roadways, walkways, and rooftops, minimising rainwater's ability to penetrate the soil turn into stormwater run-off. Many harmful effects on receiving waters from the flow of urban implicate physical, physicochemical, and biological effects such as floods, deposit, temperature, and reduced biodiversity (Marsalek et al., 2002). Existing urbanization has contributed to a severe change in the volume and quality of stormwater run-off in the current years. Urbanization increases flood run-off just as it makes watertight surfaces, sewage construction, and the storm drains speedup run-off (Youpeng et al., 2010). The drainage of stormwater has harmful substances that cause pollution, such as dangerous chemicals, metals, and nutrients that speed up eutrophication (Crasti, 2016). The rapid development of urban centres also boosts water contamination since erosions carry gasoline, motor oil, grass chemicals, and other contaminants from run-off, grass, and routes, which ultimately travel in enormous, concentrated amounts, thereby contaminating sources of water for instance, waterways.

2.4.2 Agricultural activities

Agricultural activities, such as irrigation, fertilizers, and livestock farming, have a marked effect on water quality. Soil disturbance travelling with drainage and forest ploughing can produce enormous sediments to invade the river, resulting in increased turbidity (Nisbet, 2001; Schoonover & Crim, 2015). Inadequate farming methods, coupled with imprudent utilization of fertilizers such as ammonium phosphate, can lead to runoffs, which may escalate phosphorus concentrations and ammonium in the receiving waters (Xavier de Sousa et al., 2015). Enforcement to increase agricultural systems' efficiency to meet household demand has contributed to the escalation of agriculture, making use of land and depending on pesticides and fertilizers (Shabalala & Combrink, 2012). Wastes released from livestock grazing contain high levels of nitrogen, phosphorus, and microorganisms (Otte et al., 2019; Shabalala & Combrink, 2012). Human faecal wastes constitute health problems if allowed to reach water bodies (Reeves et al., 2004).

2.4.3 Effects of industrial activities on water quality

Industrial water contamination is due to the discharge of poisonous chemicals and composites in water, making it unfit for consumption and other purposes (Chakraborti et al., 2018). Periodically waste substance implicates the extraction residues of used nutrient solutions, hazardous materials, and other organics (Anderson et al., 2013). Such wastewater is coloured, and the pH value is either high or low. The tannery's wastewater has an acid pH, with chloride content (Roy et al., 2015).

Furthermore, the tannery wastewater, if not well treated, will adversely affect the receiving water. The wastewater released from the brewery industry was generated from the beer wort preparation and fermentation (Mathias et al., 2015), washing and cleansing of machines and filters (Bokulich & Bamforth, 2013). Such wastewater possesses elevated levels of suspended solids and soaps.

2.5 Water is an index of the quality of the river ecosystem

Water environments have many environmental variables affecting them; hence, ecologists have designed various parameters for assessing the overall health condition of aquatic ecosystems. That is one of the leading causes of having numerous Water Quality Indices (WQI) for different local, regional, and international settings (Hamlet et al., 2017). In South Africa, as part of the River Health Programme, numerous indices have been implemented to control and evaluate aquatic ecosystems.

The overall conditions of the ecosystem depend on combining these indices. However, the combination of the broad array of different indicators, for instance, plants and animals, can be problematic because populations of organisms in ecosystems are affected to varying levels by community climax, disease, parasitism, and competition (Boon et al., 2013). Because of the variety of aquatic ecosystems wherein biotic and abiotic factors exist, it is difficult to evaluate an indicator representing overall health conditions. An excellent ecological indicator must measure the magnitude of stress, level of exposure to the pressure, or environmental response to the exposure (Lu et al., 2015). The indicator must include a series of variables that provide

a general idea of the ecosystem status. Therefore, water quality may be the right indicator to measure the river ecosystem's overall health since variables as turbidity and chemical concentrations are easily measured (Dzwairo et al., 2015).

Safe water is essential for the development of human resources, and typically, it is an element necessary to human production and a valuable economic sustainability tool. It has a dominant role in people's social well-being and health (RadFard et al., 2019). The quality of water depends on its composition and could be influenced by natural occurrences or human-induced (Heidarinejad et al., 2018). Groundwater bodies are vital sources of freshwater that supply water for numerous applications, such as consumption, farming, and manufacturing (Qasemi et al., 2018). Any parameter that could influence water quality, the survival of organisms, recreations, and other utilities, will affect managerial decision-making (Cavalcante et al., 2013).

An artificial neural network (ANN) is a computing system animated by studies of the brain and nervous system. ANN carries out perfect mathematical complex systems. One of its good qualities is learning capability. This process is called training the neural network (Wang, 2015). This training controls itself in developing internal sets of characteristics that it uses to categorize data.

In contrast to other methods, the ANN model allows insufficient information and sacrificial results and reduces vulnerability to outliers (Dawood et al., 2016). In the last twentieth anniversary, ANN has maintained unstable growth in most research fields (Messikh et al., 2007). The ANN is employed to investigate the water quality of river basins, especially for planning and to manage to forecast the parameters of water quality (Sengorur et al., 2015), in conformity with microbiological water-quality parameters to assess the source of contamination.

ANN application in the fields of water engineering and environmental sciences has been informed from the early 1990s. In the past few years, and have already been used to predict and forecast several water resource areas, mainly aquatic resource study, marine science, and ecological sciences (Liong et al., 1999). The utilization of data-driven methods for modelling freshwater and seawater quality has been successful in the last decades. In North Carolina, a survey studied Bayesian probability network models for directing the policy-making process about water quality in the Neuse River (Reckhow, 1999). Blockeel et al. (1999) investigated the predictability of numerous physicochemical characteristics of river water. Dzeroski et al. (2000) utilized trees with bio-data and chemical information to forecast water quality in Slovenian rivers. Feedforward networks with sigmoidal-type transfer functions have made extensive use to predict water resource parameters (Chen et al., 2020). A survey used the neural networks with active neurons in the form of a modelling technique to forecast seawater quality pointer such as temperature, pH, DO, and turbidity (Hatzikos et al., 2007). Restricted quality of water and considerable expense of environmental monitoring frequently constitute significant concerns for process-based modelling methods. The ANNs give an excellent choice since they are quick and demand much less independent variables and entry conditions than

deterministic models.

The ANN method has different benefits over the semi-empirical model since they include input information without assumptions. ANN secure a mapping for independent and dependent variables that can be employed to forecast the necessary dependent based on the desirable independent variable (Mittal et al., 2019). Any sentential function between the independent and dependent vectors can get closer to the multi-layer neuron network by selecting the correct set of connecting weights and transport functions (Lillicrap et al., 2016). ANN models have been exceptionally utilized in problems of water quality. This aims to determine the usefulness of Artificial Neural Network techniques.

2.6 Artificial neural network

The utilization of Artificial Neural Network (ANN) is steadily growing for forecasting variables of sewage works. This application cuts costs of operation and checks the firmness of the environmental balance. This chapter concentrates on putting ANN's approach with a Feedforward Back-Propagation for predicting the physicochemical parameters of the rivers and WWTPs of the municipalities.

2.6.1 Advantages of ANN

The ANN can elucidate non-linear and multiplex relationships by using input and outputs training designs from a collection of data. The methods start the relationship of non-linear between the outputs and inputs (Alizamir et al., 2017). The ANN can be revealed based on an architecture that displays connection designs between the nodes, determination of connection weights procedure, and activation function (Alizamir et al., 2017). Due to their means to master the system's dynamics from the data, the ANN has the power to find the answer to large-scale multiplex problems (Zhang et al., 2019). The feedforward neural network is commonly used for ANN. The network training is based on optimizing the weights to meet the suitable weight function to cut the errors. The procedure proceeds until the output layer's values are as near as possible to the real outputs (Nielsen, 2015). The ANN applied in different application areas may share comparable learning algorithms and speculations (Subaşı, 2010).

2.6.2 Application area of ANN

The ANNs have gained a broad-spectrum application area to apply for experienced problems. Currently, they can be employed in several branches of industry effectively. There are no restraints about ANN's application area, although they are commonly used to categorize, predict, and model areas. The ANNs are put into action for several challenges, and problem number slowly accentuates. Because of ANNs' better description capacity of trends and data patterns, they are right for predicting approaches. Several instances for the broader use of ANNs are quality assurance, financial prediction, economic prediction, and Laboratory Research (Ahmed et al., 2020; Yurtoğlu, 2005).

2.7 Essential elements of ANNs

ANNs are made up of artificial cells connected hierarchically and may work parallel (Çelik, 2018). The manufactured cells are believed analogous to neurons in the biological nervous

system; moreover, they can be referred to as process components. Every single part comprises five principal factors: inputs, weights, summation function, transfer function, and neurons' output (Özçelik, 2015). Figure 2.2 demonstrates the diagrammatic representation of an artificial neuron.

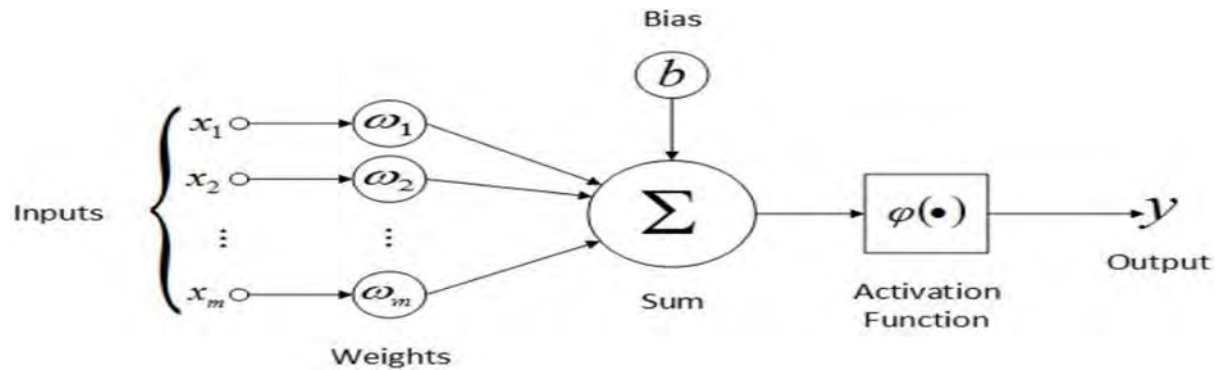


Figure 2.2: Diagrammatic representation of an artificial neuron (Oliveira et al., 2017).

2.7.1 Input functions

Inputs are components of ANN that accept information from the outer world. They lack all the other functions than to convert the information to the next step. That is, inputs are not making some mathematical operation on data, and they function as a transporter. The contributions are classified between two components of ANN that are connected with the Otherworld of the network. A neuron may have an unrestricted variety of data; however, there has to be the only output of each neuron (Özçelik, 2015).

2.7.2 Output functions

The function of output is necessary for transporting the activation function's output value to Otherworld as the network's final output value or to another associated neuron as their input values (Özçelik, 2015). Figure 2.3 shows the structure of ANN and its layers.

Artificial neural networks team up to make ANN. In a general sense, they team up in three layers and parallel in every segment to make ANN, not random. Such coatings are as follows:

2.7.2.1 Input layer: The neurons throughout this layer transport the data out from the outer world towards hidden layers; usually, in this layer, there is no data processing.

2.7.2.2 Hidden layer: Data from the input layer is processed into these layers and transmitted to the output layer. There may be multiple hidden layers.

2.7.2.3 Output layer: Process components into this layer, transported data from hidden layers are managed and output for given information into input layers. The generated output is transmitted to Otherworld as an outcome (Özçelik, 2015).

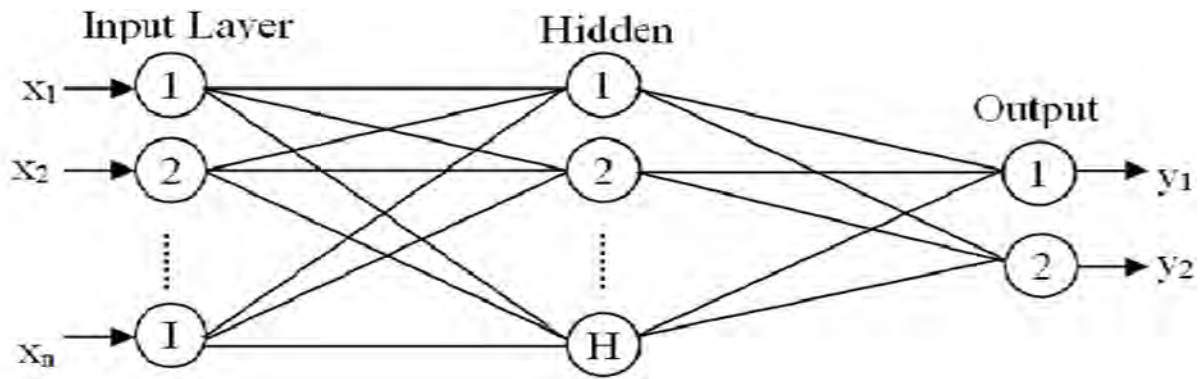


Figure 2.3: ANN structure with its layers (Aydın et al., 2014).

2.7.3 Weights

Weights are particularly significant components of mathematical neurons and ANN. Learned information by the network is saved on the weight function. The ANN may do its learning function, utilizing transforming the weights function. For this reason, synaptic weight expresses as $W = [W_i]$ $n \times 1$ comprises transformable values. Generally, the original weight value is chosen, just like the random value in the $(-1, 1)$ range (Özçelik, 2015). Weights of ANN may be considered as synapses in the biological nervous system (Richárd, 2018).

2.7.4 Summation function

The summation function is necessary to summarize every bit of information out from the Otherworld and associated weights. This function transports the generated weighted inputs (V_i) to the activation function (Godfrey, 2018; Wang et al., 2019). The θ states express the threshold value. The utility of the threshold value within this function is not mandatory. The use of the threshold value is connected with the demand for network architecture designers (Çelik, 2008). This function is commonly used in ANN (Richárd, 2018). Nevertheless, multiple services may be used, and the summation function is employed in most analyses (Çelik, 2008).

Expression of summation function: $V_i = X_1W_1 + X_2W_2 + X_3W_3 + \dots X_nW_n - \theta$

2.7.5 Activation function

The activation function is necessary to activate the specific weighted input and resolution value of the last information (Yang et al., 2013; Zainal-Mokhtar & Mohamad-Saleh, 2013). There is no general formulation for which particular activation function be applied. The excellent activation function is decided due to the efforts of the designer (Özçelik, 2015). The option of activation function depends mainly on accessible information. Below is an expression of the activation function:

$$F(V_i) = y$$

2.8 ANN class concerning the structure

The ANN architecture is split down into two categories of feedforward and feedback propagation based on the direction of the neurons' connections (DeMarse et al., 2016).

2.8.1 Feedforward network

Within the feedforward networks, the neurons are placed into the layers. The inputs are transported from one layer to the other by one-way weights (Çelik, 2018). Due to one-way links, the next layer's return to the past layer as input is impracticable (Özçelik, 2015). Examples of feedforward are Multi-Layer Perceptron and Learning Vector Quantization. In this network, the input layer transmits the data from the Otherworld to the hidden layer in the absence of any data process (Çelik, 2018). The information is processed in the hidden and output layers; therefore, network output is created. The feedforward network with such structures executes a non-linear static function (Ahmed et al., 2015). Some continuous features may be converged through this network and three layers, which may unite with desirable precision. The more commonly called backpropagation learning algorithm is applied successfully in this kind of ANNs' training (Garg et al., 2016); occasionally, they are referred to as back-propagation networks (Özçelik, 2015).

2.8.2 Feedback network

The hidden and output layers are fed to the input layer or the past hidden layers (Çelik, 2018). Thus, the input can be conveyed to the forward direction and reverse direction. This network has a heap memory (Garcia-Garcia et al., 2016); the output reflects then and past input values. Hence, they are, particularly for forecasting applications. This network has been influential in assessing different kinds of time series (Çelik, 2018). The Hopfield and Self Organization Map can be held up as an example. This network can be among the neurons in layers and among the segments. The ANN with such structures reveals a non-linear ever-changing way. The value of the output created by the network is contrasted with the anticipated outcomes of the system. For reducing an absolute error to a minimum, it must be scattered to the process components generating this error. It implies the modification of the weights of process components (Özçelik, 2015).

2.9 METHODOLOGY

This chapter presents the sampling points, full site description, and sample collection method. The research methodology is detailed, and the obtained results were compared with World Health Organisation, South African National Standard, and Target Water Quality Range guideline values to achieve the research objectives.

2.9.1 THE STUDY REGION

A clear statement of the research approach and study region is an essential element of the investigation. A comprehensive description of the study region is significant to the reviewer and prospective research worker. It illustrates the site's reviewer where the survey was conducted and the selection of that specific region. Moreover, maps represent substantial resources to geographical location. The study was conducted in three rivers, and the WWTPs

were found on these rivers' banks. Figures 2.4, 2.5, and 2.6 show different river catchments demonstrating the study region with sampling sites.

The evaluation of the physicochemical characteristics was conducted in the three District Municipalities of Makhanda, Amothole, and Buffalo City Metropolitan. The study focused on the following sources: the Bloukrans River, Tyhume River, and Buffalo River, and the WWTPs found on the banks of these rivers. Samples were collected from the upper, middle, and lower sections of each stream. For wastewater treatment plants, samples were collected from the influent and effluent. This chapter focuses on analyzing the physicochemical parameters of the WWTPs and river water of Makhanda, Alice, and King William's Town in Eastern Cape Province, South Africa. This chapter also focuses on the modelling of parameters for water quality assessment using the application of an artificial neural network.

Time distribution

Data on water quality was acquired from three seasonal sampling collections from the significant sources of mentioned-above municipalities. Table 2.3 represents the geographic database concerning seasonally sampling points of the streams and the site's full description.

Table 2.3: Geographic database of seasonally sampling points of the streams and the full description site.

<i>River</i>	<i>Site</i>	<i>Site Full Description</i>	<i>GPS Coordinate</i>	
			<i>Latitude</i>	<i>Longitude</i>
<i>Makhanda region</i>				
<i>Bloukrans</i>	<i>GU</i>	Upper site of the Bloukrans River	33.31774167	26.52194444
	<i>GM</i>	Middle site of the Bloukrans River	33.31427500	26.55166667
	<i>GL</i>	Lower site of the Bloukrans River	33.31780556	26.56833333
	<i>GE</i>	Influent site of WWTP of Makhanda region	33.31667500	26.55750000
	<i>GI</i>	Effluent site of WWTP of Makhanda region		
<i>King William's Town region</i>				
<i>Buffalo</i>	<i>BU</i>	Upper site of the Buffalo River	32.78991389	27.36916667
	<i>BM</i>	Middle site of the Buffalo River	32.89703333	27.39277778
	<i>BL</i>	Lower site of the Buffalo River	32.93447500	27.44027778
	<i>BE</i>	Influent site of WWTP of King William's Town region	32.89969722	27.40305556
	<i>BI</i>	Effluent site of WWTP of King William's Town region		
<i>Alice region</i>				
<i>Tyhume</i>	<i>AU</i>	Upper site of the Tyhume River	32.61067778	26.90944444
	<i>AM</i>	Middle site of the Tyhume River	32.79636667	26.84583333
	<i>AL</i>	Lower site of the Tyhume River	32.82713889	26.88833333
	<i>AE</i>	Influent site of WWTP of Alice region	32.79108611	26.85000000
	<i>AI</i>	Effluent site of WWTP of Alice region		

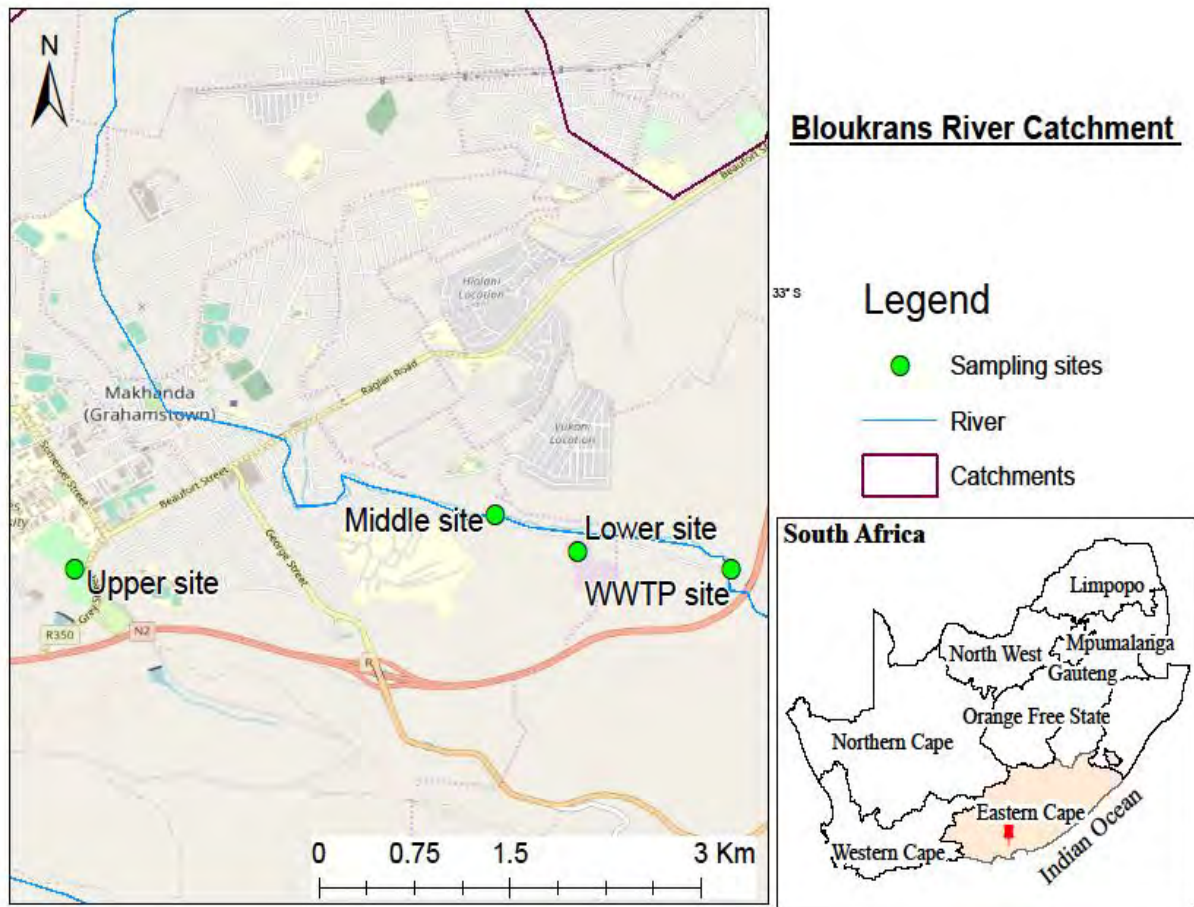


Figure 2.4: The map of the Bloukrans River Catchment demonstrating the study region with sampling sites.

¹Bloukrans River

¹ Bloukrans River is in the region of Tsitsikamma of Garden Route in South Africa. It is in between the border of the Western and Eastern Provinces. The river starts closer to Peak Formosa in the region of Plettenberg Bay. It lies on the geographical coordinates: 33° 58' 8.04" S, 23° 38' 44.16" E. The coordinates of the sites are as follows: Upper stream: 33° 19' 3.87" S, 26° 31' 19.44" E; middle stream: 33° 18' 51.39" S, 26° 33' 6.30" E; and lower stream: 33° 19' 4.10" S, 26° 34' 5.76" E. The Makhanda WWTP coordinates 33° 19' 0.03" S, 26° 33' 26.49" E.

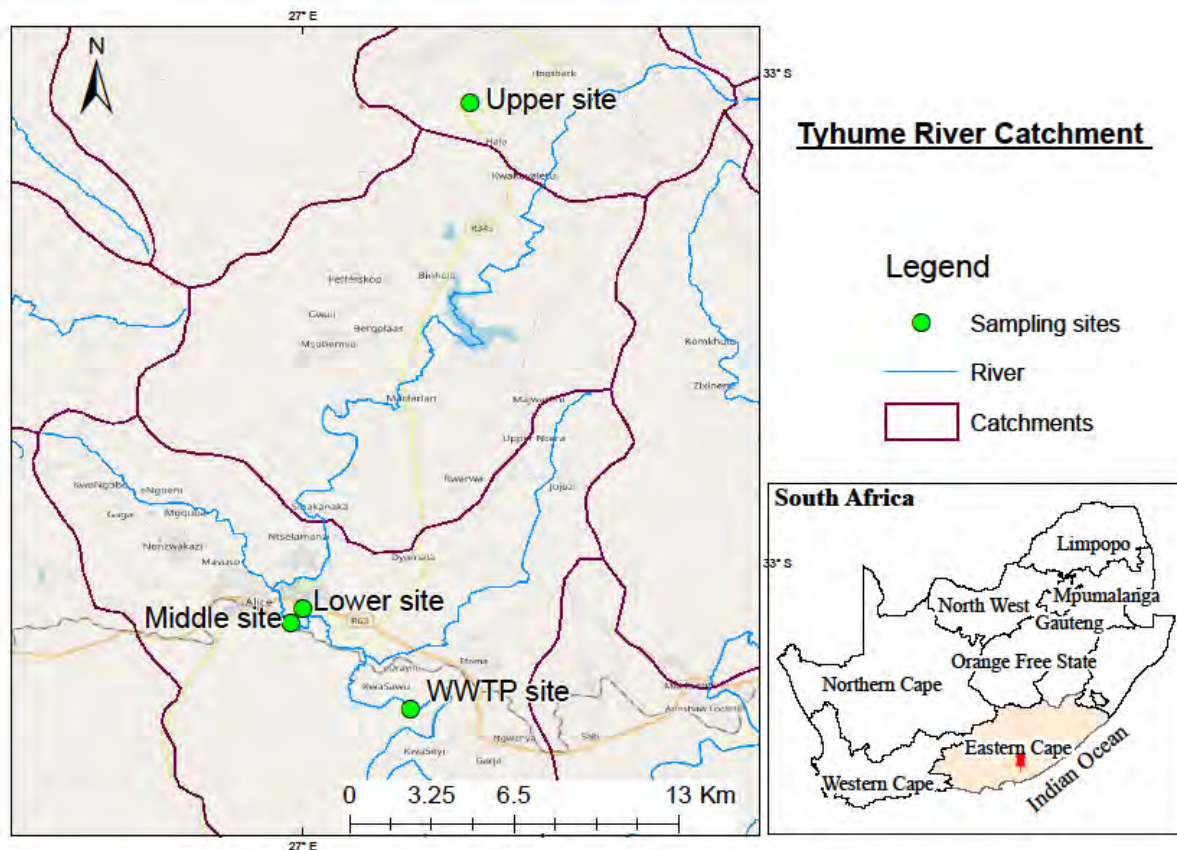


Figure 2.5: The map of the Tyhume River Catchment demonstrating the study region with sampling sites.

²Tyhume River

² Tyhume River is in the centre of the Eastern Cape Province in the Amathole District Municipality. The river originates from the forested mounts and passes down the Valley of Tyhume River and down the township called Alice, in the Eastern Cape, surrounding the University of Fort Hare's grounds. The river lies on the geographical coordinate 32° 41' 5" S, 26° 54' 18" E. The sites' coordinates are as follows: Upper stream: 33° 36' 38.44" S, 26° 54' 33.66" E. The medium flow: 33° 47' 46.92" S 26° 50' 45.13" E and the lower stream: 33° 49' 37.70" S, 26° 53' 18.03" E. Alice WWTP coordinates: 32 ° 47' 27.91" S, 26° 50' 59.83" E. Tyhume River is the vital water storage reservoir of the rural settlements. After the dam, the rivers pass along the south, then east, and southeast to amalgamate the river of Keiskamma.

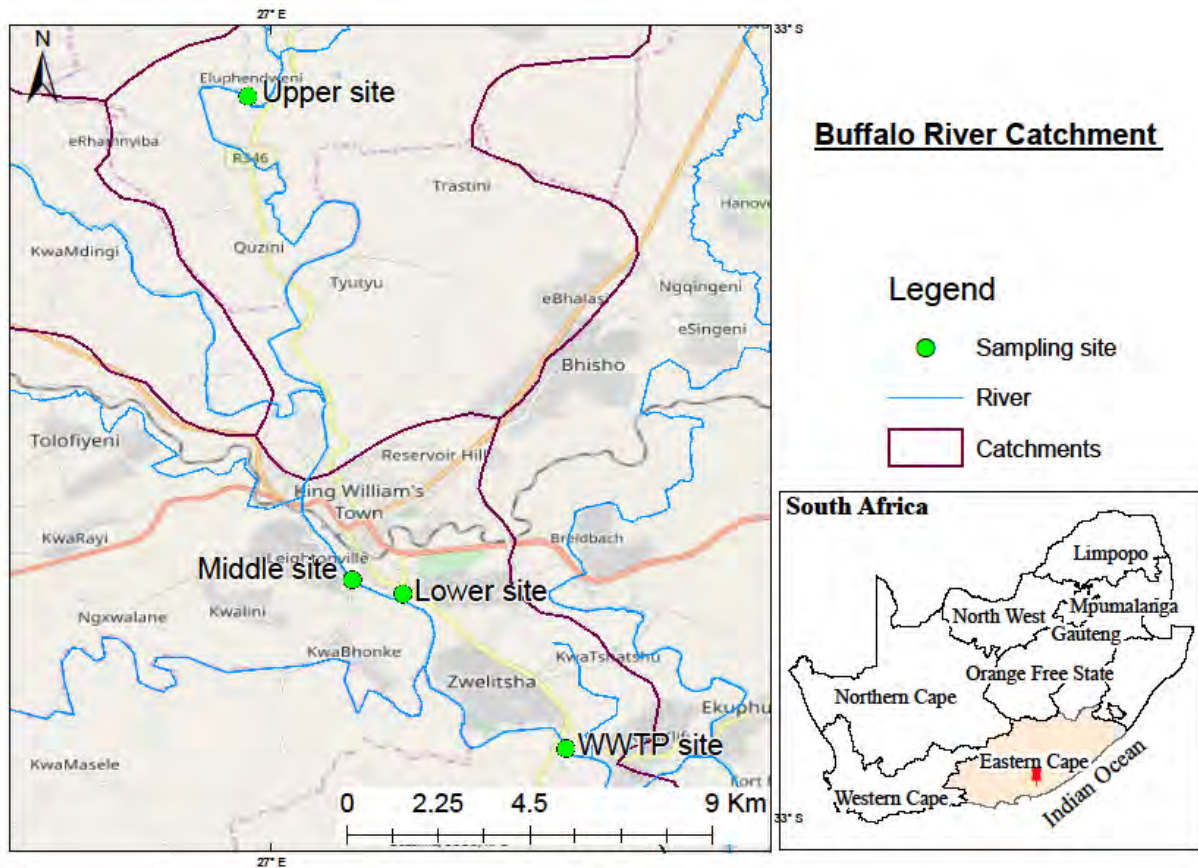


Figure 2.6: The map of the Buffalo River Catchment demonstrating the study region with sampling sites.

³Buffalo River

³ Buffalo River is a river in the Eastern Cape Province that is in the town of East London. Buffalo River is a unique river that can be sailed on in entire South Africa (DWAF, 2004). It lies on the geographical coordinate 33° 1' 43" S, 27° 51' 51" E. The coordinates of the sites are as follows: Upper stream: 32° 47' 23.69" S, 27° 22' 9.09" E; medium stream: 32° 53' 49.32" S, 27° 23' 34.07" E and the lower flow: 32° 56' 4.11" S, 27° 26' 24.98" E. King William's Town WWTP coordinates: 32° 53' 58.91" S, 27° 24' 11.09" E. Sloping at an altitude of 1200 m. The length of the river is 126 km (78 mi) (DWAF, 2004). The catchment area supports roughly 570 000 Homo sapiens in 1,287 km² (497 sq. mi) (DWAF, 2004). As a result, a year lower than 500 m³ of water is accessible to one person (DWAF, 2004). Down the Buffalo River, the four obstructions provide the primary site of King William's Town, Zwelitsha, Mdantsane, and East London (DWAF, 2004). The blockage in the sewage apparatus, insufficient treatment capacity, and poor management affect the release of relatively treated and untreated sewage in the direction of the river and dams (DWAF, 2004). Therefore, it creates a problem that contains algal blooms and intolerable high concentrations of faecal bacteria. The processing wastes are insufficiently treated or untreated in any way. Substandard water quality constitutes a substantial health risk to people residing in rural areas since multitudes are dependent on untreated river water (DWAF, 2004).

2.9.2 Approach for data analysis

Samples were carried out to Rhodes University, Faculty of Pharmacy, in the Pharmaceutical Chemistry laboratory (The Environmental Health and Biotechnology Research). The sample analysis was carried out by utilizing HANNA Multi-parameter HI 9829 and EUTECH Instruments Turbidimeter TN-100 for physical parameters. For COD, sulfate, and phosphate, the photometer AL410 parameter tool was used. For chloride, the photometer AL100 parameter tool was used. Nitrate stripes were utilized for nitrate. Multiparámetro Lovibond Senso Direct 150 tool was used for Dissolved Oxygen. The outcomes of the tests, as mentioned above, were assessed against the World Health Organization and a guideline of South African water quality following the Department of Water Affairs and Forestry, 1996. The Target Water Quality Guideline Range for phosphate, dissolved oxygen, and pH for aquatic ecosystems are subjected to discussion. There is no exclusive Target Water Quality Guideline Range for chloride, nitrate, sulfate, electrical conductivity, and turbidity concerning aquatic ecosystems. The results were also addressed against the standard terms and conditions for wastewater purification in keeping with rules (DWAF, 1984) for chloride, nitrate, sulfate, dissolved oxygen, pH, chemical oxygen demand, and turbidity. DWAF defines no particular standard terms and conditions for other water quality parameters in this study's scope. The results were also compared to the South African National Standard for drinking water (SANS-241:2015). As reported by SANS, parameters that are not within specified limits can initiate severe or chronic medical conditions in an individual.

Additionally, the statistical test was done on Microsoft Excel 2016. It is a useful parametric test to verify a considerable difference among the sampling sites using the average and standard deviation. It was carried out on entire samples to distinguish any substantial water quality amendments at the sampling points. Table 2.4 shows all the parameters of interest and the used instruments.

Table 2.4: Parameters of water quality and analytical technique for the source of water assessment.

<i>Parameters</i>	<i>Analytical technique</i>	<i>Instrument</i>
<i>pH</i>	Instrumental <i>in situ</i>	HANNA Multi-parameter HI 9829
<i>Temperature</i>		
<i>Electrical Conductivity</i>		
<i>Turbidity</i>		EUTECH Instruments Turbidimeter TN-100
<i>Sulfate</i>	Photometric technique Laboratory	Photometer AL410
<i>Phosphate</i>		
<i>Chemical Oxygen Demand</i>		
<i>Dissolved Oxygen</i>		Multiparámetro Lovibond Senso Direct 150
<i>Nitrate</i>		Nitrate strips
<i>Chloride</i>		Photometer AL100

2.9.2.1 Sample collection

Water samples were collected from three rivers in the Eastern Cape Province, South Africa, Makhanda, Alice, and King William's Town. Each river was sampled of three sites: the upper stream, middle stream, lower stream, and the wastewater treatment plants, namely, the influent (raw sewage from the surrounding) and the effluent (the final treated effluent discharged to the rivers). One litre of Scott bottles was used to acquire water samples. After collecting from the sites, bottles were put in a cooler box containing ice packs to cool the samples. The cooler box was used to keep the sample temperature at 4°C. The analysis of samples was performed within 48 hours from the time of sampling.

2.9.2.2 Physicochemical analysis

Water samples were analyzed for physicochemical parameters by utilizing a recalibrated HANNA Multi-parameter HI 9829. The physical parameters that were measured were pH, EC, temperature, and turbidity. The HANNA Multi-parameter portable meter was used to measure the pH, temperature, and EC *in situ*. Turbidimeter TN-100 (EUTECH Instrument) was used to measure turbidity in situ.

The chemical parameters that were measured were chloride (Cl), sulfate (SO_4^{2-}), nitrate (NO_3^-), phosphate (PO_4^{3-}), Dissolved Oxygen (DO), and Chemical Oxygen Demand (COD). COD, DO, phosphate, and sulfate were measured using photometer AL410, and DO was measured using Multiparámetro Lovibond Senso Direct 150. Chloride was measured by using Photometer AL100. The measurements of the samples were taken in triplicates from every site. The device was first set to zero by running it with a blank reagent.

Procedural preparation of physicochemical parameters:

Temperature, electrical conductivity, and the pH

The pH, Electrical Conductivity, and temperature of water samples were measured by utilizing the HANNA Multi-parameter HI 9829, which was dipped into the water thrice to read the measurements.

The turbidity of water samples was measured by using EUTECH Instruments Turbidimeter TN-100. Water samples were poured into the vial to the mark of 10 mL and put into a turbidity meter for the readings.

The vial was filled with 10 mL of the water sample. The bottle was placed in a chamber, and the machine was set to zero. One SULFATE T tablet was added to the water sample crushed using the stirring rod to dissolve the tablet. The vial was closed tightly and placed into the chamber for readings. After the reaction period, the result was shown in mg/L.

One nitrite-nitrate test strip was removed from the vial. The test strip was dipped into the water sample so that both pads are immersed and held for 2 to 3 seconds. The nitrate strip was removed and shaken off any excess liquid. The test pad was compared to colour chart after 30 seconds. The vial was filled with 10 mL of the water sample. The bottle was placed in a chamber, and the machine was set to zero. One PHOSPHATE HR P1 tablet was added to the

water sample crushed using the stirring rod to dissolve the tablet. One PHOSPHATE HR P2 tablet was also added to the water sample to dissolve the tablet. The vial was closed tightly and placed into the chamber for readings. After the reaction period, the result was shown in mg/L.

The vial was filled with 10 mL of the water sample. The bottle was placed in a chamber, and the machine was set to zero. One CHLORIDE T1 tablet was added to the water sample crushed using the stirring rod to dissolve the tablet. One CHLORIDE T2 tablet was also added to the water sample to dissolve the tablet. The vial was closed tightly and placed into the chamber for readings. After the reaction period, the result was shown in mg/L.

The manual temperature-compensation value adjustment procedure was followed per MultiMeter Instrument- Instruction Manual and calibration. The probe cap was removed, and the probe body was held and wholly immersed in the water sample. The data function recorded the minimum and maximum readings. Then the measurement value was displayed in mg/L. One white-capped reaction vial was opened, and 0.2 mL of deionized water was added. Another white-capped reaction vial was opened, and a 0.2 mL water sample was added. These vials were gently inverted several times to mix the contents. The bottles were heated for 120 minutes in the preheat reactor at 150°C. These tubes were removed from the heating block and allowed to cool to 60 °C. The contents were mixed by inverting each test tube several times while still warm. Then the cells were allowed to cool to ambient temperature. The clear vial of deionized water was placed in a chamber and set to zero. Then it was removed, and the water sample vial was put into a room. Some results were shown in g/L and then converted to mg/L.

Data-processing and analysis

Water quality and water status pollution were assessed based on WHO, SANS, and TWQR for calculating samples that did not abide by recommended values. Basic statistics such as average and standard deviation were used to examine and assess the diffusion of range values for every parameter.

2.9.2.3 Artificial neural network model

The ANNs are a method of problem-solving. The ANN input variables and output variables were chosen based on the engineering's judgment, where input variables significantly impact the output variables. Two types of feedforward ANN, namely, multi-layer perception (MLP) and radial basis function, were evaluated using Statistica version 13.2 software (Round Rock, Texas, USA).

For training, the dependent variables were pH, Electrical conductivity, turbidity, and dissolved oxygen, and the independent variables were temperature, sulfate, phosphate, and chloride.

Artificial neural network

ANN is an effort to imitate the neural network to create the human brain such that the computer is capable of learning things and reach a decision in a manner of humanlike. The fundamental component consists of a wide range of processing units. The biological neuron is composed of dendrites, synapses, axons, and the cell body. Dendrites receive signals; axon helps to make

synaptic connections. The shape of the neuron totals the incoming signals from dendrites. The neuron sends the impulse to the axon provided the input signals are adequate. There would be no impulse if the input signals did not reach the required level. Thus, ANN can extract patterns and draw meaning from inaccurate information (Dawood et al., 2016).

Optimal selection of ANN model

The precision of the neural network is reliant on the architecture of the system and set parameters. The most significant employment is to detect the optimal architecture of the system, which is concerning find the number of optimal layers and neurons in the initial weights that mostly bring the change to the performance of the trained neural network. The architecture of the neural network is kept constant. Below is the description of the empirical formula used.

$$M = \sqrt{i + 0} + c \dots \dots \dots (1)$$

Where m is the number of hidden layer nodes, i is the number of input sets, zero is the number of output sets, and c is a constant number 1 and 10.

Selection of input variables

One of the principal functions is to decide the model-independent variables that have significant impacts on the dependent variables. Independent variables' choice is often based upon advanced knowledge on cause and effect and statistical data analysis of possible inputs and outputs. The choice of independent variables for this network modelling is dependent on the study of statistical correlation of data in the field, prediction accuracy of variables of water quality, and theoretical knowledge.

Evaluation of ANN models performance

The basis of statistics is useful to compare the sufficiency of models. In this study, the following criteria: mean square error (MSE), root mean square error (RMSE), and coefficient of correlation (R2), were employed since they are commonly used to evaluate models of water quality. The ratio of zero and one's correlation values suggest that the observed mean is equally well as the model, perfection, and best forecaster than the model.

$$MSE = \frac{1}{n} \sum_{i=1}^n (t_i - O_i)^2 \dots \dots \dots (2)$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (t_i - O_i)^2} \dots \dots \dots (3)$$

$$R = \frac{\sum_{i=1}^n (t_i - \bar{t})(O_i - \bar{O})}{\sqrt{\sum_{i=1}^n (t_i - \bar{t}) \sum_{i=1}^n (O_i - \bar{O})}} \dots \dots \dots (4)$$

Where, t_i =target value, o_i =output value and n= total number

$$\bar{t} = \frac{1}{n} \sum_{i=1}^n t_i$$

$$\bar{o} = \frac{1}{n} \sum_{i=1}^n o_i$$

Artificial neural network and aquatic resources

One can determine if the model's predictive ability is sufficiently precise to reach decisions about data utilization regarding the sensibility of water quality parameters and differences among the predicted and experimental variables.

2.10 RESULTS AND DISCUSSION

2.10.1 Physicochemical Analyses

The seasonal physicochemical analysis from the minimum to maximum and average \pm standard deviation are presented in Tables 2.5 to 2.8 below. Data obtained from triplicates. The sampling was carried out seasonally, and the findings of this research were reported based on the seasonal mean of the sample. Table 2.9 shows water quality guidelines for WHO, TWQR, and SANS.

Table 2.5: Overall average values of water physical and chemical properties from Tyhume, Buffalo, and Bloukrans Rivers (means ± standard deviations)

<i>River</i>	<i>Site</i>	<i>Temp</i> °C	<i>pH</i>	<i>Turbidity</i>	<i>EC</i> mS/m	<i>NO³</i> mg/L	<i>COD</i> mg/L	<i>DO</i> mg/L	<i>Cl⁻</i> mg/L	<i>PO³₄</i> mg/L	<i>SO²₄</i> mg/L
<i>Bloukrans</i>	<i>GU</i>	13.24±1.25	6.22±1.27	9.14±2.13	73.42±14.52	3.33±4.44	79.70±30.91	7.25±0.21	n.d	n.d	45.84±33.8
	<i>GM</i>	15.63±1.45	7.28±0.55	30.96±8.90	230.6±100.45	3.33±4.44	49.52±36.54	6.03±1.11	69.67±0	n.d	127.3±32
	<i>GL</i>	14.75±0.85	6.41±0.92	89.82±37.45	223.5±61.15	n.d	59.66±33.76	6.27±0.58	83.33±0	0.55±0.00	156.5±36.50
	<i>GE</i>	17.93±0.88	7.16±0.34	137±120.33	209.5±54.66	n.d	42.14±7.78	5.84±0.26	9.67±0	0.35±0.08	136.8±53.41
	<i>GI</i>	21.51±0.34	7.52±0.87	206.15±99.35	226.3±52.11	n.d	206.4±34.82	4.72±1.53	n.d	0.43±0.00	63.33±62.66
<i>Tyhu</i>	<i>AU</i>	12.77±1.86	8.08±1.78	7.32±3.67	11.10±2.80	3.33±4.44	31.14±17.21	7.43±0.22	n.d	0.06±0.00	n.d
	<i>AM</i>	15.66±2.47	7.05±1.09	18.21±11.82	26.02±7.63	5.56±3.70	135.1±33.19	7.57±0.11	7.67±0	0.42±0.00	n.d
	<i>AL</i>	14.88±2.34	9.43±1.51	12.36±2.29	40.29±10.79	8.89±5.93	144.1±36.59	7.58±0.10	n.d	n.d	n.d
	<i>AE</i>	18.38±3.08	7.16±1.00	6.57±1.18	64.89±19.25	55.56±37.04	103.2±22.70	7.22±0.21	28.00±0	n.d	65.34±5.78
	<i>AI</i>	19.32±1.39	7.29±0.68	15.28±54.70	72.82±16.78	n.d	301.5±130.72	4.85±0.90	n.d	n.d	48.44±45.04
<i>Buffalo</i>	<i>BU</i>	17.24±2.60	7.46±0.98	18.17±17.84	50.35±4.52	n.d	29.05±11.03	7.26±0.17	180.67±0	n.d	9.67±0.00
	<i>BM</i>	18.22±2.92	7.71±1.35	23.27±16.46	61.74±1.54	7.78±1.48	44.65±30.02	7.02±0.32	n.d	0.30±0.16	52.50±27.2
	<i>BL</i>	17.95±3.74	7.89±1.16	14.34±7.64	75.33±1.94	25.00±5.55	54.40±24.84	7.11±0.36	n.d	n.d	119.0±3
	<i>BE</i>	19.25±2.20	7.27±0.86	23.44±18.96	85.24±21.12	5.56±7.41	86.43±43.93	6.59±0.46	n.d	0.28±0.00	64.22±19.93
	<i>BI</i>	19.50±1.68	7.27±0.87	191±40.00	102.9±27.40	n.d	877.1±997.46	4.77±0.89	16.00±0	0.13±0.00	32.84±9.17

n.d= not detected

Chloride: Limit of detection is <5 mg/L

Phosphate: Limit of detection is <0.05 mg/L

Sulfate: The limit of detection is <5 mg/L

U- Upper streams, M- middle streams, L- lower streams, E -final treated effluent discharged into rivers, I- the influent from the surrounding area

G-Bloukrans River, GI/GE- Makhanda WWTP

B- Buffalo River, BI/BE- King William's Town WWTP

A- Tyhume River, AI/AE- Alice WWTP

⁴Table 2.6: The average water physicochemical parameters of the Bloukrans River and WWTP in autumn, winter, and spring seasons.

Physical Parameter			Chemical Parameter								
Season	Site Code	Temp °C	EC (mS/m)	Turbidity (NTU)	Chloride (mg/L)	Sulphate (mg/L)	COD (mg/L)	Nitrate (mg/L)	DO (mg/L)	Phosphate (mg/L)	pH
Average ± St. Dev			Average ± St. Dev								
Autumn	GU	15.11±0.02	95.20±0.27	6.49±0.70	n.d	12.00±0.00	5.33±2.56	n.d	7.21±0.12	n.d	7.80±0.15
	GM	16.25±1.96	381.27±1.04	43.07±0.62	69.67±0.44	95.33±2.22	184.3±17.56	10.00±0.00	4.37±0.29	n.d	7.35±0.08
	GL	15.47±0.00	315.2±0.04	146.0±6.67	83.33±1.11	120.0±12.00	199.0±12.67	n.d	5.40±0.20	n.d	7.67±0.01
	GE	18.37±0.00	291.5±3.84	53.47±0.98	n.d	170.7±0.89	69.10±15.27	n.d	5.60±0.20	0.44±0.24	7.66±0.00
	GI	21.11±0.00	304.4±0.22	315.3±4.44	n.d	16.67±1.56	111.0±3.33	n.d	2.43±0.42	0.43±0.12	7.87±0.00
Winter	GU	12.53±0.10	62.13±0.11	12.34±0.35	n.d	78.00±0.67	56.33±21.56	n.d	7.57±0.04	n.d	4.32±0.00
	GM	13.45±0.03	157.7±3.67	17.60±0.47	n.d	164.7±0.89	85.33±27.78	n.d	6.83±0.24	n.d	8.04±0.12
	GL	13.47±0.25	173.1±2.80	85.03±0.62	n.d	193.0±1.33	109.3±51.11	n.d	6.90±0.20	n.d	5.02±0.04
	GE	16.60±0.00	173.9±16.02	318.0±15.33	9.67±0.89	192.0±0.00	121.7±18.89	n.d	5.70±0.00	0.38±0.01	6.13±1.23
	GI	18.11±0.01	201.0±0.27	57.13±2.04	n.d	73.3±3.11	497.67±74.22	n.d	5.87±0.31	n.d	8.34±0.02
Spring	GU	12.08±0.10	62.93±1.51	8.60±0.04	n.d	79.67±0.44	31.77±0.58	10.00±0.00	6.97±0.04	n.d	6.55±0.03
	GM	17.18±0.26	152.8±0.09	32.20±2.07	n.d	159.3±1.78	135.7±13.11	n.d	6.90±0.07	n.d	6.45±0.05
	GL	15.30±0.00	182.2±0.89	38.43±0.91	n.d	193.0±11.33	124.0±3.33	n.d	6.50±0.00	0.55±0.02	6.53±0.02
	GE	18.81±0.00	163.2±2.16	41.03±0.11	n.d	183.0±0.00	118.7±5.11	n.d	6.23±0.09	0.24±0.00	6.68±0.01
	GI	18.53±0.02	173.4±0.18	246.0±4.00	n.d	157.3±4.44	296.0±54.67	n.d	5.87±0.11	n.d	6.34±0.01

⁴ Please refer to Table 2.5 for site code and acronyms/abbreviations

⁵Table 2.7: The average water physicochemical parameters of Buffalo River and WWTP in autumn, winter, and spring seasons.

Physical Parameter					Chemical Parameter						
Season	Site Code	Temp °C	EC (mS/m)	Turbidity (NTU)	Chloride (mg/L)	Sulphate (mg/L)	COD (mg/L)	Nitrate (mg/L)	DO (mg/L)	Phosphate (mg/L)	pH
<i>Average ± St. Dev</i>					<i>Average ± St. Dev</i>						
<i>Autumn</i>	<i>BU</i>	19.61±0.37	49.93±0.49	44.93±0.96	n.d	n.d	35.37±1.84	n.d	7.40±0.00	n.d	8.03±0.21
	<i>BM</i>	20.96±0.23	63.00±0.07	47.97±0.84	n.d	n.d	19.87±7.89	10.00±0.00	7.50±0.00	0.14±0.04	8.35±0.09
	<i>BL</i>	20.60±0.06	76.37±0.16	25.80±0.33	n.d	n.d	58.73±14.51	25.00±0.00	7.43±0.04	n.d	8.86±0.28
	<i>BE</i>	22.06±0.01	116.93±0.24	7.53±0.34	n.d	70.33±0.44	57.63±15.09	25.00±0.00	6.87±0.71	n.d	8.37±0.20
	<i>BI</i>	22.03±0.01	143.97±0.51	235.0±11.33	n.d	n.d	103.1±19.91	n.d	3.67±0.44	n.d	8.20±0.03
<i>Winter</i>	<i>BU</i>	13.34±0.12	44.00±0.07	4.96±0.02	n.d	n.d	39.27±12.36	n.d	7.37±0.11	n.d	8.35±0.14
	<i>BM</i>	13.83±2.01	62.80±0.07	6.78±0.11	n.d	79.67±0.44	89.67±44.89	6.67±4.44	6.60±0.80	n.d	9.10±0.30
	<i>BL</i>	12.35±0.01	72.43±1.04	11.81±1.00	n.d	122.0±0.00	87.33±1089	33.33±22.22	7.33±0.09	n.d	8.67±0.05
	<i>BE</i>	15.96±0.01	67.40±0.07	10.91±0.41	n.d	88.00±0.00	49.33±8.89	n.d	7.00±0.20	0.28±0.00	7.45±0.12
	<i>BI</i>	16.99±0.01	85.83±0.04	207.0±4.67	n.d	23.67±1.78	2373.3±451.11	n.d	6.10±0.53	0.13±0.01	7.64±0.03
<i>Spring</i>	<i>BU</i>	18.77±1.02	57.13±19.02	4.63±0.21	180.7±1.56	9.67±0.44	12.50±4.07	n.d	7.00±0.13	n.d	5.99±0.05
	<i>BM</i>	19.86±0.13	59.43±0.04	15.07±0.13	n.d	25.33±0.44	24.40±5.33	10.00±0.00	6.97±0.16	0.46±0.03	5.69±0.06
	<i>BL</i>	20.91±0.06	77.20±0.13	5.42±1.02	n.d	116.0±0.00	17.13±3.89	25.00±0.00	6.57±0.71	n.d	6.15±0.03
	<i>BE</i>	19.74±0.00	71.40±0.00	51.87±1.09	n.d	34.33±15.78	152.3±5.56	n.d	5.90±0.73	n.d	5.98±0.00
	<i>BI</i>	19.49±0.00	78.83±0.04	131.0±2.00	16.00±0.00	42.00±2.67	155.0±6.00	n.d	4.53±1.24	n.d	5.97±0.02

⁵ Please refer to Table 2.5 for site code and acronyms/abbreviations

⁶Table 2.8: The average water physicochemical parameters of the Tyhume River and WWTP in autumn, winter, and spring seasons.

