

Water quality of the Fonteinspruit stream on the outskirts of Bloemfontein, Free State, South Africa

By

Mongezi Adoons

Submitted in fulfilment of the requirements for the Degree

Master of Health Sciences in Environmental Health

Department of Life Sciences

Faculty of Health and Environmental Sciences

Central University of Technology, Free State

BLOEMFONTEIN

2020

Declaration

I, Mongezi Adoons, identity number _____ and student number _____, do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree Master of Health Sciences in Environmental Health, is my own independent work. This work complies with the code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, Free State; and has not been submitted before to any institution by myself or any other person for the attainment of a qualification.

.....

Mongezi Adoons

2020

I certify that the above statement is correct.

.....

Dr. Leana Esterhuizen (Supervisor)

Professor Annabel Fossey (Co-supervisor)

Acknowledgements

I wish to recognise the following people and organisations for their remarkable contribution towards the completion of this study.

- ✚ My supervisor, Dr Leana Esterhuizen, thank you for your continued support and leadership. Without your persistent help, the goal of this project would not have materialized.
- ✚ My co-supervisor, Professor Annabel Fossey, thank you for the great leadership and invaluable assistance. Although the journey felt overwhelming most of the time, your guidance pushed me through. You have assisted me in shaping and honing my skills throughout.
- ✚ The Department of Life Sciences at the Central University of Technology, Free State, thank you for the support and use of the laboratory facilities and equipment.
- ✚ The National Research Fund and Free State Provincial Government for funding my research project and tuition fees.
- ✚ To Patricia and Malcolm, thank you whole-heartedly for your support and assistance with countless things throughout this study.
- ✚ I wish to acknowledge the support and great love of my entire family, especially my mom.
- ✚ Lebo, I appreciate all your great support with lots of things.
- ✚ To all my friends, thank you for the encouragement and support.

Table of Contents

Declaration.....	i
Acknowledgements	ii
Table of Contents	iii
List of Tables	x
List of Figures	xiii
Abbreviations.....	xvii
Abstract.....	xx
Chapter 1	22
Introduction.....	22
1.1 Introduction	22
1.2 Aim and objectives	25
1.3 Layout of the dissertation	25
Chapter 2.....	28
Literature Review	28
2.1 Introduction	28
2.2 Water resources in South Africa	29
2.3 Water pollution.....	31
2.3.1 Water pollution through natural processes	32

2.3.2	Water pollution through human activities	33
2.3.3	Water pollution through emerging contaminants	35
2.4	Water quality	38
2.4.1	Physical properties	40
2.4.2	Chemical properties	42
2.4.3	Microbiological properties	46
2.5	Effects of polluted stream water	47
2.5.1	Effects of polluted stream water on humans	47
2.5.2	Effects of polluted stream water on the aquatic ecosystems	48
2.6	Assessment of water quality	50
2.6.1	Water quality index	51
2.7	Assessment of emerging contaminants	52
2.8	Assessment of ecological water quality	53
2.8.1	Aquatic macroinvertebrates as indicator organisms of ecological water quality	54
2.9	Conclusions	58
Chapter 3	59

Materials and Methods.....	59
3.1 Introduction	59
3.2 Study area.....	60
3.3 Water sampling sites	60
3.4 Data collection period.....	62
3.5 Study design	65
3.6 Water quality assessment.....	66
3.6.1 Measurement of physical properties	67
3.6.2 Measurement of chemical properties	69
3.6.3 Measurement of microbiological properties	74
3.6.4 Statistical analysis of water quality.....	75
3.6.5 Calculation of Water Quality Index.....	76
3.7 Emerging contaminant assessment.....	78
3.7.1 Emerging contaminant sample preparation	78
3.7.2 Emerging contaminant sample analysis.....	79
3.8 Ecological water quality assessment.....	79

3.8.1	Aquatic macroinvertebrate sample preparation and preservation	81
3.8.2	Aquatic macroinvertebrate sample analysis	81
3.8.3	Calculation of aquatic macroinvertebrate data	82
3.8.4	Interpretation of aquatic macroinvertebrate data	82
3.9	Conclusion	83
Chapter 4	84
Results of Water Quality in the Fonteinspruit Stream	84
4.1	Introduction	84
4.2	Results: Physical water quality properties	85
4.2.1	Electrical conductivity.....	85
4.2.2	pH.....	87
4.2.3	Turbidity.....	89
4.2.4	Temperature.....	90
4.2.5	Dissolved oxygen	92
4.2.6	Chemical oxygen demand	94
4.3	Results: Chemical water quality properties	96