Physical Parameter			Chemical Parameter								
Season	Site Code	Temp °C	EC (mS/m)	Turbidity (NTU)	Chloride (mg/L)	Sulphate (mg/L)	COD (mg/L)	Nitrate (mg/L)	DO (mg/L)	Phosphate (mg/L)	pH
<i>Average ± St. Dev</i>				<i>Average ± St. Dev</i>							
<i>Autumn</i>	<i>AU</i>	14.75±0.01	15.30±0.00	12.82±1.39	n.d	n.d	82.10±42.73	n.d	7.70±0.00	n.d	8.77±0.40
	<i>AM</i>	17.27±0.01	37.47±0.36	35.93±0.31	7.67±0.44	n.d	11.57±1.84	10.00±0.00	7.70±0.00	n.d	7.67±0.01
	<i>AL</i>	17.58±0.00	56.47±0.04	15.80±1.03	n.d	n.d	100.6±34.49	25.00±0.00	7.70±0.00	n.d	10.60±0.18
	<i>AE</i>	20.32±0.08	93.77±0.42	4.79±0.16	n.d	65.67±0.44	30.47±4.51	n.d	7.13±0.42	n.d	7.61±0.07
	<i>AI</i>	21.40±0.01	98.00±0.80	233.3±54.44	n.d	13.33±2.22	198.3±1.11	n.d	3.50±0.47	n.d	8.03±0.01
<i>Winter</i>	<i>AU</i>	9.97±0.12	8.70±0.13	4.73±0.14	n.d	n.d	123.7±11.11	n.d	7.50±0.00	0.06±0.00	10.05±0.31
	<i>AM</i>	11.95±0.01	20.00±0.00	8.61±0.07	n.d	n.d	104.3±5.11	n.d	7.40±0.07	0.42±0.02	8.07±0.16
	<i>AL</i>	11.33±0.02	32.60±0.20	11.81±0.35	n.d	n.d	69.33±26.44	n.d	7.43±0.04	n.d	10.53±0.05
	<i>AE</i>	13.76±0.02	46.47±0.04	7.33±0.80	28±0.00	56.67±0.44	47.67±12.89	66.67±44.44	7.00±0.27	n.d	8.21±0.20
	<i>AI</i>	17.58±0.01	59.57±0.04	92.17±1.11	n.d	16.00±0.67	258.7±19.56	n.d	5.27±0.64	n.d	7.57±0.03
<i>Spring</i>	<i>AU</i>	13.58±0.15	9.30±0.00	4.41±0.11	n.d	n.d	33.33±13.11	10.00±0.00	7.10±0.47	n.d	5.41±0.00
	<i>AM</i>	17.75±0.02	20.60±0.07	10.08±0.32	n.d	n.d	32.67±7.78	10.00±0.00	7.60±0.00	n.d	5.42±0.07
	<i>AL</i>	15.60±0.12	31.80±0.00	9.47±1.1	n.d	n.d	9.03±0.16	10.00±0.00	7.60±0.00	n.d	7.17±0.07
	<i>AE</i>	21.06±0.14	54.43±0.09	7.58±0.58	n.d	73.67±0.44	48.27±4.71	100.00±0.00	7.53±0.09	n.d	5.65±0.02
	<i>AI</i>	18.98±0.02	60.90±0.27	128.3±6.44	n.d	116.0±2.67	162.3±1.78	n.d	5.77±1.04	n.d	6.27±0.01

⁶ Please refer to Table 2.5 for site code and acronyms/abbreviations

Table 2.9: Water quality guidelines for WHO, TWQR, and SANS

<i>Water Quality Test</i>	<i>World Health Organisation Guideline value</i>	<i>Target Water Quality Range</i>	<i>South African National Standard</i>
<i>Temperature</i>	30-32		
<i>pH</i>	6.5-8.5	6-9	≥ 5 and ≤ 9.7
<i>EC</i>		0-70	
<i>Sulfate</i>		0-200	≤ 500
<i>Nitrate</i>	50	0-6	≤ 11
<i>Phosphate</i>	0.5		
<i>Chloride</i>	0.5-2		≤ 300
<i>COD</i>	10		
<i>DO</i>	7.5	0-1	
<i>Turbidity</i>	5	0.1	≤ 1

TWQR= Target Water Quality Range; WHO GV=World Health Organisation Guide value; SANS= South African National Standard

Temperature

The temperature plays an integral part in creatures' metabolic activities, and it serves as a biologically significant component (Smitha & Shivashankar, 2013). The temperature of the water is the primary element that influences the biological activities of aquatic creatures. Freshwater fluctuates from zero to 35 °C, which depends on the source, the depth of water, the season of the year, geographic location, and sampling time. The temperature ranged from 9.97°C to 22.06°C. In autumn, the temperature ranged from 14.75°C to 20.96°C in streams and 18.37°C to 22.06°C in the WWTP. In winter, it went from 9.97°C to 13.87°C in river water and 13.76°C to 18.11°C in sewage work. While in spring, it was documented 12.08°C to 20.91°C in river water and 18.53°C to 21.06°C in WWTP.

Additionally, the lowest temperature values were found in autumn, the GU and AU. The findings indicate that there is a considerable variation in the average values between the sites. Following WHO guidelines, temperature values between 30 to 32°C are ideal for water quality. The highest temperature was observed in autumn (BM at 20.96°C, BC at 22.6°C, AE at 20.32°C, and WWTP). Furthermore, in spring, BL and AE's water temperature at 20.91°C and 21.06°C, respectively. There are no guideline values for temperature in TWQR and SANS. The temperature influences water's physical characteristics, namely, density, the solubility of gases such as carbon dioxide and oxygen, viscosity, and other things. The water temperature has adverse effects on the biosorption process of dissolved heavy metals. When the water temperature rises, the harder it becomes for aquatic life to acquire adequate oxygen to respond to their needs. Whole organisms involved with freshwater are poikilothermic, which means that they are incapable of controlling their core body temperature.

Consequently, they heavily depend on room temperature and are highly sensitive to alter water temperatures (DWAF, 1996). Thus, dramatic changes in water temperature can seriously damage aquatic creatures and give rise to lethality. Less extreme temperature variations in water bodies can have sub-lethal implications or cause deviation in the current underwater

community. Thermal pollution may result in aquatic creatures' population shifts (Shukla et al., 2013). Temperature data indicate that temperature variations are not at the stage that can influence aquatic life adversely. Figure 2.7 shows the fluctuations in water temperature among the sampling sites.

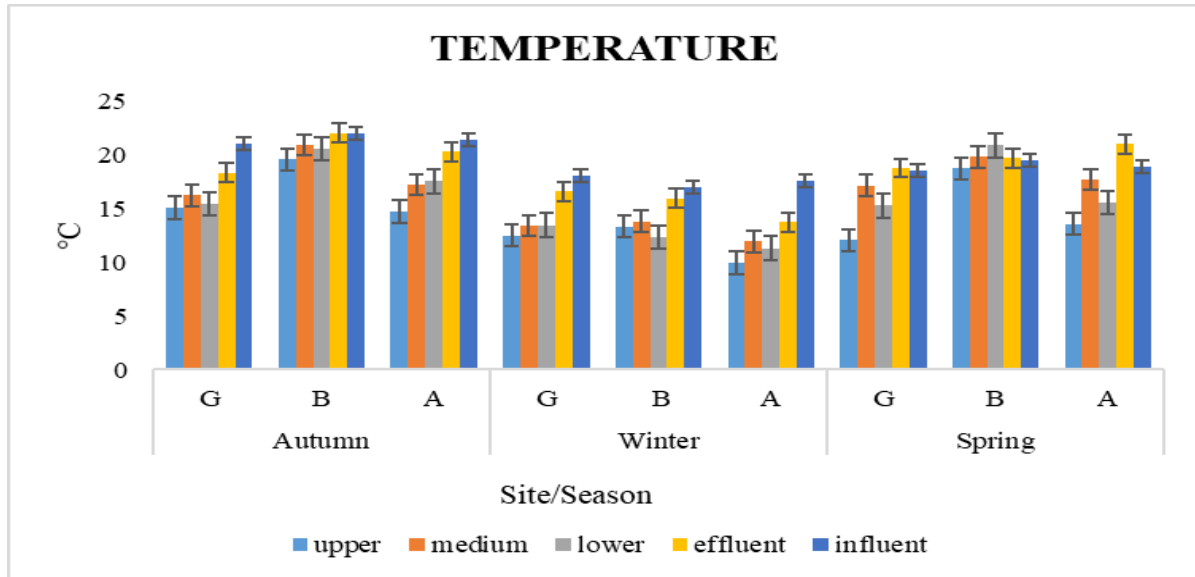


Figure 2.7: Water temperature representing all seasons of sampling.

G-Bloukrans River, GI/GE- Makhanda WWTP

B- Buffalo River, BI/BE- King William's Town WWTP

A- Tyhume River, AI/AE- Alice WWTP

pH

The pH is a parameter that demonstrates chemical properties and biological characteristics and is employed for categorizing the basicity and acidity of water. Acidic water has an extra (H⁺), and alkaline water has an additional (OH⁻). The pH is one of the most significant measures frequently conducted in natural water and bioactivities, and fluctuations influence the water pH in the surrounding atmosphere's temperature. The pH should be ideal when it is between six and nine.

The pH ranges from 4.32 to 10.60. The average pH values of season 1 are within the pH range of 6.0 to 9.0. In autumn, the river water pH ranged from 7.35 to 10.6 and 7.61 to 8.37 in WWTP. It ranged from 4.32 to 10.53 in river water and 6.13 to 8.34 in WWTP in winter. In contrast to winter, the pH value was documented from 5.41 to 7.17 in river water and 5.65 to 6.68 in WWTP. The lowest concentration was observed in winter (GU at 4.32 and GL at 5.02), spring (BU at 5.99, AU at 5.42, BM at 5.69, AM at 5.42, BE at 5.98, AE at 5.65, and BI at 5.97).

In comparison, the highest range of pH was observed in autumn (AL at 10.6) and winter (AL at 10.53). Following SANS, sites with greater than five are suitable for human consumption. The pH value in AL shows that the chances of toxicological effects related to deprotonated species rise substantially. High pH is fatal. The water may have a bitter taste. These analyses

reveal that 62.2% of these sites have no substantial impact on health because of the noxiousness of dissolved metal ions and protonated species.

Lower pH impacts on taste might be observed occasionally. The likelihood of deprotonated species' poisonous effects rises sharply concerning season autumn (AL) and season winter (AU and AL). On those grounds, the water tastes sour when the pH is above nine (DWAF, 1996). It could arise out of anthropogenic activities; for instance, industrial wastewater discharge can bring about pH changes. The alteration of pH may result from the water source's geological conditions (Ander et al., 2016). The highest pH could be under the buffering capacity of water and earth features of the river basin. As noted above, the median pH range complies with the requirements. For the water source to be fit for aquaculture, Tyhume, Buffalo, Bloukrans Rivers, and the wastewater treatment plants are fit to be consumed concerning pH. Concerning the impacts, there are none. The higher the pH, the lower the chloride's germicidal potential (Kumar et al., 2012). Figure 2.8 shows the fluctuations in water pH among the sampling sites.

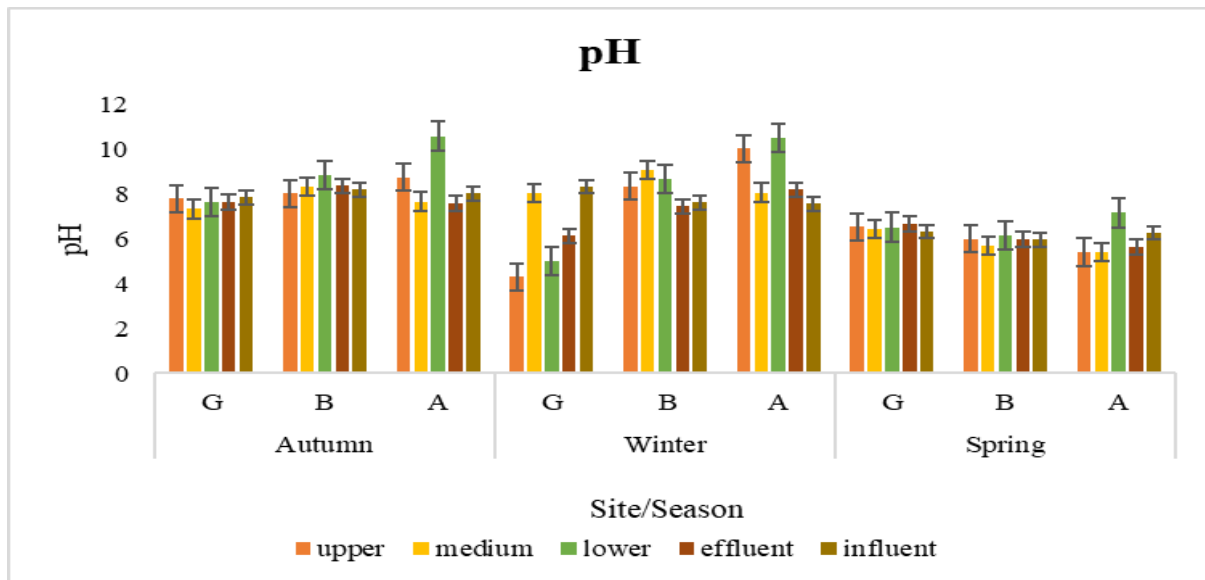


Figure 2.8: Water samples of pH representing all seasons of sampling.

Turbidity

Turbidity is also the dominant test in the determination of water quality. The concentration turbidity ranged from 4.41 to 318 NTU. Turbidity should be perfect when it is below 5 NTU. In autumn, river water turbidity ranged from 6.49 to 44.93 NTU and 4.79 to 318 NTU in WWTP. Whereas in winter went from 4.96 to 85.03 NTU in river water and 7.33 to 318 NTU in WWTP. In spring, river water turbidity ranged from 4.41 to 38.43 NTU and 7.58 to 246 NTU in WWTP. The averages of turbidity in all sites exceed the turbidity of the Target Water Quality Range of 0 to 5 NTU and SANS.

The highest turbidity was observed in autumn (GM and BM at 43.07 NTU and 47.97 NTU). In

contrast to the winter season, the turbidity of GL was 85.03 NTU. Acceptable turbidity in conformity with World Health Organization guidelines is seen in autumn (AE at 4.79 NTU), winter (GU at 4.96 NTU and AU at 4.79 NTU), and spring (GU at 4.63 NTU and AU at 4.41 NTU). The ranges are higher than 10 NTU, resulting in harsh effects. Following the data obtained, only three sites are within the criteria of the WHO, SANS, and TWQR. Within the WWTPs, influent locations have shown the highest turbidity values than effluents in all three seasons. Turbidity can differ throughout the season. Big rivers are usually less turbid in winter but increase drastically during runoff when the water transports soil within the river. GL in autumn may be due to human activities in the upstream area river. The results show no considerable variation on the average of the turbidity between the sites. There is no health-based guiding principle, notwithstanding, bacteria, fungi, viruses, and protozoa are generally fastened to particulate matters (Yahya et al., 2019).

Consequently, turbid water may be microbiologically polluted and vicariously create medical problems. The high amount of turbidity reduces the filter-runs, which leads to disease-causing organisms being most dangerous to humanitarian conditions. Consuming water that is exceptionally turbid and chlorinated can cause threats to health (WHO, 1996). The increment of water turbidity value gives rise to the disturbance of light penetration. Thus, this harms aquatic life and worsen surface water quality. The turbidity in the WWTPs may result from natural sedimentations (Smitha & Shivashankar, 2013). Besides, a considerable degree of turbidity can back up microscopic organisms by the impacts of decontamination, causing the chlorine demand and minimizing the execution of disinfecting treatments (WHO, 2012).

Turbidity in water is due to suspension substances made up of a combination of dead matters suchlike particles of soil and organic materials. The second of the two can be both biotic and abiotic factors. Substances in suspension can clog or cause harm to fish gills, reducing their resistance to disease, minimizing their growth performances, affecting the eggs and larval maturing, and effectiveness of fish catching method (Overstreet & Hawkins, 2017). Besides, substances in suspension support adsorption media for heavy metals and many dangerous organic contaminants (Lawal et al., 2017). Twenty-one percent of these results do not fulfill the WHO guideline and Target Water Quality Range criterion. Figure 2.9 shows the fluctuations of water turbidity among the sampling sites.

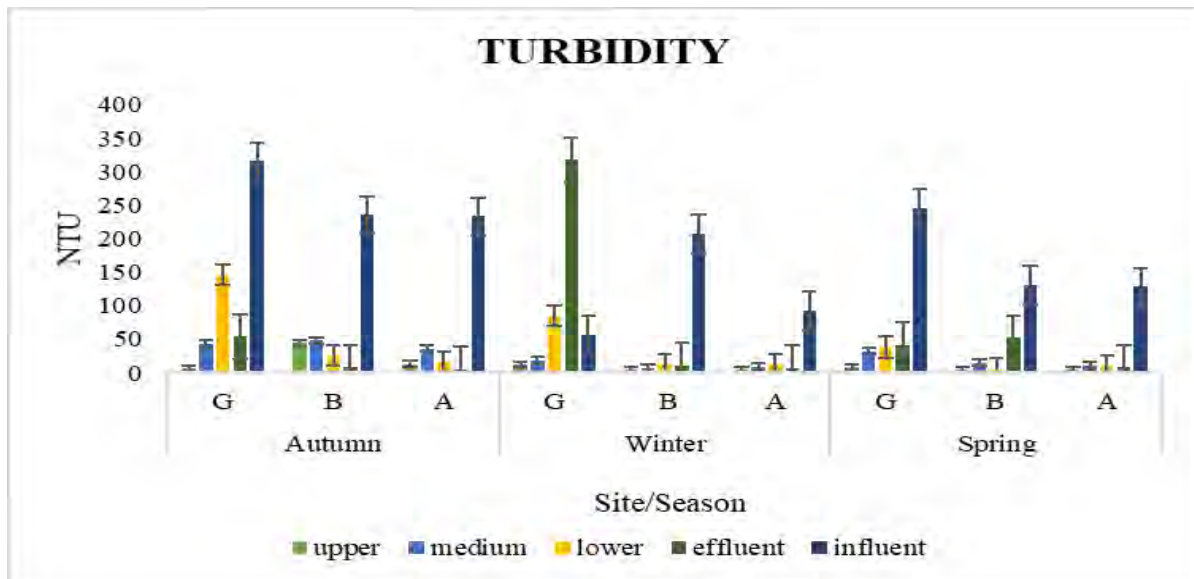


Figure 2.9: Turbidity of water samples during seasons of sampling

Electrical conductivity

Electrical Conductivity is one of the most crucial parameters, particularly in fish farming, and the suitable range of EC is 0 to 70 mS/m. The EC values imply the existence of many salts in the streams. The amount of EC ranged from 8.70 to 381.3 mS/m. In autumn, EC concentration ranged from 15.3 to 318 mS/m in streams and 98 to 304.4 mS/m. It ranged from 8.70 to 173.1 mS/m in river water and 46.47 to 201 mS/m in WWTP in winter. While in spring, EC levels ranged from 9.30 to 182.2 mS/m in river water and 54.43 to 173.4 mS/m in sewage work. The highest EC level was observed in autumn (GM at 381.3 mS/m, GL at 315.2 mS/m, GE at 291.5 mS/m, and GI at 304.4 mS/m). Additionally, it was also observed in winter (GU, GL, GE, and GI at 157.7 mS/m, 173.1 mS/m, 173.9 mS/m, and 201 mS/m, respectively). It was documented in spring in GM, GL, GE, and GI at 152.8 mS/m, 182.17 mS/m, 163.2 mS/m, and 173.4 mS/m. All sites in Tyhume River have shown EC values within the standard throughout all seasons, except for AE and AI at 93.77 mS/m and 98 mS/m, respectively. Such results indicate no health effects. All the sites of Buffalo River and the treatment plants show no health effects as well. These values fall within the range of 150 to 300 mS/m. Therefore, the intake of water does not generate damage to health in the short-term. Thus, water consumption may be accepted with the body's salt balance (DWAF, 1996). Higher values in EC point out that more chemicals have dissolved in water. The presence of nitrate ions, sulfate ions, chloride ions, and suchlike influence water conductivity. The EC values become the highest with the increase in pollution degree (Kumar & Prabhakar, 2012). The lowest EC range could be because of the most increased precipitation that minimizes the number of dissolved solids by diluting water in the dam via overflow, raising the water quantity (Hubert & Wolkersdoefer, 2015). The findings show a significant difference in the sites. Figure 2.10 shows the fluctuations of water EC among the sampling sites.

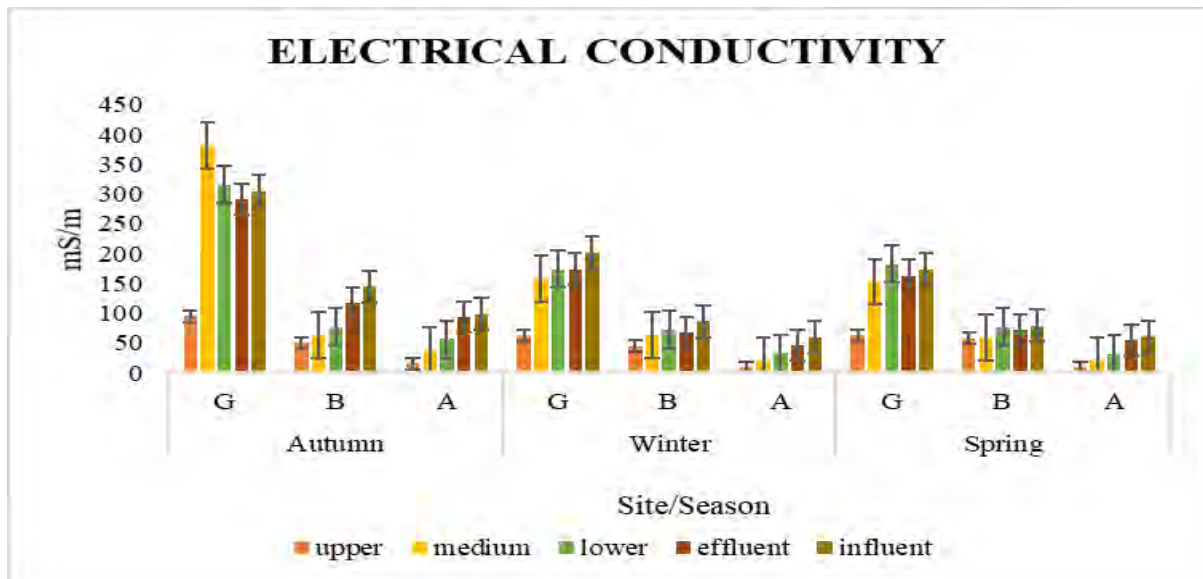


Figure 2.10: Electrical conductivity of water samples representing all seasons of sampling

Nitrate

Nitrate is the finished product of nitrogenous organic substances. High levels of nitrate in water imply the presence of pollution. The nitrate sources in water are human excreta, animal waste, industrial wastewaters, agricultural waste and fertilizers, and grass via sewer systems. Moreover, the nitrate source could result from the oxidation of alternative ways of nitrogen composites such as ammonia and nitrite into nitrate (Hisseien et al., 2015). Nitrate values ranged from not detected to 100 mg/L. In autumn, the nitrate concentration was observed from not detected to 16.67 mg/L in river water and not detected to 16.67 mg/L in sewage work. While in winter, it ranged from not detected to 33.33 mg/L in river water and not detected to 66.67 mg/L in WWTP, and spring nitrate ranged from not detected to 25 mg/L and not detected to 100 mg/L in streams and WWTP, respectively.

The highest nitrate value was observed in winter (AE at 66.67 mg/L) and spring (AE at 100 mg/L); this means that the sample is highly polluted. The increase in nitrate amount in surface waters leads to troubles such as oxygen level in water reduced and the impacts on aquatic life, flora, and algae (Gupta, 2017). Hence, showing no adverse health effects. According to the SANS guidelines, the autumn season (GU, AU, BM, AM, and AL at 10mg/L are both within limits. The permissible value in conformity with the TWQR was observed in winter (BM at 6.67 mg/L). While in autumn (BL, AL, and BE at 16.67 mg/L are within the acceptable range, as stated in WHO guideline. Therefore, the results show rare cases of methemoglobinemia in babies, which stops the oxygen level, but no results in adults (DWAF, 1996).

However, as recommended by the WHO criterion, most site values were below the permissible limit. Concentrations of nitrate that exceed 10 mg/L in potable water pose a risk to the health of infants. Acu (2000) reported that the mortality rate of fish starts at 4mg/L or higher. Besides, high levels can also promote algae growth that lowers water quality (Khatri & Tyagi, 2015). Figure 2.11 shows the fluctuations of water nitrate among the sampling sites.

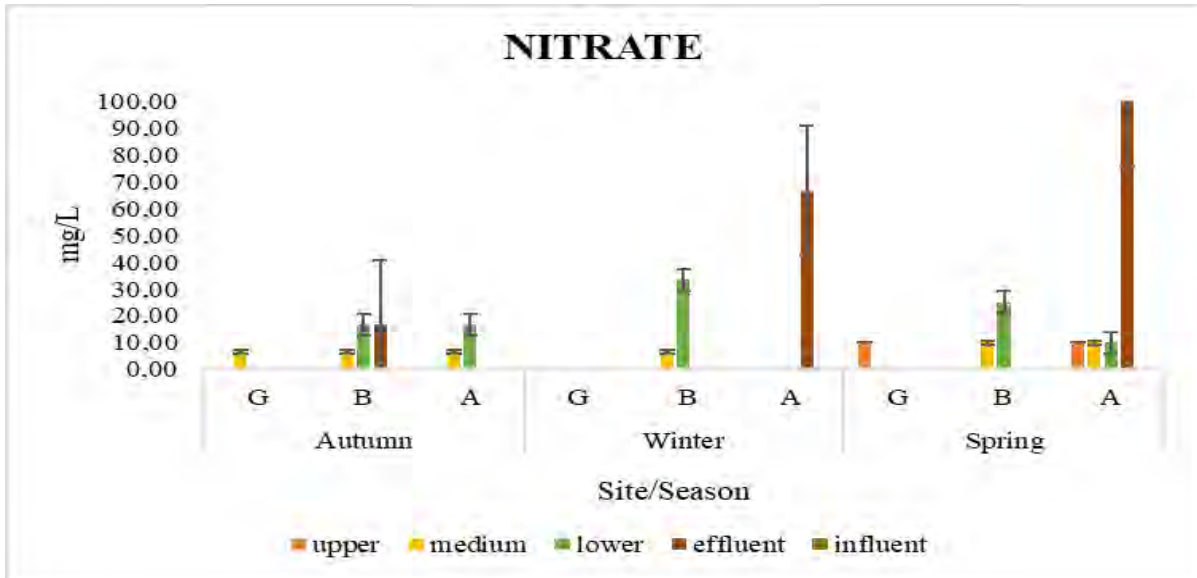


Figure 2.11: Nitrate in water samples representing all seasons of sampling.

Sulfate

Sulfate ions arise in natural waters promoting permanent hardness. Among natural anions in the water, sulfate is essential for enhanced biological conductivity (Omer, 2019). Sulfate concentrations range from not detected to 193.00 mg/L. In autumn, sulfate concentration ranged from not detected to 120 mg/L in river water and not detected to 17067 mg/L in WWTP. While in winter and spring, the level went from not detected to 193 mg/L in streams and 16 to 192 mg/L and 34.33 to 183 mg/L in sewage works, respectively. The highest sulfate concentration was observed in autumn (GE at 170.67 mg/L) and winter (GU, GL, and GE at 164.7 mg/L, 193 mg/L, and 192 mg/L, respectively). In comparison to spring, it was observed in GM, GL, and GE at 159.3 mg/L, 193 mg/L, and 183 mg/L, respectively. It shows that the levels fall under the Target Water Quality Range criterion of 0 to 200 mg/L; therefore, there are no health effects. All the sites with 4 mg/L of sulfate concentration are under the detection limit of 5 mg/L. Potential causes of high levels could be the utilization of cleansing agents and soaps by city dwellers (Smitha & Shivashankar, 2013). The sulfate values are within the acceptable range of the SANS guideline.

The intake of ample amounts of sulfate in potable water often leads to diarrhoea. Persons exposed to high levels of sulfate concentrations in their freshwater for over extended periods become adapted and stops undergoing such effects. High levels of sulfate exert mostly severe health effects (DWAF, 1996). The concentrations of water sulfate in the surface water are 5mg/l, even though the levels of several 100 mg/L can transpire where the dissolution of mineral sulfate or dismissal of sulfate-rich effluents from acid rock sewerage is conducted. Atmospheric sulfur dioxide released on the burning of fossil-fuel-based causes sulphuric-acid in rainwater; consequently, this gives rise to the return of sulfate to the water surface in the ecosystem. Sulfates enter the water from various sources like weathering and dissolution of minerals, the decay of plants and animal remains. Apart from industrial effluents from branches of industry such as sulphuric acid plants, dyes, and insecticides, domestic and municipal wastes

and sulfate-containing fertilizers remain; disposed of in water bodies rises the sulfate concentration. The acceptable limit for sulfate concentration in potable water is 250 mg/L, by WHO, and for that reason, should the sulfate concentration be above the level mentioned earlier, then the water is impure. Figure 2.12 shows the fluctuations of water sulfate among the sampling sites.

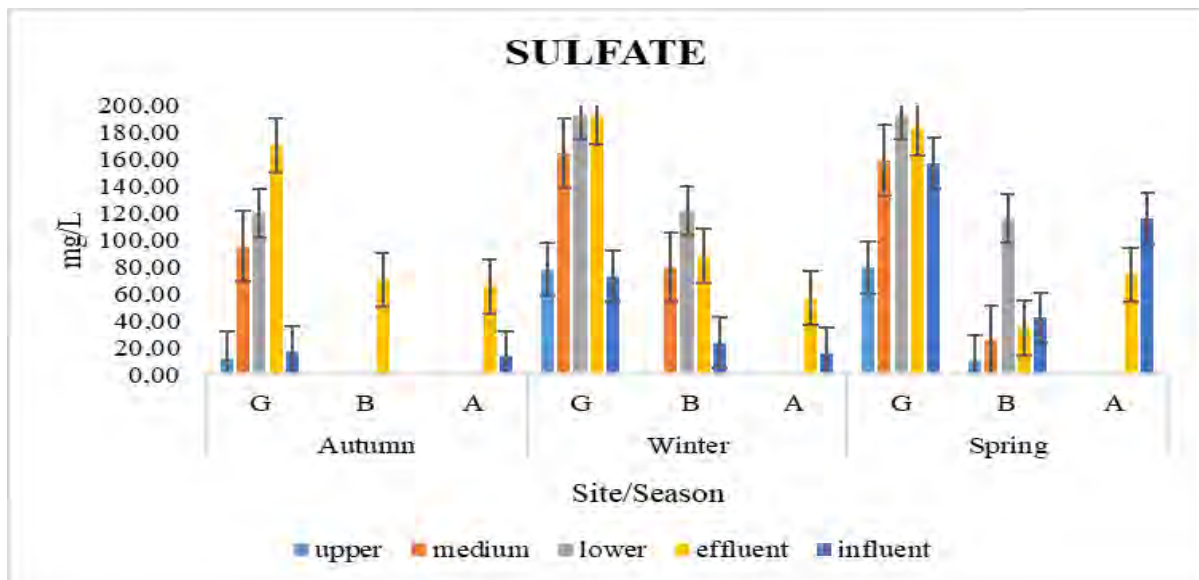


Figure 2.12: Sulfate content in water samples representing all seasons of sampling.

Phosphate

Phosphate water-resource is essential for aquatic life. Phosphate presence in water and wastewater examination has paramount importance. Phosphate in low levels is employed in waterworks to decrease scale formation, enhance water mains' carrying capacity to prevent deterioration in water mains, and eliminate iron and manganese in micro-quantities and coagulation, particularly in acid conditions (Gupta et al., 2016). The phosphate level ranged from not detected to 0.55 mg/L in seasons. The phosphate concentration ranges from not detected in these findings, which is under the lowest detection limit to 0.55 mg/L. In autumn, phosphate levels ranged from not detected to 0.14 mg/L in streams and ranged from not detected to not detected mg/L in WWTP. It ranged from not detected to 0.42 mg/L in winter and spring and from not detected to 0.55 mg/L in river water and not detected to 0.38 mg/L and not detected to 0.24 mg/L in sewage works, respectively.

The highest phosphate concentration was found in autumn (GU and GI at 0.44 mg/L and 0.43 mg/L). Also in winter (AM at 0.42 mg/L and GE at 0.38 mg/L) and spring (BM at 0.46 mg/L and GL at 0.55 mg/L). All the sites are under WHO guidelines. The reason why phosphate concentration fluctuates could be the utilization of agricultural fertilizers that contain phosphate. Phosphate is classified among the mineral nutrient that has effects on the production capability of aquatic life. The presence of high concentrations of phosphate in water

demonstrates that contamination is via industrial effluents and wastewater. Phosphate in water boosts the development of nuisance, causing microbes. Phosphorus is mostly present in the water and sewer as phosphates and falls into orthophosphates, phosphates, and organic phosphates. Such composites are available in the remains of plants and animals (Panda et al., 2018). An additional phosphate source is the specific action of bacteria that discharge phosphates from organophosphorus compounds present in remains of plants, sewer, animal excreta, cleaning products, and phosphate that contains fertilizers utilized by farmworkers for use in agriculture (Ishaq & Khan, 2013). Figure 2.13 shows the fluctuations of water phosphate among the sampling sites.

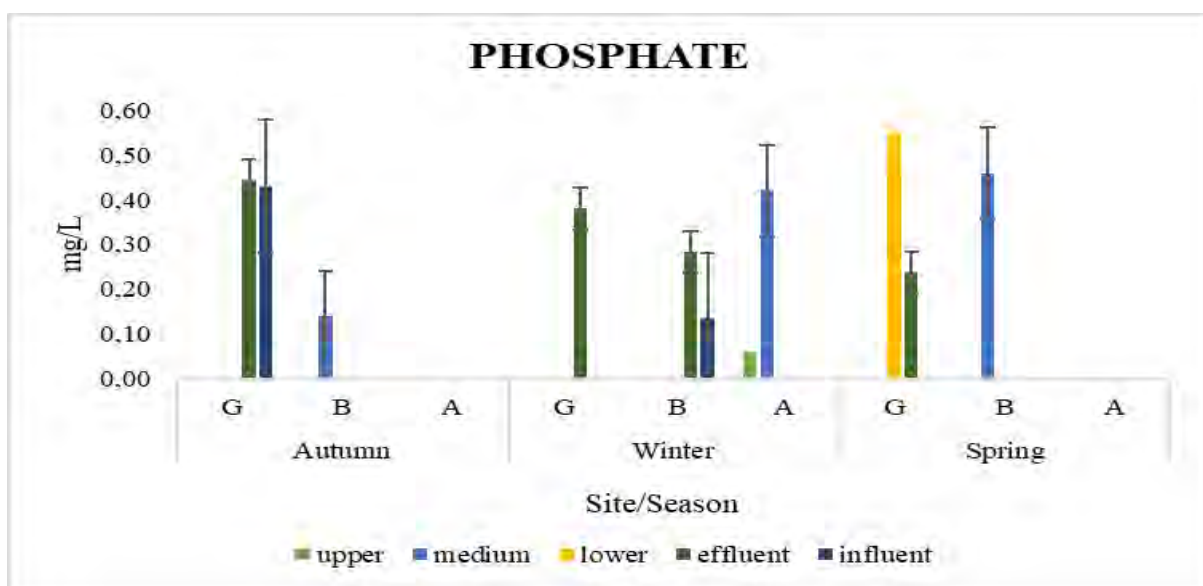


Figure 2.13: Phosphate content in water samples representing all seasons of sampling.

Chloride

Chloride ions are primarily causing-agents of the hardness of the water and naturally occurs in rivers and lakes. Chlorides enter surface water by different sources such as agricultural runoff and wastewater. The level of chloride ranged not detected to 180.67 mg/L. In autumn, the chloride concentration ranged from not detected to 83.33 mg/L in streams and not detected mg/L in WWTP. While in winter and spring, the level went from not detected to 28 mg/L and not detected to 187.67 mg/L in sewage works.

The highest level of chloride was found in autumn (GM at 69.67 mg/L and GL at 83.33 mg/L) and in winter (AE at 28 mg/L). In contrast to spring, chloride level was observed in BU at 180.67 mg/L. The rest of the sampling points are below the lowest limit of the measuring range of 5 to 200 mg/L. All the sites are within the Target Water Quality Range of 0 to 100 mg/L, except BU in spring at 180.67 mg/L. That could imply that there is a presence of organic components, possibly animal-based. Chloride ions in potable water do not pose detrimental

effects on public health. There are no health effects; however, high levels can produce an unpleasant salty taste and potential expansion in the rate of corrosion in household equipment. Chloride raises with a certain amount of eutrophication (Weldemariam, 2013). Chloride is present in every kind of water. The presence of relatively high chloride in water means contamination because of the sewer, sediments, and industrial wastewaters. Low levels of chloride in water are vital for normal cell function in plants and animals' lives.

Additionally, the chloride presence in waters is too often by the nature of lands traversed (Toure et al., 2017). WHO guide value suggests the ranges of 0.5 to 2 mg/L free residues in potable water. That means that the concentrations obtained here do not comply with WHO GV. A significant amount of chloride work with sodium forming the water to be salty and raising the value of total dissolved solids of water (Malik et al., 2012). The concentration of chloride is ordinarily low in natural waters. The drinking water standards accept the level of chloride that is below 250 mg/L. Figure 2.14 shows the fluctuations of water chloride among the sampling sites.

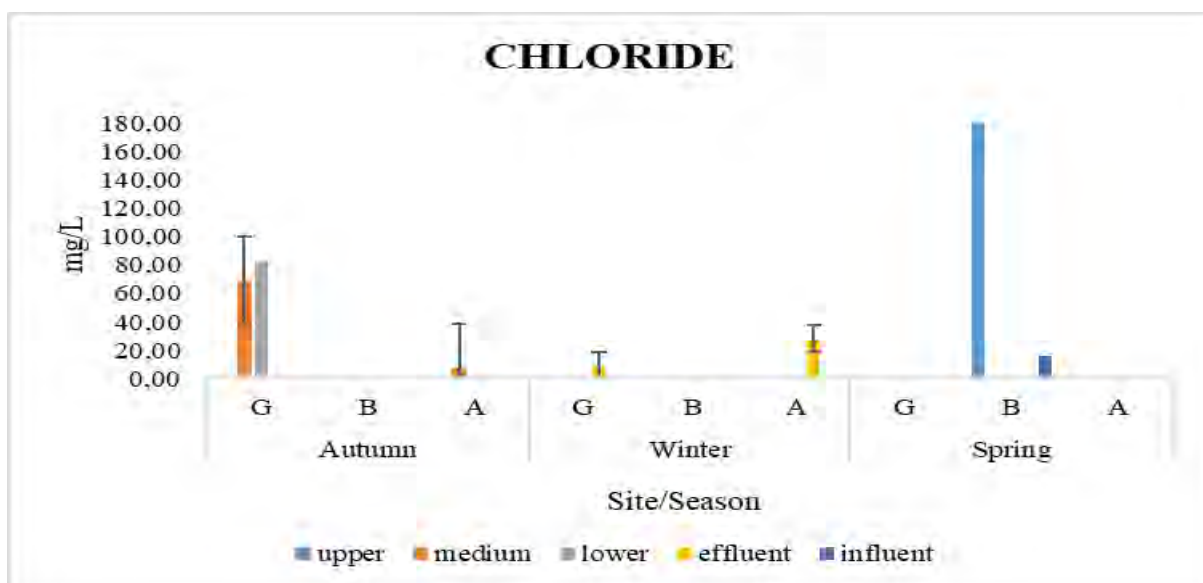


Figure 2.14: Chloride content in water samples representing all sites of sampling

Dissolved oxygen

Dissolved oxygen is an essential parameter for examining water quality in rivers and lakes, the amount of oxygen dissolved in water. Dissolved oxygen is the primary test of water contamination. Oxygen is of the utmost importance to the survival of animate beings and aquatic vegetation. Therefore, the scarcity of dissolved oxygen is not merely an indication of contamination but destructive to fish as well. Dissolved oxygen might be acquired from the external environment. Dissolved oxygen is essential for the ventilation of living creatures, and it is generated by plant photosynthesis only if adequate sunlight and nourishment are available (Muhammad et al., 2016). Poikilothermous vertebrates such as fish utilize much oxygen with

the increase in temperature. DO demonstrates the capability of the expanse of water to sustain aquatic life. It signifies the oxygen volume that is in the water. Anaerobically, in bodies of water, the final product of chemical reactions causes unpleasant colour, smell, and taste of water (Smitha & Shivashankar, 2013).

Dissolved oxygen is used up by oxidation of organic material of living agents that are in the water. Oxygen deficiency in water is likely to kill fish and aquatic animals. In general, the river running water is about 10 mg/L. The findings of this research range from 2.43 to 7.70 mg/L in both wet and dry seasons. In autumn, the DO level ranged from 4.37 to 7.70 mg/L in streams and 2.49 to 7.13 mg/L in WWTP. The DO ranges from 6.60 to 7.57 mg/L in river water and 5.27 to 7 mg/L in sewage work in winter.

The DO went from 6.97 to 7.60 mg/L in streams and 4.53 to 7.53 mg/L in WWTP in spring. The highest DO concentration was found in autumn (AU, AM, and AL at 7.70 mg/L). In spring, it was found in AM and AL at 7.60 mg/L, while in winter, it was observed in GU at 5.75 mg/L. The highest DO concentrations were found in both Tyhume river sites in both seasons. The lowest DO level was found in autumn (GI at 2.43 mg/L, GM at 4.37 mg/L) and spring (BI at 4.53 mg/L). The decrease in oxygen because of temperature increase could affect the development of the eggs that, in turn, could alter the age class structure, dominancy, and guild abundance (Muhammad et al., 2016). In spring, dissolved oxygen levels can be related to elevated activities of microbes requiring much oxygen for metabolizing and degradation of organic substances.

The fluctuations of DO concentration in the data could result from climate variability and geographical changes in the site and the sampling period. The higher the concentration of dissolved oxygen in bodies of water, the less the contaminants and conversely. The level of dissolved oxygen in water bodies increases when input sources are higher than the outputs, and the minimum level required for dissolved oxygen is 6 mg/L. For fish, breeding is 4mg/L (Panda et al., 2018). The dissolved oxygen level of every stream changes over 24h with seasonal change, like photosynthesis, temperature, and turbidity, to mention the few changes with seasons. The concentration of DO of any river basins could be influenced by discharging industrial and agricultural wastes and ultimately household wastes to the river basins as dissolved oxygen utilizes in the process of a redox reaction to steady the contaminants (Panda et al., 2018). There is no direct impact of dissolved oxygen on public health. Healthy water contains DO levels above 6.5 to 8 mg/L. Figure 2.15 shows the fluctuations of water DO among the sampling sites.

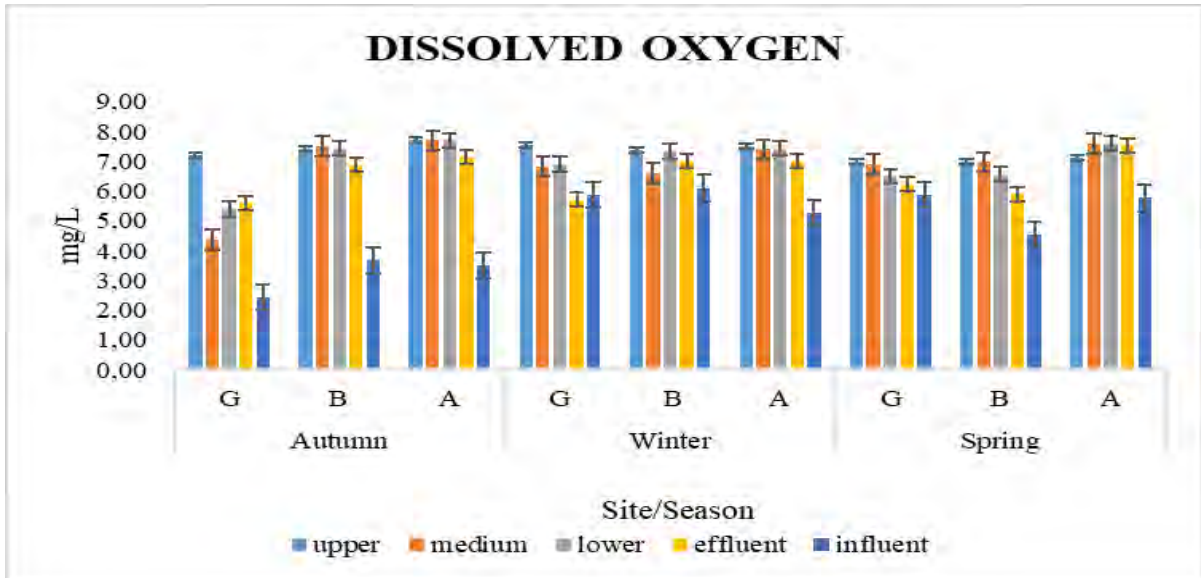


Figure 2.15: Dissolved oxygen in water samples representing all seasons of sampling.

Chemical oxygen demand

Chemical Oxygen Demand measures all organics such as biodegradable and non-biodegradable matters. COD is a chemical test that utilizes strong oxidizing chemicals; heat and the outcome are accessible in an hour (Yao et al., 2014). COD is employed to define the level of contamination of water. When the COD concentration is higher than 25 mg/L, it implies a disease presence. Besides, when the level exceeds 50 mg/L, it means intense pollution and toxic effects for aquatic organisms. COD values ranged from 5.33 to 2373 mg/L. In autumn, COD levels ranged from 5.33 to 199 mg/L in river water and 30.47 to 198.3 mg/L in sewage work. While in winter and spring ranged from 39.27 to 109 mg/L and 9.03 to 135.7 mg/L in streams, 47.67 to 2373.3 mg/L, and 48.27 to 296 mg/ in WWTP. The highest COD value was observed in autumn (GM at 184.3 mg/L, GL at 199 mg/L, AL at 100.4 mg/L, GI at 111 mg/L, BI at 103.1 mg/L, and AI at 198.3 mg/L). In winter, it was observed in AM at 104.3 mg/L, GL at 109.3 mg/L, GE at 121.7 mg/L, GI at 497.7 mg/L, BI at 2373.3 mg/L, and AI at 258.7 mg/L. In contrast to spring, COD level was observed in GM at 135.7 mg/L, GL at 124 mg/L, GE at 118.7 mg/L, BE at 152.3 mg/L, GI at 296 mg/L, BI at 155 mg/L, and AI at 162.3 mg/L. The concentration values of these findings have demonstrated significant differences amongst the sampling sites.

While the minimum COD concentrations were found in autumn (GU at 5.33 mg/L) and winter (AL at 9.03 mg/L), COD is connected with living, and non-living contaminants give rise to unfavourable circumstances for the development of microbes. High concentration values might be linked to the leaching and conveyance of wastewater from households, agricultural and industrial pollutants. As stated by WHO GV, the acceptable limit is 10 mg/L. Site GU in autumn and AL in autumn and spring comply with the permissible limit. Such condition arises from chemical substances like oxygen demand in nature originating from the washing of other cars and surface-runoff (Tawati et al., 2018). Figure 2.16 shows the fluctuations of water COD among the sampling sites.

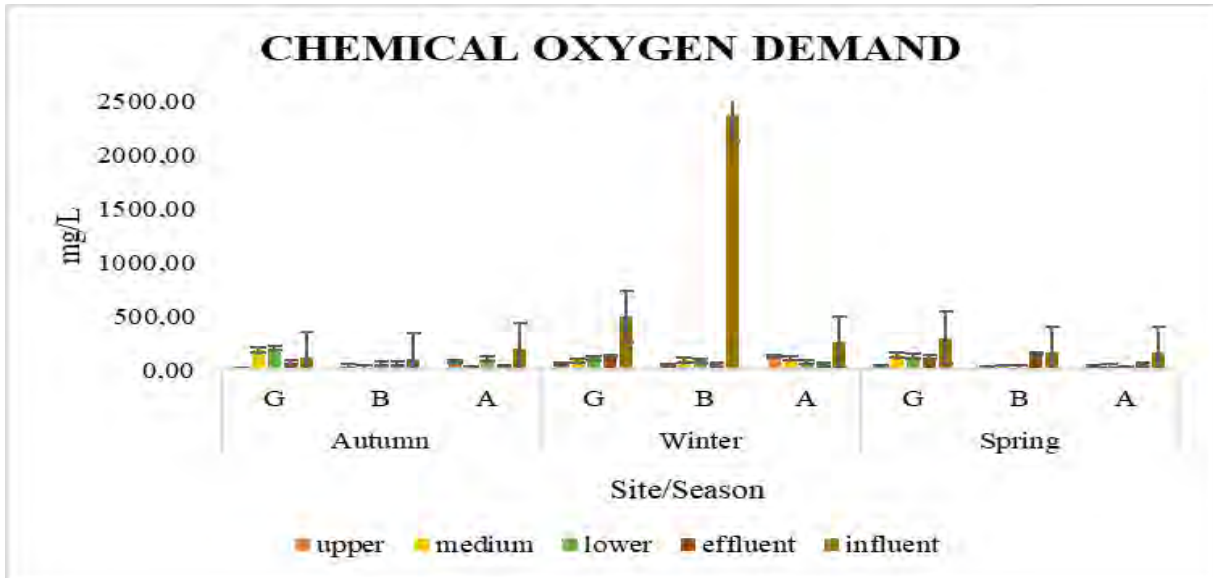


Figure 2.16: Chemical oxygen demand in water samples representing all seasons of sampling.

2.10.2 The Artificial Neural Network Model structure

Specific parameters were chosen from the ten initial settings by factorial analysis that demonstrated that the water quality of interest areas was primarily affected by specific physicochemical properties. The physicochemical parameters are chloride, sulfate, temperature, phosphate, pH, electrical conductivity, turbidity, and dissolved oxygen. The most crucial thing to do to develop a prediction model is to select the appropriate independent parameters. The feed-forward model was used with at least one hidden layer to model nonlinear and complicated functions. The artificial neural network type that was employed is Multilayer Perception (MLP). It is a feedforward model that arranges the input data sets towards the correct output sets. It is challenging to pick the number of hidden layers; however, several publications suggest that one hidden layer is reasonably well to validate the prediction (Hossain, 2014; Hung et al., 2009; Mohamed, 2019; Paneiro & Rafael, 2020). Every artificial neural network subgroup's training and validation results are tabulated in Table 2.12, 2.13, 2.14, and 2.15. Through the MLP architecture, the training set may be forecasted to a high level of precision. The MLP reached substantially less error confirming that the artificial neural network models can find the non-linear relationships among the variables. MLP is useful in figuring out the relationship between several inputs and output variables. Table 2.11 shows a summary of the active network.

The statistical variables of annual water quality parameters at the river basins of Tyhume, Buffalo, and Bloukrans Rivers and their municipal wastewater treatment plants are given in Table 2.10 below. For obtaining the best results from the performance of ANN, the network model has to be formulated systematically. The significant stage of developing the ANN model is to decide the model input variables, which have a considerable influence on the performance model. The input layers have four neurons: temperature, chloride, sulfate, and phosphate, whereas the output layers have four neurons: pH, electrical conductivity, turbidity, and dissolved oxygen.

The multi-layer feedback neural network with propagation was employed to train, test, and validate the multi-layer neural network due to its simplicity and suitability. Thus, feedforward backpropagation was selected in building the prediction model for the target output (pH, EC, turbidity, and DO).

The following data set was used: the program/model training set was employed to generate the model of ANN, validating and testing were used to confirm the model's generalization competencies. The measured data collection is divided into 70% of the training set, 10% of validation and testing sets.

Table 2.10: The annual experimental values of physicochemical parameters of streams and WWTPs of Makhanda, Alice, and King William's Town regions.

<i>Sample area</i>	<i>Temperature</i>	<i>Chloride</i>	<i>Sulfate</i>	<i>Phosphate</i>	<i>pH</i>	<i>Turbidity</i>	<i>Electrical</i>	<i>Dissolved Oxygen</i>
<i>AU</i>	12.77	n.d	n.d	n.d	8.08	7.32	11.10	7.43
<i>AM</i>	15.66	7.67	n.d	0.42	7.05	18.21	26.02	7.57
<i>AL</i>	14.88	n.d	n.d	n.d	9.43	12.36	40.29	7.58
<i>AE</i>	18.38	28.00	65.34	n.d	7.16	6.57	64.89	7.22
<i>AI</i>	19.32	n.d	48.44	n.d	7.29	15.28	72.82	4.85
<i>BU</i>	17.24	180.67	9.67	n.d	7.46	18.17	50.35	7.26
<i>BM</i>	18.22	n.d	52.50	0.30	7.71	23.27	61.74	7.02
<i>BL</i>	17.95	n.d	119.00	n.d	7.89	14.34	75.33	7.11
<i>BE</i>	19.25	n.d	64.22	0.28	7.27	23.44	85.24	6.59
<i>BI</i>	19.50	16.00	32.84	0.13	7.27	191.00	102.88	4.77
<i>GU</i>	13.24	n.d	45.84	n.d	6.22	9.14	73.42	7.25
<i>GM</i>	15.63	69.67	127.33	n.d	7.28	30.96	230.60	6.03
<i>GL</i>	14.75	83.33	156.50	0.55	6.41	89.82	223.50	6.27
<i>GE</i>	17.93	9.67	136.78	0.35	7.16	137.00	209.54	5.84
<i>GI</i>	21.51	n.d	63.33	0.43	7.52	206.15	226.27	4.72

G-Bloukrans River, GI/GE- Makhanda WWTP

B- Buffalo River, BI/BE- King William's Town WWTP

A- Tyhume River, AI/AE- Alice WWTP

Table 2.11: Summary of active network

<i>Network Name</i>	<i>R²</i>	<i>MSE</i>	<i>Training Algorithm</i>	<i>Error Function</i>	<i>Hidden Activation</i>	<i>Output Activation</i>
<i>MLP 4-5-4</i>	0.989383	39.03087	BFGS 88	SOS	Logistic	Logistic
<i>MLP 4-9-4</i>	0.993532	39.06589	BFGS 130	SOS	Tanh	Exponential

Table 2.12: Experimental values and predicted values for pH generated by MLP 4-5-4 and MLP 4-9-4. Sample: Train, Test, Validation.

<i>Sample area</i>	<i>Sample</i>	<i>Experimental pH values</i>	<i>Predicted pH values (MLP 4-5-4)</i>	<i>% Difference</i>	<i>Predicted pH values (MLP 4-9-4)</i>	<i>% Difference</i>
<i>AU</i>	Train	8.080000	8.071202	0.11	8.086230	0.08
<i>AM</i>	Train	7.050000	7.048911	0.02	7.097960	0.68
<i>AL</i>	Train	9.430000	9.330070	1.06	9.444249	0.15
<i>AE</i>	Test	7.160000	7.441819	3.94	7.393835	3.27
<i>AI</i>	Train	7.290000	7.442833	2.10	7.351340	0.84
<i>BU</i>	Train	7.460000	7.399046	0.82	7.466864	0.09
<i>BM</i>	Test	7.710000	7.441837	3.48	7.387560	4.18
<i>BL</i>	Train	7.890000	7.441830	5.68	7.621972	3.40
<i>BE</i>	Train	7.270000	7.442062	2.37	7.475793	2.83
<i>BI</i>	Train	6.270000	7.443619	18.72	7.085941	13.01
<i>GU</i>	Train	6.220000	6.220000	0.00	6.221081	0.02
<i>GM</i>	Validation	7.280000	7.426453	2.01	7.733750	6.23
<i>GL</i>	Train	6.410000	6.552220	2.22	6.384595	0.40
<i>GE</i>	Validation	7.160000	7.441715	3.93	7.994136	11.65
<i>GI</i>	Train	7.520000	7.448825	0.95	7.622527	1.36

According to the test data, the networks' percentage difference, both networks adequately understood the relationship between the data set. The percentage differences for the first test data set were comparable/similar, and that of the second set showed a difference in predictive ability. The lowest percentage difference for the second test data set was given by MLP 4-5-4, and therefore, this network best understood the relationship between the independent variables and pH of water.

Table 2.13: Experimental values and predicted values for EC generated by MLP 4-5-4 and MLP 4-9-4. Sample: Train, Test, Validation.

<i>Sample area</i>	<i>Sample</i>	<i>Experimental EC</i>	<i>Predicted EC MLP 4-5-4</i>	<i>% Difference</i>	<i>Predicted EC MLP 4-9-4</i>	<i>% Difference</i>
<i>AU</i>	Train	11.1000	12.7558	14.92	11.1286	0.26
<i>AM</i>	Train	26.0200	27.4272	5.41	41.8489	60.83
<i>AL</i>	Train	40.2900	11.9350	70.38	15.6602	61.13
<i>AE</i>	Test	64.8900	62.7317	3.33	61.9240	4.57
<i>AI</i>	Train	72.8200	86.7355	19.11	75.6735	3.92
<i>BU</i>	Train	50.3500	60.1378	19.44	51.4812	2.25
<i>BM</i>	Test	61.7400	62.7808	1.69	59.0714	4.32
<i>BL</i>	Train	75.3300	62.7223	16.74	81.7645	8.54
<i>BE</i>	Train	85.2400	67.6846	20.60	66.1918	22.35
<i>BI</i>	Train	102.8800	108.4363	5.40	101.2073	1.63
<i>GU</i>	Train	73.4200	73.9280	0.69	73.0470	0.51
<i>GM</i>	Validation	230.6000	62.9033	72.72	93.0826	59.63
<i>GL</i>	Train	223.5000	226.2581	1.23	223.6889	0.08
<i>GE</i>	Validation	209.5400	62.7630	70.05	119.5078	42.97
<i>GI</i>	Train	226.2700	214.8109	5.06	226.2055	0.03

The percentage difference for the test data on both networks was within the acceptable range. Despite these results, MLP 4-5-4 demonstrated that better predictive ability as it exhibited the lowest percentage difference.