4.3.1	Ammonia.....	96
4.3.2	Nitrate	98
4.3.3	Sulphate.....	100
4.3.4	Phosphate.....	102
4.3.5	Total hardness.....	103
4.4	Results: Microbiological water quality properties	105
4.4.1	<i>E. coli</i>	105
4.4.2	Total coliforms	107
4.5	Comparative analysis of the water sampling rounds	109
4.6	Overall water quality of the water samples.....	110
4.7	Summary	112
Chapter 5.....		114
Results of Emerging Contaminants on Water Quality in the Fonteinspruit Stream		114
5.1	Introduction	114
5.2	Results: Pharmaceutical analytes.....	115
5.2.1	Carbamazepine	115

5.2.2	Estrone.....	117
5.2.3	Estradiol.....	118
5.2.4	17a ethynylestradiol.....	119
5.3	Results: Plasticiser analytes.....	121
5.3.1	Bisphenol A.....	121
5.4	Results: Herbicide analytes.....	123
5.4.1	Atrazine.....	123
5.4.2	Metolachlor.....	124
5.4.3	Terbutylazine.....	126
5.5	Summary.....	128
	Chapter 6.....	129
	Results of Ecological Water Quality in the Fonteinspruit Stream.....	129
6.1	Introduction.....	129
6.2	Results of aquatic macroinvertebrates.....	130
6.2.1	Aquatic macroinvertebrate families.....	131
6.2.2	Sensitivity classification of aquatic macroinvertebrate families.....	133

6.2.3	Classification of the pollution condition	135
6.3	Summary	137
Chapter 7	139
Discussion and Conclusion	139
7.1	Introduction	139
7.2	Water quality in the Fonteinspruit stream.....	139
7.3	Overall water quality of the Fonteinspruit stream.....	144
7.4	Health conditions of Fonteinspruit stream	145
7.5	Limitations of the study.....	147
7.6	Suggestion of future studies.....	147
7.7	Recommendations.....	148
7.8	Practical applicability	148
7.9	Conclusion	149
Reference List	151

List of Tables

Table 3.1	Water sampling sites, coordinates, description and motivation for choice of site.	63
Table 3.2	CCME-WQI categorisation schema.....	77
Table 3.3	Modelled reference conditions for the Highveld – lower Ecoregion based on SASS5 and ASPT scores.....	82
Table 4.1	Electrical conductivity measurements in the three water sampling rounds and summary statistics.....	85
Table 4.2	The pH measurements in the three water sampling rounds and summary statistics.	87
Table 4.3	Turbidity measurements in the three water sampling rounds and summary statistics.	89
Table 4.4	Temperature measurements in the three water sampling rounds and summary statistics.	91
Table 4.5	DO measurements in the three water sampling rounds and summary statistics.	92
Table 4.6	COD measurements in the three water sampling rounds and summary statistics.....	94
Table 4.7	Ammonia measurements in the three water sampling rounds and summary statistics.	97
Table 4.8	Nitrate measurements in the three water sampling rounds and summary statistics.	98
Table 4.9	Sulphate measurements in the three water sampling rounds and summary statistics.	100

Table 4.10 Phosphate measurements in the three water sampling rounds and summary statistics. ...	102
Table 4.11 Total Hardness measurements in the three water sampling rounds and summary statistics.....	104
Table 4.12 <i>E. coli</i> measurements in the three water sampling rounds and summary statistics.....	106
Table 4.13 Total coliforms measurements in the three water sampling rounds and summary statistics.	108
Table 4.14 Statistical comparison results of physical, chemical and microbiological water quality properties in the three water sampling rounds.	109
Table 4.15 Water quality indexes and water quality ranges of water samples collected at the different water sampling sites.	111
Table 5.1 Carbamazepine concentration values in water sampling Round 1 and summary statistics.	115
Table 5.2 Estrone concentration values in water sampling Round 1 and summary statistics.....	117
Table 5.3 Estradiol concentration values in water sampling Round 1 and summary statistics. ...	118
Table 5.4 17a ethynylestradiol concentration values in water sampling Round 1 and summary statistics.....	119
Table 5.5 Bisphenol A concentration values in water sampling Round 1 and summary statistics.....	121

Table 5.6	Atrazine concentration values in water sampling Round 1 and summary statistics.	123
Table 5.7	Metolachlor concentration values in water sampling Round 1 and summary statistics.	124
Table 5.8	Terbutylazine concentration values in water sampling Round 1 and summary statistics.	126
Table 6.1	Description of aquatic macroinvertebrate families found at the 12 macroinvertebrate habitat water sampling sites.	130
Table 6.2	Aquatic macroinvertebrate families at the 12 water sampling sites.	132
Table 6.3	Aquatic macroinvertebrate families with their sensitivity scores.	134
Table 6.4	Number of taxa, SASS scores and ASPT values at the 12 water sampling sites.	135
Table 6.5	Classification of the SASS 5 score and ASPT values at the 12 water sampling sites.	136
Table 7.1	Overall summary of conditions at the water sampling sites and human-induced activities affecting the health status of the water sampling sites in the Fonteinspruit stream.	146

List of Figures

Figure 3.1	Study area of the Fonteinspruit stream and identified water sampling sites surrounded by settlements.....	61
Figure 3.2	Flow diagram of the study design of the project.....	66
Figure 3.3	Apparatuses used for the measurement of physical water quality properties a. HQ40d handheld multi-meter b. 2100Q Portable turbidity meter.	67
Figure 3.4	DR3900 spectrophotometer for the measurement of nitrate, ammonia, sulphate and total hardness.....	70
Figure 3.5	Example of the Colilert 97 MPN table used to match the counted large and small wells of <i>E. coli</i> and total coliforms.	75
Figure 4.1	Histogram showing the EC measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	87
Figure 4.2	Histogram showing the pH measurements at the 12 water sampling sites in the three water sampling rounds. The orange horizontal lines show the compliance range for pH...	88
Figure 4.3	Histogram showing the turbidity measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	90

Figure 4.4	Histogram showing the temperature measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	92
Figure 4.5	Histogram showing the DO measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	94
Figure 4.6	Histogram showing the COD measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	96
Figure 4.7	Histogram showing the ammonia measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	98
Figure 4.8	Histogram showing the nitrate measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. Orange horizontal line indicates the compliance range.	100
Figure 4.9	Histogram showing the sulphate measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds.	101
Figure 4.10	Histogram showing the phosphate measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	103

Figure 4.11 Histogram showing the total hardness measurements in water samples at the 12 water sampling sites in the three water sampling rounds.	105
Figure 4.12 Histogram showing the <i>E. coli</i> counts in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	107
Figure 4.13 Histogram showing the total coliforms counts in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.	109
Figure 5.1 Histogram showing the measurements of carbamazepine in water samples collected at the 12 water sampling sites in water sampling Round 1.....	116
Figure 5.2 Histogram showing the measurements of estrone in water samples collected at the 12 water sampling sites in water sampling Round 1.....	118
Figure 5.3 Histogram showing the measurements of 17a ethynylestradiol in water samples collected at the 12 water sampling sites in water sampling Round 1.	121
Figure 5.4 Histogram showing the measurements of bisphenol A in water samples collected at the 12 water sampling sites in water sampling Round 1.....	122
Figure 5.5 Histogram showing the measurements of atrazine in water samples collected at the 12 water sampling sites in water sampling Round 1.....	124
Figure 5.6 Histogram showing the measurements of metolachlor in water samples collected at the 12 water sampling sites in water sampling Round 1.....	126

Figure 5.7	Histogram showing the measurements of terbutylazine in water samples collected at the 12 water sampling sites in water sampling Round 1.....	127
Figure 6.1	Histogram showing the number of macroinvertebrate families at the 12 water sampling sites.....	133
Figure 6.2	Scatter plot of SASS 5 scores at the 12 macroinvertebrate habitat water sampling sites using Biological bands - Highveld lower Ecoregion.....	137

Abbreviations

AEV	Acute Effect Value
AMD	Acid Mine Drainage
ANOVA	Analyses of Variance
ASPT	Average Score per Taxon
ATP	Adenosine triphosphate
AWQUS	Aquatic Water Quality Limits for Urban Streams
B-IBI	Benthic Integrated Biotic Index
BI	Biotic Index
BPA	Bisphenol A
BMWP	British Monitoring Working Party
CCME	Canadian Council of Ministers of the Environment
CCME-WQI	Canadian Council of Ministers of the Environment's Water Quality Index
CE	Capillary Electrophoresis
CEV	Chronic Effect Value
cfu	Colony forming unit
Cl	Chloride
COD	Chemical Oxygen Demand
DO	Dissolved oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
<i>E. coli</i>	<i>Escherichia coli</i>
EC	Electrical conductivity
ECs	Emerging contaminants
EDCs	Endocrine disrupting compounds