Table 2.14: Experimental values and predicted values for turbidity generated by MLP 4-5-4 and MLP 4-9-4. Sample: Train, Test, Validation.

<i>Sample area</i>	<i>Sample</i>	<i>Experimental Turbidity</i>	<i>Predicted Turbidity MLP 4-5-4</i>	<i>% Difference</i>	<i>Predicted Turbidity MLP 4-9-4</i>	<i>%Difference</i>
<i>AU</i>	Train	7.3200	7.3200	0.00	7.4476	1.74
<i>AM</i>	Train	18.2100	7.5026	58.80	10.3960	42.91
<i>AL</i>	Train	12.3600	7.3200	40.78	8.0410	34.94
<i>AE</i>	Test	6.5700	17.3447	164.00	16.5765	152.31
<i>AI</i>	Train	151.200	147.5284	2.43	150.3962	0.53
<i>BU</i>	Train	18.1700	15.3185	15.69	13.9046	23.47
<i>BM</i>	Test	23.2700	17.4291	25.10	16.9588	27.12
<i>BL</i>	Train	14.3400	17.3287	20.84	25.6735	79.03
<i>BE</i>	Train	23.4400	29.5244	25.96	21.8990	6.57
<i>BI</i>	Train	191.0000	201.9652	5.74	192.3942	0.73
<i>GU</i>	Train	9.1400	7.3200	19.91	8.2733	9.48
<i>GM</i>	Validation	3.9600	17.2437	335.45	32.7986	728.25
<i>GL</i>	Train	89.8200	87.0369	3.10	89.3686	0.50
<i>GE</i>	Validation	137.5000	17.3304	87.40	55.9932	59.28
<i>GI</i>	Train	206.1500	206.1500	0.00	205.9516	0.10

The test data set percentage differences for both networks were above the 10% limit, suggesting that the built systems did not adequately understand the relationship between the investigated independent variables and turbidity. It may be because the studied factors not causing a significant effect on water turbidity; therefore, more elements would have to be considered in future studies.

Table 2.15: Experimental values and predicted values for dissolved oxygen generated by MLP 4-5-4 and MLP 4-9-4. Sample: Train, Test, Validation.

<i>Sample area</i>	<i>Sample</i>	<i>Experimental DO</i>	<i>Predicted DO MLP 4-5-4</i>	<i>% Difference</i>	<i>Predicted DO MLP 4-9-4</i>	<i>% Difference</i>
<i>AU</i>	Train	7.430000	7.580000	2.02	7.420167	0.13
<i>AM</i>	Train	7.570000	7.570000	0.00	7.602289	0.43
<i>AL</i>	Train	7.580000	7.580000	0.00	7.596899	0.22
<i>AE</i>	Test	7.220000	7.137646	1.14	6.746576	6.56
<i>AI</i>	Train	4.850000	4.907170	1.18	4.722991	2.62
<i>BU</i>	Train	7.260000	7.261512	0.02	7.239937	0.28
<i>BM</i>	Test	7.020000	7.133850	1.62	7.021295	0.02
<i>BL</i>	Train	7.110000	7.138362	0.40	7.022525	1.23
<i>BE</i>	Train	6.590000	6.638667	0.74	6.598599	0.13
<i>BI</i>	Train	4.770000	4.726695	0.91	4.721815	1.01
<i>GU</i>	Train	7.250000	7.580000	4.55	7.251133	0.02
<i>GM</i>	Validation	6.030000	7.142718	18.45	6.927154	14.88
<i>GL</i>	Train	6.270000	6.214789	0.88	6.331988	0.99
<i>GE</i>	Validation	5.840000	7.138329	22.23	6.721605	15.10
<i>GI</i>	Train	4.720000	4.720000	0.00	4.769756	1.05

The percentage difference for both networks' test data was with the acceptable limit of 10%, revealing that the systems understood the relationship between the data set. MLP 4-5-4 exhibited a percentage difference significantly lower (5.42% difference) than that of MLP 4-9-4 for the first data set. For the second test data, MLP 4-9-4 exhibited the smallest percentage difference, but this was only 1.60% different from that of MLP 4-5-4. These results suggest that the best predictive ability was demonstrated by MLP 4-5-4.

2.11 CONCLUSION

This research evaluated the physicochemical characteristics of 45 water samples of Buffalo River, Tyhume River, and Bloukrans River and the wastewater treatment plants of Buffalo City, Nkonkobe, and Makana Municipalities. The examination was conducted by measuring essential parameters such as temperature, pH, EC, turbidity, chloride, sulfate, phosphate, DO, COD, and nitrate. The data acquired from the examination displays that significant amounts of COD and turbidity of the streams and wastewater treatment plants are not within the Target Water Quality Range criteria, or the South African National Standard (SANS) of drinking water, and World Health Organization rubric. Except, the turbidity and COD, all the parameters were within the permissible limit. The ample amounts of these contaminants might be hazardous for the aquatic and human condition. There are no adverse effects on the survival and development of vegetation and animate being. Pharmaceuticals in water are composed of various chemical and biological compounds. Because of this character, this water can cause variations in the quality of water. When pollutants such as pharmaceuticals enter in water, they influence the quality of our lakes, streams, and rivers. Drug pollutants always find a way to fauna. Fish and aquatic animals continue to be in great danger for biological imbalances and alterations from drug pollution, which may destroy certain species. The model of ANN was used to assess the quality of water in river water and WWTPs.

Nevertheless, the little-known components controlling the quality of river water variables and the dataset's reduced size, an excellent correlation was observed among the predicted and experimented values. The model ANN has a great opportunity as a predictive tool. Most notably, we have seen that the method of MLP was able to learn the system reasonably well. The best predictive ability for water quality was shown by MLP 4-5-4, and this built network may, therefore, be used to determine water quality for any other area with the parameters studied in this work. However, the limited opportunity of this research was a limited number of samples. Although the availability of the amount of data is small, we managed to obtain good results. Should much data made available, the proposed method shall establish the best prediction. The ANN model is a golden and valid instrument that optimizes the observational network by determining important monitoring sites and predicting river water variables' quality with acceptable precision.

REFERENCES

- Ahmed, Z., Mohamed, K., Zeeshan, S., & Dong, X. (2020). Artificial intelligence with multi-functional machine learning platform development for better healthcare and precision medicine. *Database*, 2020.
- Al-Abri, M., Al-Ghafri, B., Bora, T., Dobretsov, S., Dutta, J., & Castelletto, S. et al. (2019). Chlorination disadvantages and alternative routes for biofouling control in reverse osmosis desalination. *Np journal of Clean Water*, 2(1).
- Ander, E. L., Watts, M. J., Smedley, P. L., Hamilton, E. M., Close, R., Crabbe, H., Fletcher, T., Rimell, A., Studden, M., & Leonardi, G. (2016). Variability in the chemistry of private drinking water supplies and the impact of domestic treatment systems on water quality. *Environmental geochemistry and health*, 38(6), 1313–1332.
- Anderson, A. R., Eure, T. R., Orr, M. F., Kolbe, L. J., & Woolf, A. (2017). Hazardous Chemical Releases Occurring in School Settings, 14 States, 2008-2013. *Journal of environmental health*, 80(4), E1–E7.
- Arnold, T. M., Zimmerman, R. C., Engelhardt, K. A., & Stevenson, J. C. (2017). Twenty-first century climate change and submerged aquatic vegetation in a temperate estuary: the case of Chesapeake Bay. *Ecosystem Health and Sustainability*, 3(7), 1353283.
- Aydın, M. Y, Mehmet, S. K. & Kiarash, K.O. (2014). Modelling of Driver Lane Choice Behaviour with Artificial Neural Networks (ANN) and Linear Regression (LR) Analysis on Deformed Roads. *Computer Science & Engineering: An International Journal (CSEIJ)*, 4(1), 47-57.
- Ayers, J. C., Goodbred, S., George, G., Fry, D., Benneyworth, L., Hornberger, G. ... & Akter, F. (2016). Sources of salinity and arsenic in groundwater in southwest Bangladesh. *Geochemical transactions*, 17(1), 4.
- Baharvand, S., & Mansouri Daneshvar, M. R. (2019). Impact assessment of treated wastewater on the physicochemical variables of the environment: a case of Kermanshah wastewater treatment plant in Iran. *Environ Syst Res*, 8, 18.
- Bhateria, R., & Jain, D. (2016). Water quality assessment of lake water: a review. *Sustainable Water Resources Management*, 2(2), 161-173.
- Barakat, A., El Baghdadi, M., Rias, J., Aghezzaf, B., & Slassi, M. (2016). Assessment of spatial and seasonal water quality variation of Oum Er Rbia River (Morocco) using multivariate statistical techniques. *Foreign soil and water conservation research*, 4(4), 284-292.
- Barham, P., Skibsted, L. H., Bredie, W. L. P., Frøst, M. B., Møller, P., Risbo, J., Snitkjær, P., & Mortensen, L. M. (2010). Molecular Gastronomy: A New Emerging Scientific Discipline. *Chem. Rev.*, 110, 2313–2365.
- Bashir, I., Lone, F. A., Bhat, R. A., Mir, S. A., Dar, Z. A., & Dar, S. A. (2020). Concerns and Threats of Contamination on Aquatic Ecosystems. In *Bioremediation and Biotechnology* (pp. 1-26). Springer, Cham.
- Bathiany, S., Dakos, V., Scheffer, M., & Lenton, T. M. (2018). Climate models predict increasing temperature variability in developing countries. *Science advances*, 4(5), eaar5809.
- Bezgin, E., Eryücel, C. O., & Tuncel, Z. (2019). Physicochemical properties of both drinking

- and domestic waters in Çorum. *International Journal of Science Letters*, 1(1), 42-55.
- Bertolaccini, L., Solli, P., Pardolesi, A., & Pasini, A. (2017). An overview of the use of artificial neural networks in lung cancer research. *Journal of Thoracic Disease*, 9(4), 924.
- Blockeel, H., Dzeroski, S., Grbovic, J., 1999. Simultaneous prediction of multiple chemical parameters of river water quality with a tilde. In: Proceedings of the Third European Conference on Principles of Data Mining and Knowledge Discovery. LNAI, 1704. Springer-Verlag, p. 16.
- Bokulich, N. A., & Bamforth, C. W. (2013). The microbiology of malting and brewing. *Microbiology and molecular biology reviews: MMBR*, 77(2), 157–172.
- Bond, A., Benbow, S., Wilson, J., Millard, A., Nakama, S., English, M. . . . Garitte, B. (2013). Reactive and non-reactive transport modelling in partial water-saturated argillaceous porous media around the ventilation experiment, Mont Terri. *Journal of the Rock Mechanics and Geotechnical Engineering*, 5(1), 44-57.
- Boon, E., Meehan, C. J., Whidden, C., Wong, D. H., Langille, M. G., & Beiko, R. G. (2014). Interactions in the microbiome: communities of organisms and populations of genes. *FEMS microbiology reviews*, 38(1), 90–118.
- Botheju, D., & Bakke, R. (2011). Oxygen Effects in Anaerobic Digestion – A Review. *The Open Waste Management Journal*, 4, 1-19
- Bryan, N. S., Alexander, D. D., Coughlin, J. R., Milkowski, A. L., & Buffett, P. (2012). Ingested nitrate and nitrite and stomach cancer risk: an updated review. *Food and Chemical Toxicology*, 50(10), 3646-3665.
- Cavalcante, Y., Hauser-Davis, R., Saraiva, A., Brandão, I., Oliveira, T., & Silveira, A. (2013). Metal and physicochemical variations at a hydroelectric reservoir analyzed by Multivariate Analyses and Artificial Neural Networks: Environmental management and policy/decision-making tools. *Science of the Total Environment*, 442, 509-514.
- Cawood, S., & Vos, A. (2016). Water quality as a bio-cultural screening indicator for the integrity of free heritage sites. *Water SA*, 42(4), 516.
- Çelik, B. (2008). Time Series Analysis with Artificial Neural Networks Methodology: *Theory and Application*. İstanbul.
- ÇELİK, O. (2018). A Research on Machine Learning Methods and Its Applications. *Journal of Educational Technology & Online Learning*, 1(3).
- Chakraborti, D., Singh, S. K., Rahman, M. M., Dutta, R. N., Mukherjee, S. C., Pati, S., & Kar, P. B. (2018). The Groundwater Arsenic Contamination in the Ganga River Basin: A Future Health Danger. *International Journal of the Environmental Research and the Public Health*, 15(2), 180.
- Chakraborty, R., Khan, K., Dibaba, D., Khan, M., Ahmed, A., & Islam, M. (2019). Health Implications of Drinking Water Salinity in Coastal Areas of Bangladesh. *International Journal of Environmental Research and the Public Health*, 16(19), 3746.
- Chen, Y., Song, L., Liu, Y., Yang, L., & Li, D. (2020). A Review of the Artificial Neural Network Models for Water Quality. *Applied Sciences*, 10(17), 5776.
- Corwin, D. L., & Yemoto, K. (2017). Salinity: Electrical conductivity and total dissolved solids. *Methods of soil analysis*, (msaonline2017).
- Crasti, L. (2016). Decontamination of Urban Run-Off: Importance and Methods. *Balanced Urban Development: Options and Strategies for Liveable Cities*, 85-107.

- Crini, G., & Lichtfouse, E. (2019). Advantages and disadvantages of techniques used for wastewater treatment. *Environmental Chemistry Letters*, 17(1), 145-155.
- Dallas, H. F., & Day, J. F. (2004). The effect of the water quality variables on aquatic ecosystems: a review. *Water Research Commission*, Report No. TT 224/04. Pretoria, South Africa.
- Dallas, H. F. & Ross-Gillespie, V. (2015). Sublethal effects of temperature on freshwater organisms, with particular reference to aquatic insects. *Water SA*, 41(5), 712-72.
- Dawood, A. S., Hussain, H. K., Hassan, A. (2016). Modelling of river water quality parameters using an artificial neural network: a case study.
- DeMarse, T., Pan, L., Alagapan, S., Brewer, G., & Wheeler, B. (2016). Feed-Forward Propagation of Temporal and Rate Information between Cortical Populations during Coherent Activation in Engineered In Vitro Networks. *Frontiers in Neural Circuits*, Department of Water Affairs and Forestry. (1996). South African Water Quality Guidelines (second edition). Volume 1: Domestic Use.
- Devangee, S., Kinjal, B., Jain, N. K., & Modi, H. A. (2013). Physicochemical analysis of water from various sources and their comparative studies. *IOSR Journal of Environmental Science, Toxicology, and Food Technology*, 2319-2402.
- DWAF: Department of Water Affairs and Forestry. (1996c). South Africa Water Quality Guidelines-Industrial Use, Volume 3. Government Printer.
- DWAF: Department of Water Affairs and Forestry. (2004a). National Water Resource Strategy. Chapter 2. *Pretoria, South Africa*: Government Printer.
- Dzeroski, S., Demsar, D., Grbovic, J., 2000. Predicting chemical parameters of river water quality from bioindicator data. *Applied Intelligence*, 13 (7–17)
- Dzwauro, R. B., Barratt, G. J., & Otieno, F. A. O. (2015). Ecosystem-specific water quality indices. *African Journal of Aquatic Science* 2015, 40(3), 227–234
- Edokpayi, J. N., Odiyo, J. O., & Durowoju, O. S. (2017). Impact of wastewater on surface water quality in developing countries: a case study of South Africa. *Water quality*, 401-416.
- Englande Jr, A. J., Krenkel, P., & Shamas, J. (2015). Wastewater Treatment & Water Reclamation. *Reference Module in Earth Systems and Environmental Sciences*.
- Fate, C., Permitting, N. P. D. E. S. Permitting, Negotiation Water Withdrawal Permitting, Storm Water Runoff Monitoring, and Storm Water Pollution Prevention Plan. "WATER QUALITY." Fewtrell, L. (2004). Drinking-water nitrate, methemoglobinemia, and the global burden of disease: a discussion. *Environmental health perspectives*, 112(14), 1371-1374.
- Garcia-Garcia, A., Gomez-Donoso, F., Garcia-Rodriguez, J., Orts-Escolano, S., Cazorla, M., & Azorin-Lopez, J. (2016, July). Point net: A third convolutional neural network for real-time object class recognition. In *2016 International Joint Conference on Neural Networks (IJCNN)* (pp. 1578-1584). IEEE.
- Garg, B., Kirar, N., Menon, S., & Sah, T. (2016). Performance comparison of different back propagation neural networks methods for forecasting wheat production. *CSI transactions on ICT*, 4(2-4), 305-311.
- Gheorghe, I. F., & Ion, B. (2011). The effects of air pollutants on vegetation and the role of vegetation in reducing atmospheric pollution. *The impact of air pollution on health*,

- economy, environment, and agricultural sources*, 241-280.
- Gholizadeh, M. H., Melesse, A. M., & Reddi, L. (2016). A comprehensive review of the water quality parameter estimation using remote sensing techniques. *Sensors*, 16(8), 1298.
- Gill, S., Al-Shankiti, A., Azam, F., Shahid, S. A., & Begum, H. M. (2018). A Comprehensive Review of Sewage Collection and Treatment: Historical Perspective. *Acta Scientifical Agriculture*, 2(10), 166-176.
- Godfrey, L. B. (2018). Parameterizing and Aggregating Activation Functions in Deep Neural Networks.
- Gupta, N., Pandey, P., & Hussain, J. (2017). The effect of physicochemical and biological parameters on the quality of river water of Narmada, Madhya Pradesh, India. *Water Science*, 31(1), 11-23.
- Gupta, S. R. N. (2016). Physicochemical Analysis of Drinking Water Samples from Different Regions of Nagpur and Amravati in Maharashtra State, India, *Research Journal of Chemical Sciences*, 6(7), 48-61.
- Haarhoof, J., Mamba, B., Krause, R., Van Stade, S., Nkambele, T., Dlamini, S., & Lobanga, K. P. (2013). Natural organic matter in drinking water sources: Its characteristics and treatability. *NOM WRC Report* (1883).
- Hall, R. O. (2003). A Stream's role in watershed nutrient export. *Proceedings of National Academy of Science*, 100.
- Hamid, A., Bhat, S. U., & Jehangir, A. (2020). Local determinants were influencing stream water quality. *Applied Water Science*, 10(1), 24.
- Hamilton, P. B., Rolshausen, G., Uren Webster, T. M., & Tyler, C. R. (2017). Adaptive capabilities and fitness consequences associated with pollution exposure in fish. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372(1712), 20160042.
- Hamlet, A., Guidoum, A., & Koulala, I. (2017). Status and trends of water quality in the Tafna catchment: a comparative study using water quality indices. *Journal of Water Reuse and Desalination*, 7(2), 228-245
- Haseena, M., Malik, M. F., Javed, A., Arshad, S., Asif, N., Zulfiqar, S., & Hanif, J. (2017). Water pollution and human health. *Environmental Risk Assessment and Remediation*, 1(3), 16-19.
- Hatzikos, E., Bassiliades, N., Asmanis, L., Vlahavas, I., 2007. Monitoring water quality through a telematic sensor network and a fuzzy expert system. *Expert Systems* 24(3), 143–161. 19.
- Heidarinejad, Z., Hashemzadeh, B., Feizabadi, G., Asghari, F., Radfard, M., & Akbarpour, B. et al. (2018). Data on quality indices of groundwater resource for agricultural use in the Jolfa, East Azerbaijan, Iran. *Data in Brief*, 19, 1482-1489.
- Hisseien, A, A-T., Kamga, R., & Mahamat, T. N. (2015). Physicochemical analysis of Logone River water at Moundou City in Southern Chad. *Int. J. Biol. Chem. Sci*, 8(3), 1654-64.
- Hlavsa, M. C., Roberts, V. A., Kahler, A. M., Hilborn, E. D., Wade, T. J., Backer, L. C., & Yoder, J. S. (2014). Recreational water-associated disease outbreaks—the United States, 2009–2010. *MMWR. Morbidity and mortality weekly report*, 63(1), 6.
- Hossain, K. (2014). Predictive Ability of Improved Neural Network Models to Simulate Pollutant Dispersion. *International Journal of Atmospheric Sciences*, 2014

- Hubert, E., & Wolkersdorfer, C. (2015). Establishing a conversion factor between the electrical conductivity and total dissolved solids in South African mine waters. *Water SA*, 41(4), 490.
- Ishaq, F., & Khan, A. (2013). The massive metal analysis of the river Jamuna & their relation with some physicochemical parameters. *Global J. of Environmental Research*, 7(2), 34-39.
- Kent J.A. (2003) Sulfur and Sulfuric Acid. In: Kent J.A. (Eds) Riegel's Handbook of Industrial Chemistry. Springer, Boston, MA.
- Kjelland, M. E., Woodley, C. M., Swannack, T. M., & Smith, D. L. (2015). A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioural, and transgenerational implications. *Environment Systems and Decisions*, (8), 334-350.
- Lawal, A. T., & Fantke, P. (2017). Polycyclic aromatic hydrocarbons. A review. *Cogent Environmental Science*, 3(1), 1339841
- Li, P., & Wu, J. (2019). Drinking water quality and public health. *Exposure and Health*, 11(2), 73-79.
- Lillicrap, T. P., Cownden, D., Tweed, D. B., & Akerman, C. J. (2016). Random synaptic feedback weights support error backpropagation for deep learning. *Nature communications*, 7(1), 1-10.
- Ling, T. Y., Gerunsin, N., Soo, C. L., Nyanti, L., Sim, S. F., & Grinang J. (2017). Seasonal Changes and Spatial Variation in Water Quality of a Large Young Tropical Reservoir and Its Downstream River. *Journal of Chemistry*, 2017, 1-16.
- Liong, S. Y., Lim, W. H., Paudyal, G., 1999. Real-time river stage forecasting for flood-stricken Bangladesh: a neural network approach. *Journal of Computing in Civil Engineering ASCE* 14 (1), 1-8.
- Jover-Smet, M., Martín-Pascual, J., & Trapote, A. (2017). Model of suspended solids removal in the primary sedimentation tanks for the treatment of urban wastewater. *Water*, 9(6), 448.
- Kemker, C. (2015). Dissolved Oxygen-Environmental Measurement Systems.
- Khatri, N., & Tyagi, S. (2015). The influences of the natural and anthropogenic factors on the surface and groundwater quality in rural and urban areas. *Frontiers in Life Science*, 8(1), 23-39.
- Kim, M., Weigand, M. R., Oh, S., Hatt, J. K., Krishnan, R., Tezel, U. ... & Konstantinidis, K. T. (2018). Widely used benzalkonium chloride disinfectants can promote antibiotic resistance. *Applied and Environmental Microbiology*, 84(17), e01201-18.
- Kirchmann, H., Börjesson, G., Kätterer, T., & Cohen, Y. (2016). From the agricultural use of sewage sludge to nutrient extraction: A soil science outlook. *Ambio*, 46(2), 143-154.
- Kulthanan, K., Nuchkull, P., & Varothai, S. (2013). The pH of water from various sources: an overview of the recommendation for patients with atopic dermatitis. *Asia Pacific Allergy*, 3(3), 155-160.
- Majumdar, D. (2003). The blue baby syndrome. *Resonance*, 8(10), 20-30.
- Malik, G. M., Raval, V. H., Zadafiya, S. K., & Patel, A. V. (2012). Idol immersion and Physicochemical Properties of South Gujarat Rivers. India, *Res. J. Chem. Sci.*, 2(3), 21-25.

- Malovanyy, M., Shandrovykh, V., Malovanyy, A., & Polyuzhyn, I. (2016). Comparative analysis of the effectiveness of regulation of aeration depending on the quantitative characteristics of treated sewage water. *Journal of Chemistry*, 2016.
- Manisalidis, I., Stavropoulou, E., Stavropoulos, A., & Bezirtzoglou, E. (2020). Environmental and health impacts of air pollution: A review. *Frontiers in public health*, 8.
- Manyatshe, A., Fosso-Kankeu, E., Van Der Berg, D., Lemmer, N., Waanders, F., & Tutu, H. (2016). Assessment of seasonal variation in surface water quality of the Mooi and Vaal Rivers network, South Africa. In *International Conference on Advances in Science, Engineering, Technology and Natural Resources (ICASETNR-16) Nov* (pp. 24-25).
- Mathias, T. R. D. S., Alexandre, V. M. F., Cammarota, M. C., de Mello, P. P. M., & Sérvulo, E. F. C. (2015). Characterization and determination of brewer's solid wastes composition. *Journal of the Institute of Brewing*, 121(3), 400-404.
- McCleskey, R. B., Nordstrom, D. K., & Ryan, J. N (2012). Comparison of electrical conductivity calculation methods for natural waters. *Association for the Science of Limnology and Oceanography: Methods*, 10, 952–967.
- Meride, Y., & Ayenew, B. (2016). Drinking water quality assessment and its effects on residents' health in Wondo genet campus, Ethiopia. *Environmental Systems Research*, 5(1), 1.
- Messikh, N., Samar, M., & Messikh, L. (2007). Neural network analysis of liquid-liquid extraction of phenol from wastewater using TBP solvent. *Desalination*, 208(1), 42-8.
- Mittal, M., Goyal, L. M., Sethi, J. K., & Hemanth, D. J. (2019). Monitoring the impact of the economic crisis on crime in India using machine learning. *Computational Economics*, 53(4), 1467-1485.
- Mohamed, Z.E. (2019). Using artificial neural networks for prediction and validating solar radiation. *Journal of the Egyptian Mathematical Society*, 27(1), 47.
- Muhammad, I., Muhammad, A., Niaz, S., Shams, S., Khan, A., Khan, I., & Ullah, A. (2016). PHYSICO-CHEMICAL ANALYSIS OF RIVER SWAT AT BATKHELA DISTRICT Malakand, KPK PAKISTAN. *International Journal of the Pure and Applied Zoology*, 4(1), 70-76.
- Naidoo, S., & Olaniran, A. (2013). The treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. *International Journal of Environmental Research and the Public Health*, 11(1), 249-270.
- Navarro, H., & Bennun, L. (2014). Illustrative Examples of the Limitations of Artificial Neural Networks Applied to the Analysis of Independent Stochastic Data.
- Neira, J., Ortiz, M., Morales, L., & Acevedo, E. (2015). Oxygen diffusion in soils: Understanding the factors and processes needed for modelling. *Chilean Journal of Agricultural Research*, 75, 35-44.
- Ngatia, L., Grace III, J. M., Moriasi, D., & Taylor, R. (2019). Nitrogen and Phosphorus Eutrophication in Marine Ecosystems. In *Monitoring of Marine Pollution*. IntechOpen.
- Niesel, M. A. (2015). *Neural Networks and Deep Learning* (Vol. 2018). San Francisco, CA: Determination process, 2015.
- Nisbet, T. R. (2001). The role of forest management in controlling diffuse pollution in UK forestry. *Forest Ecology Management*. No. 143:215-226.
- Nyamangara, J., Jeke, N., & Rurinda, J. (2013). Long-term nitrate and phosphate loading

- river water in the Upper Manyame catchment, Zimbabwe. *Water SA*, 39 (5), 637-642.
- Oberholster, P. J., & Ashton, P. J. (2008). The State of the Nation Report: An overview of the status of water quality and eutrophication in South African rivers and reservoirs. CSIR Report No. CSIR/NRE/WR/IR/2008/0075/C. The *Council for the Scientific and Industrial Research*: Pretoria, South Africa.
- Omarova, A., Tussupova, K., Hjorth, P., Kalishev, M., & Dosmagambetova, R. (2019). Water supply challenges in rural areas: A case study from central Kazakhstan. *International journal of environmental research and public health*, 16(5), 688.
- Omer, N. H. (2019). Water Quality Parameters. In *Water Quality-Science, Assessments and Policy*. IntechOpen.
- Oppelaar, J. J., & Vogt, L. (2019). Body fluid-independent effects of dietary salt consumption in chronic kidney disease. *Nutrients*, 11(11), 2779.
- Otte, J., Pica-Ciamarra, U., & Morzaria, S. (2019). A comparative overview of the livestock-environment interactions in Asia and Sub-Saharan Africa. *Frontiers in veterinary science*, 6, 37.
- Overstreet, R. M., & Hawkins, W. E. (2017). Diseases and mortalities of fishes and other animals in the Gulf of Mexico. In *Habitats and biota of the Gulf of Mexico: Before the Deepwater Horizon oil spill* (pp. 1589-1738). Springer, New York, NY.
- Panda, P. K., Panda, R. B., & Dash, P. K. (2018). River water pollution in India and abroad: A critical review to study the relationship between the different physicochemical parameters. *American Journal of Water Resources*, 6(1), 25-38.
- Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. *Amb Express*, 4(1), 51.
- Prest, E., Hammes, F., van Loosdrecht, M., & Vrouwenvelder, J. (2016). Biological-Stability of Drinking Water: Controlling Factors, Methods, and Challenges. *Frontiers in Microbiology*, 7.
- Proksch, E. (2018). The pH in nature, humans, and skin. *The Journal of Dermatology*, 45(9), 1044-1052.
- Popa, P., Timofti, M., Voiculescu, M., Dragan, S., Trif, C., & Georgescu, L. (2012). Study of Physicochemical Characteristics of Wastewater in an Urban Agglomeration in Romania. *The Scientific World Journal*, 2012, 1-10.
- Poschenrieder, C., Fernández, J., Rubio, L., Pérez, L., Terés, J., & Barceló, J. (2018). Transport and Use of Bicarbonate in Plants: Current Knowledge and Challenges Ahead. *International Journal of Molecular Sciences*, 19(5), 1352.
- Qasemi, M., Farhang, M., Biglari, H., Afsharnia, M., Ojrati, A., & Khani, F. et al. (2018). Health risk assessments due to nitrate levels in drinking water in villages of Azadshahr, northeastern Iran. *Environmental Earth Sciences*, 77(23).
- Quevedo, C., M., G., & Paganini, W. (2017). Management of phosphorus in water: a case study of the Tietê River, Brazil. *Rev. Ambient. Água*, 12(6), 903-917.
- RadFard, M., Seif, M., Ghazizadeh Hashemi, A., Zarei, A., Saghi, M., & Shalyari, N. et al. (2019). Protocol for the estimation of drinking water quality index (DWQI) in water resources: Artificial neural network (ANFIS) and Arc-Gis. *MethodsX*, 6, 1021-1029.
- Radhakrishnan, K., Holland, C. K., & Haworth, K. J. (2016). Scavenging dissolved oxygen via acoustic droplet vaporization. *Ultrasonic sonochemistry*, 31, 394-403.

- Rahmanian, N., Ali, S. H., Homayoonfard, N., Ali, J., Rehan, M., Sadeh, Y., & Nizami, A. S. (2015). Analysis of Physiochemical Parameters to Evaluate the Drinking Water Quality in the State of Perak, Malaysia. *Journal of Chemistry*, 31.
- Rashid, M., & Pandit, D. (2019). Determining the provision of wastewater management infrastructure in rural India from the local communities' perspectives. *Water Science and Technology*, 79(3), 489-500.
- Rebich, R. A., Houston, N. A., Mize, S. V., Pearson, D. K., Ging, P. B., & Evan Hornig, C. (2011). Sources and Delivery of Nutrients to the North-western Gulf of Mexico from Streams in the South-Central United States. *Journal of the American Water Resources Association*, 47(5), 1061–1086.
- Reckhow, K.H., 1999. Water quality prediction and probability network models. *Canadian Journal of Fisheries and Aquatic Sciences* 56, 1150–1158
- Richárd, N. (2018). The differences between Artificial and Biological Neural Networks. *Towards Data Science*.
- Ritter, J.A., 2010. *Water Quality. Fourth edition*. Printed in the United States of America.
- Roy, S., Nagarchi, L., Das, I., Mangalam, Achuthanathan, J., & Krishnamurthy, S. (2015). Cytotoxicity, Genotoxicity, and Phytotoxicity of Tannery Effluent Discharged into Palar River Basin, Tamil Nadu, India. *Journal of toxicology*, 2015, 504360.
- Saad, D., Byrne, D., & Drechsel, P. (2017). Social Perspectives on the Effective Management of the Wastewater. *Physicochemical Wastewater Treatment and Resource Recovery*.
- Samer, M. (2015). Biological and chemical wastewater treatment processes. *Wastewater Treatment Engineering*, 150.
- Schindler, D. W., Carpenter, S. R., Chapra, S. C., Hecky, R. E., & Orihel, D. M. (2016). Reducing phosphorus to curb Lake Eutrophication is a success. *Environ. Sci. Technol.* 50(17), 8923–8929.
- Schmidt, H., Seitz, S., Hassel, E., & Wolf, H. (2018). The density–salinity relation of standard seawater. *Ocean Sci.*, 14, 15–40.
- Schoonover, J. E., & Crim, J. F. (2015). An introduction to soil concepts and the role of soils in watershed management. *Journal of Contemporary Water Research & Education*, 154(1), 21-47.
- Sengorur, B., Koklu, R. & Ates, A. (2015). Water Quality Assessment Using Artificial Intelligence Techniques: SOM and ANN—A Case Study of Melen River, Turkey. *Water Qual Expo Health*, 7, 469–490
- Sengupta, P. (2013). Potential health impacts of hard water. *International journal of preventive medicine*, 4(8), 866–875.
- Shabalala, A. N. & Combrink, W. (2012). Correlation of water quality with farming activities. *WISA. Water & Sanitation, Africa*. 3s Media. SA.
- Sharma, S., & Bhattacharya, A. (2017). Drinking water contamination and treatment techniques. *Applied Water Science*, 7(3), 1043-1067.
- Shen, W., Huang, F., Zhang, X., Zhu, Y., Chen, X., & Akbarjon, N. (2018). Online chemical oxygen demand estimation-models for the photo-electro-catalytic oxidation advanced treatment of papermaking wastewater. *Water Science and Technology*, 78(1-2), 310-319.
- Shukla, P., Akshay, S., & Ashok, S. (2017). Food additives from an organic chemistry

- perspective. *MOJ Biorgananic and Organic Chemistry*, 1(3), 70-79.
- Smitha, D., & Shivashankar, P. (2013). Physicochemical Analysis of the Freshwater at River Kapila, Nanjangudu Industrial Area, Mysore, India. *International Research Journal of Environment Sciences*, 2(8), 59-65.
- Sonune, A., & Ghate, R. (2004). Developments in wastewater treatment methods. *Desalination*, 167, 55-65.
- Strifling, D. (2018). Reducing chloride discharges to surface water and groundwater: A menu of options for policymakers. *Environmental Law*, 48(1), 167-210.
- Subaşı, H. (2010). Modelling of Wastewater Treatment Performance using Artificial Neural Network. Master Thesis, *Çukurova University, Institute of Science*, Adana.
- Tawati, F., Risjani, Y., Djati, M. S., Yanuwadi, B., & Leksono, A. S. (2018). The analysis of the physical and chemical properties of the water quality in the rainy season in the Sumber Maron River-Kepanjen, Malang-Indonesia. *Resources and Environment*, 8(1), 1-5.
- Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Experientia Supplementum*, 101, 133–164.
- Toure, A., Wenbiao, D., & Keita, Z. (2017). Comparative Study of the Physicochemical Quality of Water from the wells, boreholes, and Rivers Consumed in the Commune of Pelengana of the Region of Segou in Mali. *Environ Sci. Ind. J*, 13(6), 154.
- Tytla, M. (2019). Assessment of Heavy-Metal Pollution and the Potential Ecological Risk in the Sewage Sludge from the Municipal Wastewater Treatment Plant Located in the Most Industrialized Region in Poland—Case Study. *Int. J. Environ. Res. Public Health*, 16, 2430.
- Uba, B. N., & Eze, A. (2004). Incidence of *Chromobacterium violaceum* in borehole water in Port Harcourt metropolis, Rivers State, Nigeria. *Journal of Water Supply: Research and Technology-Aqua*, 53(6), 433-439.
- United Nations Environmental Programme. (2012). Division of Technology, Industry and Economics Biosolids, Management, and Environmentally Sound Approach for Managing Sewage Treatment Plant Sludge.
- The United States Environmental Protection Agency (USEPA). (1997). Wastewater Treatment Manuals: Primary, Secondary and Tertiary treatment.
- United States of Environmental Protection Agency (U.S. EPA). (2012a). *Impaired Waters and Total Maximum Daily Loads*. Accessed May 8, 2014.
- Vadde, K. K., Wang, J., Cao, L., Yuan, T., McCarthy, A. J., & Sekar, R. (2018). The assessment of Water Quality and Identification of Pollution Risk Locations in Tiaoxi River (Taihu Watershed) China. *Water*, 10, 2-18.
- Van den Brand, T., Roest, K., Chen, G., Brdjanovic, D., & van Loosdrecht, M. (2015). Potential for practical application of sulfate-reducing bacteria in sulfate-containing domestic wastewater treatment. *The World Journal of Microbiology and Biotechnology*, 31(11), 1675-1681.
- Van Loon, A. F. (2015). Hydrological drought explained. *Wiley Interdisciplinary Reviews: Water*, 2(4), 359-392.
- Viret, F., & Grand, S. (2019). Combined Size and Density Fractionation of Soils for Investigations of Organo-Mineral Interactions. *JoVE (Journal of Visualized*

- Experiments*), (144), e58927.
- Voulvoulis, N. (2018). The water reuse from a circular economy perspective and the potential risks from an unregulated approach. *Current Opinion in Environmental Science & Health*, 2, 32-45.
- Uwidia, I. E., & Ukulu, H. S. (2013). Studies on electrical conductivity and total dissolved solids concentration in raw domestic wastewater obtained from an estate in Warri, Nigeria. *Greener Journal of Physical Sciences*, 3(3), 110-114.
- Wang, P., Zhao, J., Xiao, H., Yang, W., & Yu, X. (2019). Bacterial community composition shaped by water chemistry and geographic distance in an anthropogenically disturbed river. *Science of the Total Environment*, 655, 61-69.
- Wang, W., Liu, X., Wang, Y., Guo, X., & Lu, S. (2016). Analysis of point source pollution and water environmental quality variation trends in the Nansi Lake basin from 2002 to 2012. *Environmental Science and Pollution Research*, 23(5), 4886-4897.
- Wang, Y. (2015). Towards the abstract system theory of system science for cognitive and intelligent systems. *Complex & Intelligent Systems*, 1(1-4), 1-22.
- Wang, Z. Y., Lee, J. H., & Melching, C. S. (2014). *River dynamics and integrated river management*. Springer Science & Business Media.
- Weigelhofer, G., Hein, T., & Bondar-Kunze, E. (2018) Phosphorus and Nitrogen Dynamics in Riverine Systems: Human Impacts and Management Options. In: Schmutz S., Sendzimir J. (eds) Riverine Ecosystem Management. *Aquatic Ecology Series*, 8, Springer, Cham
- Weiner, R. F., & Matthews, R. (2003). Environmental Engineering, *fourth Ed. Butterworth-Heinemann*, USA.
- Weldemariam, M. M. (2013). Physicochemical Analysis of GudBahri River Water of Wukro, Eastern Tigray, Ethiopia. *The International Journal of Scientific and Research Publications*, 3(11), 1-4.
- WHO. (2018) Drinking water. World Health Organization fact sheets, <https://www.who.int/en/news-room/fact-sheets/detail/drinking-water>, Accessed 27 Dec 2018
- WISA (Water Institute of Southern Africa). (2002). The handbook for the operation of the Wastewater Treatment Works.
- Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: impacts across multiple levels of the organization. Philosophical transactions of the Royal Society of London. *Series B, Biological Sciences*, 365(1549), 2093–2106.
- Wose Kinge, C., & Mbewe, M. (2010). Characterization of Shigella species isolated from river catchments in the North West province of South Africa. *South African Journal of Science*, 106(11-12), 1-4.
- Wuana, R. A., & Okieimen, F. E. (2011). The Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks, and Best Available Strategies for the Remediation. *ISRN Ecology*, 11.
- Xavier de Sousa, R. T., Korndörfer, G. H., Soares, R. A. B., & Fontoura, P. R. (2015). Phosphate Fertilizers for Sugarcane Used at Pre-Planting (Phosphorus Fertilizer Application). *Journal of Plant Nutrition*, 38(9), 1444-1455.

- Yahya, R. Z., Arrieta, J. M., Cusack, M. & Duarte, C. M. (2019). Airborne Prokaryote and Virus Abundance over the Red Sea. *Frontiers in Microbiology*, 10, 1112.
- Yang, H., & Abbaspour, K. (2007). Analysis of wastewater reuse potential in Beijing. *Desalination*, 212(1-3), 238-250.
- Yang, J., & Zhang, G. (2011). The Water infiltration in urban soils and its effects on the quantity and quality of runoff. *Journal of Soils and Sediments*, 11(5), 751-761.
- Yang, S., Ting, T. O., Man, K. L., & Guan, S. U. (2013). Investigation of neural networks for function approximation. *Procedia Computer Science*, 17, 586-594.
- Yao, N., Wang, J., & Zhou, Y. (2014). The rapid determination of the chemical oxygen demand of water using a thermal biosensor. *Sensors (Basel, Switzerland)*, 14(6), 9949–9960.
- Youpeng, X., Jintao, X., Jinjia, D., Ying, C., Yixing, Y., & Xingqi, Z. (2010). Impacts of urbanization on hydrology in the Yangtze River Delta, China. *Water Science & Technology*.
- Yurtoğlu, H. 2005, Artificial Neural Network Modeling and Forecasting: The Case of Turkey for some macroeconomic variables. *Ankara: SPO Publication*
- Zainal-Mokhtar, K., & Mohamad-Saleh, J. (2013). An oil fraction neural sensor developed using electrical capacitance tomography sensor data. *Sensors*, 13(9), 11385-11406.
- Zaharia, C. (2017). Comparative overview of the primary sedimentation-based mechanical stage in some Romanian wastewater treatment systems. *IOP Conf. Series: Materials Science and Engineering*, 22.
- Zhang, L., Shen, Z., Fang, W., & Gao, G. (2019). Composition of bacterial communities in the municipal wastewater treatment plants. *Science of the Total Environment*.
- Zhang, Q., Yu, H., Barbiero, M., Wang, B., & Gu, M. (2019). Artificial neural networks enabled by nanophotonics. *Light: Science & Applications*, 8(1), 1-14.

CHAPTER III

EVALUATION OF PHARMACEUTICAL COMPOSITES' (ANTIRETROVIRAL AND CONTRACEPTIVE DRUGS) ECOLOGICAL IMPACT IN RIVER WATER AND WASTEWATER TREATMENT PLANTS

3. BACKGROUND

An overall increase in pharmaceutical intake has come in conjunction with a similar surge in the environment's pollution with the active pharmaceutical ingredient, metabolites, and transformation products (Emara et al., 2018). In the past few decades, the existence of pharmaceuticals in the surroundings has acquired greater emphasis. The academic literature has shown that pharmaceuticals invade the environment and can induce negative impacts. In the central part of surface waters, medicines can now be identified in ng/L. While in the past were in the background but hidden (Taylor & Senac, 2014). Pharmaceutical drugs are composites that are planned to make a therapeutic effect in the human system, generally active at shallow levels, can travel via biological membranes, and endure in the body system so long to dodge deactivation before having an impact. Such composites are discharged from the human body in faeces and urine compounded with metabolites and materials that are not changed (Forlani et al., 2010). Humans can be exposed to medicinal drugs that contaminate the hydrosphere via potable water or aquatic organisms' intake.

Solid Phase Extraction (SPE) is a technique available in the market, which utilizes several stationary phase chemistries to extract analytes from a broad range of liquid matrices (Berthod et al., 2014). This technique was employed for its suitability of screening medicinal products from samples. SPE benefits are speed, high selectivity, no cross-contamination, its usability, and the process in a few steps, greater precision, and low cost (Gao et al., 2019). This method does not consume time and demands only low solvents volumes for the extraction. SPE is still used to separate and purify pharmaceuticals such as organophosphorus pesticides (Sapahin et al., 2019), antibiotics (Mirzaei et al., 2017), antiretrovirals (Mwando et al., 2017), herbicides (Simon et al., 2019), carbamates (Fernandez-Ramos et al., 2014) and tebuconazole (Deng et al., 2010) in water samples. The method of SPE is extensively employed to prepare liquid samples. SPE removes interfering substances from the matrices and concentrates the analytes; thus, contaminants at small levels may be determined by LC-MS with various detectors (de Oliveira et al., 2019).

Distinct analyzing techniques have been employed to examine pharmaceuticals in sewage, showing the concentration of contamination in ng/L and ug/L. Previous investigations used SPE and liquid-liquid extraction methods before separating the target composites to reduce matrix interferences that can influence the detection and quantification (Semreen et al., 2019). Nevertheless, most studies have utilized Liquid Chromatography with tandem mass spectrometry/MS and gas chromatography or one (Chang et al., 2014; Xu et al., 2009). Both of them are vigorous techniques for analyzing pharmaceuticals for their sensitivity and selectivity. The LC-MS is a technique for studying that combines the physical separation

abilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (Kumar et al., 2016). It is widely utilized in pharmacokinetic studies of medicinal products and often in the areas of health sciences. It takes part in pharmacognosy, particularly in the areas of molecular pharmacognosy. Liquid chromatography uses fine particle-dispersed packed and running at a higher pressure and is named high-performance liquid chromatography (HPLC) (Ooh et al., 2014). Its fundamental premise is adsorption. HPLC methods are split into two separate sub-categories in reliance on stationary phases and the mobile phase polarity. HPLC does not only divide materials but also gives the data regarding how the chemical could be. In HPLC, it is complicated to be sure regarding the purity of a specific peak. Adding mass spectrometry to this will let one know about the masses of the available chemicals that could be utilized to identify them and the best practice for checking for the purity (Banerjee & Mazumdar, 2012).

Mass spectrometry is a vigorous technique for analyzing that is employed to quantify the known substances, identify new composites in a sample, and elucidate the chemical and physical characteristics of diverse particulates (Olshina & Sharon, 2016). It creates several ions from the samples, split them based on their specific mass-to-charge ratio (m/z), and files the ion type's abundance. The initial stage in mass spectrometry is creating gas-phase ions of the composites, primarily by electron ionization. The molecular ions undergo fragmentation. The ions provide data about the nature and structure of the precursor ions. If the molecular ion is available in pure composite, it will appear at the maximum number of m/z and provide the composite's molecular mass (Nicolescu, 2017).

Advanced LC-MS/MS technique has been implemented for constant resolution, quantitative and qualitative studies for various herbal products. It has also become a vigorous two-dimensional hyphenated technology used in different analytical and bioanalytical methods to analyze proteins, lipids, amino acids, and other things (Vonk et al., 2015). Kumar (2016) said, *“For certain clinical chemistry and toxicology analytes, liquid chromatography (LC) paired with tandem mass spectrometry (MS/MS) offers eloquent advantages over traditional testing by immunoassays.”*

3.1 General introduction to pharmaceuticals

Pharmaceutical drugs incorporate non-steroidal medications such as antibiotics, antiretroviral drugs (ARVs), steroidal drugs, as well as further endocrine-disrupting compounds, which are of concern (Archer et al., 2017; Schoeman et al., 2017). The influence of endocrine-disrupting compounds (EDC) that are environmentally persistent can be expected to constitute a risk to drinking water supplies (Gadupudi et al., 2019; Schoeman et al., 2017). Pharmaceuticals were not thought to form a danger to human beings' well-being through potable water and fish consumption (Bottoni et al., 2010; Haseena et al., 2017; Schoeman, 2017). Still, displayed that pharmaceutical and personal care products (PPCPs), instead of traditional importance impurities, led to water's toxicity (Schoeman et al., 2017). Some PPCPs have been shown to occur over a prolonged period during wastewater treatment because that causes risk when discharged in environmental waters (Al Aukidy et al., 2014). The WHO appraised that 2.5 million population in 2012 required antiretroviral therapy (ARVT) (Lundgren et al., 2013;

Schoeman et al., 2017). Figure 3.1 below shows the diagram of pharmaceutical composites in drinking water.

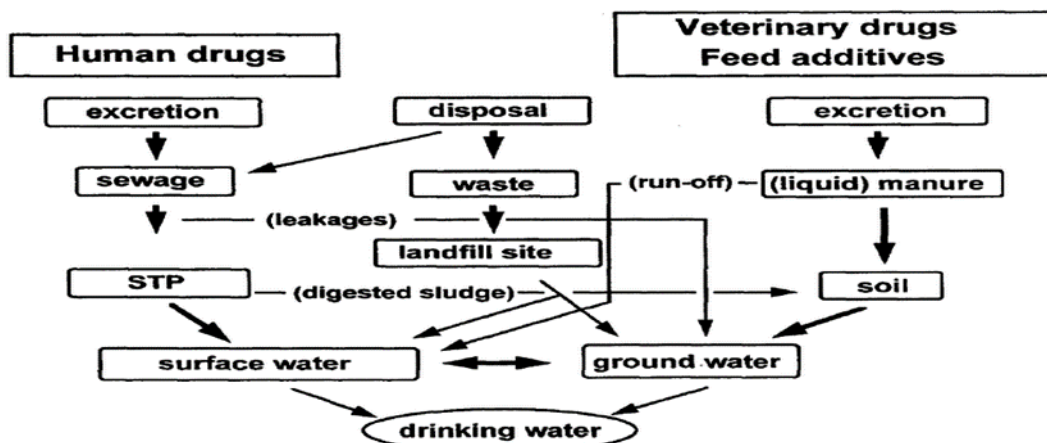


Figure 3.1: Pharmaceutical compounds in potable water (Mutiyar & Mittal, 2013).

*STP- Sewage Treatment Plant

3.2 Class of pharmaceuticals and their occurrence in bodies of water

3.2.1 Steroid hormones

Steroid hormones are a class of hormones taken from cholesterol from the group of composites called steroids. The adrenal cortex, the gonads, and the placenta in humans and animals (Ying et al., 2002) excrete natural steroids. The estrogens are mostly for female hormones essential to maintain the reproductive tissues, breasts, and epidermis' healthiness. Both animals and beings discharge the hormone steroids from the system that wind up in the environment via wastewater disposal and livestock waste disposal (Suresh & Abraham, 2018). These composites may influence the health of wildlife and beings by disturbing the endocrine system. The steroids have been discovered in WWTPs and surface water (Ebele et al., 2017; Ying et al., 2002). Olarinmoye et al. (2016) found Ethinylestradiol and 17- β -estradiol in the final treated effluent, and the raw sewage of the sewage works at a level higher than the detection limit of 0.01 $\mu\text{g/L}$.

3.2.2 Anti-malarial

On the African continent, malaria is among the first causes of death and morbidity that attacked a million population. Presently, there is a scarcity of data on the occurrence of anti-malarial drugs present in the water supply network. In extensive research, the drugs have been studied; however, the drugs have not been detected in water supply networks, or if detected, the limit of quantification was 0.39 ng/L (Madikizela et al., 2014). In Kenya, sulfadoxine has been seen at the levels of 2 $\mu\text{g/L}$ in the wastewater (K'oreje et al., 2012). The inadequate data on these drugs in SA is due to their restricted intake or usage.

3.2.3 Anti-epileptics

Anti-epileptics have been employed to treat epilepsy. The neurological disorder happens when excessive neurons are carried away in the central nervous system. Carbamazepine is a widely used drug, and it has been identified at the levels of 227 to 1028 ng/L in the raw wastewater of Belgium (Tarcomnicu et al., 2011). Diazepam has also been detected at 3 to 16 ng/L levels in the untreated sewage of Chinese sewage works (Shao et al., 2009). Moreover, the WWTP with conventional activated sludge and a membrane bioreactor has demonstrated a very inadequate elimination of carbamazepine from the effluent (Verlicchi et al., 2012). Despite the use of sophisticated water treatment methods, there have been reports whereby carbamazepine has been found in potable water. A research carried out in Japan revealed that carbamazepine was 12 to 25 ng/L (Simazaki et al., 2015). The highest level of carbamazepine was found in potable water at the levels of above 600 ng/L in Canada (Kleywegt et al., 2011).

3.2.4 Anti-retroviral

South Africa counts 7.7 million HIV-infected people, as stated by the WHO in 2017. Throughout the years, progress has been made on the examination of ARV drugs in bodies of water. It might originate from the continually rising number of individuals that get the treatment for ARV. Several studies outlined ARVs in sewage and streams (Gogoi et al., 2018; Wood et al., 2015). A survey conducted in Kenya streams showed that a higher amount of ARVs had been detected at the maximum level of 167 µg/L for lamivudine, 17 µg/L Zidovudine, and lastly, 6 µg/L for Nevirapine (K'Oreje et al., 2016). Low levels ranging from 26.5 to 430 ng/L in surface water were informed in SA. The highest concentration of the sewage works effluent was 0.350 µg/L for Nevirapine and 7.100 µg/L for Efavirenz (Schoeman et al., 2015). Schoeman et al. (2015) suggested that Nevirapine's prevalence in the environment results from being broadly used to treat HIV and prevent mother-to-child transmission.

3.2.5 Antibiotics

For most antibiotics, urine and faeces' discharge contain many active remnants (Massé et al., 2014). In high-income nations with highly sophisticated sewage networks, the release to the environment is minimized; however, communities of microbes inside the WWTPs can yet be exposed to µg/L levels of chosen antibiotics (Larsson, 2014). Most of these antibiotics gather in sludge that later lay on cropland to recycle nutrients. Immediate release from the manufacturer may be too high. On several occasions, concentrations above 1 mg/L have been identified in the final treated industrial wastewater (Sim et al., 2011). The wide dissemination of antibiotics may be on account of a shortage of wastewater treatment facilities. In certain circumstances, such as in Kwa-Zulu Natal at Durban, the sewage works assist only the city area (Segura et al., 2015).

3.2.6 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used drugs globally (Gunaydin & Blige, 2018). Below them are diclofenac, and ibuprofen is identified in the environment's areas as trace emerging contaminants (Jiang et al., 2017). It has been found that the diclofenac had harmfully influenced the species of the situation at levels of <1 ug/L. Ibuprofen counts most levels among pharmaceuticals. The ever-increasing production and

utilization of NSAIDs increase the danger of releasing them into sewage treatment works. Wang et al. (2008) concluded that these two NSAIDs tend to gather in aquatic bodies, increasing the likelihood of human exposure.

3.3 The occurrence of pharmaceuticals

3.3.1 Personal care products

Personal care products (PCPs) are still another category of emerging pollutants, including the ordered and non-ordered medical drug in humans and veterinary, and the quick and inactive elements for personal care goals (Becker & Stefanakis, 2017; Richardson et al., 2005). A couple of PCPs are shampoos, cosmetics, and steroids (Halla et al., 2018). The UV filters are familiar with the estrogenic activity and are described as part of the more often found in personal care products in underground water and different aquatic habitats (Krause et al., 2012). PCPs are discharged within the wastewater and proceeded to WWTPs, in their natural converted formations. Within the WWTPs, the fate of personal care products and their substances are transformed into carbon dioxide and water, mixing with receiving bodies of water, as both a real or mineralized product and sorption through the solids such as sludge. Most of the composite or the biological moderated transfiguration product is lipophilic (Gogoi et al., 2018).