EPA	Environmental Protection Agency
F	Fonteinspruit
GSM	Gravel, sand and mud
IAT	Immuno-analytical techniques
IBMWP	Iberian Biological Monitoring Working Party
ICI	Invertebrate Community Index
IHAS	Integrated Habitat Assessment
IHI	Index of Habitat Integrity
MA	Microbiological assays
MDGR	Millennium Development Goals Report
mg	Milligram
Mg	Magnesium
mIHAS	modified Invertebrates Habitat Assessment System
MS	Mass spectrometry
MPN	Most Probable Number
MUG 4	methylumbelliferyl- β -D-glucuronide
NH₃	Ammonia
NH₃⁻	Nitrite
NH₄⁺	Ammonium
NO₂⁻	Nitrate
NTU	Nephelometric turbidity units
°C	Degree Celsius
ONPG	θ -nitrophenyl- β -D-galactopyranoside
P	Phosphorus
PCPs	Pesticides, personal care products
PO₄³⁻	Phosphate

ppm	Parts per million
RBPs	Rapid Bioassessment Protocols
RHP	River Health Programme
SASS	South African Scoring System
SASS4	South African Scoring System version 4
SASS5	South African Scoring System version 5
SIC	Stones-in-current
SO₄²⁻	Sulphate
SOOC	Stones out of current
TDS	Total Dissolved Solids
TOC	Total organic carbon
TWQR	Target Water Quality Range
UN-DESA	United Nations Department of Economic and Social Affairs
US EPA	United States Environmental Protection Agency
VEG	Vegetation
WFD	Water Framework Directive
WHO	World Health Organization
WHO-OECD	World Health Organization and the Organisation for Economic Co-operation and Development
WQI	Water Quality Index
WWAP	World Water Assessment Programme
WWTP	Waste Water Treatment Plant
µS	Micro Siemens

Abstract

Introduction: The quality of water was measured in water samples collected at the 12 water sampling sites in the Fonteinspruit stream. The Fonteinspruit stream extends about 4.5 km in length and drains an area of about 17 km², wherein high-density residential areas, a Water Treatment Plant, industrial and agricultural areas as well as an informal settlement, are located. The contamination of the Fonteinspruit stream is attributed to human-induced activities such as improper disposal of solid waste and domestic sewage, animal manure and industrial effluent, which contain pollutants and other emerging contaminants. The contamination of this stream poses health risks to humans, animals and aquatic organisms.

Methodology: In this study, water quality was assessed in water samples collected in the Fonteinspruit stream in terms of conventional physical, chemical and microbiological properties as well as the presence of emerging contaminants, namely carbamazepine, estrone, estradiol, 17 α ethynylestradiol, bisphenol A, atrazine, metolachlor and terbuthylazine. The ecological quality of the stream was also assessed by enumerating aquatic macroinvertebrate families from which the quality of their habitats could be evaluated. A Water Quality Index was calculated for the water at each water sampling site to ascertain the overall water quality at a particular water sampling site and thereby obtain an understanding of the water quality in the Fonteinspruit stream. The South African Scoring System 5 was employed to measure the biological effects of polluted water on aquatic macroinvertebrate families. The health conditions of the water at each sampling site were classified in terms of the modelled reference conditions for the Highveld Ecoregion.

Results and Discussion: The water quality of the Fonteinspruit stream was found to be poor due to high levels of turbidity, electrical conductivity and phosphate and high bacterial counts of coliforms and *Escherichia coli*. The compliance rate was below 20% for many of the measurements, particularly for turbidity, phosphate and total number of coliform bacteria as well as the emerging contaminants,

such as carbamazepine, estrone, estradiol, 17 α ethynylestradiol, bisphenol A, atrazine, metolachlor and terbuthylazine. The presence of only pollution tolerant aquatic macroinvertebrate families strongly supported the notion that ecological conditions of the water at all 12 water sampling sites in the Fonteinspruit stream were critically impaired.

Conclusion: In conclusion, water quality of the Fonteinspruit stream was deteriorating; that human-induced activities were affecting the water quality in the stream; that the poor water quality in the Fonteinspruit stream was affecting the aquatic macroinvertebrates and their diversity. Finally, human and animal health could be at risk if they accidentally drink the contaminated water in the Fonteinspruit stream during swimming or if they eat vegetables irrigated with the contaminated water.