3.3.2 Endocrine disruptors

Endocrine disruptors are distinguished under the name of synthetic chemicals, which, once consumed in the body, can copy or hinder the hormones and result in the body's average performance (Gore et al., 2015). The Environmental Protection Agency describes the endocrine disruptors as the exterior agents, which interfere with the structure, discharge, transfer, attachment, deed, or removal of the body's natural hormones, which sustain homeostasis and growth replica, and manner (USEPA, 1997). Biological and engineering endocrine disruptors are released in the habitat by human cultural way of life, animals, and industry branches, notably through wastewater treatment plants, before moving to surface water, underground water (Gogoi et al., 2018; Lv et al., 2016). The endocrine disruptors are existing in low concentrations in wastewater. Such composites are of severe distress as their long-duration exposure and harmful effects on humans are unidentified (Gogoi et al., 2018).

3.4 Sources of pharmaceuticals

As progressed and commercialized, pharmaceutical compounds intended to be utilized in people and creatures medication usually have complicated chemical structures (Atanasov et al., 2015). Very few are made to be noxious, and several are made to be helpful. Very few are categorized as dangerous, several are endocrine modifiers, and unluckily, there is a lack of public ownership information on the complete usage of these compounds (Kümmerer, 2009). Furthermore, as soon as they invade the habitat, they are complex to eradicate. Pharmaceutical compounds initiate from the industrial places and can depart these places as completed and wrapped products designed to be used in people and creatures' medication (Ventola, 2011). Once pharmaceuticals leave the sites, their dissemination to the habitat pursues a complicated variety of possible paths that are usually away from the root places.

3.4.1 Source of individual pharmaceuticals

The primary origin of individual drugs incorporates medical facilities, retail sectors, and extended-care facilities, which release in sewage treatment works that are principally public property sewage treatment works. All of the above origins lead to unused medications like wastes (Davies & Davies, 2010; Kümmerer, 2008). As each individual represents a possible origin for pharmaceuticals to the habitat, and it follows that either they discharge at their place, work, or in another area, their dissemination is temporarily deposited into the sewage treatment works for treatment and alteration on their route to the habitat (Scheoeman et al., 2017). The expired medications or unused ones are rejected in the domestic wastes, finally ending up in waste disposal site leeching at sewage treatment works on their route to the habitat (Gogoi et al., 2018). The current study concerning contributions of several origins of pharmaceuticals on the hydrosphere recommends that patient contribution through body discharge is essential and might be above 90% (Kümmerer, 2008).

3.4.2 Sources of creature pharmaceuticals

Pharmaceuticals that invade the habitat from stockbreeding works had long been assessed to represent a useful source. They are dispossessed mainly from manure fertilization of farming land, drainage from farming land, and release from aquaculture functions and dust (WHO, 2016). They follow the same path as individual pharmaceuticals to get to similar environmental sinks, namely surface and underground waters, air, and transport their potential for polluting these environmental sinks (WHO, 2016).

On a global scale, the manufacture of seafood via aquaculture proliferates with above 90% of aquaculture (Froehlich et al., 2018). A broad range of veterinary medicinal products, including antibiotics, is utilized preventively and to regulate the outbreak of diseases in aquatic aquaculture (Gaw et al., 2014); as far as 70% of the given dietary dose, the veterinary medicinal product is lost in the surroundings. The loss mechanism involves the renal excretion of unfinished medicines, faecal discharge of drug metabolites (Gaw et al., 2014). Other aquatic creatures in the surroundings, like untamed fish, consume remaining foods and faecal matter from marine aquaculture, possibly disseminating pharmaceuticals and alteration products. A pond-based farmstead situated in coastal zones is a source of antibiotics coming in seacoasts through leaks and dismissal of wastewater that hold increased pharmaceuticals concentrations (Watts et al., 2017).

3.4.3 Source of landfills

When disposed of with household refuse, composites end up on land disposal to access the waste disposal site. When there is no wastewater gathering, this could be a source of surface water pollution or groundwater pollution (Sasakova et al., 2018).

3.4.4 Hospitals

Pharmaceuticals are also present in the hospital's effluents (Frédéric & Yves, 2014). Hospital pharmaceutical concentration in effluents is farther up the municipal sewage (Al Qarni et al., 2016). In any case, the whole substance flow is considerably less due to a far less share of wastewater from hospitals in municipal wastewater in advanced economies. Hospital effluent

dilution by municipal effluents is by far more than a factor of 100 (Edokpayi et al., 2017).

3.4.5 Private houses

Obsolete medications are often discarded down the domestic drainages. In conformity with European Union legislation, the disposal of unused medicines via domestic squander has been authorized after 1994. According to the reports, roughly one in three of whole amounts of pharmaceuticals marketed in Germany, and 25% of that was sold in Australia (Kümmerer, 2009) is discarded with domestic squander or in the sewer. A model showed that the discarding of unused drugs, either by domestic squander or through the toilet or sink, could be a notable pathway that needs stronger emphasis (Akici et al., 2018). Three-quarters of patients examined in research carried out in the United States recorded unused and expired drugs in their households, and over half had dragged down the toilet. In a study in Kuwait, nearly one-half of interviewees acquired medications by prescription three times higher in a year, and almost all had defective drugs in their houses (Abahussain et al., 2006). The rationale for having unused medicines was mainly because of a change of medication by the practitioner or self-discontinuity (Kümmerer, 2009). Their commonly agreed methodology was to either dispose of unwanted medicines in the rubbish or drag the drugs down the drainages.

3.4.6 Manufacturers

On account of proper manufacturing method regulations and regularly elevated economic interests of active compounds, the masses of discharges taking place in manufacturing have been insignificant. Kümmerer (2009) said such releases are found to be low in North America and Europe. Hence, manufacturers have still not disseminated information in this regard. Lately, it has been observed in Asia's countries' concentrations, for single composite as far as several mg/L may be discovered in effluents. Nevertheless, the input from the regional manufacturer in Norway was more significant (Kümmerer, 2009). According to Kümmerer's work, there is no information accessible on the discharge throughout storage and transportation.

3.5 Source control

The complete elimination of pharmaceutical compounds in conventional sewage treatment works is impracticable (Xing et al., 2018). They could not be dependent on unique technology for regulating pharmaceutical compounds' access to the habitat. These sewage treatment works pathways represent the valuable input- through several estimations, over 80% of human involvement is expected to overgrow while the strain for generating the most up-to-date and extra dominant order and non-order pharmaceutical increases (WHO, 2016). Such insufficiency in treatment ability causes it to regulate the vast number of pharmaceuticals invading these techniques through origin control plans to minimize the habitat's overload (Taylor & Senac, 2015; WHO, 2016). Therefore, the prevention turns into a long-period control imperative (WHO, 2016).

3.6 Consequences of pharmaceutical contaminants on the quality of water

The wastewater discharged from pharmaceutical manufacturing composed of various chemical and biological composites (Puyol et al., 2017). Due to such a nature, these wastewaters are likely to give rise to alterations in water quality. Based on the whole potable water quality parameters, several attributes, namely pH, TDS, temperature, BOD, suspended solids, COD,

and chloride, demonstrate a higher contamination level of wastewater (Chander et al., 2016).

3.7 Impacts of pharmaceutical composites in potable water on the human body

Pharmaceuticals existing in water bodies directly affect the user's health through respiratory disorders, reproductive difficulty, cancers, and congenital difficulties involving mental retardation and physical irregularities. While decreasing the agricultural area's effectiveness, modifying the agrarian structure, and giving rise to stockbreeding and fishes' high mortality rate (Chander et al., 2016). Small quantities of pharmaceutical compounds in potable water are likely to give rise to considerable harmful effects to the human body after long-term effects (Kaczala & Blum, 2016). The concentrations of pharmaceutical compounds discovered in potable water samples have various malfunctions. The discharge of EDCs onto the habitat may give rise to endocrine-associated illnesses to users' health that is rising in animals, and altering the human reproductive health involves birth effects, breast cancer, and testicular cancer (Manibusan & Touart, 2017; Nikolaou, 2007). Pharmaceutical materials utilized against breast cancer have long been spotted in surface water (Chander et al., 2016). Moreover, the chiral pharmaceutical compounds can be the best choice to minimize pharmaceutical dosage burden to the sick and secure potable water resources from pharmaceutical remedies' unneeded effect (Chander et al., 2016).

3.8 The effects of drug combinations

The impact of drug combinations on environmental pollutants remains misunderstood and needs further research. Although hazard assessment methods concentrate on one substance simultaneously, aquatic creatures living in ponds and rivers, people drinking groundwater are subjected to numerous pollutants such as microbial, organic material, inorganic material, macroscopic and thermal (Shankar et al., 2014). Toxicological research should not study the effects of sole pharmaceuticals on creatures but should examine the negative impacts of subjection to multiple pharmaceutical medicines right away (Cleuvers, 2003; Taylor & Senac, 2015).

3.9 Pharmaceutical compounds in the WWTP procedure

There are three possible results for pharmaceutical compounds to tour throughout the sewage treatment work procedures; continuity through the process and discharge in receiving waters, alterations to more hydrophobic composites and sorption to solids, and elimination after degrading minor toxic composites (Chander et al., 2016). The extent of hydrophobicity of a composite is essential when evaluating their destiny in the sewage treatment works. In contrast, more non-polar composites adsorb upon activated sludge, and more polar composites stay in the water phase, proceeding via the system unaltered and touring towards receiving water (Jeli et al., 2011). Previous studies have documented the low elimination of pharmaceutical compounds in the procedure of conventional activated sludge by comparing concentrations of pharmaceuticals in the inflow to those discharged to the outflow (Angeles et al., 2020; Kimura et al., 2005; Tambosi et al., 2010).

3.10 Antiretroviral metabolites and catch WWTP

It is common that some drugs are quickly metabolized in the human system after usage, leading

to breakdown products becoming the main factor in wastewater. After excretion in wastewater, some medications may be transformed through non-living and living components, affecting the drug's stability and fate. The consequences of drug metabolites' environment in water supply networks could be further harmful than when the parental compound is present (Acher et al., 2017). Besides the potential of drug metabolites to exert the effects on the health, if they exist in the ecosystem, the harmful material balance for the drugs and their metabolites has also been identified at various sewage works globally (Blair et al., 2015). This adverse material balance tendency is referred to as increased ARVs being discovered in the final treated effluents of the sewage work compared to raw sewage coming into the treatment facility. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are uncompetitive inhibitors that act; otherwise, they are metabolized by Cytochrome P450, for instance, Nevirapine hydroxylated metabolites, followed by glucuronidation (Andrade et al., 2011). The derived products have been found in human faeces and urine. Efavirenz is broadly metabolized, and a small portion of the uniform drug has been found in the urine of some species, including people (Andrade et al., 2011).

3.11 Removal of pharmaceuticals by the techniques of advanced chemical oxidation

As elucidated earlier, pharmaceutical medicines have been long discovered within the effluents of WWTPs, despite the fact of low levels, proposing that the conventional wastewater treatment technique cannot eliminate the pharmaceutical drugs. For this reason, the procedure of advanced chemical treatment is required to handle wastewater comprising pharmaceutical medicines. The methods for chemical oxidation suchlike ozonation, Fenton oxidation, Ultraviolet, and electrochemical oxidation, were efficient for the deterioration of poisonous natural pollutants in water solution (Wang & Wang, 2016).

3.11.1 Ozonation

Ozonation is the most often employed oxidation approach for the elimination of pharmaceuticals. Ozonation primarily counts on hydroxyl radicals' potent unselective oxidizing activity to eradicate the pharmaceutical medicines (Wang & Wang, 2016). Ozone has been put into practice as a subsequent treatment method to identify the execution in eliminating the pharmaceuticals. Wang and Wang (2016) have shown that the ozone can eradicate ample pharmaceutical medicines with the eradication efficiency exceeding 90%. The process of oxidation has demonstrated excellent elimination of pharmaceuticals. The ozonation mechanism is primarily based on the formation of hydroxyl radicals. As a result, the radical hydroxyl concentration is closely associated with the rate of ozonation of pharmaceuticals. The level of ozonation drastically rejects in the presence of the scavenger of hydroxyl radical within the system (Wang & Wang, 2016). De Wilt et al. (2018) conducted a study of removing pharmaceuticals from water; their results have shown 95% removal of caffeine, gemfibrozil, ibuprofen, and naproxen. Carbamazepine, diclofenac, and trimethoprim have shown the removal efficiency of 77%, 80%, and 49%, respectively (de Wilt et al., 2018). Another study showed the removal efficiency of caffeine, N, N-Diethyl-meta-toluamide, and cyclophosphamide at 84%, 89%, and 46%, respectively (Kim & Tanaka, 2010).

3.11.2 Fenton oxidation

Fenton oxidation employing iron salts and H_2O_2 in an acidic environment (Razzak et al., 2016)

is an essential oxidation treatment to eliminate the impurities and commonly used to treat trade effluents. In line with ozone oxidation, Fenton oxidation relies on hydroxyl radicals' powerful oxidizing capacity (Razzak et al., 2016; Wang & Wang, 2016). Along with conventional Fenton oxidation, Fenton-like systems suchlike electro-Fenton and photo-Fenton oxidation have been drawn up in the last few years. Fenton oxidation has shown its productiveness in the elimination of pharmaceuticals. Fenton oxidation focuses on breaking down the hydrogen peroxide to produce hydroxyl radicals employing various metal-based catalytic agents. In addition to ozone oxidation, Fenton oxidation can lead to the formation of poisonous secondary effects. The effect of H₂O₂ level in this process for carbamazepine degradation shown the removal rate of 100% 0.6 mg/L H₂O₂ dosages and 99.4% at 0.5 mg/L H₂O₂ dosages (Sönmez et al., 2020). Another study showed the removal efficiency of clofibric acid, diclofenac, carbamazepine, naproxen, ibuprofen, and ketoprofen at 2.6%, 2%, 3.8%, 1.4%, 2.6%, and 1.8%, respectively (Zou et al., 2020). Mackul'ak et al. (2015) observed a removal efficiency of amphetamine and tetrahydrocannabinol carboxyl acid at 80%.

3.11.3 Ultraviolet treatment

UV treatment is a highly regarded approach to decontaminate drinking water. UV disinfects are similarly used in the wastewater sector, following bioremediation and filtration of sand in the reuse of recycled water. Currently, the treatment of UV has been employed to eliminate pharmaceuticals (Kim et al., 2009). The apparatus of UV treatment is to destroy chemical bonds of contaminants by Ultraviolet light named photolysis. In any case, the UV light is occasionally productive. Vogna et al. (2004) have shown that UV photolysis was not productive for minimizing carbamazepine concentration. The concoction of UV light with H₂O₂ must be introduced to increase UV light ability to eradicate pharmaceuticals (Monge, 2011; Wang & Wang, 2016). Yuan et al. (2011) have confirmed that the concoction method is productive for breaking down contaminants. In common with Fenton and ozone oxidation, the UV/ H₂O₂ procedure is based on the generated hydrogen radicals caused by the absorption of Ultraviolet light by H₂O₂. In brief, the UV/ H₂O₂ procedure can be found to be a promising field of technology for eradicating pharmaceuticals. The results of Zupanc et al. (2013) showed high removal efficiency of diclofenac, naproxen, and carbamazepine at 77%, 86%, and 72%, respectively. In contrast, they have observed low removal efficiency of clofibric acid, ibuprofen, and ketoprofen at a rate ranging from 45% to 52% (Zupanc et al., 2013). A study showed a good removal efficiency of ketoprofen at 62% and excellent removal at 98% (Kim & Tanaka, 2010).

3.11.4 Ionizing irradiation

As part of the advanced oxidation process, gamma irradiation is an effective technic to eliminate the continual natural contaminants viz. pharmaceuticals, EDCs from water and sewage (Wang & Wang, 2016). A study has shown that few pharmaceutical medicines, such as carbamazepine and clofibric acid at 0.6 mg/L levels in biologically treated wastewater, might be decayed entirely at 2.0 kGy gamma irradiation (Kimura et al., 2012). Zheng et al. (2011) concluded that ibuprofen's elimination effectiveness dropped when pH raised from 1.45 to 11, suggesting that ibuprofen's elimination might contain a hydroxyl radical reaction. A study

conducted by Al Qarni et al. (2016) showed removal efficiency of paracetamol at >98 % and caffeine at >99% at hospital wastewater treatment plant. A study showed the removal efficiency of acetaminophen, caffeine, cotinine, morphine, and trimethoprim at >95%, while carbamazepine, sulfamethazine, and sulfamethoxazole showed lower removal efficiency of 25% (Al-Mashaqbeh et al., 2019).

3.11.5 Waste management

Waste management in coastal zones is also a source of pharmaceuticals coming into the aquatic surroundings (Lim et al., 2017). Leachate from seacoast landfills shall be a route for pharmaceuticals disposition of homes and hospital wastes entering seacoast (Gaw et al., 2014). Modern medicine effluents, livestock manure, and sewage sludge were dropped in the sea (Kroiss, 2011).

3.12 Selected pharmaceutical compounds understudy

Table 3.1 below shows the selected pharmaceutical compounds (targeted and non-targeted) and their CAS numbers, molar masses, molecular structures, pKa, and Log K_{ow} qualities.

Efavirenz (EFV) is an antiretroviral drug utilized in the treatment and prevention of HIV/AIDS. Efavirenz can be used to prevent an injury of a needle stick and possible exposure. It is taken once daily by mouth. It is a non-nucleoside reverse transcriptase inhibitor, and it operates by hindering the activity of reverse transcriptase. It is very dangerous to use before birth; however, it is recommended to be used throughout the first quarter of pregnancy. Efavirenz is defined as emerging contaminants, and it continues to exist in the environment over a prolonged period, consequently posing a potential hazard to potable water (Schoeman et al., 2017). The eradication of Efavirenz by WWTP ranges from 27 to 71% (Swanepoel et al., 2015). The concentration of Efavirenz invading the sewage works scoped between 0.0055 to 0.014 mg/L (Schoeman et al., 2017). Robson et al. (2017) found severe adverse effects on the liver of fish caused by Efavirenz's exposure. Among the most ordinary destructions to the organ involved with Efavirenz therapy is hepatic steatosis. Additionally, it is generally known to cause hepatotoxic effects in the human liver (Robson et al., 2017).

Emtricitabine (FTC) is an antiviral drug and a nucleoside reverse-transcriptase inhibitor to prevent and treat HIV from multiplying in the grown-ups and juvenile systems. It can cause a critical illness termed lactic acidosis. First signs of lactic acidosis could worsen over some time, and such conditions can be life-threatening. The common adverse effects of Emtricitabine are headache, nausea, discolouration of the skin, and sleeping disorders (Swartz et al., 2015).

Nevirapine (NVP) is a medicament used as one of the antiretroviral therapy. Nevirapine is also referenced as Viramune. It is a non-nucleoside reverse transcriptase inhibitor. Such medicines cease HIV from the multiplication by prohibiting the reverse transcriptase enzyme from working. It has often been identified in surface water nationwide (Nibamureke et al., 2019) since it is impervious to biodegradation. The process of chlorination is utilized in sewage works around the country. It gives rise to the formation of various Nevirapine degradation products; a number of them have the same antiviral activity as the starting substance (Wood et al., 2016).

It is known for causing unwelcome adverse effects in people, particularly hepatotoxicity and skin eruption (Rivero et al., 2007). Being increased continuously in the surface water due to daily intake, Nevirapine and more pharmaceuticals are consistently present in surface water. Such a case gives cause for concern about the potential consequences of Nevirapine on fish health.

Lopinavir (LPV) is an HIV protease inhibitor, is served in the treatment of HIV status (Sakuma et al., 2015). Lopinavir is dissolvable in ethanol and methanol and virtually unsolvable in water. Its side effects are heart rhythm disturbances, hypersensitivity reactions like skin eruption, and vomiting.

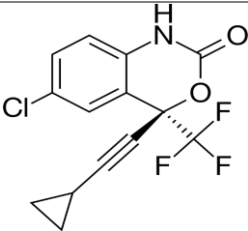
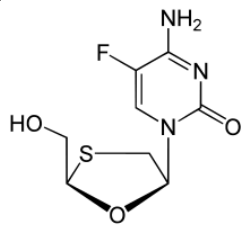
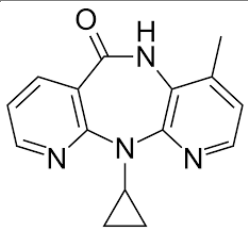
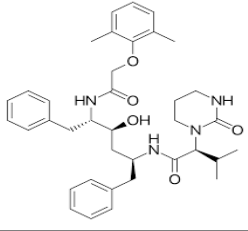
Zidovudine (ZDV) was the most generally used ARV, and it is still widely used. ARVs increase HIV-infected people's life expectancy, but the anxiety has been lifted concerning their concurrence to the environment (Ngumba et al., 2016; Russo et al., 2018). In South Africa and Germany, ZDV has been identified in the effluents of wastewater treatment plants and natural surface water (Russo et al., 2018). The existence of antiretroviral in the effluents of wastewater treatment plants and surface water indicates the ineffective of existing wastewater treatment plant processes (Herbig & Meissner, 2019). Excessive concentrations of ZDV have been identified in South Africa and Kenya. At a recent time, ZDV has also been noticed in the groundwater (K'oreje et al., 2016). ZDV is not entirely taken away in conventional treatment plants (Wood et al., 2016). A study carried out in synthetic wastewater has shown that Zidovudine is non-biodegradable, poisonous, and inhibitory to activated sludge bacteria (Russo et al., 2018).

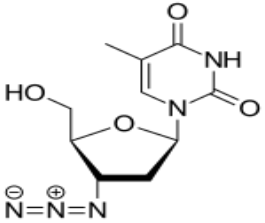
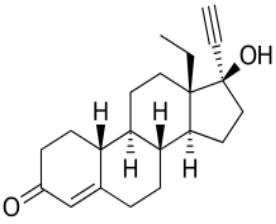
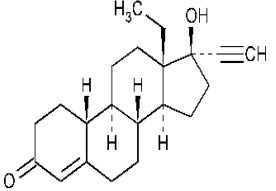
Levonorgestrel (LNG) is a synthetic chemical progestogen (Suhendi, 2016), used as prosperous and safe. It is also used to prevent pregnancy after unprotected sexual intercourse, and it is available as another possibility of hormonal replacement therapy (Suhendi, 2016). Oral Levonorgestrel is excellently absorbed and does not go through hepatic metabolism. The serum levels are achieved within 1.1 ± 0.4 hours, relying on the dose and differences that vary from one individual to another (Vandenberg et al., 2012). Suhendi (2016) has outlined Levonorgestrel's establishment, such as spectrophotometry, LC-MS/MS, and Gas chromatography-mass spectrometry/MS in human beings' plasma. The establishment of Levonorgestrel levels in water samples from environmental waste and municipal waste has also been reported using LC-MS/MS (Vulliet et al., 2007) and HPLC with a cloud point extraction (Wang et al., 2006). Like any other drug, Levonorgestrel also has side effects such as irregularities in the menstrual period and breast cancer (Regidor, 2018).

Ethinylestradiol (EE) is an estrogen medication used extensively in birth control pills and progestin. It is an orally bioactive estrogen and is among the most generally utilized drug for humankind, stockbreeding, and aquaculture activities (Aris et al., 2014). Ethinylestradiol has become a universal challenge on the habitat because of its high resistance to the procedure of degradation and the likelihood to absorb organic substances, gather in sediment, and concentrate on living things. Considerable research has reported Ethinylestradiol's capability to change sex determination, detain sexual maturation, and dwindle secondary sexual role of

exposed creatures nevertheless at low concentration by imitating its real analogue 17β -estradiol (Aris et al., 2014; Gunnotte et al., 2014). The EE is moderately soluble in ethanol, although it has low solubility in water. Humankind and stockbreeding release some hormones; thus, these hormones infiltrate the surface and groundwater system through WWTPs, septic tanks, and agricultural run-off when wastewater and manure are used as fertilizers (Aris et al., 2014). The amount of released estrogen relies on the sex, hormonal condition, menstruation phase, and employment of contraceptives and pregnancy. Once in the sewage works, EE can be stimulated in its free choice because of bacterial modification. EE stays constant in the process of activated sludge in the Sewage Treatment Plant, thereby dodging breakdown and eradication.

Table 3.1: Selected pharmaceutical compounds and their CAS numbers, molar masses, molecular structures, pKa, and Log K_{ow} qualities (Kim et al., 2019).

<i>Pharmaceutical Compounds</i>	<i>Molar Mass g/mol</i>	<i>CAS Number</i>	<i>Molecular formula</i>	<i>Water Solubility (mg/L)</i>	<i>Chemical Structure</i>	<i>pKa</i>	<i>Log K_{ow}</i>
<i>Efavirenz</i>	315.675	154598-52-4	C ₁₄ H ₉ ClF ₃ NO ₂	0.00855 mg/mL		12.52/-1.5	4.15
<i>Emtricitabine</i>	274.248	143491-57-0	C ₈ H ₁₀ FN ₃ O ₃ S	2.0 mg/mL		2.65	-0.43
<i>Nevirapine</i>	266.298	129618-40-2	C ₃₀ H ₃₀ N ₈ O ₃	100 mg/l @ neutral pH		10.37/5.06	3.89
<i>Lopinavir</i>	628.81	192725-17-0	C ₇₄ H ₉₆ N ₁₀ O ₁₀ S ₂	7.7X10 ⁻³ mg/L at 25 deg C (est.)		13.39-1.5	5.94 (est.)

<i>Zidovudine</i>	267.24242	30516-87-1	C ₁₀ H ₁₃ N ₅ O ₄	25 mg/mL at 25 °C		9.96/-3	0.05
<i>Ethinylestradiol</i>	296.403	57-63-6	C ₂₀ H ₂₄ O ₂	11.3 mg/L at 27°C		10.33/-1.7	3.67
<i>Levonorgestrel</i>	312.446	797-63-7	C ₂₁ H ₂₈ O ₂	2.05 mg/L at 25°C		17.91/-1.5	3.48

3.13 METHODOLOGY

This chapter presents a detailed description of the research methodology. The obtained results of antiretroviral drugs in water samples are discussed.

3.13.1 Standard preparation

All of the reagents, methanol (MeOH), water, and acetonitrile (ACN) HPLC grade together with hydrophilic-lipophilic balance (HLB) SPE cartridge were purchased from Milford (USA). All the reference standards (Ethinylestradiol, Levonorgestrel, Efavirenz, and Zidovudine) high purity >98% were purchased from SIGMA-ALDRICH, South Africa. The LC-MS column was purchased in the USA (Agilent). All of the reagents were prepared fresh in LC-MS quality water before administration.

Efavirenz, Zidovudine, Ethinylestradiol, and Levonorgestrel were utilized as internal standards (IS). The pharmaceuticals preparation was prepared as follows: Levonorgestrel, Zidovudine, Efavirenz, and Ethinylestradiol were weighed at 0.0125g, 0.1g, 0.005g, and 0.012g, respectively. All the standards were placed in Radioimmunoassay vials in a solid-state form. They were later sent to Central Analytical Facilities (LC-MS system) at Stellenbosch University.

3.13.2 Extraction of pharmaceuticals

The freeze-drying machine, Vir Tis –Benchtop K, was used to freeze-dry the water samples for analysis of SPE and LC-MS/MS. Water samples were firstly freeze-dried (lyophilized) at pressure state and temperature state under 0.01 °C (273.16K, 32.01 ° F), 0.0063 atm to allow ice sublimation (Nireesha et al., 2013). The initial step of lyophilization begins with preparing samples succeeded by freezing, primary, and secondary drying to acquire the ultimate dried products with the desired water content. The fundamental drying is conducted at a pressure of 10^{-4} to 10^{-5} atm and temperatures of -45 up to -20 °C.

Lyophilised samples were reconstituted in 9 mL of 10% MeOH with 1mL of 50 µg/L p-amino salicylic acids (PAS) as the internal standard. The entire 10 mL sample was then passed through an HLB SPE cartridge (Waters, Milford, USA), washed with water, and the analytes eluted off using 1mL MeOH.

3.13.3 Liquid Chromatography-Tandem Mass Spectroscopy (LC-MS/MS)

Liquid chromatography with mass-spectrometric detection was conducted using Waters Acquity UPLC linked to a Xevo TQ-S Mass Spectrometer. Compounds were analyzed by UPLC-MS/MS using the Acquity BEH C18 column (100 mm x 2.1 mm, 1.7 µm). Deionized water (H₂O), formic acid (HCOOH), and acetonitrile (CH₃CN) were used for the preparation of mobile phases, Solvent A and B.

Solvent A was prepared with liquid chromatography-mass spectrometer quality H₂O, and also 0.1% HCOOH, and solvent B was made with Liquid Chromatography-Mass Spectrometer quality CH₃CN and even HCOOH 0.1%. The acquisition was formed in the selected reaction monitoring time (SRM) mode. Table 3.2 demonstrates the instrumental conditions of LC-

MS/MS, and Table 3.3 shows the gradient elution for the aimed analyte of the mobile phase. Table 3.4 shows the physicochemical characteristics of individual drugs.

3.13.4 Instruments of analysis and detection facilities

LC-MS/MS is increasingly being used in pharmaceutical analysis. It is the preferred approach for studying the metabolism of the drug by its excellent specificity and sensitivity. Moreover, it provides improved accuracy and precision. It also stipulates molar mass and fragmentation designs to elucidate structure (Pratima & Ramesh Gadikar, 2018). Ionization method utilized hinges on the structure and the pH of the interest of the drug. Electrospray ionization (ESI) ion (positive or negative) is best for strongly pH. Compared to others, ESI has a great deal of sensitivity.

LC-MS/MS was used to analyze analytes from estrogen, progesterone, and antiretroviral therapeutic classification, which carry diverse chemical and physical characteristics. Liquid chromatography coupled mass spectroscopy is a necessary condition for analyzing a vast range of polar pollutants in samples of water when investigating the fate and occurrence of such composites in the areas of the environment and sewage works. Historically, LC-MS-MS has been employed in the areas of the environment to identify and quantify the results. Before the separation of analytes, the samples were pre-treated to eliminate the sample matrices and terminate the obtrusive elements. The stage is also essential to separate and enhance the analytes before the instrumental determination (Falaki, 2019). The Oasis HLB cartridges have been chosen because they have higher extraction capacity and can be employed to adjust the pH to make handling easier (Caban et al., 2015). We used the SPE method to extract and pre-concentrate the analytes originating from the upper, middle, and lower streams and raw sewage (Influent) and final treated effluents (Effluent). The analysis was carried out by employing LC-MS/MS in ES+ ionization mode. The limits of detection of different analytes ranged in ng/L concentrations.

Table 3.2: The instrumental conditions of LC-MS/MS

<i>Parameter</i>	<i>Input</i>
LC Column	Waters Acquity UPLC BEH C18, 2.1x100mm, 1.7µm
Mobile	Solvent A (0.1%) formic acid in the water, Solvent B (0.1%) formic acid in acetonitrile
Inlet	Water
Flow rate	0.300 mL/min
Injection volume	0 µL
Ionization mode	ES+ (Electrospray ionization, positive ion mode)

Table 3.3: Gradient elution for the aimed analyte of the mobile phase

<i>Step</i>	<i>Time (min)</i>	<i>Flow (ml/min)</i>	<i>%A</i>	<i>%B (Solvent)</i>
1	Initial	0.30	100.0	0.0
2	0.50	0.30	100.0	0.0
3	9.00	0.30	0.0	100.0
4	10.00	0.30	0.0	100.0

5	10.50	0.30	100.0	0.0
6	13.00	0.30	100.0	0.0

Table 3.4: Physicochemical characteristics of individual drugs

Class	Drug	Excretion (Metabolite)		Water solubility (mg m/L)	Total (mg/day)	Dosage table	Bioavailability
		In urine	In faeces				
Nucleoside reverse transcriptase inhibitors (NRTI)	Emtricitabine	86	-	112	200	1	93
	Efavirenz	-	-	0.00855	600	1	-
Non-nucleoside reverse transcriptase inhibitors (NNRTI)	Nevirapine	<3	-	0.7046	400	2	>90
	Lopinavir	-	-	7.7E-06	-	1	-

The data was extracted from the drug bank website. Void cells with a hyphen (-) mean no available data on the specific ARV.

3.13.5 Statistical analyses

The minimum, maximum, mean, standard error, and standard deviation were calculated using Statistica 13.4 (TIBCO Software Inc., Palo Alto, CA., USA).

3.14 RESULTS AND DISCUSSION

All the tables of the results present the compounds that were targeted and non-targeted. Levonorgestrel and Ethinylestradiol were not identified in water samples. Table 3.5 below demonstrates pharmaceutical concentrations (ng/L) at different sites determined by Solid Phase Extraction and analysis by UPLC-ESI-MS/MS. Please refer to Appendix A (Figure A1 to A4) for the standard spectrum of Lopinavir, Emtricitabine, Efavirenz, and Nevirapine.

The calibration curve linearity for both analytes was possible with a regression coefficient of both analytes. Figure 3.2 shows the Wastewater treatment plant of Alice, Makhanda, and King William's Town regions. Contrarily, Figure 3.3 shows the streams of Bloukrans, Tyhume, and Buffalo. Table 3.6 shows the Optimized Multiple-Reaction Monitoring (MRM) parameters of ARVs by UPLC-ESI-MS/MS. Retention time, precursor ion, product-ion, R2 in different matrices (rivers and sewage works).

Table 3.5: Pharmaceutical concentrations (ng/L) at different sites determined by Solid Phase Extraction and analysis by UPLC-ESI-MS/MS.

<i>River</i>	<i>Site</i>	<i>Lopinavir</i>	<i>Emtricitabine</i>	<i>Nevirapine</i>	<i>Efavirenz</i>
		<i>Retention Time (min)</i>			
		7.56	2.85	4.53	7.53
		<i>Concentration (ng/L)</i>			
<i>Makhanda region</i>					
<i>Bloukrans</i>	<i>GU</i>	n.d	n.d	n.d	40.6
	<i>GM</i>	806.4	5858	643.4	11225.6
	<i>GL</i>	454.4	3738	183.6	7691.8
	<i>GI</i>	1058.6	5605.8	10047.2	17816.2
	<i>GE</i>	288.4	5407.4	19.8	11034
<i>King William's Town region</i>					
<i>Buffalo</i>	<i>BU</i>	2.4	n.d	18.8	378.6
	<i>BM</i>	31	41.2	9614.8	1398.6
	<i>BL</i>	1141.8	2190.8	34.8	27534.6
	<i>BI</i>	1547.8	6059	39	52651.4
	<i>BE</i>	107.4	n.d	2.4	3702.6
<i>Alice region</i>					
<i>Tyhume</i>	<i>AU</i>	1.8	n.d	7	334.2
	<i>AM</i>	204.8	723.4	23.8	9311
	<i>AL</i>	978.4	n.d	52	48506
	<i>AI</i>	752.6	18757.4	44.2	55844.6
	<i>AE</i>	374.8	5636.4	41	21551.4
<i>The occurrences in all sites</i>		14	10	14	15
<i>Mean concentration detected in ng/L*</i>		545.9	5401.7	1480.8	16747

n.d- Not Detected (Indicates that it was below the detection level)

*Mean concentrations identified from water samples

Site: U- Upper stream, M- middle streams, L- lower streams, E -final treated effluent discharged into rivers, I- raw sewage from the surrounding area.

Region: G- Bloukrans River, GI/GE- Makhanda WWTP

B- Buffalo River, BI/BE- King William's Town WWTP

A- Tyhume River, AI/AE- Alice WWTP

Drug: LPV- Lopinavir, NVP- Nevirapine, EFV- Efavirenz, FTC- Emtricitabine

Table 3.6: Optimized MRM parameters of ARVs by UPLC-ESI-MS/MS. Retention time, precursor ion, product-ion, R^2 in different matrices (rivers and sewage works). The calibration curve linearity for both analytes was possible with a regression coefficient of both analytes (Appendix A).

<i>Analyte</i>	<i>Retention time (min)</i>	<i>Precursor ion (m/z)</i>	<i>Product Ion (m/z)</i>	<i>R²</i>
<i>Lopinavir</i>	7.56	629	447.3	0.998173
<i>Emtricitabine</i>	2.85	247	-	0.999088
<i>Nevirapine</i>	4.54	266	226.1	0.999672
<i>Efavirenz</i>	7.52	316	243.9	0.994173

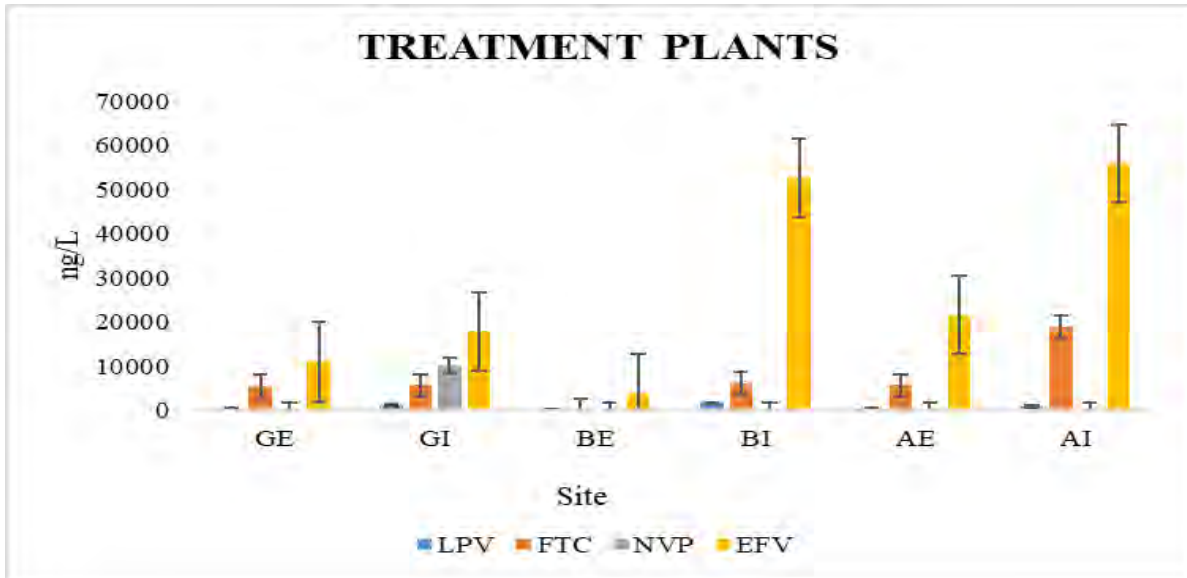


Figure 3.2: The concentration of LPV, FTC, NVP, and EFV in the WWTPs of Alice, Makhanda, and King William’s Town regions.

*EFV-Efavirenz, LPV-Lopinavir, FTC-Emtricitabine, NVP-Nevirapine

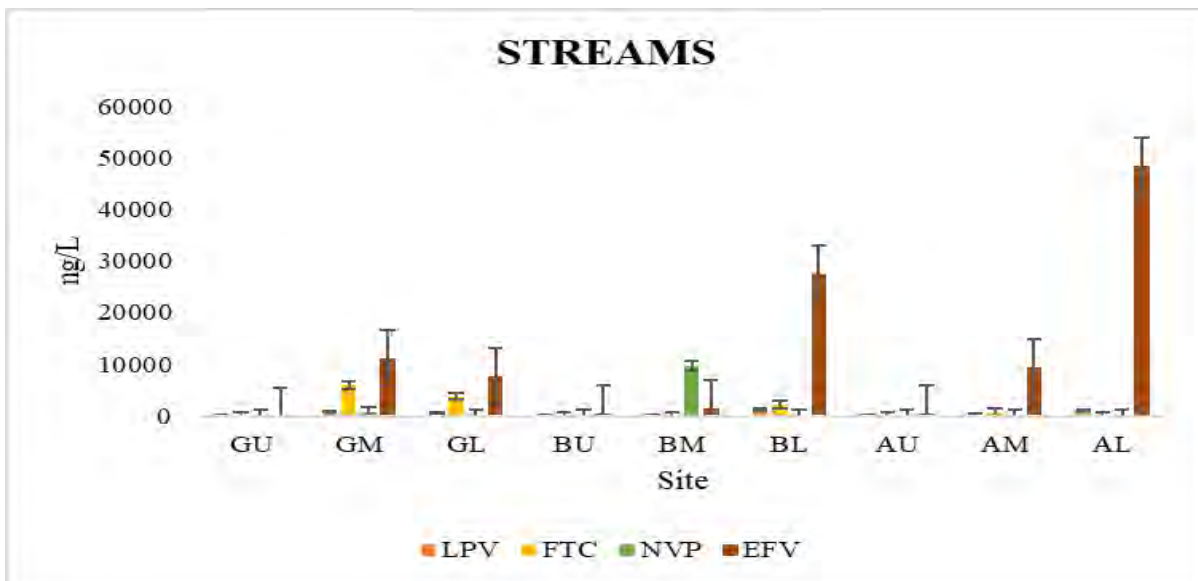


Figure 3.3: The concentration of LPV, FTC, NVP, and EFV in the streams of Alice, Makhanda, and King William’s Town regions.

*Please, refer to Figure 3.2 for drug code

3.14.1 Identification approach

Little et al. (2013) concluded that the known-unknown materials are known in publications or given by relevant data but unknown to the researcher. The hidden targets set out the study's workstream—identifying Efavirenz and Nevirapine using non-target screening. The disparity

between these two approaches is the usefulness of Triple Quadrupole Mass Spectroscopy and Multiple Reaction Monitoring. For expected particles (suspected-target screening) as opposed to the monitoring of mass range by the precise mass spectrometer, in particular, ToF-system (non-target screening; Letzel et al., 2015; Loos et al., 2016).

The main target compounds were one estrogen, one progestogen, and two antiretroviral drugs. However, the target estrogen and progestogen compounds were not detected in any of the analyzed samples. Our results are similar to the study of King et al. (2016), where Levonogestrel was not identified in the river water above the detection limit of 2.5 ng/L. The measured concentrations of Levonogestrel and Ethinylestradiol were below the detection limit of 0.05 ng/L. Only one study, Leusch et al. (2014), has made efforts to quantify Levonogestrel's concentration in Australia's effluent, and the results were recorded to be below the detection limit of 5 ng/L.

Levonogestrel was not only detected in any samples of our sites but was also not detected in the Spanish rivers nor Australia (Kuster et al., 2008). Together with Ethinylestradiol, Levonogestrel is a useful substance in the contraceptive pill; it is appalling that it was not identified. The reason for not being detected could result from the quantification limit of the analysis methods used. The potential reason why Levonogestrel was not recognized is that it may be metabolized by the body thoroughly than Ethinylestradiol, or the substance was not very responsive to identify Levonogestrel at environmentally appropriated concentrations.

3.14.2 Qualities of pharmaceuticals

The dissemination of pharmaceuticals between the liquid state and the solid-state is influenced by the drug's physicochemical characteristics and their partitioning coefficients (Grey et al., 2016; Hua, 2019). Most medicines are extremely ionic by nature and have low solubility. The most significant ratios, which identify the dissemination, are the octanol/water partition coefficient (K_{ow}) and logarithmic acid dissociation constant (pKa). The octanol-water partition ratio is the most common way of expressing a compound's lipophilicity. It is defined as the ratio of the concentration of a solute in water-saturated octanol to its concentration in an octanol-saturated aqueous phase. The pKa value is one method used to indicate an acid's strength and is the negative log of the acid dissociation constant or pKa value. A lower pKa value indicates a stronger acid. The coefficient values of the pharmaceuticals have been put together in Table 3.1.

Most pharmaceuticals exist as ions with several charges, for instance, positive and negative, whereas few exist in the absence of any charges. Some pharmaceutical medicines are neutral or hydrophobic. The greater the composite is, the higher it will be separated in lipids and hence be discovered in the waste of STP and living things. The parameters such as temperature and pH of the habitat impact the pharmaceutical's ionizing extent, also the category of functional sets present (Rummel et al., 2017). The pKa explains the acidic qualities of pharmaceuticals. Pharmaceuticals that are vigorous acids have low pKa values. Pharmaceuticals with a positive charge, composites with fundamental conditions, are probably discovered in suspended pieces, dredge, and soil, although the negatively charged liquefies into surface water (Okaiyeto et al.,

2016).

3.14.3 The existence of ARVs in the upper to downstream

ARV medications have been identified at inconstant concentrations in various water supplies. In the current study, a significant number of samples have quantifiable concentrations. Efavirenz's levels were determined in all sites, except for, Lopinavir, Emtricitabine, and Nevirapine, respectively. Lopinavir and Nevirapine were not detected in the upper stream (GU) of the Bloukrans River. In contrast, Emtricitabine was not detected throughout all rivers' top sampling sites and at the lower flow (AL) of the Tyhume River. The lowest concentration found was 1.8 ng/L of Lopinavir, and the highest level was 48506 ng/L of Efavirenz. Lopinavir was detected at the highest concentration of 1141.8 ng/L in BL, while Emtricitabine was higher at 5858 ng/L in the GM site. The AL site had a considerably higher concentration of Nevirapine at 27534.6 ng/L. In contrast, Efavirenz occurred in the BM, GL, AL, GM, and BL sites at 1398.6 ng/L, 7691.8 ng/L, 9311 ng/L, 11225.6 ng/L, 27534.6 ng/L, and 48506 ng/L, respectively. The results showed that Efavirenz occurred at high concentrations across all the sites. This observation occurs because Efavirenz has a long half-life and increased persistence in the environment for a prolonged duration. Nevirapine has been detected in most water samples; it can be deduced that this compound is used extensively. Hence, its occurrence can be ascribed to its regular medicinal use and its continuous use and release in the environment. Furthermore, another factor of Nevirapine persistency is that it is commonly utilized in HIV treatment and maternal-child transmission prevention.

3.14.4 Concentrations of ARVs in the influent and effluent of WWTPs

The analysis of wastewater is a valuable method to estimate and monitor the regional use of drugs. The present results show that over-the-counter prescribed medications contribute to water bodies' pollution since the sewage works are not efficient in eradicating them before discharging to the outflow. The treatment plants' effluent is then a vital source of drug loading in different receiving water (Barancheshme & Munir, 2018).

Table 3.8 shows the concentration of the drugs in the river samples. Efavirenz has the highest mean level in the samples with 11824.56 ng/L. The FTC and NVP follow it with 1394.6 ng/L and 1175.356 ng/L, respectively. Lopinavir has the least concentration of 402.3333 ng/L. Table 3.7 shows the mean levels in the wastewater samples. The highest mean level of the drug observed was EFV at 27100.03 ng/L, followed by NVP and FTC at 1698.933 ng/L and 6911 ng/L, respectively. The least mean concentration observed was LPV at 688.2667 ng/L.

The concentrations of ARVs differed greatly between the three WWTPS (Table 3.8). The values of concentrations fluctuate from <LOQ to 11034 ng/L (effluent), <LOQ to 17816.2 ng/L (Influent) in Makhanda WWTP; <LOQ to 3702.6 ng/L (effluent), <LOQ to 52651.4 ng/L (influent) in King William's Town WWTP; and <LOQ to 21551.4 ng/L (effluent), LOQ to 55844.6 ng/L (influent) in Alice WWTP. In any case, Efavirenz was detected the highest at 55844.6 ng/L in Alice WWTP, while the concentrations of Lopinavir and Emtricitabine varied across all three WWTPs. Nevirapine was found at lower concentrations in all WWTPs except for the GI site, while Emtricitabine was not detected in King William's Town's outflow (BE). Elevated concentrations of biologically active compounds have demonstrated a decrease in the

competence of WWTPs. Nevirapine's continuous detection in the influent may involve Nevirapine's reduced breakdown at low pH used in wastewater treatment (Wood et al., 2016).

Table 3.7: Univariate statistics for the treatment plant of Makhanda, Alice, and King William's Town regions

	<i>LPV</i>	<i>FTC</i>	<i>NVP</i>	<i>EFV</i>
<i>N</i>	6	6	6	6
<i>Min Conc.</i>	107,4	0	2,4	3702,6
<i>Max Conc.</i>	1547,8	18757,4	10047,2	55844,6
<i>Mean Conc.</i>	688,27	6911	1698,93	27100,03
<i>Std. error</i>	221,78	2545,676	1669,67	8947,358
<i>Stand. Dev</i>	543,26	6235,61	4089,83	21916,46

LPV-Lopinavir, FTC-Emtricitabine, NVP-Nevirapine, EFV-Efavirenz

Table 3.8: Univariate statistics for streams of Bloukrans, Buffalo, and Tyhume Rivers

	<i>LPV</i>	<i>FTC</i>	<i>NVP</i>	<i>EFV</i>
<i>N</i>	9	9	9	9
<i>Min Conc.</i>	0	0	0	40,6
<i>Max Conc.</i>	1141,8	5858	9614,8	48506
<i>Mean Conc.</i>	402,3	1394,6	1175,34	11824,56
<i>Std. error</i>	153,83	708,18	1057,14	5434,83
<i>Stand. Dev</i>	461,4919	2124,536	3171,431	16304,49

The concentration of Efavirenz entering the sewage works fluctuated from 17816.2 to 55844.6 ng/L, while the highest increase was found in the AE site at 21551.4 ng/L. There was a slight removal of Lopinavir (50.2%), Emtricitabine (70%), and Efavirenz (61.4%) in Alice treatment plants' outpouring. While Efavirenz has shown an insignificant reduction in the effluent of Makhanda WWTP (GE), Lopinavir and Efavirenz accumulate in King William's Town WWTP's influent (BI).

Nevirapine's slight fluctuations were observed at the two WWTPs in the influent sample of Makhanda WWTP (GI). Efavirenz is a component of the cocktail prescribed to patients living with HIV and is a stand-in for Nevirapine in the presence of contraindications (Department of Health, 2013).

Additionally, Efavirenz's excretion rate is above Nevirapine's excretion rate, which gives rise to a concentration of Efavirenz coming into the treatment plants. However, there is no significant difference in Emtricitabine and Nevirapine levels between GE and AE influent and effluent (5605.8 to 5407.4 ng/L and 44.2 to 41 ng/L, respectively). There is 93% removal of Lopinavir and Efavirenz and 94% removal of Nevirapine, while there was 100% removal of Emtricitabine in the final treated effluent of King William's Town WWTP (BE). There is a 99.8% removal efficiency of Nevirapine in GE. There was a minimal removal of Efavirenz (38%) in GE, the very low removal efficiency of Emtricitabine (3.5%). Explicitly, this indicates that the overwhelming majority of present treatment plant routines are deficient in eliminating

such pollutants. The utilization of Nevirapine in South Africa is extraordinarily high because of the high burden of HIV experienced. Furthermore, the particle is never ceasing in the environment. Therefore, besides considering the composite amount released into the background, we must find the composite's behaviour in the course of the sewage purification and its biodegradability when researching its occurrence in the environment.

The removal efficiency of medical drugs from sewage is, in fact, reliant on a series of factors like the condition of climate, the kind of the sewage treatment plant, its operational state, and the age of activated sludge utilized in the treatment facility. However, the most crucial factor is the bulk of medical drugs' physicochemical characteristics: the acidic and higher solubility in the water with an extremely low solid-liquid partition (Gupta et al., 2018). In general, medicinal drugs are polar and hydrophilic and have low Log K_{ow} ; therefore, they will prevail in the aqueous phase (Schoeman et al., 2017). Low Log K_{ow} leads to pharmaceuticals and personal care products leaching from dregs, mud, and soil.

Therapeutic drug concentration resulting from excretion in domestic wastewater is insignificant since any drug is utilized by a tiny percentage of people daily, apart from endemic and epidemic diseases. Generally known, a person has to consume eight glasses of water a day, resulting in a higher dilution of bodily waste and urine. Many medicinal drugs are successfully eradicated in the course of wastewater purification. Thereupon, concentrations that go beyond 10 $\mu\text{g/L}$ are not often noticed in treated domestic waster, at which active pharmaceutical ingredient is ordinarily identified in levels of ng/L .

3.14.5 Antiretroviral medications in the streams of water

Efavirenz was the antiviral pharmaceutical detected in water streams with the highest levels of 48506 ng/L . This outcome was significantly different from that reported in the streams of water in other papers. As an instance, in a study by Wood et al. (2015) conducted in SA, Efavirenz was not even detected in the Orange River. These outcomes indicate the diverse utilization of antiviral pharmaceuticals in various areas.

3.14.6 Antiretroviral drugs intake

At present, the amount of individual drug used up for each epidemiological region is unidentified, which means that it is hard to estimate or predict the exact amount of the drug being released into the environment. We rely on a quick view of the overall value of ARV amounts used up and excreted daily by making use of WHO as a point of reference. Suppose to be the case; if 19.8 million citizens on the antiretroviral program have been using these drugs, it would be equal to 21.78 tons of drugs being used up. This value would be equivalent to 3.96 tons of Emtricitabine and 11.18 tons of Nevirapine (Ncube et al., 2018). Let alone South Africa, with its most significant antiretroviral program, would have used up 4.32 tons of drug combination daily. Nations such as Kenya, Uganda, to mention a few, estimated to would have used up much medication daily (Ncube et al., 2018). Refer to Table 3.4; Emtricitabine has a bioavailability of 93%, meaning that 3.96 tons of the drug are used daily; therefore, 0.28 tons of Emtricitabine is not in any way enter the blood circulation.

Additionally, 3.16 tons of Emtricitabine is released in the urine. It is to say that 3.44 tons of

Emtricitabine is discharged into the environment in the course of excretion every single day. With the earth's surface, streams estimated at 2120 km³ (Čajka & Swiatlowski, 2018) are equivalent to the mean worldwide concentration of 1.47 ng/L of Emtricitabine discharged into the streams daily. Rimayi et al. (2018) reported the level of Emtricitabine at 13 ng/L in streams. Efavirenz and Nevirapine have been both found in higher concentrations in the AL streams that may imply that most citizens are on HIV treatment. Furthermore, Efavirenz was detected the highest in the AI of Alice treatment facility.

3.14.7 Remediation of antiretroviral drugs in the environment

A research carried out by Prasse et al. (2015) reported that Zidovudine and Emtricitabine had been ultimately mineralized and taken-up by microorganisms. In Germany, an investigation on the degradation kinetics for antiretroviral in the course of bioremediation of wastewater (Funke et al., 2016) evidenced that antiretroviral go through quick biotransformation via the terminal hydroxyl group's oxidation to carboxyl transformation products (Ncube et al., 2018). In their survey, Zidovudine and Emtricitabine have been identified in the raw sewage. In contrast, their carboxyl transformation products have been the main constituent in the final treated effluent after biotransformation. Such transformation products are persistent under the state of the environment and have been identified in surface water bodies receiving the final treated effluents and freshwater. Abafe et al. (2018) pointed out that the conventional anaerobic digestion of wastewater succeeded by chlorination, resulting in over ninety-nine elimination of Zidovudine from the outflow. In contrast, Efavirenz, Lopinavir, and Nevirapine remain constant. Wood et al. (2016) have proved the consistency of Nevirapine under the process of chlorine. The method of water chlorination is to add the compound chlorine to the water. This process is the latest phase in the treatment facilities. Wood et al. (2016) outcomes have proven that by-products had antiviral qualities and present in samples of surface water receiving sewerage. They concluded that Nevirapine was broken down in the process of chlorination at alkali than in acidic pH. A study has shown the execution of anaerobic fluidized membrane bioreactor versus activated carbon under aerobic carbon for Emtricitabine (Ncube et al., 2018). The elimination value of Emtricitabine under bioreactor was 63%.

3.14.8. In respect to other surveys

Efavirenz and Nevirapine determination in the effluent and influent of the WWTP samples is so vigorously. Efavirenz has been identified at different surface water; the levels were extremely low quantitation (Wood et al., 2015). Nevirapine has been identified throughout 24 samples but has been quantified in only nine samples, and 1480ng/L was the highest concentration reported (Schoeman et al., 2015). Robson et al. (2017) found that the level of Efavirenz was detected at 10.3 ng/L in river site one, river site two at 5.1 ng/L, river site four at 2.0 ng/L, and lastly in river site five at 1.6 ng/L. In comparison, other river sites had Efavirenz concentration below the limit of quantification of 1.0 ng/L. Gauteng's survey reported Efavirenz's level entering the treatment plants fluctuated from 5500 to 14 000 ng/L (Schoeman et al., 2017). Schoeman et al. (2015) reported that Efavirenz concentration in the aquatic environments was 7100 to 17 400 ng/L. Swanepoel et al. (2015) reported Nevirapine and Zidovudine levels in the treatment plant's final treated effluent at 0.4 ng/L and 0.3 ng/L, respectively. In Kenya's aquatic environments, Efavirenz's concentration was detected at 20 to

560 ng/L (Wood et al., 2017).

In Germany's sewerage, Nevirapine has been identified during several to hundreds of ng/L, and the final treated effluent of Kenya has been found at about one ng/L (Peng et al., 2014). A survey in Finland revealed the same concentration but with a 50% decrease in the effluent level (Schoeman et al., 2017). In this regard, differences result from the intake of ARV medications in various nations. Ngumba et al. (2016) found Zidovudine and Nevirapine's concentration in the effluent and influent fluctuating from 13 to 62 ng/L and 8 to 37 ng/L in each instance. The highest level in the effluent and influent was Zidovudine, then followed by Nevirapine. In Kenya, Nevirapine was identified from 30 to 5620 ng/L (K'oreje et al. 2016), and in South Africa, it was detected from <LOQ to 177 ng/L. The concentration of Nevirapine was discovered in Kenya was approximately 100 times greater. It might be based on the high prevalence of HIV/AIDS in Kenya, where antiretroviral drugs formed 15% of all of the medicinal products used up in Nairobi (Ternes et al., 2004). The concentration of Emtricitabine was detected at 361 ng/L in the aquatic environment (Wood et al., 2017). Wood et al. (2017) reported that the concentration of Lopinavir was detected at 130 to 305 ng/L.

3.14.9 Health consequences

Globally, extensive research has found that pharmaceuticals and personal care products exist in water supply sources; however, there is no convincing proof of population health effects. Lack of conclusive evidence under no circumstances means that the population is protected from the detrimental impacts of unceasing exposure to drug waste in the water. For instance, low levels of antiretroviral used up via potable water can lead to the drug-resistance position once used up by HIV-infected individuals who are not taking the treatment or are oblivious they are infected. Further investigations will be necessary to determine the effects of continuous exposure to different combinations of drugs. What would happen if anti-epileptics, anti-malarial, to mention the few, blend with antiretroviral medications.

3.14.10. The ongoing changes

The primary route of antiretroviral drugs in surface water bodies after excrement from the human body is via the discharge of unprocessed or inadequately processed effluent from the sewage works. Indecent sanitation and illicit dumping of industrial and household refuse is an additional source of the potential of antiretroviral drugs in the areas of the environment. The record for antiretroviral medicines identified in water samples mentioned shows that identifying antiretroviral medications in the effluent of the sewage works and environmental chambers is recent. Additionally, the degree of ARVDs in environmental water samples in our continent is outnumbering other places. It could be correlated to the frequency of occurrence of Acquired immunodeficiency syndrome. The discharge of unprocessed or less processed effluents and restricted wastewater dilution is because of the effects of drought (weak precipitation) can be an element. It is worsened by the limited execution of available operations of sewage works against drugs, which leads to the discharge of polluted effluents to surfaces of water (Funke et al., 2016). Inadequacy of appropriate sanitary facilities in some areas of the country can result from increasing the number of antiretroviral drugs on the water's surface because of the direct disposal of faecal material and urine in the ground transported in the rivers

in the event of rain. In this study, Nevirapine and Efavirenz conspicuously show up in river water and sewage works with high levels at 52651.4 ng/L and 27534.6 ng/L, respectively. Being the most identified drug in all river water and sewage works, Efavirenz was the highest in sewage work effluent than other studies conducted in South Africa.

3.14.11 Statistical results

Table 3.9 shows the correlation of the sites regarding ARV contamination in the wastewater samples. Samples AE and AI show the highest relationship of 0.9967, indicating the drugs' relative concentration under investigation. Other water samples, such as GE and AI, BI, and AE, also show similarities higher than 90%. Samples GE and GI show the least correlation (0.74457). Table 3.10 shows the relationship of the sites regarding ARV contamination in the wastewater samples. Samples AL and GU; AM and BL; AU and BU; AL and AU; and BU and GM and show the highest correlation of 0.99983; 0.99982; 0.99963; 0.99963; and 0.9995. Other samples such as AL and BU; AL and BL; AL and AM; AM and AU; AU and BL; BU and GU; BL and GU; and AM and GU show a similarity higher than 90%. Samples BM and BU; AU and BM; BM and GU; AL and BM; AM and BM; BL and BM; BM and GM; and BM and GL show the least correlation of -0.15472; -0.1816; -0.19864; -0.20701; -0.24331; -0.42121; and -0.42773, respectively.

Table 3.9: Correlation coefficient of ARVDs for the treatment plant of Makhanda, Alice, and King William's town

	<i>GE</i>	<i>GI</i>	<i>BE</i>	<i>BI</i>	<i>AE</i>	<i>AI</i>
<i>GE</i>	1					
<i>GI</i>	0,74457	1				
<i>BE</i>	0,87221	0,84536	1			
<i>BI</i>	0,92174	0,84108	0,99364	1		
<i>AE</i>	0,97111	0,82361	0,96364	0,98757	1	
<i>AI</i>	0,98724	0,80478	0,93877	0,97156	0,9967	1

G-Bloukrans River, GI/GE- Makhanda WWTP

B- Buffalo River, BI/BE- King William's Town WWTP

A- Tyhume River, AI/AE- Alice WWTP

Table 3.10: Correlation coefficient of ARVDs for streams of Bloukrans, Buffalo, and Tyhume Rivers

	<i>GU</i>	<i>GM</i>	<i>GL</i>	<i>BU</i>	<i>BM</i>	<i>BL</i>	<i>AU</i>	<i>AM</i>	<i>AL</i>
<i>GU</i>	1								
<i>GM</i>	0,87592	1							
<i>GL</i>	0,88781	0,9995	1						
<i>BU</i>	0,99899	0,8616	0,87344	1					
<i>BM</i>	-0,19864	-0,42121	-0,42773	-0,15472	1				
<i>BL</i>	0,99779	0,90196	0,91313	0,99402	-0,25513	1			
<i>AU</i>	0,99984	0,86957	0,88152	0,99963	-0,1816	0,99647	1		
<i>AM</i>	0,99783	0,90497	0,91566	0,99452	-0,24331	0,99982	0,99667	1	
<i>AL</i>	0,99983	0,87111	0,88352	0,99852	-0,20701	0,99757	0,99959	0,99727	1

3.15 CONCLUSION

Four antiretroviral drugs were identified and quantified in the streams, the effluents, and the treatment plants' influents. The targeted and non-targeted ARV drugs were quantified at the concentration levels above the Limit of Quantification (0.05 ng/L). It is evident of the widespread of ARV in our country. Overall, the sewage works' techniques applied on the sewage do not seem to be effective in eradicating antiretroviral drugs. The degradation products and kinetics of antiretroviral medications must be addressed in the future. Strict supervision of ARVs amount and concentration in physical surroundings should be conducted to reduce the possibility of secondary health-related matters. Users must realize that every pill of ARV has long-term implications in the surrounding. Variations in water quality have an immediate impact on supply security and continuity and subsequently on social dimensions.

REFERENCES

- Abafe, O. A., Späth, J., Fick, J., Jansson, S., Buckley, C., Stark, A., Pietruschka, B., & Martincigh, B. S. (2018). LC-MS/MS determination of antiretroviral drugs in influents and effluents from wastewater treatment plants in KwaZulu-Natal, South Africa. *Chemosphere*, *200*, 660-670.
- Abahussain, E. A., Ball, D., E. & Matowe, W., C. (2006). Practice and opinion towards the disposal of unused medication in Kuwait. *Med Prin. Pract*, *15*, 352-357.
- Akici, A., Aydin, V., & Kiroglu, A. (2018). Assessment of the association between drug disposal practices and drug use and storage behaviours. *Saudi pharmaceutical journal: SPJ: the official publication of the Saudi Pharmaceutical Society*, *26*(1), 7–13.
- Al Aukidy, M., Verlicchi, P. & Voulvoulis, N. (2014). A framework for the assessment of the environmental risk posed by pharmaceuticals originating from hospital effluents. *Science of the Total Environment*, *493*, 54-64.
- Al-Mashaqbeh, O., Alsafadi, D., Dalahmeh, S., Bartelt-Hunt, S., & Snow, D. (2019). Removal of Selected Pharmaceuticals and Personal Care Products in Wastewater Treatment Plant in Jordan. *Water*, *11*, 2004.
- Al Qarni, H., Collier, P., O'Keeffe, J., & Akunna, J. (2016). Investigating the removal of some pharmaceutical compounds in hospital wastewater treatment plants operating in Saudi Arabia. *Environmental science and pollution research international*, *23*(13), 13003–13014.
- Andrade, C. H., Freitas, L. M. D., & Oliveira, V. D. (2011). Twenty-six years of HIV science: An overview of anti-HIV drug metabolism. *Brazilian Journal of Pharmaceutical Sciences*, *47*(2), 209-230.
- Angeles, L. F., Mullen, R. A., Huang, I. J., Wilson, C., Khunjar, W., Sirotkin, H. I., ... & Aga, D. S. (2020). Assessing pharmaceutical removal and reduction in toxicity provided by advanced wastewater treatment systems. *Environmental Science: Water Research & Technology*, *6*(1), 62-77.
- Archer, E., Wolfaardt, G. M. & van Wyk, J. H. (2017). Pharmaceutical and personal care products (PPCPs) as endocrine-disrupting contaminants (EDCs) in South African surface waters. *Water SA*, *43*(4).
- Aris, A. Z., Shamsuddin, A. S., & Praveena, S. M. (2014). Occurrence of 17 α -ethynylestradiol (EE2) in the environment and effect on exposed biota: a review. *Environment international*, *69*, 104-119.
- Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P. ... Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology advances*, *33*(8), 1582–1614.
- Banerjee, S., & Mazumdar, S. (2012). Electrospray ionization mass spectrometry: a technique to access the information beyond the molecular weight of the analyte. *International journal of analytical chemistry*, *2012*.
- Barancheshme, F., & Munir, M. (2018). Strategies to Combat Antibiotic Resistance in the Wastewater Treatment Plants. *Frontiers in microbiology*, *8*, 2603.
- Becker, J. A., & Stefanakis, A. I. (2017). Pharmaceuticals and Personal Care Products as Emerging Water Contaminants. In *Pharmaceutical Sciences: Breakthroughs in*

- Research and Practice* (pp. 1457-1475). IGI Global.
- Berthod, L., Roberts, G., & Mills, G. A. (2014). A solid-phase extraction approach for the identification of pharmaceutical–sludge adsorption mechanisms. *Journal of pharmaceutical analysis*, 4(2), 117-124.
- Blair, B., Nikolaus, A., Hedman, C., Klaper, R., & Grundl, T. (2015). Evaluating the degradation, sorption, and negative mass balances of pharmaceuticals and personal care products during wastewater treatment. *Chemosphere*, 134, 395-401.
- Bottoni, P., Caroli, S., & Caracciolo, A. B. (2010). Pharmaceuticals as priority water contaminants. *Toxicological & Environmental Chemistry*, 92(3), 549-565.
- Caban, M., Stepnowski, P., Kwiatkowski, M., Maszkowska, J., Wagil, M., & Kumirska, J. (2015). Comparison of the usefulness of SPE cartridges for the determination of β -blockers and β -agonists (basic drugs) in aqueous environmental samples. *Journal of Chemistry*, 2015.
- Čajka, P., & Swiatlowski, T. (2018). Current status of drinking water sources in the world. *Acta Scientifica Academiae Ostroviensis*, 2(12), 244-274.
- Chander, V., Sharma, B., Aswal, R.S., Singh, R. & Dobhal, R. (2016). Pharmaceutical Compounds in Drinking Water. *Journal of xenobiotics*, 6(1), 5574.
- Chang, Y. X., Wang, C. P., Li, J., Bai, Y., Luo, Q., He, J. ... & Gao, X. M. (2014). LC-MS/MS determination and pharmacokinetic study of Columbianadin in rat plasma after intravenous administration of pure Columbianadin. *Chemistry Central Journal*, 8(1), 1-6.
- Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and molecular biology reviews: MMBR*, 74(3), 417–433.
- Deng, Z., Hu, J., Qin, D., & Li, H. (2010). Simultaneous analysis of hexaconazole, myclobutanil, and tebuconazole residues in apples and soil by SPE clean up and GC with nitrogen–phosphorus detection. *Chromatographia*, 71(7-8), 679-684.
- De Wilt, A., van Gijn, K., Verhoek, T., Vergnes, A., Hoek, M., Rijnaarts, H., & Langenhoff, A. (2018). Enhanced pharmaceutical removal from water in a three-step bio-ozone-bio process. *Water Research*, 138, 97-105.
- Ebele, A. J., Abdallah, M. A. E., & Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerging Contaminants*, 3(1), 1-16.
- Edokpayi, J., Odiyo, J., & Durowoju, O. (2017). Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa. *Water Quality*.
- Emara, Y., Siegert, M., Lehmann, A., & Finkbeiner, M. (2018). Life Cycle Management in the Pharmaceutical Industry Using an Applicable and Robust LCA-Based Environmental Sustainability Assessment Approach. *Designing Sustainable Technologies, Products, and Policies*, 79-88.
- Falaki, F. (2019). Sample Preparation Techniques for Gas Chromatography. In *Gas Chromatography-Derivatization, Sample Preparation, Application*. IntechOpen.
- Fernandez-Ramos, C., Šatínský, D., & Solich, P. (2014). A new method for the determination of carbamate and pyrethroid insecticides in water samples using on-line SPE fused core column chromatography. *Talanta*, 129, 579-585.
- Forlani, L., Legnani, A. & Zani, P. (2010). Urban air pollution: Particulate matter in Bologna.

- Particulate matter in Bologna, *Toxicological & Environmental Chemistry*, 92(3), 413-419.
- Frédéric, O., & Yves, P. (2014). Pharmaceuticals in hospital wastewater: Their Ecotoxicity and contribution to the environmental hazard of the effluent. *Chemosphere*, 115, 31-39.
- Froehlich, H. E., Runge, C. A., Gentry, R. R., Gaines, S. D., & Halpern, B. S. (2018). Comparative terrestrial feed and land use of an aquaculture-dominant world. *Proceedings of the National Academy of Sciences of the United States of America*, 115(20), 5295–5300.
- Funke, J., Prasse, C., & Ternes, T. A. (2016). Identification of transformation products of antiviral drugs formed during biological wastewater treatment and their occurrence in the urban water cycle. *Water Research*, 98, 75-83.
- Gadupudi, C. K., Rice, L., Xiao, L., & Kantamaneni, K. (2019). Endocrine Disrupting Compounds Removal Methods from Wastewater in the United Kingdom: A Review, *Sci*, 1(15), 1-10.
- Gao, L., Qin, D., Huang, X., Wu, S., Chen, Z., Tang, S., & Wang, P. (2019). Determination of pesticides and Pharmaceuticals from Fish Cultivation Water by parallel solid-phase extraction (SPE) and liquid chromatography–quadrupole time-of-flight mass spectrometry (LC-QTOF-MS). *Analytical Letters*, 52(6), 983-997.
- Gaw, S., Thomas, K. V., & Hutchinson, T. H. (2014). Sources, impacts, and trends of pharmaceuticals in the marine and coastal environment. *Philosophical transactions of the Royal Society of London Series B. Biological Sciences*, 369(1656), 20130572.
- Gogoi, A., Mazumder, P., Tyagi, V., Tushara Chaminda, G., An, A., & Kumar, M. (2018). Occurrence and fate of emerging contaminants in the water environment: A review. *Groundwater for Sustainable Development*, 6, 169-180.
- Gore, A. C., Chappell, V. A., Fenton, S. E., Nadal, A., Prins, G. S., Toppari, J., & Zoeller, R. T. (2015). EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine Reviews*, 36(6), E1-E150.
- Gunaydin, C., & Bilge, S. S. (2018). Effects of Nonsteroidal Anti-Inflammatory Drugs at the Molecular Level. *The Eurasian Journal of Medicine*, 50(2), 116–121.
- Gupta, D., Bhatia, D., Dave, V., Sutariya, V., & Varghese Gupta, S. (2018). Salts of Therapeutic Agents: Chemical, Physicochemical, and Biological Considerations. *Molecules (Basel, Switzerland)*, 23(7), 1719.
- Halla, N., Fernandes, I. P., Heleno, S. A., Costa, P., Boucherit-Otmani, Z., Boucherit, K., Rodrigues, A. E., Ferreira, I., & Barreiro, M. F. (2018). Cosmetics Preservation: A Review on Present Strategies. *Molecules (Basel, Switzerland)*, 23(7), 1571.
- Haseena, M., Malik, M. F., Javed, A., Arshad, S., Asif, N., Zulfiqar, S., & Hanif, J. (2017). Water pollution and human health. *Environmental Risk Assessment and Remediation*, 1(3), 16-19.
- Herbig, F. J., & Meissner, R. (2019). Talking dirty-effluent and sewage irreverence in South Africa: A conservation crime perspective. *Cogent Social Sciences*, 5(1), 1701359.
- Hua, S. (2019). Physiological and Pharmaceutical Considerations for Rectal Drug Formulations. *Frontiers in pharmacology*, 10, 1196.
- Jiang, C., Geng, J., Hu, H., Ma, H., Gao, X., & Ren, H. (2017). Impact of selected non-steroidal anti-inflammatory pharmaceuticals on microbial community assembly and activity in

- sequencing batch reactors. *PloS one*, *12*(6), e0179236.
- Kaczala, F., & Blum, S. E. (2016). The Occurrence of Veterinary Pharmaceuticals in the Environment: A Review. *Current Analytical Chemistry*, *12*(3), 169–182.
- Kim, I., Naoyuki, Y., & Hiroaki, T. (2009). Performance of UV and UV/H₂O₂ processes for the removal of pharmaceuticals detected in secondary effluent of a sewage treatment plant in Japan. *J. Hazard. Mater.*, *66*(2), 1134-40.
- Kim, I., & Tanaka, H. (2010). Use of Ozone-Based Processes for the Removal of Pharmaceuticals Detected in a Wastewater Treatment Plant. *Water Environment Research*, *82*(4), 294-301.
- Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B. A., Thiessen, P. A., Yu, B., Zaslavsky, L., Zhang, J., & Bolton, E. E. (2019). PubChem 2019 update: improved access to chemical data. *Nucleic acids research*, *47*(D1), D1102–D1109. <https://doi.org/10.1093/nar/gky1033>
- Kimura, A., Osawa, M., & Taguchi, M. (2012) Decomposition of persistent pharmaceuticals in wastewater by ionizing radiation. *Radiat. Phys. Chem.*, *81*(9), 1508-12.
- Kimura, K., Hara, H., & Watanabe, Y. (2005). Removal of pharmaceutical compounds by submerged membrane bioreactors (MBRs). *Desalination*, *178*(1-3), 135-140.
- Kleywegt, S., Pileggi, V., Yang, P., Hao, C., Zhao, X., Rocks, C., Tchach, S., Cheung, P., & Whitehead, B. (2011). Pharmaceuticals, hormones, and bisphenol A in untreated source and finished drinking water in Ontario, Canada - occurrence and treatment efficiency. *Science of the Total Environment*, *409*, 1481-1488.
- K'oreje, K.O., Vergeynst, L., Ombaka, D., De Wispelaere, P., Okoth, M., Van Langenhove, H., & Demeestere, K. (2016). Occurrence patterns of pharmaceutical residues in wastewater, surface water, and groundwater of Nairobi and Kisumu city, Kenya. *Chemosphere*, *149*, 238-244.
- Krause, M., Klit, A., Blomberg Jensen, M., Søbørg, T., Frederiksen, H., Schlumpf, M., Lichtensteiger, W., Skakkebaek, N. E., & Drzewiecki, K. T. (2012). Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. *International Journal of Andrology*, *35*(3), 424-36.
- Kroiss, H., Rechberger, H., & Egle, L. (2011). Phosphorus in Water Quality and Waste Management. *Integrated Waste Management - Volume II*.
- Kumar, P. R., Dinesh, S. R., & Rini, R. (2016). LCMS—a review and a recent update. *J Pharm Pharm Sci*, *5*, 377-391.
- Kümmerer, K. (2009). Antibiotics in the aquatic environment--a review--part I. *Chemosphere*, *75*(4), 417-434.
- Kuster, M., de Alda, M. J. L., Hernando, M. D., Petrovic, M., Martín-Alonso, J., & Barceló, D. (2008). Analysis and occurrence of pharmaceuticals, estrogens, progestogens, and polar pesticides in sewage treatment plant effluents, river water, and drinking water in the Llobregat river basin (Barcelona, Spain). *Journal of hydrology*, *358*(1-2), 112-123.
- Larsson, D. G. (2014). Antibiotics in the environment. *Upsala journal of medical science*, *119*(2), 108-112.
- Letzel, T., Bayer, A., Schulz, W., Heermann, A., Lucke, T., Greco, G. ... & Letzel, M. (2015). LC-MS screening techniques for wastewater analysis and analytical data handling

- strategies: Sartans and their transformation products as an example. *Chemosphere*, 137, 198-206.
- Leusch, F. D., Khan, S. J., Gagnon, M. M., Quayle, P., Trinh, T., Coleman, H. ... & Reitsema, T. (2014). Assessment of wastewater and recycled water quality: a comparison of lines of evidence from in vitro, in vivo and chemical analyses. *Water Research*, 50, 420-431.
- Lim, F. Y., Ong, S. L., & Hu, J. (2017). Recent Advances in the Use of Chemical Markers for Tracing Wastewater Contamination in Aquatic Environments: A Review. *Water*, 9(43), 1-26.
- Little, J. L., Cleven, C. D., & Howard, A. S. (2013). Identifying "Known Unknowns" in Commercial Products by Mass Spectrometry.
- Loos, G., Van Schepdael, A., & Cabooter, D. (2016). Quantitative mass spectrometry methods for pharmaceutical analysis. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 374(2079), 20150366.
- Lundgren, J. D., Babiker, A. G., Gordin, F. M., Borges, Á. H. & Neaton, J. D. (2013). When to start antiretroviral therapy: the need for an evidence base during early HIV infection. *BMC medicine*, 11, 148.
- Lv, X., Xiao, S., Zhang, G., Jiang, P., & Tang, F. (2016). Occurrence and removal of phenolic endocrine-disrupting chemicals in the water treatment processes. *Scientific reports*, 6, 22860.
- Mackul'ak, T., Mosný, M., Grabic, R., Golovko, O., Koba, O., & Birošová, L. (2015). Fenton-like reaction: A possible way to efficiently remove illicit drugs and pharmaceuticals from wastewater. *Environmental Toxicology and Pharmacology*, 39(2), 483-488.
- Madikizela, L.M., Muthwa, S.F., & Chimuka, L. (2014). Determination of triclosan and ketoprofen in river water and wastewater by solid-phase extraction and high-performance liquid chromatography. *S. Afr. J. Chem*, 67, 143-150.
- Manibusan, M., K. & Touart, L., W. (2017). A comprehensive review of regulatory test methods for endocrine adverse health effects. *Critical Reviews in Toxicology*, 47(6), 440-488.
- Massé, D. I., Saady, N. M., & Gilbert, Y. (2014). Potential of Biological Processes to Eliminate Antibiotics in Livestock Manure: An Overview. *Animals: an open-access journal from MDPI*, 4(2), 146–163.
- Mirzaei, R., Yunesian, M., Nasser, S., Gholami, M., Jalilzadeh, E., Shoeibi, S. ... & Mesdaghinia, A. (2017). An optimized SPE-LC-MS/MS method for antibiotics residue analysis in-ground, surface, and treated water samples by response surface methodology-central composite design. *Journal of Environmental Health Science and Engineering*, 15(1), 21.
- Monge, Z. F. (2011). Advanced oxidation of drinking water using ultraviolet light and alternative solid forms of hydrogen peroxide.
- Mutiyar, P., & Mittal, A. (2013). Pharmaceuticals and Personal Care Products (PPCPs) Residues in Water Environment of India: *A Neglected but Sensitive Issue*.
- Mwando, E., Masele, A., Sepako, E., & Sichilongo, K. (2017). A method employing SPE, MRM LC-MS/MS and a THF–water solvent system for the simultaneous determination of five antiretroviral drugs in human blood plasma. *Analytical Methods*, 9(3), 450-458.
- Ncube, S., Madikizela, L. M., Chimuka, L., & Nindi, M. M. (2018). Environmental fate and

- Ecotoxicological effects of antiretroviral: A current global status and future perspectives. *Water Research*, 145, 231-247.
- Nibamureke, U. M. C., Barnhoorn, I. E. J., & Wagenaar, G. M. (2019). Nevirapine in African surface waters induces liver histopathology in *Oreochromis Mossambicus*: A laboratory exposure study. *African Journal of Aquatic Science*, 44(1), 77-88.
- Nicolescu, T. O. (2017). Interpretation of mass spectra. *Mass Spectrometry*, 24-78.
- Nikolaou, A., Meric, S., & Fatta, D. (2007). Occurrence patterns of pharmaceuticals in water and wastewater environments. *Analytical and bioanalytical chemistry*, 387(4), 1225-1234.
- Nireesha, G. R., Divya, L., Sowmya, C., Venkatesha, N., Babu, M. N., & Lavakumar, V. (2013). Lyophilisation/Freeze Drying - An Review. *INTERNATIONAL JOURNAL OF NOVEL TRENDS IN PHARMACEUTICAL SCIENCES*, 3(4), 87-98.
- Okaiyeto, K., Nwodo, U. U., Okoli, S. A., Mabinya, L. V., & Okoh, A. I. (2016). Implications for public health demands alternatives to inorganic and synthetic flocculants: bio flocculants as essential candidates. *Microbiologyopen*, 5(2), 177-211.
- Olarinmoye, O., Bakare, A., Ugwumba, O., & Hein, A. (2016). Quantification of pharmaceutical residues in wastewater impacted surface waters and sewage sludge from Lagos, Nigeria. *J. Environ. Chem. Eco toxicology*, 8(3), 12-24.
- Olshina, M. A., & Sharon, M. (2016). Mass spectrometry: a technique of many faces. *Quarterly reviews of biophysics*, 49.
- Ooh, K. F., Ong, H. C., Wong, F. C., Sit, N. W., & Chai, T. T. (2014). High-performance liquid chromatography profiling of health-promoting phytochemicals and evaluating antioxidant, anti-lipoxygenase, iron chelating, and anti-glucosidase activities of wetland macrophytes. *Pharmacognosy Magazine*, 10(Suppl 3), S443.
- Peng, X., Wang, C., Zhang, K., Wang, Z., Huang, Q., Yu, Y., & Ou, W. (2014). Profile and behaviour of antiviral drugs in aquatic environments of the Pearl River Delta, China. *Science of the Total Environment*, 466-467, 755-761.
- Prasse, C., Wenk, J., Jasper, J. T., Ternes, T. A., & Sedlak, D. L. (2015). Co-occurrence of Photochemical and Microbiological Transformation Processes in Open-Water Unit Process Wetlands. *Environ. Sci. Technol*, 49(24), 14136-14145.
- Pratima, N. A., & Ramesh, Gadikar, R. A. (2018). Liquid Chromatography-Mass Spectrometry and Its Applications: A Brief Review. *Arch. Org. Chem. Sci*, 1, 26-23.
- Puyol, D., Batstone, D. J., Hülsen, T., Astals, S., Peces, M., & Krömer, J. O. (2017). Resource Recovery from Wastewater by Biological Technologies: Opportunities, Challenges, and Prospects. *Frontiers in microbiology*, 7, 2106.
- Razzak, N. R. B., & Hossain, D. (2016). Characterization and advanced oxidation treatment of dyeing effluent by Fenton's reagent. *Water Resources*, 43(3), 559-564.
- Regidor, P. A. (2018). The clinical relevance of progestogens in hormonal contraception: Present status and future developments. *Oncotarget*, 9(77), 34628.
- Richardson, B. J., Lam, P. K. S., & Martin, M. (2005). Emerging chemicals of concern: Pharmaceuticals and personal care products (PPCPs) in Asia, with particular reference to Southern China. *Marine Pollution Bulletin*, 50(9), 913-920.
- Rimayi, C., Odusanya, D., Weiss, J. M., de Boer, J., & Chimuka, L. (2018). Contaminants of emerging concern in the Hartbeespoort Dam catchment and the Umgeni River estuary

- 2016 pollution incident, South Africa. *Sci. Total Environ*, 627, 1008–1017
- Rivero, A., Mira, J. A., & Pineda, J. A. (2007). Liver toxicity induced by non-nucleoside reverse transcriptase inhibitors. *Journal of antimicrobial chemotherapy*, 59(3), 342-346.
- Robson, L., Barnhoorn, I. E. J., & Wagenaar, G. M. (2017). The potential effects of efavirenz on *Oreochromis mossambicus* after acute exposure. *Environmental Toxicology and Pharmacology*, 56, 225-232.
- Rummel, C. D., Jahnke, A., Gorokhova, E., Kühnel, D., & Schmitt-Jansen, M. (2017). Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. *Environmental Science & Technology Letters*, 4(7), 258-267.
- Sakuma, S., Matsumoto, S., Ishizuka, N., Mohri, K., Fukushima, M., Ohba, C., & Kawakami, K. (2015). Enhanced boosting of oral absorption of lopinavir through electrospray co-encapsulation with ritonavir. *Journal of pharmaceutical sciences*, 104(9), 2977-2985.
- Sapahin, H. A., Makahleh, A., & Saad, B. (2019). Determination of organophosphorus pesticide residues in vegetables using solid-phase micro-extraction coupled with gas chromatography–flame photometric detector. *Arabian Journal of Chemistry*, 12(8), 1934-1944.
- Sasakova, N., Gregova, G., Takacova, D., Mojziso, J., Papajova, I., & Venglovsky, J. et al. (2018). Pollution of Surface and Ground Water by Sources Related to Agricultural Activities. *Frontiers in Sustainable Food Systems*, 2.
- Schoeman, C., Dlamini, M., & Okonkwo, O. J. (2017). The impact of wastewater treatment works in Southern Gauteng, South Africa, on Efavirenz and Nevirapine discharges into the aquatic environment. *Emerging Contaminants*, 3(2), 95-106.
- Schoeman, C., Mashiane, M., Dlamini, M., & Okonkwo, O. J. (2015). Quantification of selected antiretroviral drugs in wastewater treatment works in South Africa using GC-TOFMS. *Journal of Chromatography & Separation Techniques*, 6(4), 1.
- Segura, P. A., Takada, H., Correa, J. A., El Saadi, K., Koike, T., Onwona-Agyeman, S. ... & dos Santos Junior, A. M. (2015). Global occurrence of anti-infectives in contaminated surface waters: Impact of income inequality between countries. *Environment international*, 80, 89-97.
- Semreen, M. H., Shanableh, A., Semerjian, L., Alniss, H., Mousa, M., Bai, X., & Acharya, K. (2019). Simultaneous determination of pharmaceuticals by solid-phase extraction and liquid chromatography-tandem mass spectrometry: A case study from Sharjah sewage treatment plant. *Molecules*, 24(3), 633.
- Shankar, S., Shanker, U., & Shikha. (2014). Arsenic Contamination of Groundwater: A Review of Sources, Prevalence, Health Risks, and Strategies for Mitigation. *The Scientific World Journal*, 2014, 18.
- Shao, B., Chen, D., Zhang, J., Wu, Y., & Sun, C. (2009). Determination of 76 pharmaceutical drugs by liquid chromatography-tandem mass spectrometry in slaughterhouse wastewater. *Journal of Chromatography A*, 1216(47), 8312-8318.
- Simazaki, D., Kubota, R., Suzuki, T., Akiba, M., Nishimura, T., & Kunikane, S. (2015). The occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health. *Water Research*, 76, 187-200.
- Simon, E., Schifferli, A., Bucher, T. B., Olbrich, D., Werner, I., & Vermeirssen, E. L. (2019).

- Solid-phase extraction of estrogens and herbicides from environmental waters for bioassay analysis—effects of sample volume on recoveries. *Analytical and bioanalytical chemistry*, 411(10), 2057-2069.
- Sönmez, G., Bahadır, T., & Işık, M. (2020) Removal of selected pharmaceuticals from tap water by the Fenton process. *International Journal of Environmental Analytical Chemistry*.
- Suhendi, A. 2016, Validation of High Performance of Liquid Chromatography Method for the Determination of Levonorgestrel and Ethinyl Estradiol in Tablets, *J. Pharm. Sci. & Res*, 8(1), 6-9.
- Suresh, A., & Abraham J. (2018) Bioremediation of Hormones from Waste Water. In: Hussain C. (Eds) *Handbook of Environmental Materials Management*. Springer, Cham, 1-31.
- Swanepoel, C., Bouwman, H., Peters, R. & Bezuidenhoudt, C. (2015). Presence, Levels and Potential Implications of HIV Antiretrovirals in Drinking, Treated, and Natural Waters. *Water Research Commission Report K5/2144*.
- Swartz, J. E., Vandekerckhove, L., Ammerlaan, H., De Vries, A. C., Bierman, W. F. W., ... & Levy, I. (2015). Efficacy of tenofovir and Efavirenz in combination with lamivudine or Emtricitabine in antiretroviral-naïve patients in Europe. *Journal of Antimicrobial Chemotherapy*, 70(6), 1850-1857.
- Tambosi, J. L., Yamanaka, L. Y., José, H. J., Moreira, R. D. F. P. M., & Schröder, H. F. (2010). Recent research data on the removal of pharmaceuticals from sewage treatment plants (STP). *Química Nova*, 33(2), 411-420.
- Tarcomnicu, I., van Nuijs, A. L., Simons, W., Bervoets, L., Blust, R., Jorens, P. G., ... & Covaci, A. (2011). Simultaneous determination of 15 top-prescribed pharmaceuticals and their metabolites in influent wastewater by reversed-phase liquid chromatography coupled to tandem mass spectrometry. *Talanta*, 83(3), 795-803.
- Taylor, D., & Senac, T. (2014). Human pharmaceutical products in the environment – the "problem" in perspective. *Chemosphere*, 115, 95-99.
- Ternes, T. A., Herrmann, N., Bonerz, M., Knacker, T., Siegerist, H., & Joss, A. (2004). A rapid method to measure the solid–water distribution coefficient (K_d) for pharmaceuticals and musk fragrances in sewage sludge. *Water Research*, 38(19), 4075-4084.
- Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R., Jr, Lee, D. H., Shioda, T., Soto, A. M., vom Saal, F. S., Welshons, W. V., Zoeller, R. T., & Myers, J. P. (2012). Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine Reviews*, 33(3), 378–455.
- Ventola, C. L. (2011). Direct-to-Consumer Pharmaceutical Advertising: Therapeutic or Toxic? *P & T: a peer-reviewed journal for formulary management*, 36(10), 669–684.
- Vonk, R. J., Wouters, S., Barcaru, A., Vivó-Truyols, G., Eeltink, S., de Koning, L. J., & Schoenmakers, P. J. (2015). Post-Polymerization Photografting on methacrylate-based monoliths for separation of intact proteins and protein digests with comprehensive two-dimensional liquid chromatography hyphenated with high-resolution mass spectrometry. *Analytical and bioanalytical chemistry*, 407(13), 3817-3829.
- Wang, J., & Wang, S. (2016). Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: a review. *Journal of Environmental Management*, 182, 620-640.
- Wang, L., Cai, Y. Q., He, B., Yuan, C. G., Shen, D. Z., Shao, J., & Jiang, G. B. (2006).

- Determination of estrogens in water by HPLC–UV using cloud point extraction. *Talanta*, 70(1), 47-51.
- Wang, S., Holzem, R. M., & Gunsch, C. K. (2008). Effects of pharmaceutically active compounds on a mixed microbial community originating from a municipal wastewater treatment plant. *Environmental science & technology*, 42(4), 1091-1095.
- Watts, J. E., Schreier, H. J., Lanska, L., & Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: sources, sinks, and solutions. *Marine drugs*, 15(6), 158.
- Wood, T. P., Duvenage, C. S., & Rohwer, E. (2015). The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. *Environmental Pollution*, 199, 235-243.
- Wood, T. P., Du Preez, C., Steenkamp, A., Duvenage, C., & Rohwer, E. R. (2017). Database-driven screening of South African surface water and the targeted detection of pharmaceuticals using liquid chromatography – high-resolution mass spectrometry. *Environ. Pollut*, 2(30), 453-462.
- Xing, Y., Yu, Y., & Men, Y. (2018). Emerging investigators series: occurrence and fate of emerging organic contaminants in wastewater treatment plants with an enhanced nitrification step. *Environ. Sci.: Water Res. Technol.*, 4(10), 1412-1426
- Xu, R. N., Vaca, P., Rieser, M. J., & El-Shourbagy, T. A. (2009). Highly Sensitive LC-MS-MS Analysis of a Pharmaceutical Compound in Human Plasma Using Monolithic Phase-Based On-line Extraction. *Journal of chromatographic science*, 47(6), 473-477.
- Ying, G. G., Kookana, R. S., & Ru, Y. J. (2002). Occurrence and fate of hormone steroids in the environment. *Environment International*, 28(6), 545-551.
- Zou, R., Angelidaki, I., Yang, X., Tang, K., Andersen, H., & Zhang, Y. (2020). Degradation of pharmaceuticals from wastewater in a 20-L continuous flow bio-electro-Fenton (BEF) system. *Science of the Total Environment*, 727, 138684.
- Zupanc, M., Kosjek, T., Petkovšek, M., Dular, M., Kompare, B., & Širok, B. et al. (2013). Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic cavitation, and UV treatment. *Ultrasonics Sonochemistry*, 20(4), 1104-1112

CHAPTER IV

THE PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA IN AQUATIC ENVIRONMENTS (WASTEWATER TREATMENT PLANTS AND RIVER WATER)

4.1 BACKGROUND

Antimicrobial chemicals exhibit greater antimicrobial efficacious and selective toxicity to enable their use as anti-infectious agents (Hao et al., 2014). Antimicrobial-resistant bacteria are extensive in environmental compartments such as oceans, streams, reservoirs, effluents and have yet been reported in drinking water sources (Martin & Santos, 2018). Water-supply systems harbours antibiotics, biocidal products, heavy metals, and other substances that select antimicrobial resistance within these waterborne microbial gene pools (Haberecht et al., 2019).

The production and distribution of antibiotic resistance between bacteria are considered a threat to the world, health of the population, animals, and environment. Water is a significant bacterial habitat on the ground. It is the primary way of spreading microbes in its essence and has become accepted as the substantial artificial lake of Antibiotic Resistance (AR) (Michael et al., 2013). Among human-induced surroundings, sewage plants are critical receptors and suppliers of human-originated AR (Vaz-Moreira et al., 2014). The indication of faecal pollution, *Escherichia coli*, and species of enterococcus control AR in urban wastewater. Higher resistance occurrence values were noticed for antibiotics with extensive utilization, namely, sulphonamides and tetracycline for *E. coli* and Erythromycin for enterococcus (Vaz- Moreira et al., 2014).

The growing body of support on the variety, metabolic and operation abilities of microbiota involved in the human organism indicate that microbial consortia take part in illnesses and health statuses; however, their role is still not implicit (Eloe-Fadrosch & Rasko, 2013). The emergence of antibiotic-resistant bacteria in potable water could be essential due to the negative consequences on the population's health. In these circumstances, transference can directly be of water bacteria to people or indirectly through the transference of resistance genes from water bacteria (Bengtsson-Palme et al., 2018). By nature, antibiotic-resistant bacteria and antibiotic resistance genes in water bodies are selected for and enriched by antibiotics at wastewater, agricultural runoff that arise out of the prevalent and greater use of antibiotics (Zhang et al., 2009). Previously, the issue of water quality in relation to microbes in potable water has concentrated on the emergence of pathogens in potable water distribution channels (Ashbolt, 2015; Xi et al., 2009). Various investigations have identified antibiotic resistance bacteria in water supply networks (Pavlov et al., 2004; Schwartz et al., 2003; Zhang et al., 2020); however, many surveys have focused on indicator organisms. The fate of antibiotic resistance genes in the water supply network is relatively unknown; lately, it was proposed that antibiotic resistance genes are emerging contaminants (Pruden et al., 2006; Snow et al., 2016).

Bacteria have a central role in aquatic ecology. As reducers, they take part in the decomposition and circulation of organic substances (Newton et al., 2011). These ecological importance

procedures have promoted research into communities of bacteria in water (Krevš & Kučinskienė, 2017). The results of some surveys showed the inadequacy of a relationship between the parameters of the environment and population of bacteria (Dutta & Dutta, 2016). Their qualitative and metabolic potential is determined by different living and non-living components such as temperature and trophic state.

Additionally, human activities in the river basin and along banks can influence aquatic ecosystems (Krevš & Kučinskienė, 2018). Concerning stressors, bacterial species have developed the potential to enter the state where they stay physiologically active. However, they do not grow to divide (viable but non-culturable state–VBNC). Such a function is noticed in many organisms that cause diseases and can increase human health's possible danger. In the VBNC state, bacteria present low metabolic activity (Li et al., 2014). Such nature is essential, bearing in mind the role of bacteria in the termination and transformation of organic substances in aquatic ecology. As a result, the investigation on bacteria must be carried out by employing modern fluorescent-based approaches, which also help distinguish between viable and non-viable cells. To determine bacterioplankton, quantitative structure helps understand microbes' role in decomposing organic substances because viable bacteria take part in matter circulation (Senjarini et al., 2013).

Biochemical identification systems have been provided for the identification of bacteria over the last decade. Among the methods, the API 20E identification procedure was initially designed to identify the family Enterobacteriaceae (Devenish & Burnum, 1982). The API 20E is a plastic strip with mini test tubes with dry chemicals (Okinda et al., 2014), which are inoculated with bacteria's suspension. It has been proven a quick, trustworthy, and precise technique to achieve the objective. The API 20E has become available in the UK, 1971, and the kit remains unchanged since that time nor, as yet is known, have their biochemical specifications (Holmes et al., 1978). Several researchers assessed the API 20E method to identify the Enterobacteriaceae, and both have reported considerable measure of agreement with traditional approaches in biochemical reactions and identification (Brooks et al., 1974; Nord et al., 1974; Washington et al., 1971). After the incubation of the tray, a seven-digit code is calculated from colour reactions. It is then used in comparison to Analytical Profile Index to obtain the identity of bacteria. This approach's trustworthiness is reliable, and we find methods like these in intensive usage in a lot of foods and clinical tests. Gram-negative bacteria are significant disease-causing microorganisms; their detection has been a considerable focus by various researchers (Costa et al., 2014; Doyle et al., 2012; Gavini et al., 1985; Mladenović et al., 2018). The microbial identification technique of bacterial species clinical tests is necessary because it directs us to select antimicrobial therapy to notice.

Antimicrobial susceptibility testing (AST) is a testing method in identifying which antimicrobial treatment is efficient for affected people. The AST helps assess curative health services described by health centres and state programs to control and prevent communicable diseases (Bayot & Bragg, 2020). Commercially antibiotic disks are placed on the agar followed by incubation of 35°C for 16 to 24 hours before determining the results. Various international agencies explain minimum inhibitory concentrations (MIC) of different AST. The criterion of

MIC judges if the antimicrobial agent is resistant, susceptible, or intermediate.

The Clinical and Laboratory Standards Institute (CLSI) ensures the broadest criteria following pharmacokinetic-pharmacodynamic (PK-PD) properties and mechanisms of resistance (Khan et al., 2019). Water microbial pollution is among the highest considerations governing the quality of water. The concern of microbial water pollution is becoming increasingly sophisticated when, in conjunction with antibiotic-resistant bacteria diffusion (Azzam et al., 2017). Among disease-causing agents diffused in water sources, enteric bacteria such as *E. coli*, *Shigella*, and *Salmonella* species are more often found to be accountable for types of diseases. Compound this situation further; such bacteria have been reported to be resistant to a variety of antibiotics (Dekker & Frank, 2015; Pandey et al., 2014; Poonia et al., 2014). Pathogens with a high degree of resistance were isolated from environmental areas; runoff water is the primary reservoir of antibiotics and antibiotic-resistant bacteria in the ecological zones. Antibiotic resistance continues to be a universal challenge, and its increase has been demonstrated over the past decades. Opportunistic bacterial pathogens such as *Klebsiella*, *E. coli*, *Pseudomonas*, and *Aeromonas* species are present in water supply networks (Kaur et al., 2019). The increase in bacterial resistance in polluted areas of the environment results in a high morbidity and mortality rate. As stated by the Infectious Diseases Society of America, AMR between microbes causes significant risks to the population's health (Infectious Diseases Society of America, 2011). The resolution of the antibiotic drug's effectiveness against particular disease-causing organisms should be necessary for appropriate treatment.

DNA extraction is a method employed to isolate DNA from the cell nucleus and is the initial stage of diagnostic procedures utilized to detect microorganisms in the environmental areas (Pindi et al., 2013). DNA isolation must result in proper extraction with high quality and quantity of clean and free DNA. The DNA process's removal involves organic, nonorganic, and adsorption systems (Gupta, 2019).

Polymerase chain reaction (PCR) is an in vitro enzymatic process (Verma et al., 2014). The DNA region is replicated to produce millions to billions of copies of a specific sequence in a couple of hours. Kary Mullis invented PCR in 1983 and licensed it in 1985 (Kadri, 2019). The PCR can amplify a particular DNA sequence, and it has been prepared to detect pathogens in environmental waters, foodstuff, and any biological materials, either solid or liquid. Isolated DNA is either nucleotides bases (Adenine, guanine, cytosine, and thymine) or double-stranded. PCR for bacterial identification can be employed to verify the isolation of bacteria chosen straight from the agar plate. PCR comprises several phases: denaturation by heat, annealing of primers to the target sequence, the extension of double-stranded DNA, and these processes are made for 30 to 40 cycles (Gupta, 2019). PCR implied a trusted approach to detecting coliforms pathogens in potable water (Isfahani et al., 2017). Lately, PCR utilizing universal or specific primers was first employed to amplify 16S rRNA bacterial gene before being sequenced, helping to identify the unknown or new bacterial spp (Adzitey et al., 2013). Among the highest medical purposes of the classical PCR, the approach is the discovery of disease-causing microorganisms. PCR is done by utilizing source DNA from the diversity of tissues and organisms; among these are saliva, microorganisms, skin, and other things (Garibyan &

Avashia, 2013). A group of 16S rRNA real-time PCR primers prepared to have similar optimal annealing temperature and showed specific nature of medically significant bacteria: *E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Rozman & Turk, 2016). Abulreesh et al. (2011) showed that PCR could be employed to verify *Campylobacter species* in faeces of duck and environmental water. PCR is a very understandable and accessible method to use, and it generates results very quickly. PCR techniques allow quick, specific, and identification and sensitive detection of possible disease-causing agents in environmental waters (Ahmed et al., 2008).

The 16S ribosomal DNA sequencing has become a fast-developing research area for species-level bacterial community profiling (Cho et al., 2019). MiSeq sequencing is cost-effective and has been shown to make an experimental high-throughput 16S amplification product data (Tremblay et al., 2015). MiSeq systems 16S rDNA bacterial identification are molecular biology methods based upon sequencing the bacterial 16S rDNA genes (Tawfik et al., 2018). Environmental and community genomics analysis of 16S rDNA genes employing next-generation sequencing is a modernistic molecular biology method that is progressively used in various settings. Sequencing and comparing bacterial genomes and 16S rDNA gene phylogeny have affirmed the 16S rDNA gene's representativity in bacteria's phylogeny (Manjul & Shirkot, 2018). The 16S environmental and community genomics utilize the next-generation sequence to target the bacterial species of interest and detect present microorganisms. The 16S environmental and community genomics is often employed to profile microbial communities in the surroundings and human bowels (Mallick et al., 2019).

Next-generation sequencing (NGS) was initially being termed “massively-parallel sequencing,” “deep,” or “high-throughput,” and is a flexible technique applied to viruses, bacteria, fungi, parasites, animal vectors, and human hosts (Gwinn et al., 2019). NGS determines the sequence of either RNA or DNA for studying genetic diversity associated with biological phenomena. One of the advantages of NGS is that it does not require much knowledge concerning disease-causing microorganisms; therefore, NGS is beneficial in detecting pathogens (Weinstock et al., 2016). NGS has become more cost-effective, quicker, and it can see the usual and unusual genetic diversity at once. NGS can enhance clinical and public health microbiology (Gwinn et al., 2017). Some NGS adoptions to communicable diseases are lineage-tracing, drug-resistance testing of viruses or culture isolates, and microbiome studies (Gu et al., 2019). The application of NGS has been applied to genetics and precision medicine (Pereira et al., 2017).

Further fair use of NGS is to identify microbes in forensic practices and study microbial populations within a food chain in an ecosystem (Mayo et al., 2014). Solieri et al. (2013) have explained that NGS's approach has been exploited to investigate the microbial profiles of different foods to boost optimize maturation or protection purposes of identifying untargeted microbes that produce spoilage. In metagenomics, NGS's use has made it possible to discover microorganisms, which did not grow in medium culture. Furthermore, it examines the full range of pathogenic organisms in the specimen without the need for different primers (Weinstock et al., 2016). NGS's technology has been employed in the hospital physical

environment to show microbial organisms' classification or inanimate objects (Comar et al., 2019).

Among the most useful indicator of the quality of water can be the existence or absence of biota. Several organisms are a potential indicator for contaminants' presence according to their endurance for a specific pollutant.

4.2 General view of Antibiotics

Antibiotics are composites produced by microorganisms and subject to inhibit the growth of bacteria. Synthetic or semisynthetic medicines used to treat bacterial infections are also called antibiotics. Antibiotics hinder specific critical processes of bacterial cells or metabolism. Recognition of target site and antibiotic level at target is essential to achieve significant inhibition of bacterial activity (Martinez & Baquero, 2014). Antibiotics are categorized into five main groups in Table 4.1 based on their mode of action.

Table 4.1: Antibiotic grouping by their mode of action

Group	
<i>Cell Wall Synthesis</i>	Penicillins Cephalosporins Vancomycin Beta-lactamase Inhibitors Carbapenems Aztreonam Polymyxin Bacitracin
<i>Protein Synthesis Inhibitors</i>	Inhibit 30s Subunit Aminoglycosides (gentamicin) Tetracycline Inhibit 50s Subunit Macrolides Chloramphenicol Clindamycin Linezolid Streptogramins
<i>DNA Synthesis Inhibitors</i>	Fluoroquinolones Metronidazole
<i>RNA synthesis Inhibitors</i>	Rifampin
<i>Mycolic Acid synthesis inhibitors</i>	Isoniazid Ethionamide Thiacetazone
<i>Folic Acid synthesis inhibitors</i>	Sulfonamides Trimethoprim

Note. Data from a group of cell wall synthesis from Kırmusaoğlu et al. (2019), for protein synthesis inhibitors from Kapoor et al. (2017), for DNA and RNA synthesis inhibitors from O'Rourke et al. (2014), and folic acid synthesis inhibitors from Fernández-Villa (2019).

Chemical contamination, industrial operations, mining, and active agriculture have led to

selecting antibiotic resistance determinants in the environment. The selection is preferred because of the environmental advantage to the bacteria in their natural habitat (Wright et al., 2008). Bacteria have obtained different mechanisms to adapt to environmental change. Timoney et al. (1978) and Pal et al. (2015) reported that the co-selection is often connected to the identical plasmid. Dumping of household refuse and industrial refuse has harmed public health and the environment. The toxicity of heavy metals, namely, copper, lead, mercury, chromium, harms plants, animals, and aquatic life if amassed in the soil at high levels (Tamil et al., 2012). The existence of heavy metals in the land is accountable for their continuance in the food web. Hence, it can increase the antibiotic resistance genes between the populations of bacteria. For example, the strains of bacteria isolated from tannery liquid waste like *Flavobacterium spp* have shown tolerance to copper, zinc, and mercury (Tamil et al., 2012). *Pseudomonas* species isolated from waste have shown patience antagonistically towards all antibiotics apart from Ciprofloxacin.

Additionally, bacteria have demonstrated tolerance to heavy metals, for instance, chromium, lead, and copper (Chadha, 2012). The discharge of industrial refuges resulted in the pollution of rivers and lakes. Zhang et al. (2011) have shown that the strain of *Providencia stuartii* isolated from the polluted river was resistant to all drugs as well as carbapenems. The utilization of various types of antibiotics has significantly increased the risk of antimicrobial discharge in the environment and, finally, the potential of developing resistance in pathogenic bacteria (Chadha, 2012; Zhang et al., 2011).

4.3 The effect of Antibiotic Resistance on public health

The impact of antibiotic resistance on mortality and the cost of public health is hard enough to determine, and few surveys are addressing this matter. The United States Center for Disease Control and Prevention (CDC) estimated that over two million individuals are yearly affected with antibiotic-resistant infections, comprising at least 23 000 deceased because of illness (CDC, 2013). Annually in Europe, the considerable number of diseases and mortality because of most common multidrug-resistant bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* have been measured at 400 000 and 25 000 in 2007 (ECDC, 2009; Prestinaci et al., 2015).

Various modern medicine areas rely on the accessibility of effective antibiotic medication, treatment of cancer chemotherapy, transplantation of organs, ICU for neonates, and multiple functions shall not be carried out in the absence of the effective antibiotic drug (Podolsky, 2018). In reality, infection resulting from multidrug-resistant bacterial strains are among the principal reasons affecting morbidity and mortality in patients experiencing such proceedings. Neshar and Rolston (2014) reported a high infection rate of antibiotic resistance in cancer patients with chemotherapy-related neutropenia. Kawecki et al. (2014), on infections after orthotopic liver transplantation, have demonstrated a high proportion of antibiotic-resistant bacteria isolates.

Additionally, the economic implications of antibiotic resistance are hard to measure. Increased resistance causes high costs associated with high-priced antibiotics, specialized equipment,

prolonged hospitalization, and isolation processes for patients (Ventola, 2015). Societal costs involve mortality and productivity losses.

4.4 Components contributing to the occurrence of antibiotic resistance

Antibiotic Resistance takes place when microbes are exposed to antibiotic medicinal products. Under the selective pressure of antibiotics, susceptible bacteria are destroyed or prevented, and naturally, resistant bacteria gain more opportunities to live or multiple. Besides, antibiotics misuse and antibiotics misuse contribute to increasing antibiotic resistance (Ventola, 2015). Figure 4.1 below shows the development and spread of antibiotic resistance.