Chapter 1

Introduction

1.1 Introduction

Water is an essential natural resource required by all living organisms for their survival. Less than 1% of the earth's water resources are readily available to humans as fresh water in the form of surface or groundwater (Zia et al., 2013). Surface water is water on the surface of the planet, such as in a river, lake, wetland, or ocean. Non-saline fresh water sources are mainly used for human use. With the ever-increasing global populations, the demand for water remains high. As a result, about 1.1 billion people do not have access to an adequate supply of water (Ayandiran et al., 2018). In South Africa, many of the surface water sources have declined, mostly because of changing weather patterns. The uneven distribution of water, increased usage and wastage as well as water pollution, have all given rise to the water crisis around the world (Van der Hoven et al., 2017).

The rivers represent the major source of water for human consumption, irrigation and industrial purposes. In South Africa, most rivers are small and are referred to as streams. Streams are considered to be the first- and second-order channels of a watercourse within a river network (Ding et al., 2016). Rivers and streams transport water, nutrients, inorganic and organic materials and sediments from uplands to downstream systems (Ferreira Marmontel et al., 2018). In addition, rivers and streams contribute to the biodiversity by supporting a number of biological organisms, such as aquatic macroinvertebrates, including algae (Singh & Saxena, 2018).

In South Africa, water from rivers, streams and dams is used for a wide variety of purposes. Although the vast majority of the water is used for agricultural purposes, water is also used for domestic purposes, such as cooking, washing and drinking. Other uses of water include recreational use, such as swimming, high diving and fishing. Several communities also use water to perform cultural rituals.

The quality of the water in many South African rivers and streams has declined in recent times (Rossouw, 2011). This decline can be attributed to natural processes and, more recently, to anthropogenic activities, which are linked to population expansion, urban and industrial growth as well as agricultural activities (Garcia et al., 2017; Pasternak et al., 2017). As a result of human and industrial activities, emerging contaminants (ECs) have recently been discovered in several streams, thereby further degrading the quality of the water (Talib & Randhir, 2017). These contaminants include large quantities of medicines, disinfectants, laundry detergents, pesticides, dyes and paints that are flushed, washed or discarded in the drainage system (Grassi et al., 2012).

Poor quality water has adverse effects on humans and the environment. Humans, in particular, are at risk of being exposed to waterborne pathogens that cause waterborne infectious diseases. Many waterborne infectious diseases associated with polluted water include cholera, typhoid and hepatitis A. It has been estimated that over 3 million people die from preventable water-related diseases each year (World Health Organization [WHO] & the Organisation for Economic Co-operation and Development [OECD], 2003). Diarrhoeal diseases account for over 2 million deaths annually and many of these deaths involve children under the age of five (WHO–OECD, 2003). The continuing presence of contaminants in the water can also affect the survival of aquatic organisms and ultimately reduce their diversity.

Rivers and streams are continually being enriched with nutrients from the environment. These nutrients often come from agricultural lands and households as wastewater or runoff. The major constituents of agricultural wastewater or runoff include animal manure, which is applied to the soil as the fertiliser to increase crop production, and pesticides and herbicides, which are applied to protect the vegetation (Trang et al., 2017). Fertilisers are rich in phosphorous, potassium and nitrogen, and when deposited in the soil can then be carried with the surface water runoff into the rivers and streams (Trang et al., 2017). Municipal wastewater may carry industrial or domestic effluent containing

nutrients, such as ammonia and nitrogen. The introduction of nutrients into rivers and streams may result in eutrophication, which may lead to the production of algal blooms and the reduction of dissolved oxygen (Guldhe et al., 2017).

Substantial changes in the quality of water may result in major disturbances on aquatic life. These changes can be brought about by various key stressors, which include climatic conditions and stream flow as well as the physical, chemical and biological processes, most of which can be attributed to human activities, such as alteration of land cover, deforestation and pollution (Matthews & Bernard, 2015; Trang et al., 2017). High levels of nutrients in the water promote excessive growth of toxic algal blooms and green plants. When the algae die, they become food for bacteria that decompose them. With more food available in the water, bacterial populations increase and thus use more of the dissolved oxygen. Low levels of dissolved oxygen affect the survival of fish and other aquatic organisms and may result in the reduction of their numbers in such conditions (Guldhe et al., 2017).