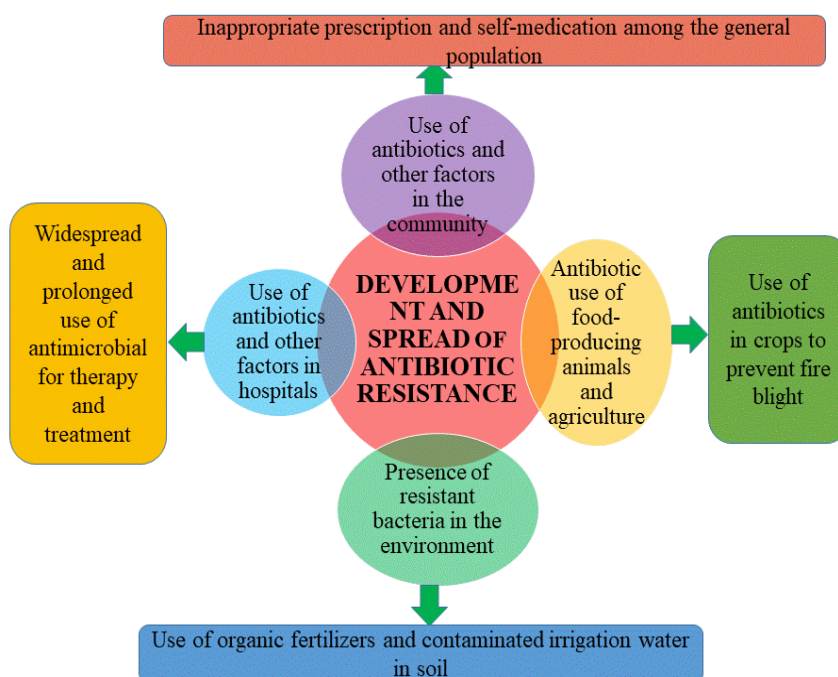


Figure 4.1: Components associated with the occurrence and dissemination of antibiotic resistance. The identification of four broad areas related to the growth of antibiotic resistance: i) the compartments of the environment, ii) livestock breeding, iii) and agriculture, and iv) human medicine in society and hospitals.

4.5 The surrounding and dissemination of resistance

Over recent years, the significance of the situation in antibiotic resistance distribution has been broadly recognized. Water is a basic form of bacteria spread between various environments' compartments (Singer et al., 2016). Large volumes of antibiotics are discharged into communal wastes because of partial metabolisms in humans or the disposition of used drugs (Prestinaci et al., 2015). The current information demonstrated that antibiotic-resistant bacteria and antibiotic-resistant genes could be discovered in samples of effluents. The states of WWTPs are beneficial for the spread of resistant bacteria. Different surveys have announced high levels

of tetracycline and sulphonamide-resistant bacteria and sulphonamide-resistant genes in sewage works (Bouki et al., 2013; Novo et al., 2013).

4.6 The emergence of resistance in species of bacteria usually cause human infections

An increased number of disease-causing organisms are resistant to one or several antimicrobial drugs. Consequently, some frequent infections turned out to be highly complex and sometimes hardly possible to treat. Pneumonia was curable after introducing penicillin; presently, it requires a second and third-line antibiotic (Wilson, 2001). Cystitis is prevalent in women and was easily treatable using oral medication, and now it requires complex antibiotic treatments that demand incremental costs on patients and healthcare systems (Chen et al., 2009). The species of bacteria that illustrate antibiotic-resistant pathogenic organisms resulting in infections in several environments. *Klebsiella pneumoniae* causes diseases prevalent in hospitals within susceptible persons (Viale et al., 2013). In intensive care units and neonates settings, *K. pneumoniae* may spread between patients, causing hospital-acquired infection outbreaks. In the past years, a considerable number of multidrug-resistant *K. pneumoniae* has rapidly increased. Resistance mediated by extended-spectrum beta-lactamases involves penicillin, cephalosporin, and aztreonam. Delgado-Valverde et al. (2013) demonstrated the occurrence of extended-spectrum beta-lactamases-producing *K. pneumoniae* of 38.9%, 8.8%, and 21.5% in Europe, the USA, and the Asia-Pacific region, respectively. The WHO announced that third-generation cephalosporin resistance in *K. pneumoniae* was higher than 60% in other nations (WHO, 2014). The WHO demonstrated a shocking rate of carbapenem resistance in *K. pneumoniae*, over 50% in other European and Eastern Mediterranean countries (ECDC, 2014). Infection risk and interferences targeted to hinder the spread of these incurable disease-causing organisms are very necessary.

4.7 The spread of antibiotic resistance in potable water distribution channels

Clean water distribution systems may enhance the distribution and persistence of antibiotic resistance. Bacteria in potable water distribution channels develop in pipe biofilm, loose deposits, and suspended solids. Figure 4.2 below illustrates the transport of antibiotic-resistant bacteria and antibiotic resistance genes in potable water distribution systems.

4.7.1 Suspended solids

The central origin of suspended particles in potable water distribution channels is biofilm, corrosion, precipitation, sediment suspension, to mention a few. Spite of being an annoyance in the quality of clean water, as it creates discolouration of water, suspended particles may induce significant troubles because of playing a role as the leading site of bacterial attachment and growth in the course of treatment of water and distribution (Fang et al., 2018). Particle-attached bacteria present a danger to the health of the population if they are pathogenic. Having a high likelihood to reach potable tap water and their variety of microbes and richness is more elevated than non-symbiotic bacteria (Parveen et al., 2011; Sanganyado & Gwenzi, 2019). Therefore, the likelihood of genetic material encoding antibiotic resistance is higher in particle-attached bacteria since their cells are moderately close. The existence of antibiotics in potable water distribution channels could enhance the adsorption of pathogenic antibiotic resistance bacteria onto suspended particles because of the formation of extracellular polymeric materials,

thereby raising their motility and threat of human exposure (Sanganyado & Gwenzi, 2019).

4.7.2 Loose deposits

Surveys on antibiotic resistance to loose deposits are still inadequate; however, the community of microbes in loose sediments forms 80% of the whole bacteria in potable water distribution channels (Chakhtoura et al., 2018). Free deposits can gather in the clean water distribution channels settling down in pipelines, where they can be carried via potable water distribution channels through bed transportation and re-suspension (Liu et al., 2014). All the loose deposits settle down, and they gather organic materials, inorganic nutrients, and extracellular polymeric materials, which make a niche microenvironment for the dwelling of bacteria and disseminate antibiotic resistance (Bohórquez et al., 2017). Lui et al. (2014) found persistent communities of bacteria in loose deposits, piping biofilm, suspended solids, and bulk water everywhere in potable water distribution channels; however, loose sediments had a tremendous variety of bacterial communities. Besides, they discovered the population and range of communities of bacteria in free deposits depended on the local amount of loose sediments in the potable water distribution channels (Liu et al., 2014).

4.7.3 Biofilms

Biofilms serve as antibiotic resistance reservoirs in water systems and enhance resistance via the following mechanisms: below; 1) Serves as novel macro habitat that promotes the continuance of antibiotic resistance bacteria, 2) Supply barriers to antibiotics, and 3) Support the genetic material exchange between microorganisms with biofilm (Sanganyado & Gwenzi, 2019). Bacteria can regrow in pipes due to the subsequent decrease in chlorine residual and increase microbial nutrients, causing the spread of antibiotic resistance bacteria (Bergeron et al., 2015). The re-growth of communities of microbes influences the temperature, duration of resistance, and pipeline materials. Extended chlorine exposure was demonstrated to improve antibiotic-resistant *Pseudomonas aeruginosa*; however, constant chlorine exposure eliminated antibiotic resistance bacteria (Mao et al., 2018). Contrary, a recent study proved that disinfecting potable water utilizing chloramine did not increase antibiotic resistance in biofilm *P. aeruginosa* (Sanganyado & Gwenzi, 2019). Presumably, chloramine can minimize glucose metabolism and diversity of microbes in bulk water but not precisely the biofilm because of the presence of extracellular polymeric materials that can play as a barrier to diffusion (Mi et al., 2015).

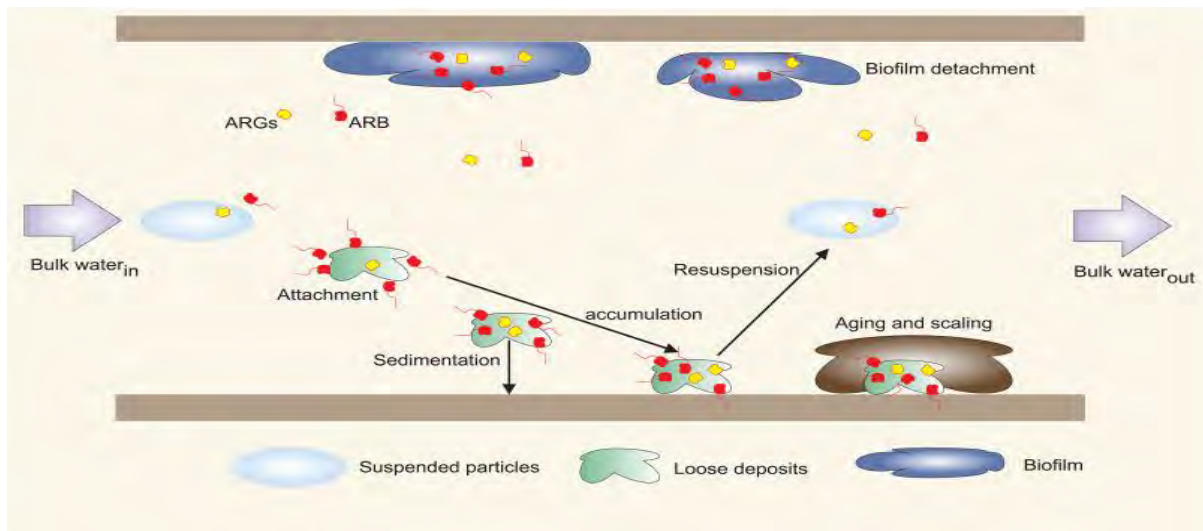


Figure 4.2: Transport of antibiotic-resistant bacteria and antibiotic resistance genes in the potable water distribution system (Sanganyado & Gwenzi, 2019).

4.8 Antibiotic mechanisms and the prior existence of antimicrobial resistance genes

Antibiotic Resistance is an antique bacterial trait present in soil bacteria and carried on plasmids (Allen & Stanton, 2014). Recent investigation has discovered resistance in ancient permafrost, isolated caves, and human samples kept for a hundred years (Perry et al., 2016). The findings of this investigation give the direct test data that antimicrobial is archaic.

4.9 Antimicrobial resistance co-selection by heavy metals

Antimicrobial resistance can be ascribed to the selective pressure by extensive use and misapplication of antimicrobials. Nonetheless, challenges have been laid under increasing evidence about antimicrobial resistance co-selection among bacteria exposed to biocidal products are utilized as disinfectants, preservatives, antiseptics, and several cationic heavy metals contained in animal diets in the form of food supplements, growth-enhancing and therapeutic agents for stockbreeding (Wales & Davies, 2015). Such metals can be distributed in grasslands to promote the growth and protection of the crop.

Heavy metals are ubiquitous and occasionally at high levels in some settings when they are utilized in farm products for various applications. Heavy metals can remain in the environment for more extended periods. Most veterinary antimicrobial composites can be metabolized and cleared from the food-producing animals in a week or months (Cheng et al., 2019). The bioavailability of regularly feed-used minerals is generally relatively low in animals, and the un-absorbed heavy metals are discharged in the faeces at high levels than in feeds (Medardus et al., 2014). The relationship between heavy metal tolerance and antimicrobial resistance had previously been washed in recent decades. It has been reported that copper was resistance to Erythromycin (Chenia & Jacobs, 2017). Heavy metals can facilitate horizontal gene transfer. Lately, Zhang et al. (2018) proposed that sub-inhibitory levels of heavy metals speed up Horizontal Gene Transfer (HGT) of plasmid-mediated antibiotic resistance genes in a water environment by supporting the conjugative transfer of genes between *E. coli* strains. Other

research demonstrated that through copper stock at 10 and 100 mg/L loadings on bacteria from potable water bio-filters, bacterial resistance to Erythromycin and Kanamycin was significantly increased (Cheng et al., 2019). Figure 4.3 explains the Co-occurrence mechanisms of heavy metal and antibiotic resistance.

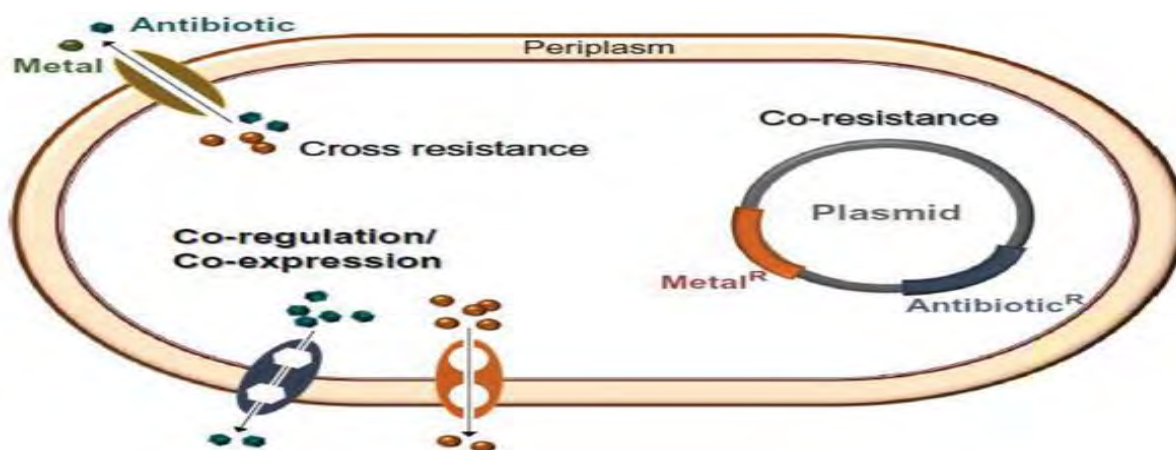


Figure 4.3: Co-occurrence mechanisms of heavy metal and antibiotic resistance (Barancheshme & Munir, 2018).

4.10 Antimicrobial co-selection by biocidal products

Biocidal products can be used as antiseptics on body zones, as disinfectants on machines and exteriors in various environments, for instance, hospitals and farmlands, as decontamination agents on carcass surfaces following the massacre, and as preservatives in medicinal products and cosmetics. Studies have been conducted on the antimicrobial resistance co-selection in bacterial isolates from food-animals and fish farming. It was suggested that exposure to Chlorhexidine Digluconate heightens the danger for resistance to different antimicrobials (Kampf et al., 2016). Other studies have involved health care environments regarding antimicrobial co-selection by biocidal products (Wales & Davies, 2015). When aerobic microbial communities were exposed to Benzalkonium Chloride, the community-wide Minimum Inhibitory Concentration (MIC) values of Benzalkonium Chloride, Ciprofloxacin, Tetracycline, and Penicillin G all increased (Tandukar et al., 2013). There are many hypothetical and test experiments that some biocidal products may co-select for antimicrobial resistance, primarily by the strong relationship of biocide resistance determinants to antimicrobial resistance determinants. However, there is a deficiency in indicating that the utilization of biocidal products causes this antimicrobial resistance co-selection in the food web (Hardy et al., 2018).

4.11 Measurement of the concentration of water antibiotic

The development in instrumental analytical chemistry, utilizing electrophoretic and

chromatographic methods, as LC-MS provides the opportunity to identify the variety of antibiotic concentrations in ng/L after SPE (Díaz-Cruz & Barceló, 2006). In preference, immunochemical methods are helpful for cost-effective rapid screening (Baquero et al., 2008).

4.12 Current Wastewater purification failure

Wastewater is released day by day from some areas and community segments. Globally, 113 nations have existing wastewater production data, 103 countries on sewage treatment, and 62 nations on wastewater use (Sato et al., 2013). Notwithstanding treatment, antibiotic residuals, antibiotic-resistant bacteria, and resistance genes continue to exist in the wastewater, and the sewage treatment plants are considered hotspots of resistance increment (Michael et al., 2013). The wastewater from homes, facilities of animal husbandry, and wastewater treatment plants effluents are discharged into near water bodies, which could be utilized for irrigation goals. An investigation has demonstrated that other antibiotics have long half-lives in agricultural soil: 55 to 578 days and 120 to 2310 days for Tetracycline and Ciprofloxacin, respectively (Bound et al., 2006; Walters et al., 2010). Conventional wastewater treatment facilities usually have biological degradation, for instance, utilizing activated sludge, while advanced facilities have tertiary treatment methods, namely; reverse osmosis, ozonation, and advanced oxidation technologies like Fenton oxidation heterogenous-photocatalysis with TiO₂ (Tamhankar & Lundborg, 2019). Such procedures do not correctly eradicate antibiotic remains, antibiotic-resistant bacteria, and resistance genes from the wastewater. For instance, according to some information, antibiotic residues, antibiotic-resistant bacteria, and resistance genes continue to exist after the conventional treatment (Galler et al., 2018). Besides, after the processes of advanced procedures are presently used, the challenge is not entirely resolved; as an example, research demonstrated that even after the treatment of ozonation, roughly 20% of sulfonamides, trimethoprim, and macrolides continues in the effluent (Hollender et al., 2009).

4.13 Classes of antibiotics under the study

Table 4.2 shows the selected antibiotic drugs and their properties.

4.13.1 Macrolide antibiotics and mechanism resistance

Macrolides are frequently in use in medicinal products for human and veterinary purposes. The lactone rings are usually 14-, 15-, or 16-membered. They are in service to treat a series of problems like gastrointestinal disturbance, allergic reactions, and hepatotoxic effects (Keskar & Jugade, 2015). Erythromycin and Clarithromycin are classifying under 14-membered antibiotics.

The most important means of resistance of bacteria to macrolides is post-transcriptional methylation of the 23S bacterial ribosomal RNA (Fyfe et al., 2016). The acquisition of resistance is by plasmid-mediated or chromosomal, for instance, via mutation and cross-resistance to macrolides, lincosamides, and streptogramins. Two kinds of acquisition of resistance seldom seen involve the production of drug-inactivating enzymes and ATP-dependent efflux proteins (Munita & Arias, 2016).

Macrolides are protein synthesis inhibitors; they inhibit protein biosynthesis of bacteria by preventing peptidyl transferase from adding the growing peptide attached to tRNA to the next

amino acid (Kannan et al., 2014) and preventing ribosomal translation. Another mechanism is premature dissociation of the peptidyl-tRNA from the ribosome (Polikanov et al., 2018). Macrolides bind reversibly to the P site on the 50S Subunit of the ribosome of bacteria. This action is bacteriostatic (Lobritz et al., 2015). Macrolides are concentrated within leukocytes and are conveyed into the infection site.

4.13.1.1 Erythromycin

Erythromycin is the initial antibiotic under macrolides. It is accessible in various forms such as tablets, oral suspension, gels, and injections, and it has a melting point of 191°C and a dissociation constant $pK_a = 8.9$ (Rang et al., 2003). It is soluble in alcohol, acetonitrile and slightly soluble in ethylene dichloride and amyl acetate.

4.13.2 Fluoroquinolone antibiotics and mechanism resistance

Quinolone antibiotics are a broad-spectrum bactericidal category that shares a bicyclic core structure related to the substance 4-quinolone. They are in use for human and veterinary medicinal products for the treatment of bacterial infections. Almost all quinolone drugs in use are fluoroquinolones with fluorine atoms in chemical structure and are efficient against Gram-negative and Gram-positive bacteria.

Fluoroquinolone is widely used for human and veterinary medicinal products because of its efficiency against Gram-negative and Gram-positive bacteria. The resistance of Fluoroquinolone is still increasing and is the biggest challenge experienced in the clinical setting. Livermore et al. (2013) reported that the percentage of *E. coli* isolates in the United Kingdom resistant to fluoroquinolones ascended from 6 to 20% from 2001-2006 and still at about 17% for all the other decades. Similar increases have been noticed in different species; for instance, the proportion of fluoroquinolone-resistant *Klebsiella pneumoniae* isolates in Italy has always increased annually, with about a five-fold increase from 11% in 2005 to 50% in 2012 (Gagliotti et al., 2011). Resistance to the fluoroquinolones is collective and may take place through a wide array of mechanisms.

Fluoroquinolones hinder the bacterial enzyme DNA gyrase that nicks double-stranded DNA, introduces negative supercoils, and reseals the nicked end (Sharma et al., 2010). It is essential to inhibit too many supercoiling of the strands when they split to allow replication and transcription (Schmidt et al., 1998). Ciprofloxacin hinders the DNA gyrase activity, a vital adenosine triphosphate-hydrolyzing topoisomerase II enzyme, or inhibits gyrase detachment from DNA. The topoisomerases apply bactericidal activity to cooperate with the DNA (Walters et al., 1999). Throughout the procedure of replication and transcription, helicase unwinds the DNA double helix. The uncoiling procedure creates tension because of excess supercoiling of the remaining DNA double helix. This tension requires being relieved if the system is to continue (Sharma et al., 2010). The topoisomerase II enzyme enables the relaxation of supercoiled DNA by breaking all strands of the DNA chain, crossing them over, and finally resealing them (Patrick, 2003).

4.13.2.1 Ciprofloxacin

Ciprofloxacin is an antibiotic that treats the infections of bacteria such as bladder infections. It terminates bacterial growth by preventing reproduction and mend the genetic material. The US Food and Drug Admin. (FDA) approved it in 1987. It is accessible in oral and intravenous preparations (Fasugba et al., 2015). It has dissociation constant pKa of 0.6 and 8.8 (Sun et al., 2002). It has low solubility in oil and water that contributes to slow permeation to lipoidal cell membranes.

4.13.3 Beta-lactam antibiotics and mechanism resistance

Beta-lactam antibiotics contain a beta-lactam ring in their structure of the molecule. They are penicillin, cephalosporin, monobactams, carbapenems, and carbacephems, and they are part of the increasingly used antibacterial group throughout the world. The first generation of β -lactam antibiotics as penicillin, next to the cephalosporin, then carbapenems, and monocyclic β -lactams are utilized to treat communicable diseases resulting from pathogenic bacteria (Konaklieva, 2014).

Resistance to β -lactam is a severe and growing problem, besides public care problems. With emerging resistance for antibiotics, it is reasonable to check resistance mechanisms since it can help determine which medicine to prescribe in various situations and means of overcoming the same (Pandey & Cascella, 2019). Hence, the resistance of bacteria to β -lactam expresses via beta-lactamase productions. Other resistance mechanisms involve the inactivation by beta-lactamase production, reduced penetration to the desired site, the transformation of desired site PBPs (penicillin-binding proteins), and efflux periplasmic space via unique pumping mechanisms (Ibrahim et al., 2019).

Peptidoglycan is an essential factor of the cell-wall of the bacteria that gives mechanical stability to it. The beta-lactam antibiotics hinder the final stage in peptidoglycan synthesis by acylating the Trans peptidase involved in cross-linking peptides to make peptidoglycan. The penicillin-binding proteins are targets for the action of beta-lactam drugs. The binding disrupts terminal transpeptidation and causes terminal transpeptidation and the process of autolytic within the cell of bacteria (Bush & Bradford, 2016; Eckburg et al., 2019).

4.13.3.1 Amoxicillin

Amoxicillin is part of widely used in the primary care setting. FDA approved Amoxicillin to treat urinary tract infections, ear, nose, and throat infections, lower respiratory tract infections (Akhavan & Vijhani, 2019). Occasionally, it is in use with Clarithromycin to treat ulcers resulting from *Helicobacter pylori* infection. It is accessible in capsules, tablets, suspensions, and injectable solutions (Unutkan et al., 2018). It is soluble in water and methanol, moderately soluble in ethanol (Ahmed et al., 2011), and has pKa values of 2.67, 7.11, and 9.55 at 37°C.

4.13.4 Tetracycline antibiotics and mechanism resistance

Tetracycline is a class of broad-spectrum antibiotic composites with standard core structures isolated from various types of *Streptomyces* bacteria or produced semi-synthetically from those isolated composites (Fair & Tor, 2014). Tetracycline antibiotics are growth inhibitors instead

of the infective agent's killers and are efficacious against increasing microbes (Langdon et al., 2016). They keep the crucial part of medicine; however, their utility has fallen with the onset of antibiotic resistance (Chopra & Roberts, 2001). Tetracycline antibiotics prevent the protein synthesis of bacterial and human cells.

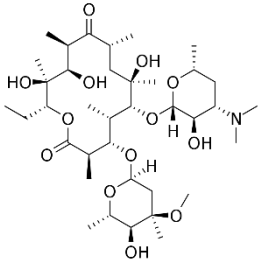
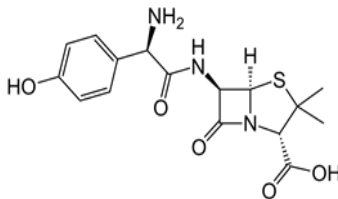
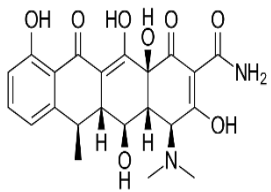
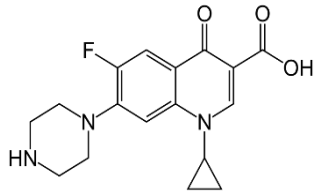
Cells become resistant to tetracycline by enzymatic inactivation of tetracycline, efflux, protection of ribosomes, reduced permeability, and ribosome mutation. Inactivation is the less common type of resistance (Forsberg et al., 2015), where NADPH-dependent oxidoreductase changes the drug at their oxidative soft spot leading to an inactivation of the Tetracycline antibiotic. In the efflux mechanism, different resistance genes encode a membrane protein that pumps the tetracycline out of the cell by exchanging a proton for a tetracycline cation complex (Lashinsky et al., 2017). This exchange results in reduced cytoplasmic concentration of tetracycline (Grossman, 2016). In ribosomal protection, a resistance gene encodes a protein with different effects according to which gene is transferred (Munita & Arias, 2016).

Tetracycline acts by binding to the ribosome's 30S subunit at the A-site (Chukwudi, 2016). In the course of protein biosynthesis, the new t-RNA with the amino acid makes an effort to bind to the ribosome's A-site. Nonetheless, as the A-site is blocked by tetracycline, the aminoacyl-tRNA cannot attach to it. Therefore, without the tRNA's sequential attachment at the A-site, protein biosynthesis cannot occur. By preventing protein, biosynthesis tetracycline induces bacterial cells (Griffin et al., 2010). Dr Gary demonstrates the action of the mechanism of tetracycline as “*The tetracycline (Tetracycline, Doxycycline, Demeclocycline, Minocycline) block bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodons of the charged tRNAs cannot align properly with the codons of the mRNA.*”

4.13.4.1 Doxycycline

Doxycycline is a broad-spectrum antibiotic of the group of tetracycline. It is used to treat infections of a high overall effect, including malaria, sexually transmitted infections, rickettsial illnesses, skin infections, and acne (Cross et al., 2016). It is highly lipid-soluble and has a pKa value of 3.09. It is yellow and crystal in powder. It is useful in the treatment of bio-threat and outbreak-associated infections (Ruhe & Menon, 2007).

Table 4.2: Selected antibiotic drugs and their properties (Kim et al., 2019).

<i>Pharmaceutical Compounds</i>	<i>Molar Mass g/mol</i>	<i>Chemical Structure</i>	<i>Molecular Formula</i>	<i>Water Solubility (mg/L)</i>	<i>CAS Number</i>	<i>Colour/Form</i>
<i>Erythromycin</i>	733.9		C ₃₇ H ₆₇ NO ₁₃	Soluble in water at 2mg/ml	114-07-8	Ointment, tablet, powder
<i>Amoxicillin</i>	365.4		C ₁₆ H ₁₉ N ₃ O ₅ S	3.43X10+3 mg/L at 25 °C (est)	26787-78-0	Crystals from water
<i>Doxycycline</i>	444,4		C ₂₂ H ₂₄ N ₂ O ₈	50 mg/mL	17086-28-1	The yellow, crystalline powder
<i>Ciprofloxacin</i>	331.346		C ₁₇ H ₁₈ FN ₃ O ₃	<1mg/mL	85721-33-1	Ointment drops

4.14 Threats to health and hazard assessment

Antibiotic Resistance can lead to improved virulence, pathogenicity, the spread of diseases, and transference resulting in extended morbidity and hospitalization and the same to mortality (Berendonk et al., 2015). WHO (2017) stated that once bacterial infections develop resistance to “first-line” antibiotics, human sicknesses' command is problematical and expensive; this could need the utilization of last-resort medicines or the creation of new ones. As mentioned above, antibiotic resistance is a world concern; therefore, human health risks are possibly more visible in developing nations than in developed nations (Sanganyado & Gwenzi, 2019). The reason is that developing countries have insufficient healthcare structures consisting of the scarcity of essential services and therapies. Such conditions make the mechanism of positive feedback that encourages the persistence and expansion of antibiotic resistance, with far-reaching implications on the population's health. The surveys on the effect of antibiotic resistance in potable water are still inadequate and restricted to epidemiology (Sanganyado & Gwenzi, 2019).

4.15 METHODOLOGY

This chapter presents the preparation and detailed description of the research methodology. The obtained results are discussed.

4.15.1 Preparation of nutrient agar

The 31 grams of the dry medium was dissolved in the appropriate volume of distilled water in a 1000 mL bottle. The powder was dissolved by heating with frequent agitate and boiling for 1 minute. The medium was sterilized by being autoclaved at 121 °C for 15 minutes. The medium was dispensed into Petri dishes to solidify. The medium was dated and stored in a cool dark place. The pH of nutrient agar was kept between 7.2 to 7.6 at ambient temperature.

Materials and equipment for agar preparation

1. Test samples
2. Nutrient Agar
3. Sterile Petri dishes
4. Magnifying glass
5. Pipettes of different sizes
6. Flame
7. Hot water bath at 45 °C

4.15.1.1 The procedure of pour plate and spread plate techniques

A bottle of molten agar was poured into sterilized Petri dishes. Water samples were diluted (from 10^{-1} to 10^{-8}), and 0.1 mL of each aliquot was plated on the nutrient agar. The plates were incubated for 37-degree centigrade for 24 to 48 hours (Ruangpan & Tendencia, 2004). After the period of incubation, the containers were examined, and the bacterial colonies were also selected.

4.15.1.2 Colony count technique

The magnifying glass was employed for colony counting. Each colony dot was counted only once. The petri dish was set on a grid background, and colonies were counted in every grid cell. The colonies were calculated from the back of the Petri dish. Generally, three plates were calculated at least thrice. However, all the containers that contained 30 to 300 colonies were selected for colony counting for a robust conclusion. Petri dishes with too numerous or fewer colonies were re-plated.

4.15.2 Identification of bacterial isolates

Bacterial isolates were examined and identified based on their morphological and biochemical properties. Additionally, the bacterial strains' biochemical properties were determined by employing the Analytical Profile Index API 20E system (BioMerieux, Marcy l'Etoile, France).

4.15.2.1 Materials and reagents

1. Agar plates of bacterial species
2. 0.85% NaCl solutions (5 mL)
3. Sterile Pasteur pipettes + bulbs
4. API 20E test strips
5. Mineral oil
6. 10% FeCl₃, Barrett's reagents
7. A and B, Kovac's reagent

4.15.2.2 Preparation of bacterial suspension in saline tube

A large colony was inoculated (2 to 3mm) of the bacterium into the 0.85% NaCl solutions. The suspension was homogenous and free from clumps of floating bacteria.

4.15.2.3 Set up of API 20E Biochemical Test Strip

A single isolated colony was picked from a pure culture, and a suspension of it was made in sterile distilled water. The API 20E strip that contained dehydrated bacterial media was taken into 20 compartments. Bacteria then reacted with it, and different colours of bacteria helped identify the species level of bacteria. The Pasteur pipette was brought to fill up to the brim with the bacterial suspension. Sterilized oil was added to arginine/antidiuretic hormone, lysine, ornithine, Na thiosulphate, and urea compartments. Drops of sterilized water were put in the API strip tray, and the API strip tray was closed. The API strip tray was marked with the identification number, and the API strip tray was incubated at 37 °C for 18 to 24 hours.

4.15.3 Antibiotic susceptibility testing using the disk diffusion technique

The bacterial isolates were tested for resistance to four antibiotics. Antimicrobial susceptibility testing was done on Mueller-Hinton agar (MHA) by the standard disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2011) to Erythromycin, Amoxicillin, Ciprofloxacin, and Doxycycline. The swabs were dipped into bacterial suspensions to inoculate the MH agar plates by spreading on the agar's surface and incubated for 24 hours at 37 °C.

4.15.3.1 Mueller-Hinton agar (MHA)

The disk diffusion approach was performed by using MHA. The MHA was used for antibiotic susceptibility testing because it is a non-selective and non-differential medium. Hence, practically all organisms will grow on it. It has the starch to absorb toxins extricated from microorganisms, not to get involved with antibiotics. Since it is a free agar, this enables the best diffusion of medicine, leading to a more accurate inhibition zone.

The 38 grams of the medium was suspended in a 1000 mL bottle of distilled water. The medium was dissolved by heating with frequent agitate and boiling for 1 minute. It was then autoclaved at 121 °C for 15 minutes and cooled to ambient temperature. The cooled MHA was poured into the sterilized Petri dishes. It was then allowed to cool at ambient temperature and stored at 2 to 8 °C. Four to five isolated colonies were touched with a sterilized inoculating loop. The organism was suspended in 2 mL of saline solution. The solution was vortexed for a smooth suspension. The turbidity of the solution was adjusted to a 0.5 McFarland standard. The suspension was used within 15 min of preparation.

The sterile swab was dipped into the inoculum tube. The excess fluid was removed with firm pressure by rotating the swab against the side of the cell. The dry surface of the MH agar was inoculated by streaking the swab thrice over the whole surface. The plate was rotated roughly for 60 degrees every time to make even distribution of the inoculum. The swab was used to rim the plate to remove excess fluid. The swab was discarded into the appropriate container. The dish was then allowed to cool at ambient temperature for 3 to 5 min to dry out the agar plate's surface.

4.15.3.2 Preparation of antibiotic stock solution and antibiotic disks

Commercially 15µg Erythromycin, Ciprofloxacin 10µg, Amoxicillin 10µg, and Doxycycline 30µg of antimicrobial susceptibility test discs were placed on the surface of the agar by making use of forceps to dispense the disk. The disks were gently pressed with forceps to ensure complete contact with the agar surface.

The temperature of 35°C ± 2°C was maintained. The plates were incubated for 18 hours. After incubation, the metric ruler was used to measure the diameter of the zone of inhibition for each antibiotic used. The results were read, and the measurement obtained from the single medicine was compared with the standard table to determine the sensitivity zone.

4.15.4 Polymerase Chain Reaction (PCR) and gene sequencing

4.15.4.1 PCR procedural steps

The reaction was mixed. The liquid was collected to the bottom of the tube by a quick spin. The cells were transferred to the PCR machine, and thermocycling began.

Materials

1. Forward and Reverse primers
2. PCR Buffers
3. PCR tubes

4. 96 well plate
5. Taq DNA polymerase
6. Nuclease-free distilled H₂O

4.15.4.2 The extraction of DNA, amplification of PCR, and 16S rDNA gene sequencing

Before the commencement of DNA extraction, the nutrient broth was freshly prepared to reinvigorate the preserved bacterial plate streaking. After the transference of bacterial isolate to the nutrient broth, the plate was incubated at 37 °C overnight. Total DNA of bacterial isolates was extracted from the cultures using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005). The 16S rDNA target region was amplified using the following primers: MiSeq16S-515F (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA-3') and MiSeq16S-926R (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCGYCAATYMTTTRAGTTT-3'). The PCR reaction was conducted in a 25µL response that contained 13µL of Master Mix (0.5µL of each forward and reverse primer, 12.5µL of Taq DNA polymerase), 5µL of DNA template, and the volume was added to 25µL of nuclease-free H₂O. The thermal cycling parameters were followed as per Table 4.3. The PCR products were purified using NucleoSpin® Gel and PCR Clean-up kit from MACHEREY-NAGEL (Germany), Catalogue No. 740609.10/.50/.250, according to the appropriate protocols in the manufacturer's instructions. After the amplification, the PCR products were resolved on gel electrophoresis at 80V for 90 min (in 2.0% agarose gel (SeaKem LE Agarose, USA), 5 µL of ethidium bromide) in a 1X tris-acetate (TAE) buffer at pH 8. Therefore, the products were visualized under the SimpliAmp Thermal Cycler (Applied Biosystems™, ThermoFisher Scientific). The amplification products were sent for Next-generation sequencing to the South African Institute for Aquatic Biodiversity (SAIAB). The final PCR product of DNA concentration (1 µL) after the clean-up was measured by exploiting NanoDrop 2000/2000c Spectrophotometers (Thermo Scientific™) nuclease-free distilled H₂O was used as blank.

Table 4.3: Thermal Cycling Parameters for amplification of 16S rDNA

<i>PCR Steps</i>	<i>Temperature (°C)</i>	<i>Duration (Seconds)</i>	<i>Number of Cycles</i>
<i>Initial Denaturation</i>	98	300	1
<i>Denaturation</i>	98	45	5
<i>Annealing</i>	44	30	
<i>Extension</i>	72	60	
<i>Denaturing</i>	98	45	18
<i>Annealing</i>	50	30	
<i>Extension</i>	72	60	
<i>Final Extension</i>	72	300	1

PCR added dual indices and Nextera XT Illumina sequencing adaptors as per the manufacturer specifications. Amplicon libraries purified using AMPure XP beads (Agencourt), quantified using the Qbit 4 fluorometer (Qubit dsDNA HS Assay, ThermoFisher Scientific), and the pooled libraries validated using the Bioanalyzer 2100 (Agilent) and DNA High Sensitivity DNA chips (Agilent). The pooled libraries underwent sequencing using the MiSeq system (Illumina Inc.) with MiSeq Reagent kit v2 Nano (Illumina) and a 10% PhiX spike (Naik et al., 2020).

Forward and reverse sequences were all merged in one file. Mothur v.1.44.0 (<http://github.com/mothur/mothur/releases/tag/1.44.0>) was exploited to process FASTQ files. The SOP (Standard Operating Procedure) developed by Schloss et al. (2009) was followed to reduce sequences errors. The chimera.vsearch command was used to remove the chimeras. The cleaned sequences were aligned to a reference alignment (silva.seed_v138). By constructing OUT (Operational Taxonomic Unit), the sequences were clustered into OTUs. The sequences were clustered to the default cutoff of 0.03. Alpha diversity was analysed, and rarefaction curves were generated according to the number of observed OTUs. The heatmap abundance of every OUT was generated. A dendrogram was generated by using jclass and thetacy calculators for showing the similarities of the samples to each other. The input files (shared file, metadata file, and cons.taxonomy file) were generated for phyloseq package in R Studio. All the necessary R packages were loaded and installed. The generated files were imported into the mothur folder. The metadata file was imported, and all the imported files were set to the correct format. Bar graphs, Heatmaps, Diversity indices, Analysis of variance, PCoA (Principal Coordinate Analysis), and NMDS (Non-Metric Multidimensional Scaling) were plotted.

4.16 RESULTS AND DISCUSSION

4.16.1 Microbial analysis

Wastewater contains millions of microorganisms per millilitre; part of them are not harmful. The presence of unhealthy microbes occurs when wastewater contains wastes that originate from people who are infected with diseases. Bacteria are single-celled organisms due to their structural cell and how they absorb food (Lyons & Kolter, 2015). In 30 minutes or less, single bacterial cells grow and divide into two new cells. They quickly reproduce in a supportive environment (temperature and pH) so that bacterial culture can contain twenty million cells per millilitre from a day (de Carvalho, 2018). Such speedy reproduction of visible bacterial colonies on nutritive media offers a chance to detect and count the number of bacteria available in the water. There are different types amongst several bacterial species; one class depends on food metabolism. They are aerobic, anaerobic, and facultative bacteria. Bacteria grow and reproduce slowly at shallow temperatures. When temperature increases, growth and reproductive rate double in every additional 10°C (Kumar & Libchaber, 2013). Many of these bacterial species have a temperature of approximately 35°C. Bacteria cause many dangerous waterborne diseases such as typhoid and paratyphoid fever, Shigellosis, and cholera (Kabwama et al., 2017). Table 4.4 below displays the overall annual values of bacterial colonies (Mean \pm St. Dev), and Figure 4.4 shows the chart of bacterial colony count representing all sites of sampling.

4.16.2 Counting of bacterial colonies

Microbial investigative techniques depend on the precise definition of colony counting units (CFUs). Systematically, this was accomplished by aliquoting a tiny portion of liquid culture medium and plate out various serial dilutions on to Petri dish that contains nutrient agar. After the incubation period in proper conditions for microbes, the colonies were counted to examine CFU numbers. It was used to calculate the CFUs on the plates manually. In this study, the manual counting technique for bacterial colonies was carried out. The most significant developments generated the approval of 30 to 300 as a more reasonable number of CFUs on the plates to be counted was examined. Two separated people conducted this technique through the instrumentality of a magnifier tool. Three data series were brought together in triplicate plates of serial dilutions ranging from 10² to 10³ of 45 water samples for assessing precision.

Table 4.4: Number of bacterial colonies in Bloukrans, Tyhume and Buffalo Rivers and WWTPs.

<i>Site</i>	<i>Min</i>	<i>Median</i>	<i>Max</i>	<i>Mean± St. Dev</i>
<i>GU</i>	3.63E+04	3.83E+04	4.17E+04	3.88E+04±1.93
<i>GM</i>	1.01E+06	5.13E+05	5.17E+05	6.81E+05±2.21E+05
<i>GL</i>	1.13E+06	5.23E+05	5.50E+05	7.36E+05±2.65E+05
<i>GE</i>	5.40E+05	5.50E+05	9.70E+05	6.87E+05±1.89E+05
<i>GI</i>	1.07E+06	1.51E+06	1.58E+06	1.39E+06±2.31E+05
<i>AU</i>	3.60E+04	4.13E+04	4.63E+04	4.12E+04±3.48E+03
<i>AM</i>	3.17E+04	4.33E+04	6.97E+04	4.82E+04±1.43E+04
<i>AL</i>	3.73E+04	4.13E+04	7.93E+04	5.27E+04±7.18E+04
<i>AE</i>	1.03E+04	3.40E+05	3.50E+04	1.59E+05±1.20E+05
<i>AI</i>	1.47E+06	4.73E+05	4.87E+05	8.09E+05±4.39E+05
<i>BU</i>	3.67E+04	4.17E+04	6.60E+04	4.81E+04±1.19E+04
<i>BM</i>	3.40E+04	4.40E+04	6.13E+04	4.63E+04±1.00E+04
<i>BL</i>	1.08E+05	3.33E+04	8.83E+04	7.67E+04±2.89E+04
<i>BE</i>	3.50E+05	3.80E+04	5.60E+04	1.48E+05±1.35E+05
<i>BI</i>	3.57E+05	3.73E+05	9.33E+05	5.54E+05±2.53E+05

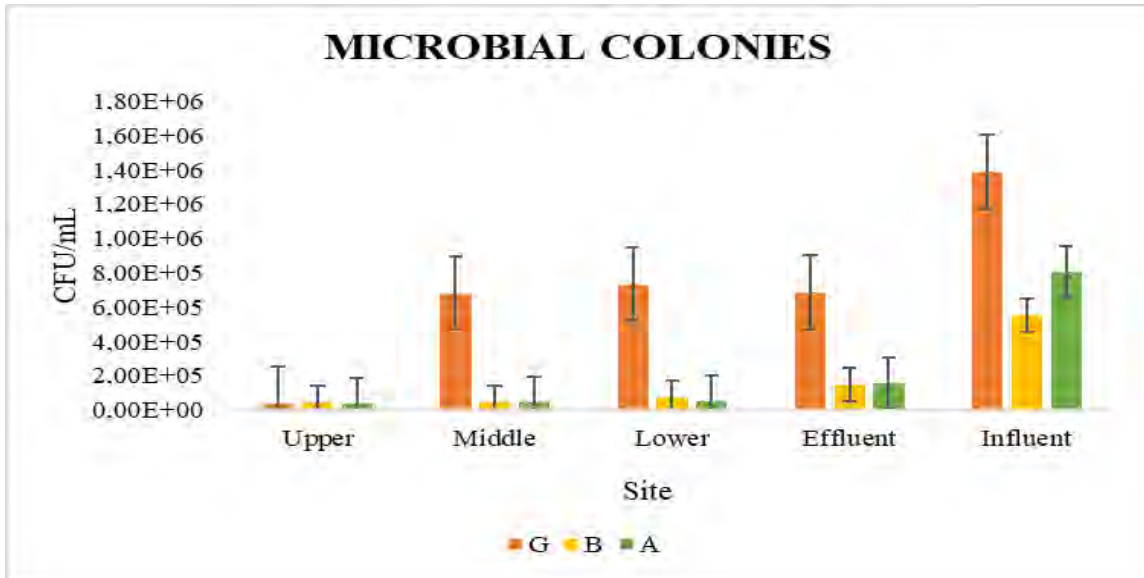


Figure 4.4: Bacterial colony count representing all sampling sites of Tyhume, Bloukrans and Buffalo Rivers and WWTPs found along the riverbanks

*G-Bloukrans, B-Buffalo, A-Tyhume

It had become clear that the colonies of bacteria vary following the sampling sites. The statistical evaluations of bacterial colonies from primary culture demonstrated that the water samples in all the wastewater treatment plants have an immense microbes load. The highest numbers of the bacterial colonies were documented with an estimation of $8.09E+05$ CFU/mL, $7.36E+05$ CFU/mL, $7.67E+05$ CFU/mL, $6.87E+05$ CFU/mL, and $6.81E+05$ CFU/mL in AI, GL, BL, GE, and GM samples, respectively. In contrast, the lowest number of colonies was documented at $1.39E+06$ CFU/mL in GI, $1.48E+05$ CFU/mL in BI, and $1.58E+05$ CFU/mL in AE.

That could be due to the fast dissemination of microorganisms that lend a hand to the degradation of organic materials existing in industrial wastewaters. A reduced number of colonies of bacteria were found with an estimate of $1.93E+06$ CFU/mL in GI. Generally, the temperature is crucial in microbes' growth and survival, hence the bacterial community structure (Zhang et al., 2019).

Nonetheless, the temperature had not been the significant environmental consideration that influenced the bacterial community's composition or dissemination. There were minor variations in temperatures between the sampling sites (12.77 to 21.51 °C). Liu et al. (2018) reported that the impact is little when the thermal temperature changes slightly. Figure 4.5 below demonstrates the community of bacteria in river water.

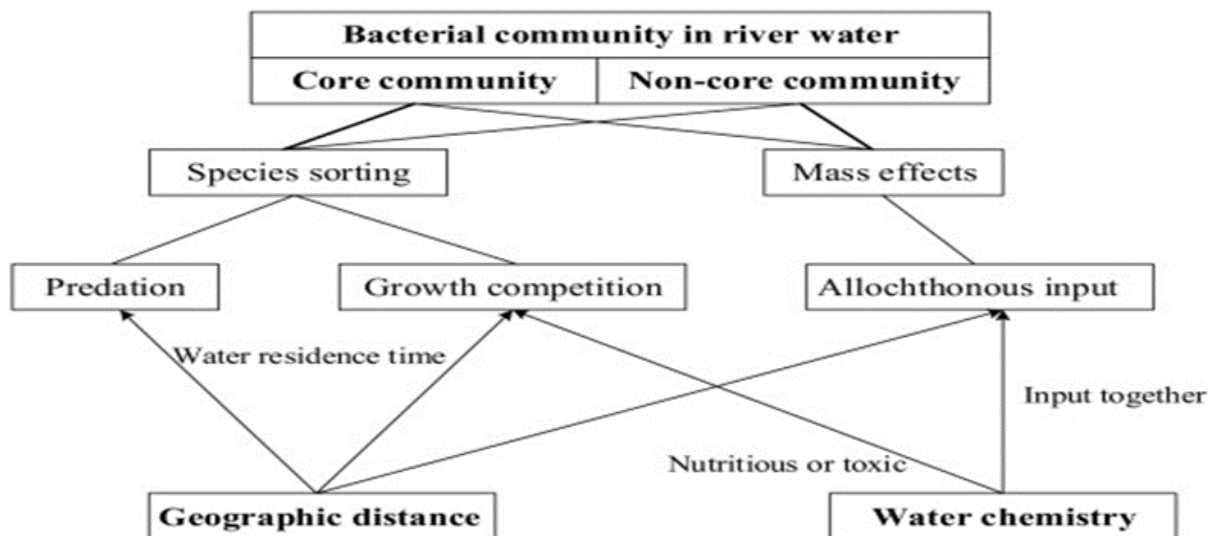


Figure 4.5: Conceptual graphic of the effect of chemistry or water and geographical extent on the community of bacteria streams from the upper stream to downstream (Wang et al., 2019).

Waterborne diseases resulting from different bacteria have been the root cause of epidemics. In the developing world, for instance, waterborne diseases infected millions of people on the African continent. As reported by WHO (2014), annually, roughly three and a half million people, mainly babies, perish due to water-related diseases. Under the United Nations Children’s Fund evaluation, four thousand kids die every day because of unclean water (UNICEF, 2014). Enhancing the quality of water can minimize the universal burden of disease by roughly 4%.

Bacterial pollution is a critical factor in river impairments. The origin of impairments and threats to health caused by waterborne bacteria are widely announced. In the United States, bacterial pollution is the primary reason for river water contamination. US Environmental Protection Agency (2012a) concluded that bacterial influxes in the streams from farmlands are the primary reason for stream impairments. Bacteria probably enter the streams from several potential sources, including the arrival of bacterial-polluted groundwater, direct disposition of faeces from cattle, discharge of polluted sanitary sewerage flows, and effluent sewage works. In rainy weather, bacteria in streams are affected by fresh input from catchments and underground flow. Kiefer et al. (2012) reported that re-suspension of bacteria from deposits might significantly increase bacterial levels. The conveyance of microorganisms is affected by the conditions of Hydrometeorology. The pollution of surface water microbes rises when heavy precipitations with water combined sewage overflows or farmland runoffs (Delpla et al., 2011). Heavy rainfall could result in river basins deluging with a steep rise of the water's turbidity, which is an accurate indication of possible pollution by aquatic bacteria (Jung et al., 2014). A study carried out in France demonstrated that groundwater affected by surface water might result from digestive tract infections at an endemic level, particularly in precipitation events in the karst environment (Jung et al., 2014).

4.16.3 Identification of bacterial isolates

For other compartments such as amygdalin, arabinose, and melibiose, the colour changes may be read right away after 24 hours; however, some needed the reagents before reading. The following reagents, such as tryptophan deaminase, indole, Voges-Proskauer 1 and 2, were added to particular compartments. The API scale was used to mark either negative or positive under the colour chart. The scores for the positive wells were added in every triplet. The profile of reaction of combinations then produced the seven-digit code that was used to identify the organisms. The organisms were determined by using API Apiweb (<https://apiweb.biomerieux.com>). Table 4.6 shows the bacterial isolates of water samples from selected rivers.

Table 4.6: Bacterial isolates of water samples from selected rivers

<i>Site</i>	<i>Significant taxa</i>	<i>% ID</i>	<i>Net significant taxa</i>	<i>Nearest % ID</i>
<i>Bloukrans River and Makhnda WWTP</i>				
<i>GU</i>	<i>-Yersinia pestis</i>	36.5	<i>-Pantoe ssp 1</i>	6.9
	<i>-Ewingella Americana</i>	28.6		
	<i>-Pasteurella pneumotropica</i>	15.3		
<i>GM</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneumotropica</i>	5.9
<i>GL</i>	<i>-Pasteurella pneumotropica</i>	75.1	-	-
	<i>-Providencia staurtii</i>	10.4		
	<i>-Shigella spp</i>	7.5		
	<i>-Pseudomonas oryzihabitans</i>	4.0		
	<i>-Escherichia coli 2</i>	1.8		
	<i>-Burkholderia cepacia</i>	42.0		
	<i>-Aeromonas salmonella ssp salmonicida</i>	22.0		
	<i>-Non-fermenter spp</i>	10.4		
	<i>-Stenotrophomonas maltophilia</i>	6.2		
<i>GE</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneuotropica</i>	5.9
<i>GI</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneuotropica</i>	5.9
<i>Tyhume River and Alice WWTP</i>				
<i>AU</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneuotropica</i>	5.9
<i>AM</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneuotropica</i>	5.9
<i>AL</i>	<i>-Chromobacterium violaceum</i>	97.7	<i>-Aeromonas salmonicida ssp salmonacida</i>	1.7
<i>AE</i>	<i>-Burkholderia cepacia</i>	42.0	-	-
	<i>-Aeromonas salmonicida ssp salmonicida</i>	22.0		
	<i>-Pasteurella pneumotropica/Mannheimia haemolytica</i>	6.6		
	<i>-Stenotrophomonas maltophilia</i>			
	<i>- Non-fermenter spp</i>	6.2		
		10.4		
<i>AI</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneuotropica</i>	5.9
<i>Buffalo River and King William's Town WWTP</i>				
<i>BU</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneuotropica</i>	5.9
<i>BM</i>	<i>-Shigella spp</i>	81.5	<i>-Pasteurella pneuotropica</i>	5.9
<i>BL</i>	<i>-Klebsiella pneumonia ssp</i>	69.0	<i>-Klebsiella pneumonia ssp</i>	4.4

	<i>rhinoscleromatis</i>	23.1	<i>ozaenae</i>	6.2
	- <i>Pasteurella pneumotropica</i>		- <i>Citrobacter freundii</i>	
BE	- <i>Shigella spp</i>	81.5	- <i>Pasteurella pneuematropica</i>	5.9
BI	- <i>Shigella spp</i>	81.5	- <i>Pasteurella pneuematropica</i>	5.9

API 20E system is a powerful approach for the identification of bacteria. Identifying bacterial isolates by the employment of the API 20E approach diagnosed Gram-negative organisms with 14 different genera. It becomes clear that the results obtained above show that the API 20E approach performed relatively well in the laboratory. The identified families are as follows: *Chromobacterium*, *Shigella*, *Stenotrophomonas*, *Escherichia*, *Aeromonas*, *Providencia*, *Burkholderia*, *Mannheimia*, *Pseudomonas*, *Ewingella*, *Yersinia*, *Citrobacter*, *Klebsiella*, and *Pasteurella*. These are precisely a member of the family Enterobacteriaceae. Genus *Shigella* was the most often occurring bacteria in BU, BM, BE, BI, AU, AM, AI, GM, GL, GTE, and GI at 81.5%, respectively. While in GU, BL, AL, and AE were not present. The second occurring genera were *Aeromonas* and *Pasteurella*. The cause of high bacterial diversity may result from different possibilities of pollutants from the environment because of a lack of knowledge and attention.

Typically, the progressive elimination of causal organisms was viewed in different regions of the sewage works. However, there were differences in terms of designs and efficiency of every plant to remove pathogens. Both the raw wastewater and final treated effluent contained *Shigella spp*. GL has genera *Escherichia* and *Aeromonas*. AE contains the genus *Aeromonas*; these pathogenic microorganisms in the water are a problem because rural communities use surface water for consumption and recreation activities. The latest review demonstrated the effect of waterborne diseases in rural communities due to sources of potable water with inadequate microbiological quality (Momba et al., 2009).

Usually, river water harbours many enteropathogens from domestic sewage discharges, rainfall runoffs from farmlands, and human faecal wastes, breeding animals due to untreated river water (Wose Kinge & Mbewe, 2010). Genus *Shigella* is a bacterium that induces severe diarrhoea. The examination of the commonness of *Shigella spp* in domestic wastewater is necessary to give baseline values because sewers are part of significant contributions of faecal pollution to various bodies of water. The commonness of *Shigella spp* differs with geographic positioning (Garcia-Aljaro et al., 2017). This species is sensitive to chlorination at background values and can survive up until 96 hours in the river. In China, the commonness of *Shigella spp* in treatment plant samples obtained from hospitals and housing estates, most samples were positive (Garcia-Aljaro et al., 2017). A study carried out the commonness of *Shigella spp* from lakes to streams in Bangladesh (Faruque et al., 2002). Other studies show the presence of *Shigella spp* in samples of surface water and sewage works, which demonstrates that surface water can convey *shigella* strains. Therefore, there is a potential of an ongoing source of pollution into the streams (Mogamedi et al., 2007; Wose Kinge & Mbewe, 2010). This species' presence in river water samples implies other causal organisms' probability, thus confirming faecal pollution in the river basin sampled. Shigellosis keeps on being a significant health

problem globally.

Aeromonas is prevalent in the aquatic ecosystem, and being present in the aquatic environment is not weird (Hossain et al., 2013). It is an emerging causal organism and, therefore, can induce medical problems. The occurrence of *Aeromonas spp* in rivers is significant regarding the social and economic importance of supply sources. The profusion of *Aeromonas spp* in the stream may result from environmental reasons such as ammonia and nitrates from manures and detergents (Bello et al., 2016). *Aeromonas spp* is frequently isolated from streams, rivers, water tables, potable water, and various foods (Zaky et al., 2011). A study reported the *Aeromonas spp* as the more commonly known species that live in water bodies (Igbinosa et al., 2012).

API 20E showed satisfactory *Chromobacterium violaceum* at 97.7 % identity at the lower site of Tyhume River. *Chromobacterium violaceum* is a pathogenic organism that causes chromobacteriosis; it is uncommon for humanity but may presume the epidemic level provided there is terrible water pollution by pathogens. *Chromobacterium violaceum* is an abundant element of the soil and water microbiota in the subtropics area across the globe (Batista & da Silvia Neto, 2017). The prevalence of *Chromobacterium violaceum* has been recorded in Nigeria's watering holes (Uba & Eze, 2004). The appearance of *Chromobacterium violaceum* in any samples of water and other causal organisms makes the water unsuitable for drinking despite lots of heterotrophs.

4.16.4 Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed in all river sites and WWTPs. Only four antibiotics were exposed to antibiotic susceptibility testing. The zone of inhibition was considered under the National Committee for Clinical Laboratory Standards guidelines referring to antibiotic-disc concentration (ug/L). Table 4.6 above represents the zone of inhibition of the data acquired and is compared with the Zone Diameter Standard and the Minimum Inhibitory Concentration (MIC) Interpretive Standard.

The results obtained clearly show that Erythromycin was resistant in the following sites: BM, BE, BI, and AM, whereas Doxycycline has demonstrated resistance in BM, BE, BI, GL, and AE sites. Amoxicillin has shown resistance in the BM, BE, BI, GU, GM, GL, GE, GI, AU, AM, AL, AE, and AI. In other words, 87% of Amoxicillin was resistant throughout the sampling sites. Amoxicillin was the only antibiotic that has shown the highest resistance to the four tested antibiotics. Our results are similar to the Efstratiou (2018) study, whereby Amoxicillin showed a high degree of resistance.

In contrast, Erythromycin was susceptible in sites BM, BL, AU, AL, and AE; Ciprofloxacin showed susceptibility to Buffalo river and WWTPs sites Bloukrans river and WWTPs, and Tyhume rivers and WWTPs except for AL. Doxycycline has demonstrated sensitivity to BU, GU, GM, AU, AM, and AI, while Amoxicillin has shown BU and BL susceptibility. The Erythromycin has shown intermediate resistance in sites BU, BL, GU, GE, GI, AI, and Ciprofloxacin shown Intermediate Resistance to AL. In contrast, Doxycycline has shown Intermediate Resistance to BL, GE, GI, and AL.

The experiments demonstrated a high degree of resistance to Amoxicillin and Doxycycline and

slightly to Erythromycin. It is multidrug resistance; hence, resistance occurred to two or more drugs (Mulu et al., 2017). The efficiency of antibiotic treatment of infections will deteriorate because of antibiotic resistance in the environment. Furthermore, Ciprofloxacin remains the best suitable drug for utilization. Ciprofloxacin showed susceptibility in 93% of sampling sites. This statement is supported in the study of Reuben and Owuna (2013), that, indeed, Ciprofloxacin is the best appropriate medication for *E.coli* and *Shigella*. The presence of antibiotic-resistant bacteria in river water is of health importance due to the risk of supporting multiple drug-resistant organisms in people through potential colonization of the digestive tract and the conjugal transfer of antibiotic resistance normal-flora. Thus, resulting in more multiple antibiotic resistance organisms (Tagoe et al., 2011). Kappell et al. (2015) supported that the widespread resistance to Erythromycin was identified throughout studied sources. The emergence of resistance to classes of macrolide is quite common in urban environments. The prevalent antibiotic-resistant bacteria present a significant problem, and the use of water containing such drug-resistant bacteria can lengthen the therapy of waterborne diseases. In other words, the treatment of waterborne diseases with such drugs may not be appropriate and will call for new drugs.

Moreover, the resistance of Erythromycin may indicate that there has been improper use of these antibiotics. The high drug resistance further reveals detrimental effects on the treatment with such a group of drugs (Kinge et al., 2010). The visible matter is that the risk of antibiotic-resistant bacteria is unlimited to rivers, and they may pollute agricultural lands that are sprayed with polluted water. A significant amount of antibiotic-resistant genes and antibiotics can be dominant sewage treatments, waste sites, and surface waters. On this point, a study reported the pollution of agricultural lands by antibiotic-resistant genes, for instance, *sull*. Notably, the presence of antibiotic-resistant genes is most dangerous for fruits (Sun et al., 2017). The identified antibiotic-resistant bacteria level in agricultural lands is superior to receiving water resources (Aali et al., 2019). Hence, the entry of high levels of antibiotic-resistant bacteria and different species of antibiotic-resistant bacteria, namely, *Pseudomonas spp.*, *Collimonas spp.*, and *Thiobacillus spp.* to the surroundings may exert pressure on resources of water and ecosystems (Iwane et al., 2001). A survey reported that the isolated *Pseudomonas aeruginosa* and *E. coli* from pond water had shown the successful transmission of plasmid from antibiotic-resistant genes, from the manufacturing plants' wastewater to plasmid less antibiotic susceptible bacteria (Shakibaie et al., 2009). Several investigations have displayed a load of antibiotic-resistant bacteria in clean water (Xi et al., 2009). Thereby controlling the emergence and dissemination of antibiotic resistance, the treatment plants' procedures must eradicate antibiotic-resistant genes besides inactivating pathogenic agents (Barancheshme & Munir, 2018).

The WHO (2016) reported that annually, roughly 700 000 humans die from infections that are resistant to antibiotics. In 2014, a survey conducted in the UK alerted that by 2050, antimicrobial-resistant bacterial infections might be the primary cause of mortality around the world (Shallcross et al., 2015). Antibiotic pollution, wherein excessive antibiotics enter environmental compartments and persuade bacteria that arise living over there, assists speed along with the growth of resistant strains. However, it interrupts the sensitive environmental stability in rivers and lakes, altering bacterial communities' structure, which can influence

ecological processes since bacteria have a crucial role in river ecosystems, such as aiding to cycle nutrients. Based on the obtained data, improvement of the effectiveness of the treatment plants' processes such as ultraviolet and ozonation is necessary for better removal of pathogenic agents in sewage treatment. It is an emergency for protection in the field of public health (Bouki et al., 2013). Finally, it is evident from the findings of this study that multiple ARB can prosper in water that acts as reservoirs and may provide a pathway to multidrug-resistant pathogens to gain access to human populations. Water treatment is essential to destroy pathogens and prevent antibiotic resistance from resistant bacteria to susceptible bacteria.

Table 4.7: Zone of inhibition of the data acquired and is compared with the Zone Diameter Standard and the Minimum Inhibitory Concentration (MIC) Interpretive Standard for Buffalo, Tyhume, and Bloukrans Rivers and WWTPs.

<i>Test/ Report group</i>	<i>Disk content</i>	<i>Antimicrobial agent</i>	<i>Sites</i>	<i>Buffalo</i>	<i>Bloukrans</i>	<i>Tyhume</i>	<i>Zone Criteria (nearest whole mm)</i>	<i>Diameter Interpretive</i>	<i>MIC (µg/mL)</i>	<i>Interpretive</i>	<i>Criteria</i>	
				<i>B</i>	<i>G</i>	<i>A</i>	<i>S</i>	<i>I</i>	<i>R</i>	<i>S</i>	<i>I</i>	<i>R</i>
<i>A</i>	<i>10µg</i>	<i>Erythromycin</i>	Upper	I	I	S	≥23	14-22	≤13	≤0.5	1-4	≥8
			Middle	R	S	R						
			Lower	I	S	S						
			Effluent	R	I	S						
			Influent	R	I	I						
<i>B</i>	<i>5µg</i>	<i>Ciprofloxacin</i>	Upper	S	S	S	≥21	16-20	≤15	≤1	2	≥4
			Middle	S	S	S						
			Lower	S	S	I						
			Effluent	S	S	S						
			Influent	S	S	S						
<i>O</i>	<i>30µg</i>	<i>Doxycycline</i>	Upper	S	S	S	≥14	11-13	≤10	≤4	8	≥16
			Middle	R	S	S						
			Lower	I	R	I						
			Effluent	R	I	R						
			Influent	R	I	S						
<i>B</i>	<i>10µg</i>	<i>Amoxicillin</i>	Upper	S	R	R	≥18	14-17	≤13	≤8/4	16/8	≤32/16
			Middle	R	R	R						
			Lower	S	R	R						
			Effluent	R	R	R						
			Influent	R	R	R						

*S: susceptible. I: intermediate. R: resistant

U- Upper, M- Middle, L- Lower, E- final treated effluent discharged into rivers, I- raw sewage from the surrounding area

4.16.5 Sequencing analysis

The surface of waters composed of either natural or human-induced water bodies (canals, rivers, oceans, lakes, and seas) play a part in ecosystem services and carry on being the drinking water source (Jin et al., 2018). Bacteria may be detrimental to people and wildlife by becoming pathogenetic and carrying antibiotic resistance genes (Numberger et al., 2019).

Variations on the surface of waters produce changes in the bacterial community, affecting surface water quality. The NGS was applied to distinguish the microbial community structure and diversity of 15 water samples collected from different sites.

Bacterial Communities

The 16S rRNA (ribosomal deoxyribonucleic acid) gene sequencing analysis is widely used in metagenomics. This gene is universal to all bacteria, and it consists of approximately 1500 bp (base pairs) (Tawfik et al., 2018). The 16S rRNA sequencing has been able to detect other microorganisms in samples, thus showing the strong sensitivity of sequencing. We examined the WWTP and river water samples by using the 16S rRNA sequences to identify microorganisms. Refer to Appendix A (Table A1 for sample codes).

The samples were grouped into OTUs and clustered at 97% identity (Xu et al., 2020). The 12 common OTUs are presented in Figure 4.6. The OTUs were determined to calculate the diversity and richness of the bacterial community. The microbial community in all sites showed a high abundance of OTU0001 and OTU0002. The variance in location Tyhume (AE, AI, AL, AM, AU) and BE are shown in the NMDS plot in Figure 4.7.

Among the four detected phyla (Figure 4.8), two were dominant, more especially *Firmicutes*. The high abundance of *Firmicutes* was detected being the most phylum in all samples and *Bacteriodota* in Sample_GE and Sample_GI and Bacilliales being the genus in all samples, followed by *Proteobacteria* and *Actinobacteria*. Zhao et al. (2017) observed similar results, where *Firmicutes* were dominant in water and sediments. Paruch et al. (2019) observed the highest abundance of *Firmicutes* in the Norwegian waterbodies' urban stream. Another study has shown similar results of high abundance of *Firmicutes* in watersheds (Unno et al., 2010). In the Yangtze River, Sun et al. (2017) observed similar findings of a high abundance of *Firmicutes*. There is a linear correlation between the 16S rRNA gene copy number and bacterial growth rate (Mente et al., 2018). *Firmicutes* and *Proteobacteria* phyla are well-known to have the highest 16S rRNA gene copy numbers (Kormas, 2011).

The phylum *Bacteriodota* showed high abundance in sample_GI. The phyla *Firmicutes* and *Bacteriodota* prove that they can remain in the treatment plants. Their occurrence in high abundance may result from contamination of human faeces. *Firmicutes* being the main of faecal microbial communities, Sun et al. (2017) conclude that *Firmicutes* in samples could result from the pollution of faecal material. The phylum Bacteria was assigned to unclassified.

Actinobacteria belong to Gram-positive bacteria containing guanine and cytosine high content in their DNA, but some have a low content of G+C (Anandan et al., 2016). *Actinobacteria* can be terrestrial or aquatic. Freshwater *Actinobacteria* are universally disseminated in lakes and rivers and washed in terrestrial environments (Anandan et al., 2016). *Actinobacteria* was

recently reported as autochthonous bacteria in lakes, rivers, streams, and wetlands (Lee et al., 2016). *Actinobacteria* are rich by their very nature, in the water, and on the ground. They are also found in soil and human digestive systems. *Actinobacteria* are generally known because agriculture and forest rely on their input to soil systems (Lewin et al., 2016), and they are decomposers of vegetative matter by their nature. A study proved that *Actinobacteria* could be isolated from freshwater locations (Anandan et al., 2016). Aquatic *Actinobacteria* can have distinct aspects from terrestrial; thus, generating novel bioactive composites, including new drugs (Nafis et al., 2019).

Bacteroidetes are Gram-negative bacteria frequently occurring in environmental areas such as soil, saltwater, digestive tubes, and animal skins. Given that *Bacteroidetes* are identified in the microbial community of many different environments, they play a positive part in the deterioration of organic substances. These bacteria are particle-associated and dependent on organic substances' presence (Fiedler et al., 2018). *Bacteroidetes* are gaining recognition as an essential compartment of the bacterioplankton in aquatic zones (Thomas et al., 2011). A similar study has shown *Bacteroidetes* and *Proteobacteria's* predominance in nine sampling locations of water areas (Pommier et al., 2007). The infection of *Bacteroidetes* may have destructive consequences in both wild and farmed fish (Scotti et al., 2017). Furthermore, *Bacteroidetes* may influence diverse vegetation and algae. A study in food connection reported the abundance of *Bacteroidetes* in lakes of water column (Thomas et al., 2011).

Firmicutes are Gram-positive bacteria that form a significant part of the human microbiome. *Firmicutes* are also referred to as a basic set of the low G+C group (Galperin, 2013) by comparison with *Actinobacteria*. Most of the *Firmicutes* produce endospores that are resistant to dryness and can survive in harsh environments.

Proteobacteria are Gram-negative bacteria, including *Escherichia*, *Salmonella*, *Vibrio*, *Helicobacter*, *Yersinia*, and *Legionellales* (Mohamadkhani, 2018; Stackebrandt et al., 1988). *Proteobacteria* are amongst the largest phylum in domain bacteria (Rizzatti et al., 2017). Generally, based on the 16S rRNA gene, this phylum is divided into six classes; however, two subclasses were observed in this study: *Alphaproteobacteria* and *Gammaproteobacteria*.

In aquatic environments, *Proteobacteria* is easily moved from location to location, then to favourable conditions. *Proteobacteria* favours average temperature to grow and develop vigorously. Other members require a host to remain alive; some create mutualistic symbiotic relations with flora, while others are parasitic, consequently bearing the infection or damaging the host (Belkaid & Hand, 2014). In addition to various illnesses to plant and animal species, *Proteobacteria* are comprised of human pathogens, which are intestinal and extra-intestinal diseases (Rizzatti et al., 2017). However, in the gastrointestinal tract, it is helpful in shaping and modulating the human immune system. *Proteobacteria* are connected with chronic illnesses such as inflammatory bowel diseases and ulcerative colitis (Matsuoka & Kanai, 2015).

The detection of different phylum could result from a different sampling method, analytical artefacts during PCR, and filtering sequences or identification (Tawfik et al., 2018; Zhou et al., 2019). The phylum-level variations show significant shifts in the microbial population's

composition because of contribution from wastewater and sediment resuspension (Ulrich et al., 2016).

At the class level (Figure 4.9), five classes were observed, with three being abundant. The Bacilli class has shown high abundance in samples, whereas *Alphaproteobacteria* and *Gammaproteobacteria* showed low abundance in samples. Sample_GI, Sample_BM, and sample_BU were enriched with *Alphaproteobacteria*, and *Gammaproteobacteria* was observed throughout the sampling sites. Bacilli abundance was higher than *Alphaproteobacteria* and *Gammaproteobacteria*. The aquatic sediment was enriched with *Gammaproteobacteria* and presented low diversity (Wang et al., 2012). *Alphaproteobacteria* and *Gammaproteobacteria*, as the abundant class, make the bacterioplankton community and favour salty water; however, they are extensively disseminated in streams and lakes (Liu et al., 2012). At the class level, the Bacteria were assigned to unclassified.

At the family level (Figure 4.10), we observed a high abundance of *Bacillaceae* throughout the samples. The two families of Bacilli and *Bacillales* are assigned unclassified.

At the genus level (Figure 4.11), we observed a high abundance of *Bacillus* across all the samples. Most of the sequences seem to be belonging to kingdom bacteria. Therefore, the findings imply that bacteria are the predominant taxonomy. Fazi et al. (2008) reported *Bacillus* in the de novo synthesis of spore germination in dried river sediment. In this study, the *Bacillales* and *Bacilli* were assigned to unclassified.

Alpha diversity and species richness

Bacterial alpha diversity was assessed based on observed, Chao1, ACE, Shannon, Simpson, nvSimpson, and Fisher indices (Figure 4.12).

Shannon's index was developed for systems of communication, and it depends on system analysis. Shannon is extensively used in diversity indices and aids in comparing diversity between communities and different classes. We exploited Shannon to measure the species diversity in a community. Shannon indices range is between zero and five. In our survey, we observed a low diversity of communities. All the samples have low diversity below 0.30 except for sample_GI, sample_GE, and sample_BM. Since Shannon is not a self-independent index, it should be compared with the Simpson diversity index. This index also measures the diversity in a community and dominance in the area. We observed lower scores within the Simpson diversity index, such as 0.14, 0.16, and 0.18, thus presenting the lowest diversity.

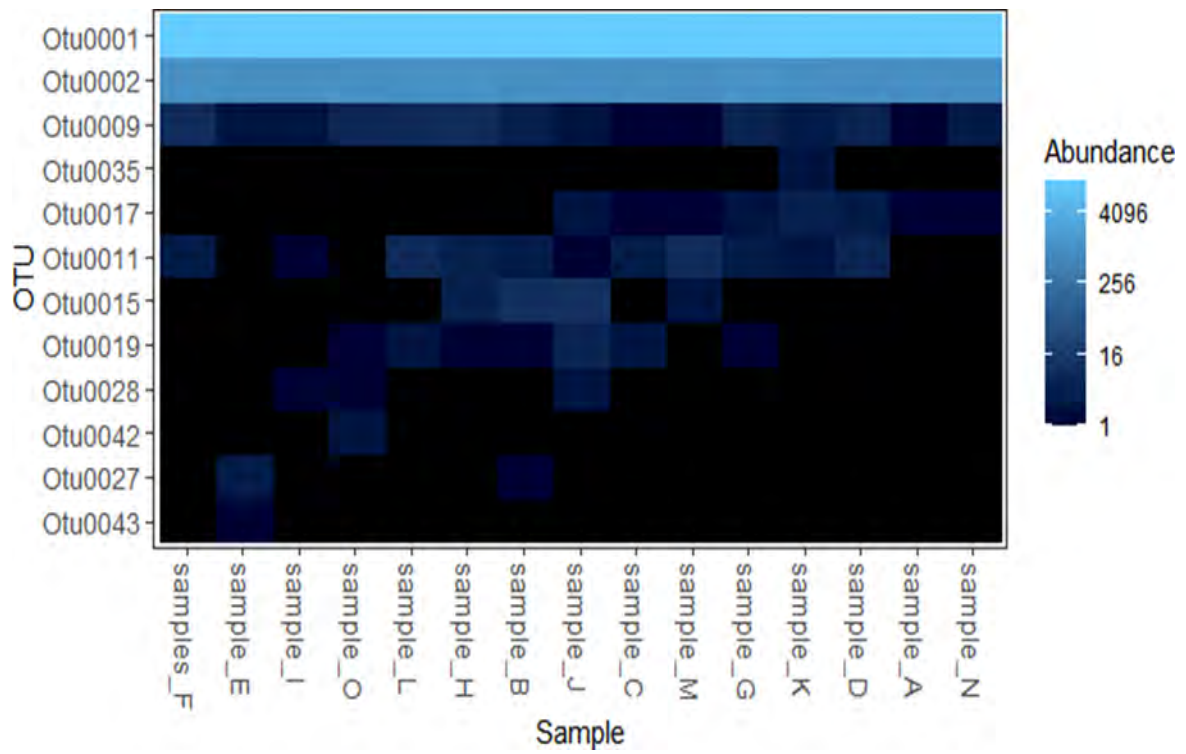


Figure 4.6: Abundance of OTUs found in all water samples

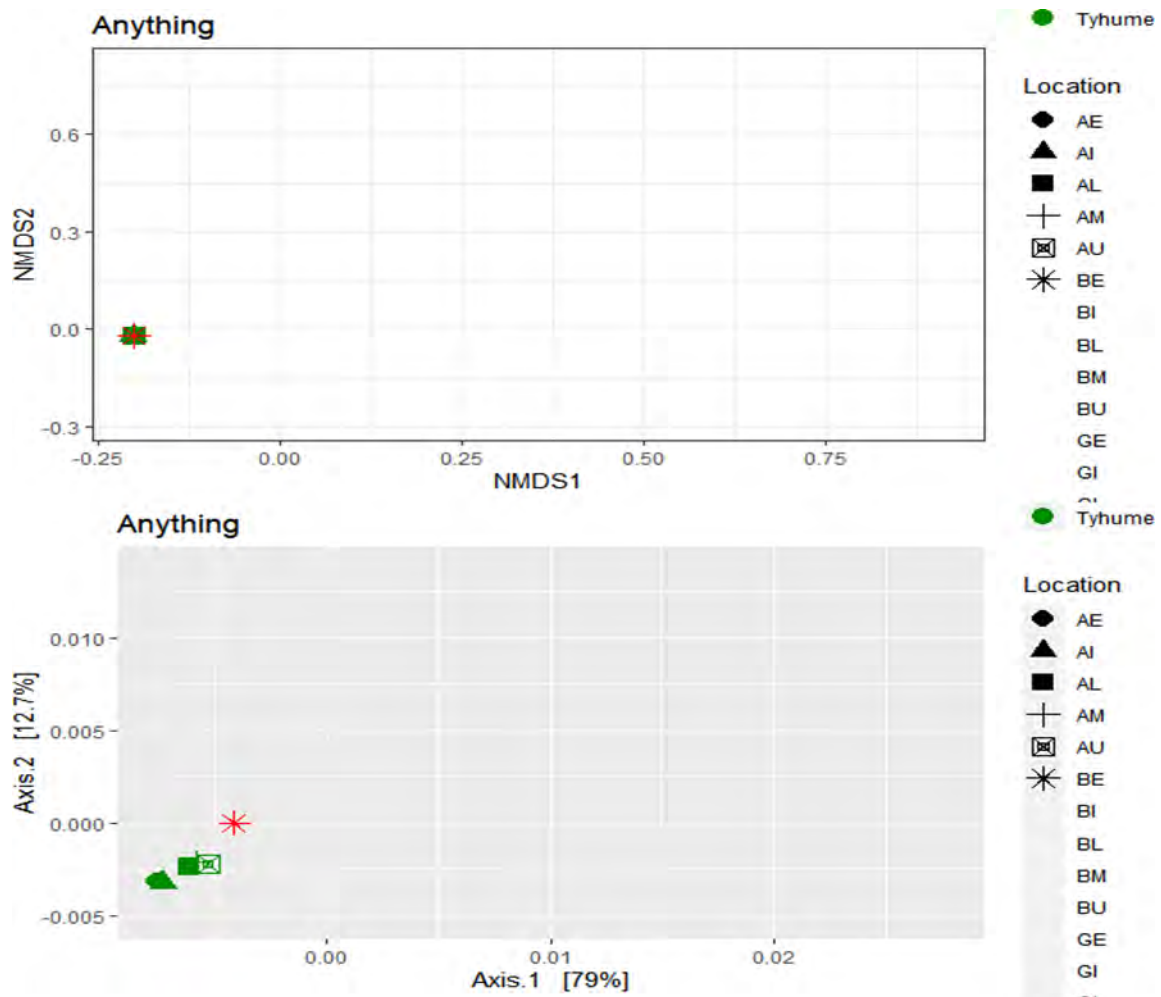


Figure 4.7: NMDS plots of water samples. The NMDS plots are based on bacterial OTUs in the water samples.

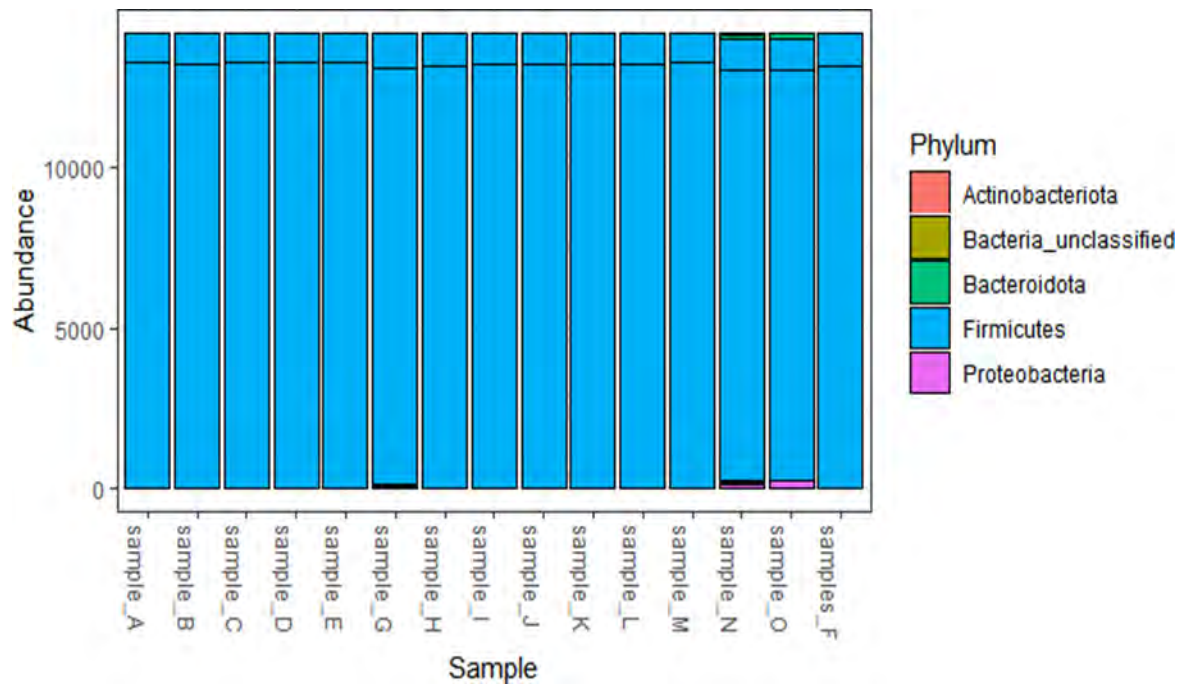


Figure 4.8: Bacterial community composition at the phylum level

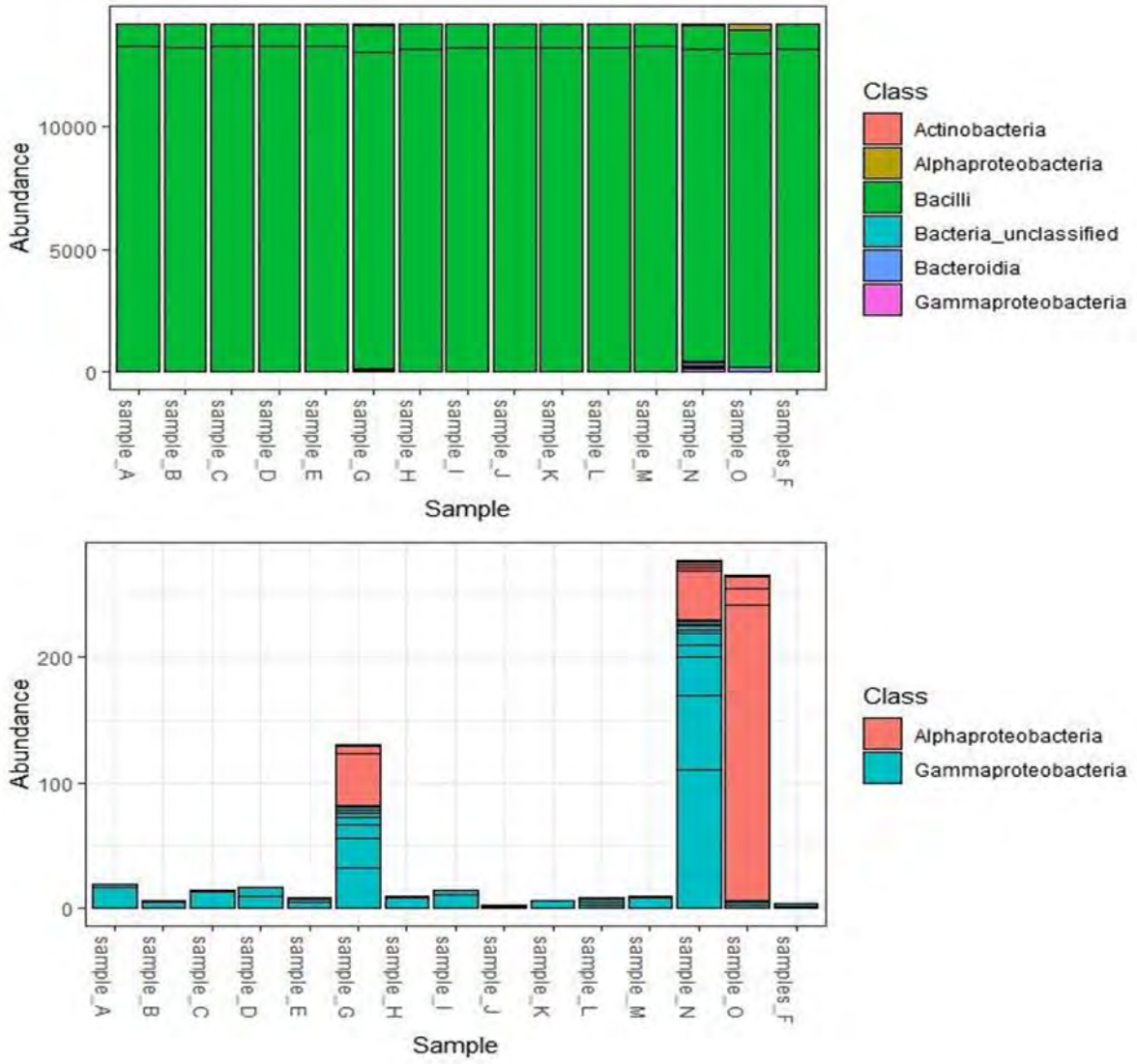


Figure 4.9: Bacterial community composition at class level

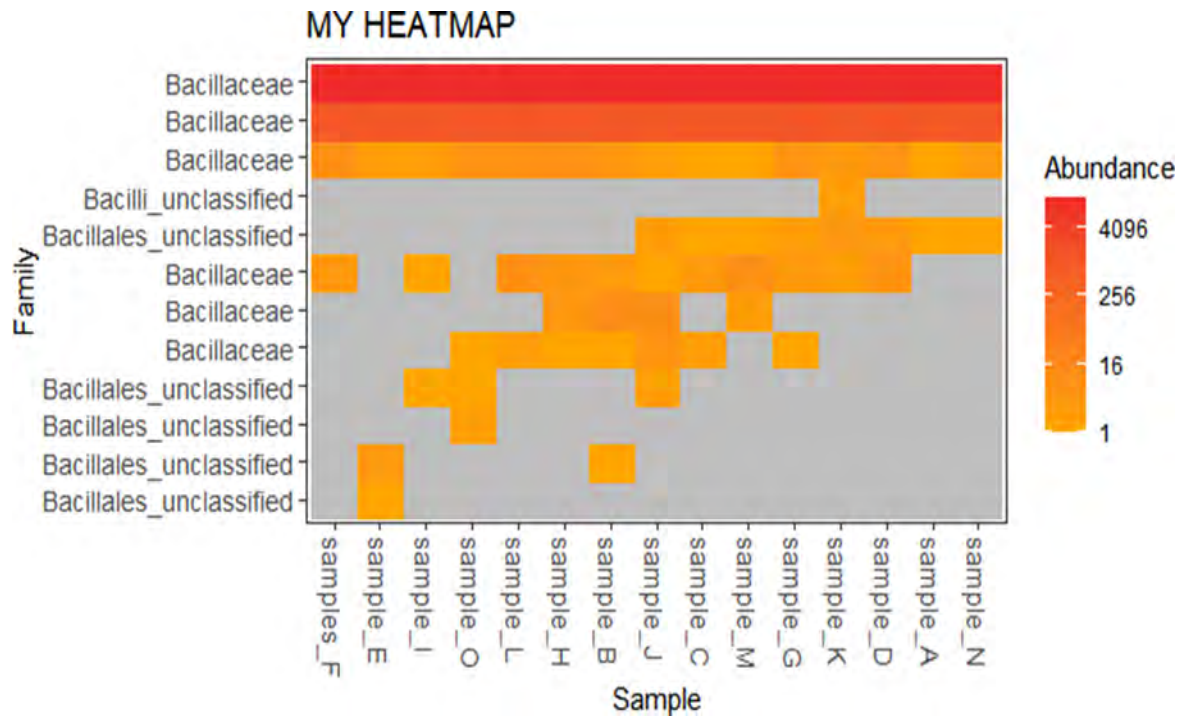


Figure 4.10: Bacterial community composition at the family level

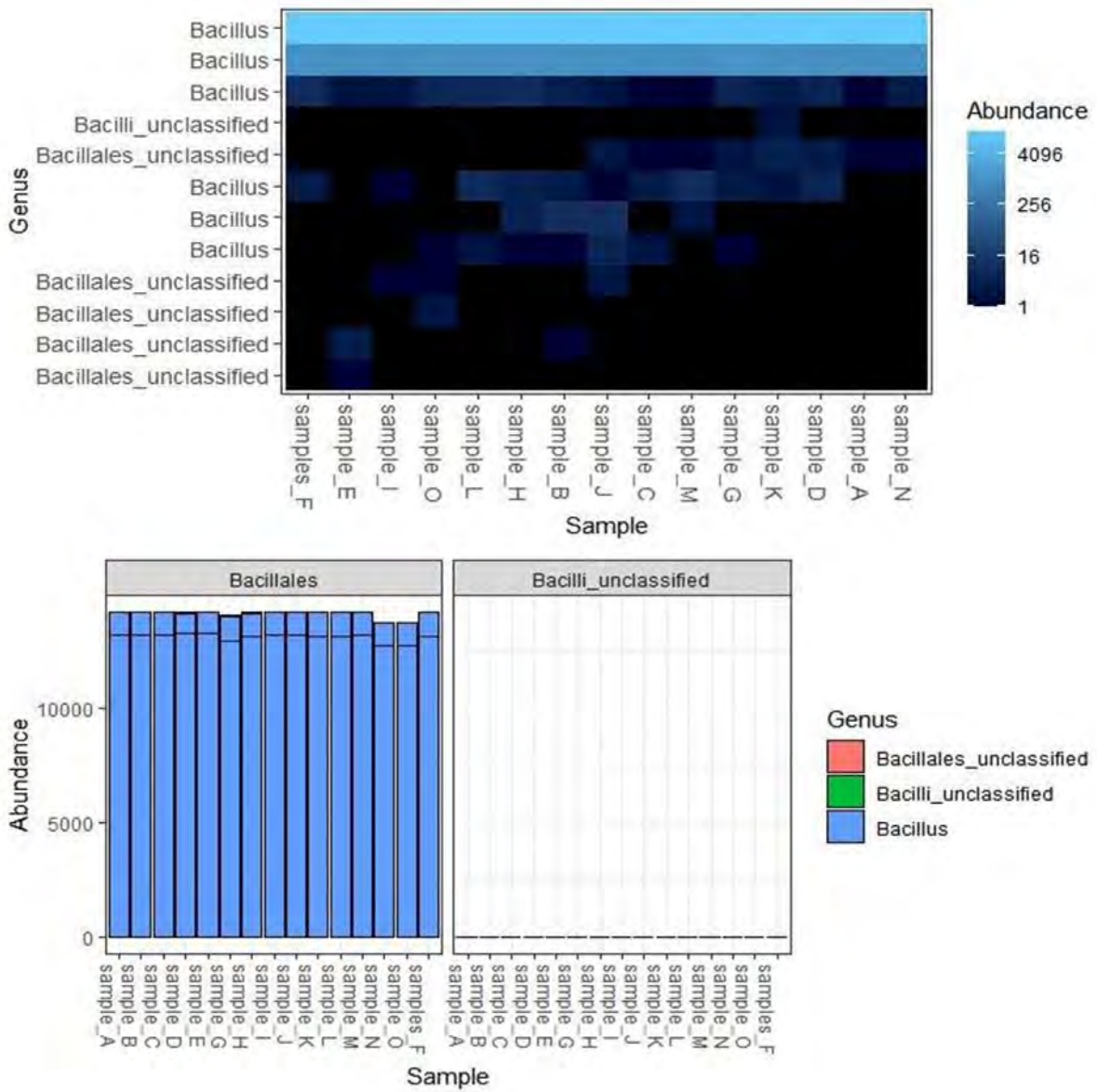


Figure 4.11: Bacterial community composition at the genus level

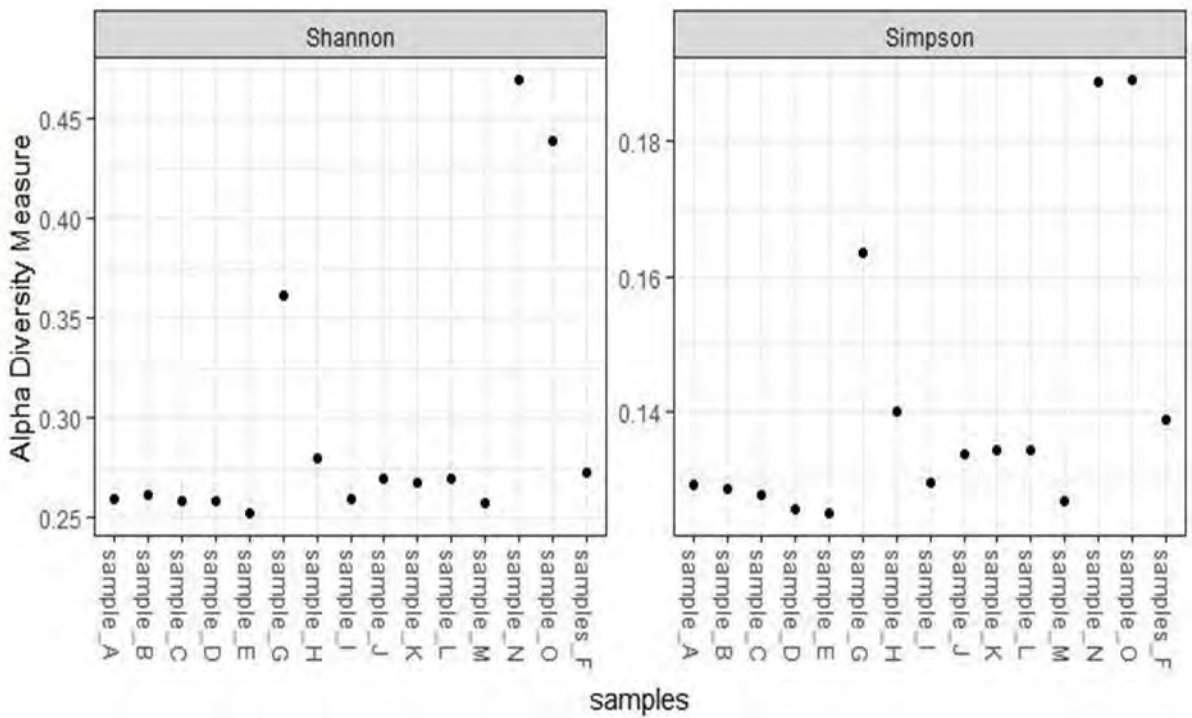
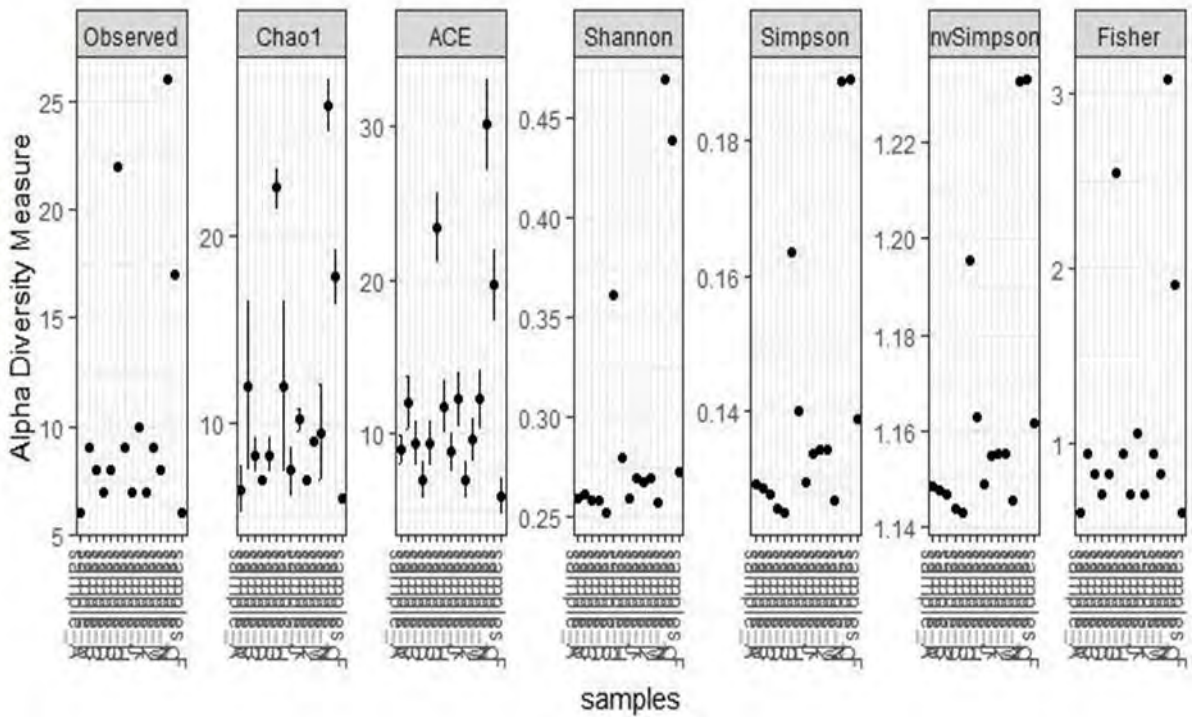


Figure 4.12: Comparison of sample_AU to sample_GI samples using Alpha Diversity indices

4.17 CONCLUSION

Microbiological contamination is a primary water quality concern globally. Our WWTP and river water samples are contaminated with bacteria; therefore, it is seen as unsafe for human consumption in line with drinking water standards. We conclude that sources of water are not safe for direct drinking and utility of food production. The critical issues that influence river

water quality are a high-density population in various human activities and rapid population growth. High counts of bacterial colonies can be due to domestic and leisure-time activities in partaking. The API 20E is a powerful technique for bacterial identification that can be used in a clinical microbiology laboratory which, cannot afford the automated systems. The overuse of antibiotics in health facilities is assumed the first part connected with the increase of new resistances. Unsuitable characteristics of water quality due to the presence of antibiotic-resistance bacteria constitute adverse effects on health conditions. High incidence of multiple ARB in water could constitute a menace to populations who consume such water. Waterborne diseases in South Africa are a health concern; therefore, it is of utmost importance to regularly check water quality. Measurements of water quality control must be established to ensure suitable treatments for water quality. It is also advisable to develop a plan of action to utilize drugs and regulations on waste treatment in hospitals, WWTPs, and municipals.

The PCR assay and 16S rDNA gene sequencing are precise, reliable, and sensitive for detecting microbes. The 16S rRNA (DNA) gene sequencing has been used extensively to examine the bacterial phylogeny and taxonomic group in bacterial taxonomy association between microbes and microbial identification. The utilization of NGS in the contamination of water with pathogens presents a broad and mutually supportive way. Further investigations must be carried out to evaluate the level of drugs in water and possible dangers closely associated with individual consumption of contaminated water.

REFERENCES

- Aali, R., Baragh, S., Asgari, E., Fard, R. F., Izanloo, H., Hosseinpoor, S., Olia, J. B. H., Naseri, R. & Rabori, M. M. (2019). Tracking of chloramphenicol, erythromycin, and sulfamethoxazole antibiotic-resistant bacteria from untreated wastewater effluents to receiving river. *Environmental Health Engineering and Management Journal*, 6(2), 89-86.
- Abulreesh, H. H., Paget, A. T., & Goulder, R. (2011). EXPLORATION OF WATERFOWL-ENVIRONMENT-CAMPYLOBACTER INTER-RELATIONSHIPS AT AMENITY PONDS. *Environmental Research Journal*, 5(1), 315-320.
- Adzitey, F., Huda, N., & Ali, G. R. R. (2013). Molecular techniques for detecting and typing of bacteria, advantages, and application to foodborne pathogens isolated from ducks. 3 *Biotech*, 3(2), 97-107.
- Ahmed, M., Suresh Babu, G., & Shetty, A. S. K. (2011). Development and validation of A moxicillin by RP- HPLC method in bulk drug and pharmaceutical dosage forms. *International Journal of ChemTech Research*, 3, 1037–1041.
- Ahmed, W., Huygens, F., Goonetilleke, A., & Gardner, T. (2008). Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. *Applied and environmental microbiology*, 74(17), 5490-5496.
- Akhavan, B. J., & Vijhani, P. (2019). Amoxicillin. In *StatPearls [Internet]*. StatPearls Publishing.
- Allen, H. K., & Stanton, T. B. (2014). Altered egos: antibiotic effects on food animal microbiomes. *Annu Rev Microbiol.*, 68, 297–315.
- Ashbolt, N. J. (2015). Microbial contamination of drinking water and human health from community water systems. *Current environmental health reports*, 2(1), 95-106.
- Azzam, M. I., Ezzat, S. M., Othman, B. A., & El-Dougdoug, K. A. (2017). Antibiotics resistance phenomenon and virulence ability in bacteria from the water environment. *Water Science*, 31(2), 109-121.
- Baquero, F., Martínez, J., & Cantón, R. (2008). *Antibiotics and antibiotic resistance in water environments*
- Barancheshme, F. & Munir, M. (2018). Strategies to Combat Antibiotic Resistance in the Wastewater Treatment Plants. *Frontiers in Microbiology*, 8, 2603.
- Batista, J. H., & da Silvia Neto, J. F. (2017). *Chromobacterium violaceum* Pathogenicity: Updates and Insights from Genome Sequencing of Novel *Chromobacterium* Species. *Frontiers in Microbiology*, 8.
- Bayot, M. L., & Bragg, B. N. (2020). Antimicrobial Susceptibility Testing. In *StatPearls [Internet]*. StatPearls Publishing.
- Bhateria, R., & Jain, D. (2016). Water quality assessment of lake water: a review. *Sustainable Water Resources Management*, 2, 161-173.
- Bello, P. H. S., Mustapha, A., Ismail, H.Y., Isa, M. A. & Mangga, H. K. (2016). Detection of Aeromonas Species from Water Sources in Northeastern Nigeria. *International Journal of Innovative Science, Engineering & Technology*, 3(2), 2348-7968.
- Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. (2018). Environmental factors influencing the development and spread of antibiotic resistance. *FEMS microbiology*

- reviews*, 42(1), fux053.
- Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., Martinez, J. L. (2015). Tackling antibiotic resistance: the environmental framework. *Nat. Rev. Microbiol*, 13, 310-317.
- Bergeron, S., Boopathy, R., Nathaniel, R., Corbin, A., & LaFleur, G. (2015). Presence of antibiotic-resistant bacteria and antibiotic resistance genes in raw source water and treated drinking water. *International Biodeterioration & Biodegradation*, 102, 370-374.
- Bohórquez, J., McGenity, T. J., Papaspyrou, S., García-Robledo, E., Corzo, A., & Underwood, G. J. (2017). Different types of diatom-derived extracellular polymeric substances drive changes in heterotrophic bacterial communities from intertidal sediments. *Frontiers in microbiology*, 8, 245.
- Bouki, C., Venieri, D., & Diamadopoulou, E. (2013). Detection and fate of antibiotic-resistant bacteria in wastewater treatment plants: a review. *Ecotoxicology and Environmental Safety*, 91, 1–9
- Bound, J. P., Kitsou, K., & Voulvoulis, N. (2006). Household disposal of pharmaceuticals and perception of risk to the environment. *Environ. Toxicol. Pharmacol*, 3, 301–307.
- Brooks, K. A., Jens, M., & Sodeman, T. M. (1974). A clinical evaluation of the API microtube system for identification of Enterobacteriaceae. *The American Journal of Medical Technology*, 40(2), 55-61.
- Bush, K., & Bradford, P. A. (2016). Beta-lactams and beta-lactamase inhibitors: An overview. *Cold Spring Harbor Perspectives in Medicine*, 6, PII: a025247.
- Cabral, J. P. (2010). Water microbiology. Bacterial pathogens and water. *International journal of environmental research and public health*, 7(10), 3657-3703.
- Chadha, T. (2012). Antibiotic-Resistant Genes in Natural Environments, *Agrotechnology*, 1(1), 1-3.
- Chen, L. F., Chopra, T., & Kaye, K. S. (2009). Pathogens were resistant to antibacterial agents. *Infect Dis Clin North Am*, 23(4), 817–45.
- Chenia, H. Y., & Jacobs. A. (2017). Antimicrobial Resistance, Heavy metal resistance, and integron content in bacteria isolated from a South African tilapia aquaculture system. *Dis. Aquat Organ*, 2126(3), 199–209.
- Cheng, G., Ning, J., Ahmed, S., Huang, J., Ullah, R., An, B. ... & Yuan, Z. (2019). Selection and dissemination of antimicrobial Resistance in Agri-food production. *Antimicrobial Resistance & Infection Control*, 8(1), 158.
- Cho, Y., Lee, M. H., Kim, H. S., Park, M., Kim, M. H., Kwon, H., ... & Lee, D. S. (2019). Comparative analysis of Sanger and next-generation sequencing methods for 16S rDNA analysis of post-mortem specimens. *Australian Journal of Forensic Sciences*, 51(4), 426-445.
- Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and molecular biology reviews: MMBR*, 65(2), 232–260.
- Chukwudi, C. U. (2016). rRNA Binding Sites and the Molecular Mechanism of Action of the Tetracyclines. *Antimicrobial agents and chemotherapy*, 60(8), 4433–4441.

- Comar, M., D'Accolti, M., Cason, C., Soffritti, I., Campisciano, G., Lanzoni, L., Bisi, M., Volta, A., Mazzacane, S., & Caselli, E. (2019). Introduction of NGS in Environmental Surveillance for Healthcare-Associated Infection Control. *Microorganisms*, 7(12), 708.
- Costa, C. F. M., Monteiro Neto, V., Santos, B. R. D. C., Costa, B. R. R., Azevedo, A., Serra, J. L. ... & Kuppinger, O. (2014). Enterobacteria identification and detection of diarrheagenic *Escherichia coli* in a Port Complex. *Brazilian Journal of Microbiology*, 45(3), 945-952.
- Cross, R., Ling, C., Day, N. P., McGready, R., & Paris, D. H. (2016). Revisiting Doxycycline in pregnancy and early childhood--time to rebuild its reputation?. *Expert opinion on drug safety*, 15(3), 367–382.
- D'Costa, V. M., King, C. E., Kalan, L., Morar, M., Sung, W. W., & Schwarz, C., et al. (2011). Antibiotic Resistance is ancient. *Nature*, 477(7365), 457–61.
- De Carvalho, C. C. C. R. (2018). Marine Biofilms: A Successful Microbial Strategy with Economic Implications. *Front. Mar. Sci.*
- Dekker, J. P., & Frank, K. M. (2015). Salmonella, Shigella, and yersinia. *Clinics in laboratory medicine*, 35(2), 225-246.
- Delgado-Valverde, M., Sojo-Dorado, J., Pascual, A., & Rodriguez-Bano, J. (2013). Clinical management of infections caused by multidrug-resistant Enterobacteriaceae. *Therapeutic Advances in Infectious Diseases*, 1(2), 49–69.
- Delpla, I., Baurès, E., Jung, A., & Thomas, O. (2011). Impacts of rainfall events on runoff water quality in an agricultural environment in temperate areas. *Science of the Total Environment*, 409(9), 1683-1688
- Devenish, J. A., & Barnum, D. A. (1982). Evaluation of the API 20E system for the identification of gram-negative non-fermenters from animal origin. *Canadian Journal of Comparative Medicine*, 46(1), 80.
- Díaz-Cruz, M., S., & Barceló, D. (2006). Determination of antimicrobial residues and metabolites in the aquatic environment by liquid chromatography-tandem mass spectrometry. *Anal Bioanal. Chem*, 386, 973–985.
- Doyle, D., Peirano, G., Lascols, C., Lloyd, T., Church, D. L., & Pitout, J. D. (2012). Laboratory detection of Enterobacteriaceae that produce carbapenemases. *Journal of clinical microbiology*, 50(12), 3877-3880.
- Dutta, H., & Dutta, A. (2016). The microbial aspect of climate change. *Energy, Ecology and Environment*, 1(4), 209-232.
- Eckburg, P. B., Lister, T., Walpole, S., Keutzer, T., Utley, L., Tomayko, J., Kopp, E., Farinola, N. & Coleman, S. (2019). Safety, Tolerability, Pharmacokinetics, and Drug Interaction Potential of SPR741, an Intravenous Potentiator, after Single and Multiple Ascending Doses and When Combined with β -Lactam Antibiotics in Healthy Subjects. *Antimicrob. Agents Chemother*, 63(9).
- European Centre for Disease Prevention and Control, the European Medicines Agency. (2009). ECDC/EMA Joint Technical Report- The bacterial challenge: time to react.
- Efstratiou, M. A., Bountouni, M., & Kefalas, E. (2018). The spread of Antibiotic Resistance in Aquatic Environments: *E. coli* as a Case Study. *Proceeding*, 2, 693.
- ECDC, E. (2013). Antimicrobial resistance surveillance in Europe in 2012. *Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) Stockholm*.

- Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in medicinal chemistry*, 6, 25–64.
- Fang, T., Cui, Q., Huang, Y., Dong, P., Wang, H., Liu, W., & Ye, Q. (2018). Distribution comparison and risk assessment of free-floating and particle-attached bacterial pathogens in urban recreational water: Implications for water quality management. *Science of the Total Environment*, 613-614, 428-438.
- Faruque, S. M., Khan, R., Kamruzzaman, M., Yamasaki, S., Ahmad, Q. S., Azim, T., & Sack, D. A. (2002). Isolation of *Shigella dysenteriae* type 1 and *S. flexneri* strains from surface waters in Bangladesh: comparative molecular analysis of environmental *Shigella* isolates versus clinical strains. *Appl. Environ. Microbiol*, 68(8), 3908-3913.
- Fasugba, O., Gardner, A., & Mitchell, B. G. *et al.* (2015). Ciprofloxacin resistance in the community- and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies. *BMC Infect Dis* 15, 545.
- Fazi, S., Amalfitano, S., Piccini, C., Zoppini, A., Puddu, A., and Pernthaler, J. (2008). Colonization of overlaying water by bacteria from dry river sediments. *Environ. Microbiol.* 10, 2760–2772.
- Fernández-Villa, Aguilar, & Rojo. (2019). Folic Acid Antagonists: Antimicrobial and Immunomodulating Mechanisms and Applications. *International Journal of Molecular Sciences*, 20(20), 4996.
- Forsberg, K. J., Patel, S., Wencewicz, T. A., & Dantas, G. (2015). The Tetracycline Destructases: A Novel Family of Tetracycline-Inactivating Enzymes. *Chemistry & Biology*, 22(7), 888–897.
- Fyfe, C., Grossman, T. H., Kerstein, K., & Sutcliffe, J. (2016). Resistance to Macrolide Antibiotics in Public Health Pathogens. *Cold Spring Harbor perspectives in medicine*, 6(10), a025395.
- Gagliotti, C., Balode, A., Baquero, F., Degener, J., Grundmann, H., Gür, D., Jarlier, V., Kahlmeter, G., Monen, J., Monnet, D. L., Rossolini, G. M., Suetens, C., Weist, K., Heuer, O, & EARS-Net Participants (Disease Specific Contact Points for AMR). (2011). *Escherichia coli* and *Staphylococcus aureus*: bad news and good news from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS), 2002 to 2009. *Euro Surveill*, 16(11), PII: 19819.
- Galler, H., Feierl, G., Petternel, C., Reinthaler, F., Haas, D., Habib, J., Kittinger, C., Luxner, J., Zarfel, G. (2018). Multi resistant Bacteria Isolated from Activated Sludge in Austria. *Int. J. Environ. Res. Public Health*, 15, 479.
- Garcia-Aljaro, C., Momba, M., and Muniesa, M. (2017). Pathogenic members of *Escherichia coli* & *Shigella* species Shigellosis. In: J.B. Rose and B. Jiménez-Cisneros, (Eds) Global Water Pathogen Project. <http://www.waterpathogens.org> (A. Pruden, N. Ashbolt, and J. Miller (Eds) Part 3 Bacteria).
- Garibyan, L., & Avashia, N. (2013). Research techniques made simple: polymerase chain (PCR). *The Journal of investigative dermatology*, 133(3), e6.
- Gavini, F., Leclerc, H., & Mossel, D. A. (1985). Enterobacteriaceae of the “coliform group” in drinking water: Identification and worldwide distribution. *Systematic and applied microbiology*, 6(3), 312-318.
- Griffin, M. O., Fricovsky, E., Ceballos, G., & Villarreal, F. (2010). Tetracyclines: a pleiotropic

- family of compounds with promising therapeutic properties. Review of the literature. *American journal of physiology. Cell physiology*, 299(3), C539–C548.
- Grisey, E., Belle, E., Dat, J., Mudry, J., & Aleya, L. (2010). Survival of pathogenic and indicator organisms in groundwater and landfill leachate through coupling bacterial enumeration with tracer tests. *Desalination*, 261(1-2), 162-168.
- Grossman T. H. (2016). Tetracycline Antibiotics and Resistance. *Cold Spring Harbor perspectives in medicine*, 6(4), a025387.
- Gupta, N. (2019). DNA extraction and polymerase chain reaction. *Journal of Cytology*, 36(2), 116-117.
- Gu, W., Miller, S., & Chiu, C. Y. (2019). Clinical Metagenomic Next-Generation Sequencing for Pathogen Detection. *Annual review of pathology*, 14, 319–338.
- Gwinn, M., MacCannell, D., & Armstrong, G. L. (2019). Next-generation sequencing of infectious pathogens. *Jama*, 321(9), 893-894.
- Gwinn, M., MacCannell, D. R., & Khabbaz, R. F. (2017). Integrating advanced molecular technologies into public health. *Journal of clinical microbiology*, 55(3), 703-714.
- Haberecht, H. B., Nealon, N. J., Gilliland, J. R., Holder, A. V., Runyan, C., Oppel, R. C., ... & Antony, L. (2019). Antimicrobial-resistant *Escherichia coli* from environmental waters in northern Colorado. *Journal of environmental and public health*, 2019.
- Hardy, K., Sunnucks, K., Gil, H., Shabir, S., Trampari, E., & Hawkey, P., et al. (2018). Increased usage of antiseptics is associated with reduced susceptibility in clinical isolates of *Staphylococcus aureus*. *MBio*, 9(3), e00894–18.
- Harmel, R. D., Karthikeyan, R., Gentry, T., & Srinivasan, R. (2010). Effects of agricultural management, land use, and watershed scale on *E. coli* concentrations in runoff and streamflow. *Transactions of the ASABE*, 53(6), 1833-1841.
- Hao, H., Cheng, G., Iqbal, Z., Ai, X., Hussain, H. I, Huang, L., et al. (2014). Benefits and risks of antimicrobial use in food-producing animals. *Front. In Microbiology*, 5, 288.
- Hollender, J., Zimmermann, S. G., Koepke, S., Krauss, M., McArdell, C.S., Ort, C., Singer, H., von Gunten, U., & Siegrist, H. (2009). Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration. *Environmental Science and Technology*, 43, 7862–7869.
- Holmes, B., Willcox, W. R., & Lapage, S. P. (1978). Identification of Enterobacteriaceae by the API 20E system. *Journal of clinical pathology*, 31(1), 22-30.
- Hossain, M. J., Waldbieser, G. C., Sun, D., Capps, N. K., Hemstreet, W. B., Carlisle, K.,... & Schroeder, S. (2013). The implication of Lateral Genetic Transfer in the Emergence of *Aeromonas hydrophila* Isolates of Epidemic Outbreaks in Channel Catfish. *PLoS ONE*, 8(11), e80943.
- Ibrahim, M. E., Abbas, M., Al-Shahrai, A. M., & Elamin, B. K. (2019). Phenotypic Characterization and Antibiotic Resistance Patterns of Extended-Spectrum β -Lactamase- and AmpC β -Lactamase-Producing Gram-Negative Bacteria in a Referral Hospital, Saudi Arabia. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 2019:6054694.
- Igbinosa, I. H., Igumbor, E. U., Aghdasi, F., Tom, M., & Okoh, A. I. (2012). Emerging *Aeromonas* species infections and their significance in public health. *TheScientificWorldJournal*, 2012, 625023.

- Infectious Diseases Society of America (IDSA). (2011). Combating antimicrobial resistance: policy recommendations to save lives. *Clinical Infectious Diseases*, 52(suppl_5), S397-S428.
- Isfahani, B. N., Fazeli, H., Babaie, Z., Poursina, F., Moghim, S., & Rouzbahani, M. (2017). Evaluation of polymerase chain reaction for detecting coliform bacteria in drinking water sources. *Advanced biomedical research*, 6.
- Ishino, S., & Ishino, Y. (2014). DNA polymerases as useful reagents for biotechnology—the history of developmental research in the field. *Frontiers in microbiology*, 5, 465.
- Iwane, T., Urase, T., & Yamamoto, K. (2001). The possible impact of treated wastewater discharge on the incidence of antibiotic-resistant bacteria in river water. *Water Science and Technology*, 43(2): 91-9.
- Jeffrey North, E., Jackson, M., & E Lee, R. (2014). New approaches to target the mycolic acid biosynthesis pathway for the development of tuberculosis therapeutics. *Current pharmaceutical design*, 20(27), 4357-4378.
- Kabwama, S. N., Bulage, L., Nsubuga, F., Pande, G., Oguttu, D. W., Mafigiri, R., & Zhu, B., P. (2017). A large and persistent outbreak of typhoid fever caused by consuming contaminated water and street-vended beverages: Kampala, Uganda, January - June 2015. *BMC public health*, 17(1), 23.
- Kadri, K. (2019). Polymerase Chain Reaction (PCR): Principle and Applications. In *Perspectives on Polymerase Chain Reaction*. IntechOpen.
- Kampf, G. (2016). Acquired resistance to chlorhexidine - is it time to establish an 'antiseptic stewardship' initiative? *Journal of Hospital Infection*, 94(3), 213–27.
- Kannan, K., Kanabar, P., Schryer, D., Florin, T., Oh, E., Bahroos, N., Tenson, T., Weissman, J. S. & Mankin, A. S. (2014). The general action of translation inhibition by macrolide antibiotics. *Proceedings of the National Academy of Sciences of the United States of America*, 111(45), 15958–15963.
- Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology*, 33(3), 300-305.
- Kappell, A. D., DeNeis, M. S., Ahjua, N. H., Ledebor, N. A., Newton, R. J., & Hristova, K., R. (2015). Detection of multidrug-resistant *Escherichia coli* in the urban waterways of Milwaukee, WI. *Frontiers in Microbiology*.
- Kaur, R., Singh, D., Kesavan, A. K., & Kaur, R. (2019). Molecular characterization and antimicrobial susceptibility of bacterial isolates present in the tap water of public toilets. *International Health*, 12(5), 472–483.
- Kawecki, D., Pacholczyk, M., Lagiewska, B., Sawicka-Grzelak, A., Durluk, M., Mlynarczyk, G., & Chmura, A. (2014). Bacterial and fungal infections in the early post-transplantation period after liver transplantation: Etiologic agents and their susceptibility. *Transplantation Proceedings*, 46(8), 2777-2781.
- Keskar, M. R., & Jugade, R. M. (2015). Spectrophotometric Investigations of Macrolide Antibiotics: A Brief Review. *SAGE journals*.
- Khan, Z. A., Siddiqui, M. F., & Park, S. (2019). Current and emerging methods of antibiotic susceptibility testing. *Diagnostics*, 9(2), 49.
- Kiefer, L. A., Shelton, D. R., Pachepsky, Y., Blaustein, R., & Santin-Duran, M. (2012).

- Persistence of *Escherichia coli* introduced into streambed sediments with goose, deer, and bovine animal waste. *Letters in applied microbiology*, 55(5), 345-353.
- Kinge, C. N. W., Ateba, C. N., & Kawadza, D. T. (2010). Antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho locality, north-west province, South Africa. *South African Journal of Science*, 106(1-2), 44-49.
- Kirmusaoğlu, S., Gareayaghi, N., & S. Kocazeybek, B. (2019). Introductory Chapter: The Action Mechanisms of Antibiotics and Antibiotic Resistance. *Antimicrobials, Antibiotic Resistance, Antibiofilm Strategies, and Activity Methods*.
- Konaklieva, M. I. (2014). Molecular targets of beta-lactam-based antimicrobials: Beyond the usual suspects. *Antibiotics*, 3, 128.
- Krevš, A., & Kučinskienė, A. (2018). Microbial decomposition of sedimentary organic matter in small temperate lakes. *Fundamental and Applied Limnology*, 191, 239–251.
- Krevš, A., & Kučinskienė, A. (2017). Influence of invasive *Acer negundo* leaf litter on benthic microbial abundance and activity in the littoral zone of a regulated river in Lithuania. *Knowledge and Management of Aquatic Ecosystems*, 418, 26.
- Kumar, P., & Libchaber, A. (2015). Pressure and Temperature Dependence of Growth and Morphology of *Escherichia coli*: Experiments and Stochastic Model. *Biophysical Journal*, 105(3), 783-793.
- Langdon, A., Crook, N., & Dantas, G. (2016). The effects of antibiotics on the microbiome throughout development and alternative approaches for therapeutic modulation. *Genome medicine*, 8(1), 39.
- Lashinsky, J. N., Henig, O., Pogue, J. M. *et al.* (2017). Minocycline for the Treatment of Multidrug and Extensively Drug-Resistant *A. baumannii*: A Review. *Infect Dis Therapy*, 6, 199–211.
- Li, L., Mendis, N., Trigui, H., Oliver, J. D., & Faucher, S. P. (2014). The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in microbiology*, 5, 258.
- Liu, G., Bakker, G. L., Li, S., Vreeberg, J. H. G., Verberk, J. Q. J. C., Medema, G. J., Liu, W. T., & Van Dijk, J. C. (2014). Pyrosequencing reveals bacterial communities in unchlorinated drinking water distribution system: an integral study of bulk water, suspended solids, loose deposits, and pipe wall biofilm. *Environmental Science and Technology*, 48, 5467-5476.
- Liu, P., Klose, M., & Conrad, R. (2018). Temperature effects on the structure and function of the methanogenic microbial communities in two paddy soils and one desert soil. *Soil Biology and Biochemistry*, 124, 236-244.
- Liu, Z. H., Huang, S. B., Sun, G. P., Xu, Z. C., and Xu, M. Y. (2012). Phylogenetic diversity, composition, and distribution of bacterioplankton community in the Dongjiang River, China. *FEMS Microbiol. Ecol.* 80, 30–44.
- Livermore, D. M., Hope, R., Reynolds, R., Blackburn, R., Johnson, A. P., Woodford, N. (2013). Declining cephalosporin and fluoroquinolone non-susceptibility among bloodstream Enterobacteriaceae from the UK: links to prescribing change? *Journal of Antimicrobial Chemotherapy*, 68(11), 2667-2674.
- Lobritz, M. A., Belenky, P., Porter, C. B. M., Gutierrez, A., Yang, J. H., Schwarz, E. G., Dwyer, D. J., Khalil, A. S., & Collins, J. J. (2015). Antibiotics and bacterial respiration.

- Proceedings of the National Academy of Science*, 112(27), 8173-8180.
- Lyons, N. A., & Kolter, R. (2015). On the evolution of bacterial multicellularity. *Current opinion in microbiology*, 24, 21–28.
- Mallick, H., Franzosa, E. A., McIver, L. J., Banerjee, S., Sirota-Madi, A., Kostic, A. D. ... & Huttenhower, C. (2019). Predictive metabolomic profiling of microbial communities using amplicon or metagenomic sequences. *Nature communications*, 10(1), 1-11.
- Manjul, A. S., & Shirkot, P. (2018). 16S rRNA gene sequencing for bacterial identification of pullulanase synthesizing thermophilic bacteria contributing to big data. *IJCS*, 6(2), 2769-2773.
- Martin, M. S., Santos, I. C., Carlton, D. D., Stigler-Granados, P., Hildenbrand, Z. L. & Schug, K. A. (2018). Characterisation of bacterial diversity in contaminated groundwater using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Science of the Total Environment*, 622-623, 1562–1571.
- Martinez, J. L., & Baquero, F. (2014). Emergence and spread of antibiotic resistance: setting a parameter space. *Ups J Med Sci.*, 119, 68-77.
- Mao, G., Song, Y., Bartlam, M., & Wang, Y. (2018). Long-term Effects of Residual Chlorine on *Pseudomonas aeruginosa* in Simulated Drinking Water Fed with Low AOC Medium. *Front Microbiology*, 9, 1-10.
- Mayo, B., TCC Rachid, C., Alegría, Á., MO Leite, A., S Peixoto, R., & Delgado, S. (2014). Impact of next-generation sequencing techniques in food microbiology. *Current genomics*, 15(4), 293-309.
- Medardus, J. J., Molla, B. Z., Nicol, M., Morrow, W. M., Rajala-Schultz, P. J., & Kazwala R, et al. (2014). In-feed use of heavy metal micronutrients in US swine production systems and its role in the persistence of multidrug-resistant salmonellae. *Applied and Environmental Microbiology*, 80(7), 2317–25.
- Mi, Z., Dai, Y., Xie, S., Chen, C., & Zhang, X. (2015). Impact of disinfection on drinking water biofilm bacterial community. *Journal of Environmental Sciences*, 37, 200-205.
- Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T. ... & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. *Water Research*, 47(3), 957-995.
- Mladenović, K. G., Muruzović, M. Ž, Žugić Petrović, T., Stefanović, O. D., & Čomić, L. R. (2018). Isolation and identification of Enterobacteriaceae from traditional Serbian cheese and their physiological characteristics. *Journal of Food Safety*, 38(1), e12387.
- Mogamedi, K. L. M., Goyvaerts, E. M. A., Venter, S. N., & Sibara, M. M. (2007). Optimization of the PCR-invA primers for the detection of *Salmonella* in drinking and surface waters following a pre-cultivation step. *Water SA*, 33(2).
- Momba, M., Osode, A., & Sibewu, M. (2009). The impact of inadequate wastewater treatment on the receiving water bodies – Case study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province. *Water SA*, 32(5).
- Mulu, W., Abera, B., Yimer, M., Hailu, T., Ayele, H., & Abate, D. (2017). Bacterial agents and antibiotic resistance profiles of infections from different sites that occurred among patients at Debre Markos Referral Hospital, Ethiopia: a cross-sectional study. *BMC Res Notes*, 10(1), 254.
- Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. *Microbiology*

spectrum, 4(2).

- Nesher, L., & Rolston, K. V. (2014). The current spectrum of infection in cancer patients with chemotherapy-related neutropenia. *Infection*, 42(1):5–13.
- Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D., & Bertilsson, S. (2011). A Guide to the Natural History of Freshwater Lake Bacteria. *Microbiol. Mol. Biol. Rev.*, 75, 14–49.
- Nord, C. E., Lindberg, A. A., & Dahlbäck, A. (1974). Evaluation of five test-kits-API, AuxoTab, Enterotube, PathoTec, and R-B-for identification of Enterobacteriaceae. *Medical Microbiology and Immunology*, 159(3), 211-220.
- Novo, A., Andre, S., Viana, P., Nunes, O. C., & Manaia, C. M. (2013). Antibiotic Resistance, antimicrobial residues and bacterial community composition in urban wastewater. *Water Res*, 47(5), 1875-87.
- Okinda, N., Mulwa, E., & Revathi, G. (2014). A five-year review of API20e bacteria identification system's performance at a teaching hospital. *East African medical journal*, 91(3), 73-76.
- O'Rourke, A., Beyhan, S., Choi, Y., Morales, P., Chan, A. P., Espinoza, J. L. ... & Nierman, W. C. (2020). Mechanism-of-action classification of antibiotics by global transcriptome profiling. *Antimicrobial agents and chemotherapy*, 64(3).
- Pal, C., Bengtsson-Palme, J., Kristiansson, E., & Larsson, D. J. (2015). Co-occurrence of resistance genes to antibiotics, biocides, and metals reveals novel insights into their co-selection potential. *BMC genomics*, 16(1), 964.
- Pandey, N., & Cascella, M. (2020). Beta-Lactam Antibiotics. *StatPearls [Internet]*.
- Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. *Amb Express*, 4(1), 51.
- Parveen, B., Reveilliez, J. P., Mary, I., Ravet, V., Bronner, G., Domaizon, I., & Debroas, D. (2011). Diversity and dynamics of free-living and particle-associated Betaproteobacteria and Actinobacteria concerning phytoplankton and zooplankton communities. *FEMS Microbiology. Ecology*, 77(3), 461-476.
- Patrick, G. L. An Introduction to Medicinal Chemistry. (2003). Oxford: Oxford University Press, 379–435
- Pavlov, D., de Wet, C. M., Grabow, W. O., & Ehlers, M. M. (2004). Potentially pathogenic features of heterotrophic plate count bacteria isolated from treated and untreated drinking water. *International Journal of Food Microbiology*, 92, 275-287.
- Pereira, M., Malta, F., Freire, M., & Couto, P. (2017). Application of next-generation sequencing in the era of precision medicine. In *Applications of RNA-Seq and Omics Strategies: From Microorganisms to Human Health* (pp. 293-318). InTechOpen, London, England, UK.
- Perry, J., Waglechner, N., & Wright, G. (2016). The prehistory of antibiotic resistance. *Cold Spring Harbour Perspective in Medicine*, 6(6), a025197.
- Pindi, P. K., Srinath, R. R., & Shiva Shanker, A. (2013). Novel approaches of genomic DNA isolation for identification of Cultivable Bacteria. *Jundishapur Journal of Microbiology*, 6(10).
- Podolsky, S. H. (2018). The evolving response to antibiotic resistance (1945–2018). *Palgrave Communications*, 4(1), 1-8.
- Pohl, H. G., Groah, S. L., Pérez-Losada, M., Ljungberg, I., Sprague, B. M., Chandal, N., ... &

- Hsieh, M. (2020). The urine microbiome of healthy men and women differs by urine collection method. *International Neurourology Journal*, 24(1), 41.
- Polikanov, Y. S., Aleksashin, N. A., Beckert, B., & Wilson, D. N. (2018). The Mechanisms of The action of Ribosome-Targeting Peptide Antibiotics. *Frontiers in molecular biosciences*, 5, 48.
- Poonia, S., Singh, T. S., & Tsering, D. C. (2014). Antibiotic susceptibility profile of bacteria isolated from natural sources of water from rural areas of East Sikkim. *Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine*, 39(3), 156.
- Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global, multifaceted phenomenon. *Pathogens and global health*, 109(7), 309-318.
- Pruden, A., Pei, R., Storteboom, H., & Carlson, K., H. (2006). Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environmental Science and Technology*, 40, 7445-7450.
- Rang, H. P., Dale, M. M., Ritter, J. M., & Moore, P. K. (2003). Pharmacology. Fifth Ed. Edinburg: Churchill, Livingstone.
- Reuben, R. C. & Owuna, G. (2013). Antimicrobial resistance patterns of *Escherichia Coli* O157: H7 from Nigerian fermented milk samples in Nasarawa State, Nigeria. *International Journal of Pharmaceutical Science Invention*, 2, 38-44.
- Rodrigues, C., & Cunha, M. Â. (2017). Assessment of the microbiological quality of recreational waters: indicators and methods. *Euro-Mediterranean Journal for Environmental Integration*, 2(25).
- Rozman, U., & Turk, S. Š. (2016). PCR technique for the microbial analysis of inanimate hospital environment. *Polymerase Chain Reaction for Biomedical Applications. UK: InTech*, 119-134.
- Ruhe, J. J., & Menon, A. (2007). Tetracyclines as an oral treatment option for patients with community-onset skin and soft tissue infections caused by methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents Chemotherapy*, 51(9), 3298-303.
- Sanganyado, E., & Gwenzi, W. (2019). Antibiotic Resistance in drinking water systems: Occurrence, removal, and human health risks. *Science of the Total Environment*, 669,785-797.
- Sato, T., Qadir, M., Yamamoto, S., Endo, T., & Zahoor, A. (2013). Global, regional, and country-level need for data on wastewater generation, treatment, and use. *Agricultural Water Management*, 130, 1–13.
- Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., ... & Sahl, J. W. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and environmental microbiology*, 75(23), 7537-7541.
- Schmidt, F. J., Hofmann, B., Hansen, B., Scheuring, S., Luckefahr, M., Klootwijk, M., et al. (1998). Relationship between ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, and moxifloxacin (BAY 12-8039) MICs and mutations in *grlA*, *grlB*, *gyrA*, and *gyrB* in 116 unrelated clinical isolates of *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, 41, 481–4.
- Schwartz, T., Kohnen, W., Jansen, B & Obst, U. (2003). Detection of antibiotic-resistant b

- acteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiology Ecology*, 43, 325-335.
- Senjarini, K., Karsten, U., & Schumann, R. (2013). Application of Fluorescence Markers for the Diagnosis of Bacterial Abundance and Viability in Aquatic Ecosystem. *J. Microbiol, Res*, 3, 143–147.
- Shakibaie, M. R, Jalilzadeh, K. A, & Yamakanamardi, S M. (2009). Horizontal transfer of antibiotic resistance genes among gram harmful bacteria in sewage and lake water and the influence of some physicochemical parameters of water on the conjugation process. *J Environ Biol*, 30(1), 45-9.
- Shallcross, L. J., Howard, S. J., Fowler, T., & Davies, S. C. (2015). Tackling the threat of antimicrobial resistance: from policy to sustainable action. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, 370(1670), 20140082.
- Singer, A. C., Shaw, H., Rhodes, V., & Hart, A. (2016). Review of Antimicrobial Resistance in the Environment and Its Relevance to Environmental Regulators. *Frontiers in microbiology*, 7, 1728.
- Snow, D. D., Cassada, D. A., Bartelt–Hunt, S. L., Li, X., D'Alessio, M., Zhang, Y. ... & Sallach, J. B. (2016). Detection, occurrence, and fate of emerging contaminants in agricultural environments. *Water Environment Research*, 88(10), 913-929.
- Solieri, L., Dakal, T. C., & Giudici, P. (2013). Next-generation sequencing and its potential impact on food microbial genomics. *Annals of Microbiology*, 63(1), 21-37.
- Stephen, F. A., Madden, A. A., Sch¨, A., S., Jinghui Zhang, F., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res*, 25, 3389-3402.
- Sun, H., He, X., Ye, L. *et al.* (2017). Diversity, abundance, and possible sources of fecal bacteria in the Yangtze River. *Appl Microbiol Biotechnol* 101, 2143–2152.
- Sun, J., Sakai, S., Tauchi, Y., Deguchi, Y., Chen, J., Zhang, R., & Morimoto, K. (2002). Determination of lipophilicity of two quinolone antibacterials, Ciprofloxacin, and grepafloxacin, in the protonation equilibrium. *European Journal Pharmaceutics and Biopharmaceutics*, 54(1), 51-58.
- Sun, J., Zeng, Q., Tsang, D. C., Zhu, L. Z., & Li, X. D. (2017). Antibiotics in the agricultural soils from the Yangtze River Delta, China. *Chemosphere*, 189, 301-8.
- Tagoe, D. N. A., Nyarko, H., Arthur, S. A., & Birikoranf, E. (2011). A Study of Antibiotic Susceptibility Pattern of Bacteria Isolates in Sachet Drinking Water Sold in the Cape Coast Metropolis of Ghana. *Research Journal of Microbiology*, 6(2), 153-158.
- Tamil, A., Anjugam, E., Devi, R. A., Kannappan, S., & Chandrasekaran, B. (2012). Isolation and Characterisation of the Bacteria from Tannery Effluent Treatment Plant and Their Tolerance to Heavy Metals and Antibiotics. *ASIAN J. EXP. BIOL. SCI*, 3(1), 34-41.
- Tandukar, M., Oh, S., Tezel, U., Konstantinidis, K. T., & Pavlostathis, S. G. (2013). Long-term exposure to benzalkonium chloride disinfectants resulted in a change of microbial community structure and increased antimicrobial resistance. *Environ Sci. Technol.*, 47(17), 9730–8.
- Tamhankar, A.S., & Lundborg, C. S. (2019). Antimicrobials and Antimicrobial Resistance in the Environment and Its Remediation: A Global One-Health Perspective. *Int. J. Environ. Res. Public Health*, 16(23), 4614.

- Tawfik, S. A., Azab, M. M., Ahmed, A. A. A., & Fayyad, D. M. (2018). Illumina MiSeq sequencing for preliminary analysis of microbiome, causing primary endodontic infections in Egypt. *International journal of microbiology*, 2018.
- Timoney, J. F., Port, J., Giles, J., & Spanier, J. (1978). Heavy Metal and Antibiotic Resistance in the Bacterial Flora of Sediments of New York Bight. *Applied and Environmental Microbiology*, 36(3), 465-472.
- Tremblay, J., Singh, K., Fern, A., Kirton, E. S., He, S., Woyke, T. ... & Tringe, S. G. (2015). Primer and platform effects on 16S rRNA tag sequencing. *Frontiers in microbiology*, 6, 771.
- Tumar, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA 6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biol. Evol.*, 30, 2725-2729.
- Uba, B. N., & Eze, A. (2004). Incidence of *Chromobacterium violaceum* in borehole water in Port Harcourt metropolis, Rivers State Nigeria. *Journal of Water Supply: Research and Technology-Aqua*, 53(6), 433-439.
- Unutkan, T., Bakırdere, S., & Keyf, S. (2018). Development of an Analytical Method for the Determination of Amoxicillin in Commercial Drugs and Wastewater Samples, and Assessing its Stability in Simulated Gastric Digestion. *Journal of Chromatographic Science*, 56(1), 36-40.
- Vaz-Moreira, I., Nunes, O. C., & Manaia, C. M. (2014). Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. *FEMS microbiology reviews*, 38(4), 761-778.
- Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P & T: a peer-reviewed journal for formulary management*, 40(4), 277-283.
- Verma, K., Dalal, J., & Sharma, S. (2014). Scientific concepts of polymerase chain reaction (PCR). *International Journal of Pharmaceutical Sciences and Research*, 5(8), 3086.
- Viale, P., Giannella, M., Lewis, R., Treccarichi, E. M., Petrosillo, N., & Tumbarello, M. (2013). Predictions of mortality in drug-resistant *Klebsiella Pnuemoniae* bloodstream infections. *Expert Rev Anti Infect Ther*, 11(10), 1053-63.
- Wales, A. D., & Davies, R. H. (2015). Co-selection of resistance to antibiotics, biocides, and heavy metals, and its relevance to foodborne pathogens. *Antibiotics (Basel)*, 4(4), 567-604.
- Walters, E., McClellan, K., & Halden, R. U. (2010). Occurrence and loss over three years of Seventy-two pharmaceuticals and personal care products from biosolids-soil mixtures in the outdoor mesocosms. *Water Res*, 44, 6011-6020.
- Walters, J. D., Zhang, F., & Nakkula, R. J. (1999). Mechanism of fluoroquinolone transport by human neutrophils. *Antimicrobial Agents Chemother*, 43, 2710-2715.
- Wang, Y., Sheng, H. F., He, Y., Wu, J. Y., Jiang, Y. X., Tam, N. F., & Zhou, H. W. (2012). Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of Illumina tags. *Applied and environmental microbiology*, 78(23), 8264-8271.
- Washington, J. A., 2nd, Yu, P. K., & Martin, W. J. (1971). Evaluation of the accuracy of multitest micro method system for identification of Enterobacteriaceae. *Applied Microbiology*, 22(3), 267-269.
- Weinstock, G. M., Goldberg, B., Ledebor, N., Rubin, E., Sichtig, H., & Geyer, C. (2016).

Applications of Clinical Microbial Next-Generation Sequencing.

- World Health Organization. (2014). *Antimicrobial resistance: global report on surveillance*. World Health Organisation.
- Wilson, R. (2001). Bacteria, antibiotics, and COPD. *European Respiratory Journal*, 17(5), 995-1007.
- Wose Kinge, C., & Mbewe, M. (2010). Characterization of Shigella species isolated from river catchments in the North West province of South Africa. *South African Journal of Science*, 106(11-12), 1-4.
- Wright, M. S., Baker-Austin, C., Lindell, A. H., Stepanauskas, R., Stokes, H. W., & McArthur, J. V. (2008). Influence of industrial contamination on mobile genetic elements: class 1 integron abundance and gene cassette structure in aquatic bacterial communities. *The ISME Journal*, 2, 417-428.
- Xi, C., Zhang, Y., Marrs, C. F., Ye, W., Simon, C., Foxman, B., & Nriagu, J. (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. *Appl. Environ. Microbiol*, 75(17), 5714-5718.
- Zaky, M. M., Salem, M. A., Persson, K. M., & Eslamian, S. (2011). Incidence of Aeromonas species isolated from water and fish sources of Lake Manzala in Egypt. *International Journal of Hydrology Science and Technology*, 1(1-2), 47-62.
- Zduńczyk, Z. (2019). Functioning of the intestinal ecosystem: From new technologies in microbial research to practical poultry Feeding—a review. *Annals of Animal Science*, 19(2), 239-256.
- Zhang, L., Shen, Z., Fang, W., & Gao, G. (2019). Composition of bacterial communities in the municipal wastewater treatment plants. *Science of the Total Environment*.
- Zhang, H. G., Ma, S. S., Li, Q. F., Fu, X. J., Zhang, Y., & Qu, K. M. (2011). Analysis of the changes of microbial community structure on bio-carrier of recirculating aquaculture systems (RAS). *Europe PMC*, 32(1), 231-239.
- Zhang, M., Xu, M., Xu, S., Zhang, L., Lin, K., Zhang, L. ... & Zhou, H. (2020). The response of the Bacterial Community and Antibiotic Resistance in Overnight Stagnant Water from a Municipal Pipeline. *International Journal of Environmental Research and Public Health*, 17(6), 1995.
- Zhang, Y., Gu, A. Z., Cen, T., Li, X., He, M., & Li, D., et al. (2018). Sub-inhibitory concentrations of heavy metals facilitate the horizontal transfer of plasmid-mediated antibiotic resistance genes in the water environment. *Environ Pollut*, 237, 74–82.
- Zhang, X. X., Zhang, T., & Fang, H. H. (2009). Antibiotic resistance genes in the water environment. *Appl. Microbiol. Biotechnol*, 82,397-414.
- Zhou, J. L., Xu, J., Jiao, A. G., Yang, L., Chen, J., Callac, P., ... & Wang, S. X. (2019). Patterns of PCR Amplification Artifacts of the Fungal Barcode Marker in a Hybrid Mushroom. *Frontiers in Microbiology*, 10, 2686.

CHAPTER V

GENERAL CONCLUSION AND RECOMMENDATION

Water quality is of vital interest to people because water quality is directly associated with people's well-being. The risk of waterborne diseases is even emerging in developed nations. The sources of water are becoming polluted with pollutants originating from urban and industrial development. Water is used carelessly, and the water quality worsens because people perform different activities that are detrimental to its quality. Several toxic wastes are released from industrial sectors directly into the bodies of water. The quality of potable water is not satisfactory because industrial wastewater and industrial raw water get into lakes and rivers. Also, sewer waters get into water surfaces. Water quality deterioration is due to plant protection agents, toxic waste dump, domestic refuse, decayed organic substances, and chemicals. The study aimed to determine the prevalence and effects of pharmaceutical compounds in the rivers (streams) of Makhanda, Alice, and King William's Town and WWTPs found along these riverbanks. The aim was accomplished by determining the WWTP and river water's physical, chemical, and biological characteristics. In addition to this, five objectives were developed to achieve the aim of this study. The findings of seasonal changes and physical and chemical tests of Bloukrans, Buffalo, and Tyhume Rivers and WWTPs found along the riverbanks give us a hand in concluding about the quality of sampled water.

Nonetheless, the quality of water is influenced by rock type in particular zones. Data from the survey has shown that inorganic materials such as chloride, nitrate, sulfate, and phosphate may enrich water bodies and support the growth of algae that block the water's surface and turn the water body anoxic. The shortage of oxygen could yield to the fatality of aquatic organisms. Livestock farmers at the riverbank need to refrain from using excessive fertilizers to discharge in the stream directly. The immediate release of industrial and household sewage water in a river untreated is a severe risk to streams' water quality.

The microbiological quality of the above rivers and WWTPs was unsatisfactory and will constitute a severe health hazard to users who consume such water untreated. The colony counts identified bacteria overstepped the acceptable limits for potable water. Our suggestion is based on the obtained results; the significance of heightened interest for domestic pollution, environmental remediation control, and sensitization regarding water pollution should be imperative. Enhancing the quality of water would assist in the ceasing of transmissible enteric bacteria through polluted water. The state of the sampled areas' physical and chemical parameters calls for human health concern since some variables were not within the acceptable range of WHO, SANS, and TWQR guidelines.

Wastewater is used for agricultural irrigation since the resources of water are limited. When freshwater supply is scarce, farmers use wastewater for irrigating their crops, especially vegetables, because they need sufficient water. The greatest challenge is that toxicity symptoms could arise from contaminated plants with poisonous substances and the concentrations are significantly high enough. However, it is recognized that a severe threat resulting from recycled

wastewater is the possible existence of pathogenic organisms that pose risks for transmission of infections to mammals should they be exposed to infectious agents. The pH, COD, and TSS are parameters that adversely affect the growth of the bacterial population. Moreover, since wastewater is nutrient-rich, it is evident that vast quantities of the disease-causing agents are present and elevates the danger of infections coming from them.

Sewer water is being treated to eradicate disease-causing organisms and stop transmittable diseases, although complete eradication is not warranted. The durability of microbes in soil and below the surface water is a complicated matter that is dependent on the kind of pathogen, the type and state of the land, characteristics of water, and the survival of the natural microbial community. Pathogenic organisms in sewage can induce diarrhoea, migraine, spasms, and other things. Hence, these pathogenic organisms could result in a severe hazard to the health of acutely immunocompromised people.

There is a need to strengthen awareness about the extent of antimicrobial resistance concerns. The universal attention shows that AMR is not an unnoticed issue; every country encounters antibiotic resistance. Antibiotic-resistant bacteria are found in humans, nutritious substances, animals, and the environment, such as water, soil, and air. AMR endangers efficient prevention and treatment of infections resulting from bacteria. Consequently, drugs cease to be effective, and diseases endure in the human system, increasing transmission risk to others. In the absence of effective medications, the accomplishment of operation and chemotherapy would be at risk. People infected with ARB are in danger of fatality compared to people infected with non-resistant strains of bacteria. AR increases medical costs due to prolonged hospitalization, and more ICUs are in demand. According to the WHO report, resistance in *K. pneumonia* has been disseminated in all country areas.

Furthermore, resistance in *E. coli* has become widespread. Polymyxin E is the ultimate antibiotic for the treatment of infections resulting from Enterobacteriaceae. However, its opposition has been identified in various nations of the world, and diseases become incurable. The probiotics may be of help to reduce the load of AMR worldwide.

The ANN model has shown to be a practical approach to the prediction of water quality parameters. In further work, the focal point will be on the optimization model by merging several parameters and more dimensions of information to predict targeted variables to strengthen the model's precision. ANN can be helpful to save the resources for forecasting water quality parameters of massive data. Therefore, the ANN model will be suitable to predict the surface water quality at a manageable rate. Municipal governments must take into account to use of this model to manage the surface water resources efficiently.

Throughout the work, a method was prepared for determining the target and non-target screening quantities in streams and WWTPs. The qualitative research detected four antiretroviral drugs. The rivers and WWTPs were found to have been contaminated with increased levels of targeted and non-targeted composites. These analytes' quantification divulged dangers to health, as all concentrations have been above the announced thresholds. Nevertheless, the levels of concentrations were above the published maximum pollutant level

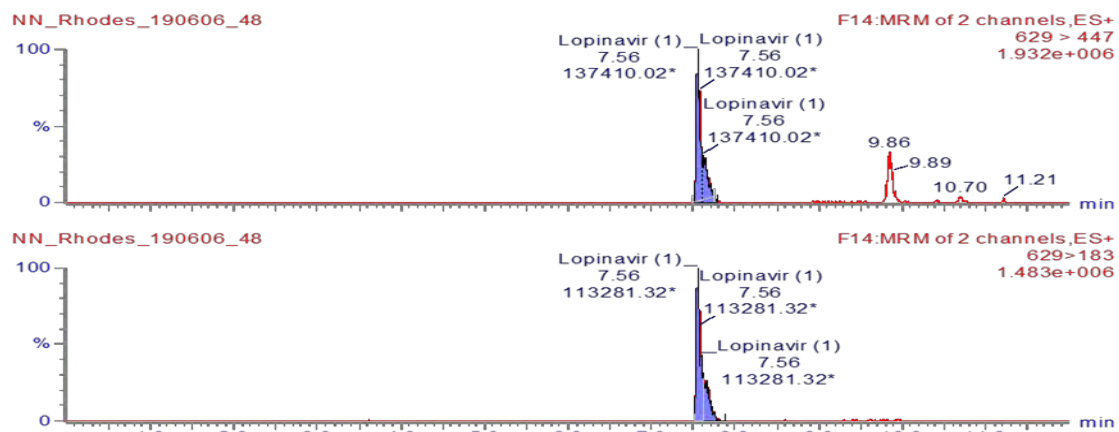
threshold. The wide variety of antiretroviral drugs consistently identified in streams and WWTPs indicates that qualitative research must be carried out most often to monitor antiretroviral drug levels in the rivers WWTPs that may affect public health. Liquid chromatography, coupled with mass spectrometry (LC-MS/MS), is a powerful analytical method that merges two-dimensions for the separation method and has shown its reliability and robustness in determining ARV drugs in river water and WWTPs. However, it was ineffective in the determination of contraceptive medications. Pharmaceuticals in the environmental field may adversely affect the health of the population at a world level. Decentralized Water Supply and Sanitation solutions, particularly in rural sectors, may help alleviate the immediate release of wastewater into surface waters.

There is very little laboratory Ecotoxicology information on pharmaceuticals' effects on aquatic creatures and an apparent scarcity of field information. There is also an evident lack of pharmaceutical medicines in aquatic surroundings in several regions. Prevention of pollution is among the most efficient instruments to protect the community and the surrounding. Obstruction of infection increases the supportable development, cuts out costs, and eradicates unwelcome squanders. Prevention of water contamination is simple, inexpensive, and effective compared with making an effort to remove its harm. The more contamination is minimized, the less we have to cleanse. Patient education must be introduced to suitable dumping of unwanted and expired drugs across the countries. The policy of contamination and surveillance of streams to diminish the unlawful release should be upgraded. For enhancing the efficiency of ozonation, extensive research is required to carry out investigations on the influence of sewage matrices on hydroxyl/ radical formation. Greater emphasis must be given to the ecological fate of poisonous secondary effects of ozonation and Fenton oxidation. For enhancing the application of this concoction method, further research is required to carry out investigations on ideal conditions such as pH, amount of hydroxyl peroxide, and temperature for a particular pollutant. Considering that eliminating pharmaceuticals during the WWTP processes is complicated due to the composition and heterogeneity prevention from the source is best to consider the impact of forms like organic materials and metal ions on eliminating drugs by ultraviolet/ hydroxyl peroxide must be examined in more detailed information. Depletion of human exposure to drugs via potable water may be reached through the amalgamation of precautionary measures, for instance, receiving community guidance and the consumer sector to promote an appropriate disposition of unwanted drugs and lower the entry of pharmaceuticals into the natural environment.

Additionally, it is essential to increase public awareness of the drinking-water quality crisis from a health perspective. For instance, transmitting to the community the possible dangers to health from the vulnerability of low levels of pharmaceuticals in potable water will aid them to get a better idea of crisis concerning additional risks viz. water-borne pathogens. There must be an initiation of a “take back” program where the pharmaceutical industry will be obliged to take back other drugs if no longer in use. Strict quality control measures must be implemented to ensure appropriate water and sewer water processing in these and different sewage plants.

Appendix A

A spectrum of target and non-target pharmaceutical compounds



Compound name: Lopinavir (1)
Coefficient of Determination: $R^2 = 0.998173$
Calibration curve: $4839.23 * x$
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None

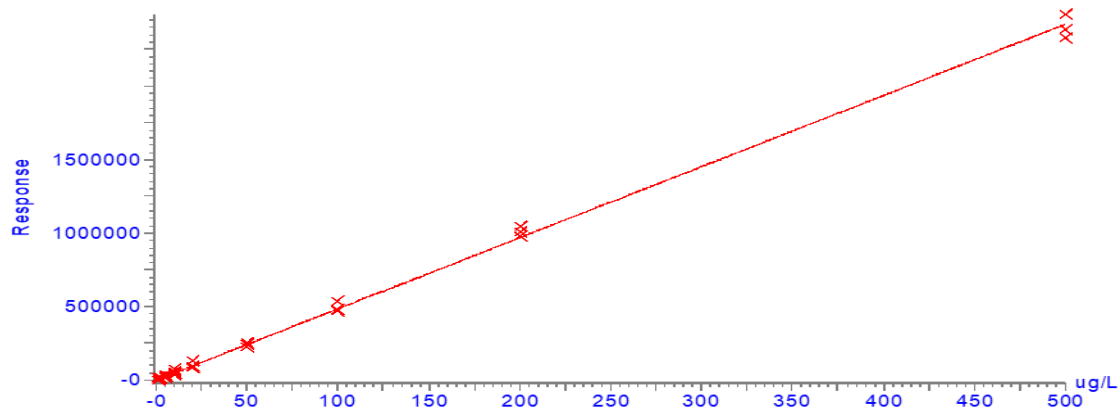
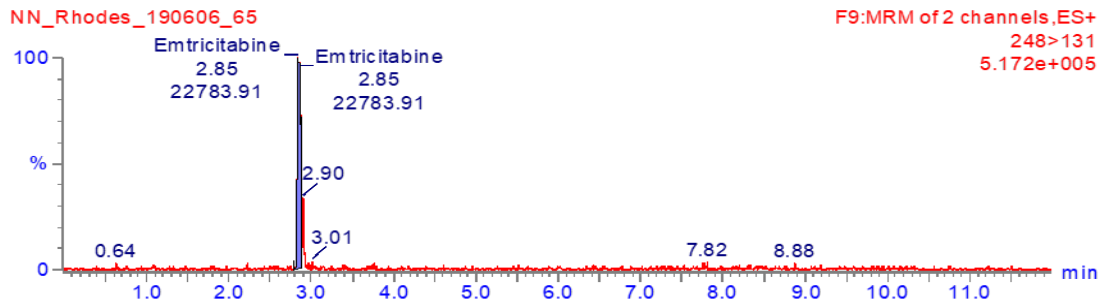
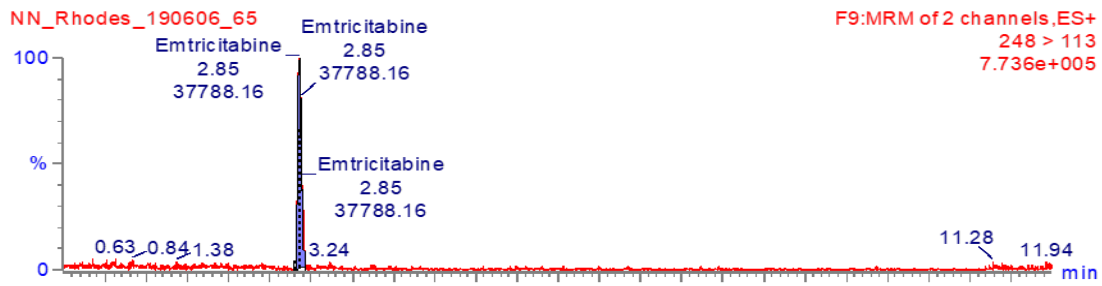


Figure A1: The spectrum of Lopinavir standard



Compound name: Emtricitabine
 Coefficient of Determination: R² = 0.999088
 Calibration curve: $-0.0968764 * x^2 + 211.09 * x$
 Response type: External Std, Area
 Curve type: 2nd Order, Origin: Force, Weighting: Null, Axis trans: None

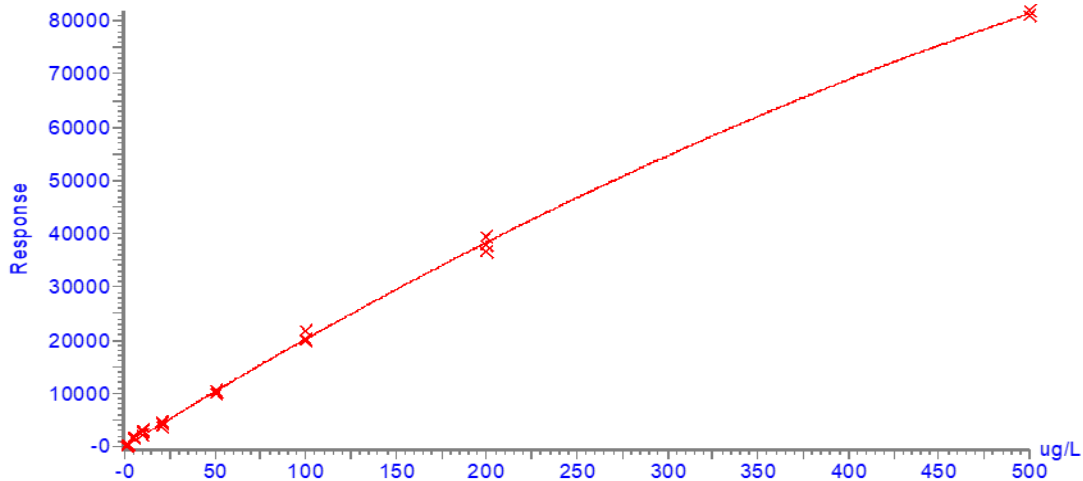
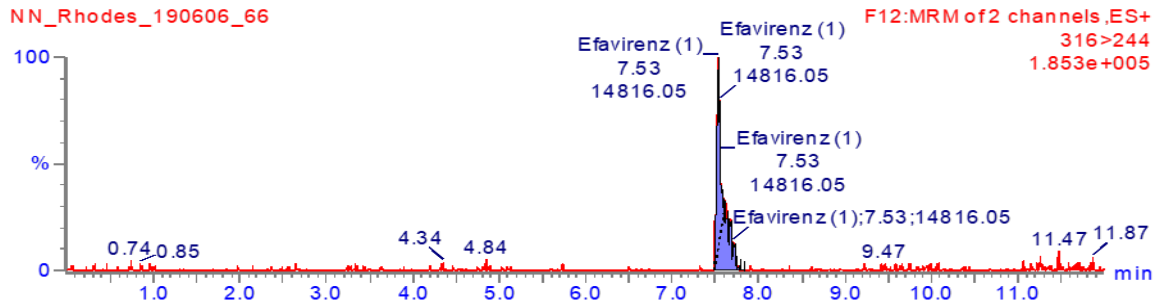
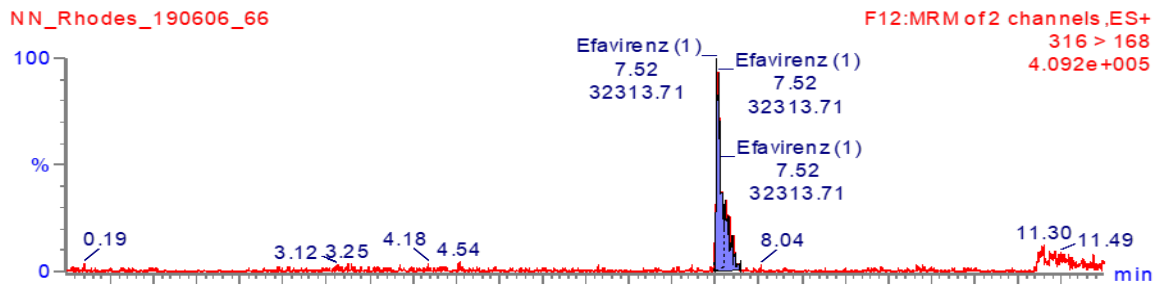


Figure A2: The spectrum of Emtricitabine standard



Compound name: Efavirenz (1)
 Coefficient of Determination: $R^2 = 0.994173$
 Calibration curve: $63.5271 * x$
 Response type: External Std. Area
 Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None

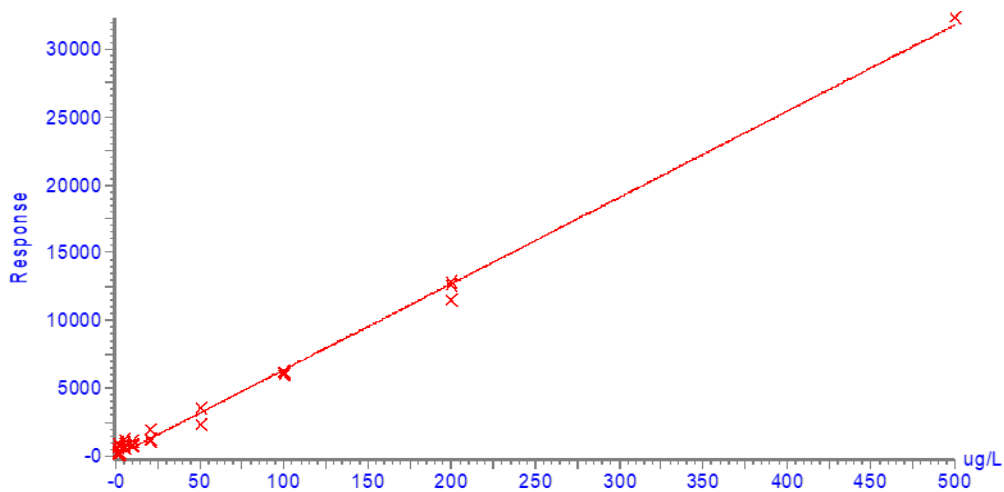
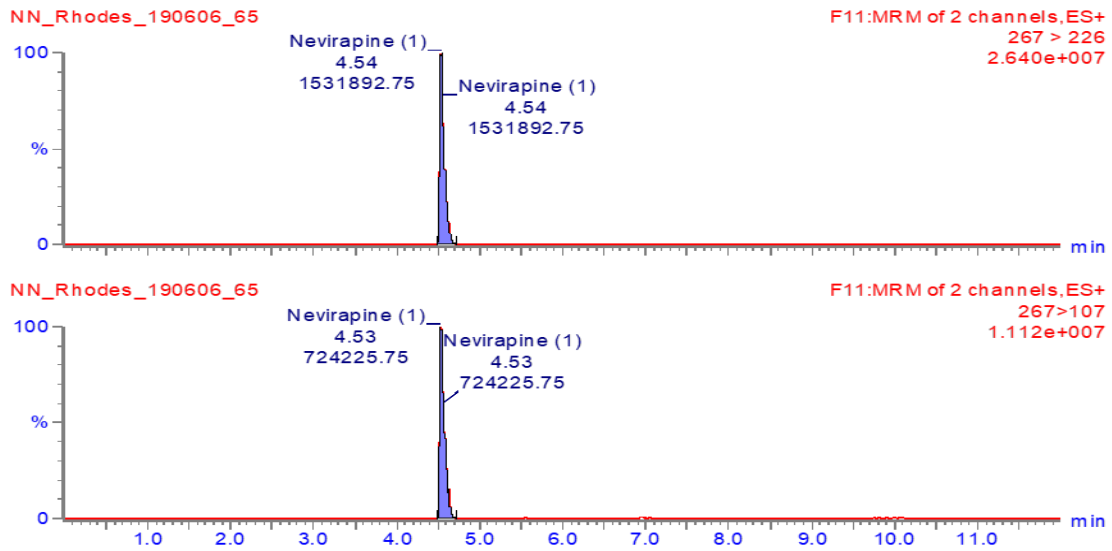


Figure A3: The spectrum of Efavirenz standard



Compound name: Nevirapine (1)
 Coefficient of Determination: $R^2 = 0.999672$
 Calibration curve: $7536.27 * x$
 Response type: External Std, Area
 Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None

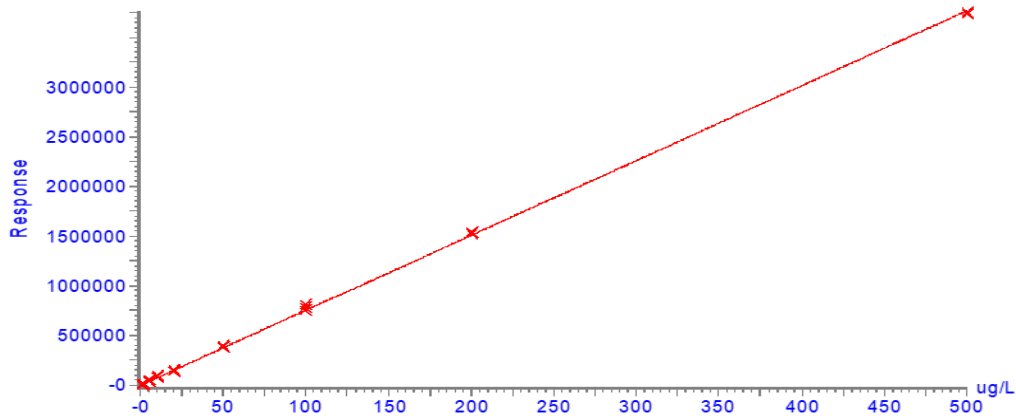


Figure A4: The spectrum of Nevirapine standard

CODING OF SAMPLES UNDER SEQUENCING

Table A1: Sample codes for gene sequencing.

<i>Sample_A</i>	AU
<i>Sample_B</i>	AM
<i>Sample_C</i>	AL
<i>Sample_D</i>	AE
<i>Sample_E</i>	AI
<i>Samples_F</i>	BU
<i>Sample_G</i>	BM
<i>Sample_H</i>	BL
<i>Sample_I</i>	BE
<i>Sample_J</i>	BI
<i>Sample_K</i>	GU
<i>Sample_L</i>	GM
<i>Sample_M</i>	GL
<i>Sample_N</i>	GE
<i>Sample_O</i>	GI