The Fonteinspruit stream is one of the streams found in the Bloemfontein area. The Fonteinspruit stream is approximately 4.5 km and flows into the Blou dam. Blou dam is a small reservoir and joins the Bloemspruit at the lower end of the catchment. The Bloemspruit further joins the Renosterspruit stream before flowing into the Modder River on the outskirts of Bloemfontein. The Fonteinspruit stream drains an area of about 17 km², which is characterised by rapidly developing residential areas, including Erlich Park, Heidedal, Batho, Bochabela, Phahameng and several informal sections of Mangaung. Along the Fonteinspruit stream there are also several industries, which include metal, building, food and engineering industries (Pretorius et al., 2002).

Several studies have shown that the Fonteinspruit stream is highly polluted. Pretorius et al. (2002) revealed that there was considerable bacterial pollution, high levels of dissolved inorganic nitrogen and dissolved solids in the water in the Fonteinspruit stream. Nyoh (2015) also demonstrated that

areas surrounding the Fonteinspruit stream are often plagued by sewer blockages, resulting in the municipal sewage flowing into the stream.

1.2 Aim and objectives

Both Pretorius et al. (2002) and Nyoh (2015) studies strongly suggested that the water quality in the Fonteinspruit stream was continually being degraded by human activities, such as pollution through leaking and blocked sewage pipes, farming, widespread littering and the existence of illegal dumping sites in the vicinity. This study was, therefore, undertaken to assess the water quality in the Fonteinspruit stream to establish whether the water quality in the stream was still undergoing degradation or whether some intervention has occurred to improve the quality of the water. The study was comprised of two major areas of investigation. In one investigation, the quality of the water in the Fonteinspruit stream was analysed, while in the other investigation, the impact of the stream water quality on the aquatic macroinvertebrate populations and habitats was analysed.

To meet this aim, the following objectives were formulated:

1. To identify suitable sampling sites along the Fonteinspruit stream.
2. To analyse the quality of the water in terms of physical, chemical, and microbiological properties.
3. To analyse the quality of the water in terms of the emerging contaminants.
4. To analyse the quality of the water in terms of its influence on aquatic macroinvertebrates population and their habitats.

1.3 Layout of the dissertation

This dissertation comprises seven chapters.

Chapter 1: Introduction

In Chapter 1, the problem statement, aim and objectives are presented.

Chapter 2: Literature Review

In Chapter 2, a comprehensive review of the literature is provided. This literature review covers topics such as water resources in South Africa, water pollution, water quality, effects of polluted stream water, assessment of water quality, assessment of emerging contaminants on water quality, and assessment of ecological water quality.

Chapter 3: Materials and Methods

In this Chapter, a summary of the materials and methods used in carrying out the research are provided.

Chapter 4: Results of water quality in the Fonteinspruit stream

In this Chapter, the results of the water quality assessment at the respective water sampling sites in the Fonteinspruit stream, together with the interpretation of the results, are presented.

Chapter 5: Results of emerging contaminants on water quality in the Fonteinspruit stream

In this Chapter, the results of the emerging contaminants on the water quality at the respective water sampling sites in the Fonteinspruit stream, together with the interpretation of the results, are presented.

Chapter 6: Results of ecological water quality in the Fonteinspruit stream

In this Chapter, the results of the ecological water quality assessment at the respective water sampling sites in the Fonteinspruit stream, together with the interpretation of the results, are presented.

Chapter 7: Discussion and Conclusion

In this Chapter, an overall discussion of the main findings of the assessment of the water quality in the Fonteinspruit stream, together with the main conclusion of the study, is presented.

References The references in this dissertation have been prepared using the reference manager Mendeley.

Chapter 2

Literature Review

2.1 Introduction

All living organisms require water for their growth and maintenance of their biological activities. About 70% of the earth's surface is covered by water. Of this water, approximately 2.5% is renewable freshwater, or by volume, that is about 34.5 million cubic kilometres (Liang et al., 2018; World Water Assessment Programme [WWAP], 2019). Large quantities of the earth's freshwater is inaccessible as it is trapped in the ice caps, which equates to approximately 200,000 cubic kilometres of usable freshwater on earth (WWAP, 2019).

Water sources include surface water in lakes, springs or rivers as well as subsurface water, mostly accessed through wells. One of the major challenges of the 21st century is access to safe drinking water (Mhlongo et al., 2018; WWAP, 2019). Water scarcity affects every continent and hinders the sustainability of natural resources, as well as economic and social development of many populations. The Millennium Development Goals Report (MDGR) of 2015 recorded that water scarcity affects more than 40% of people around the world, and is expected to increase (United Nations Department of Economic and Social Affairs [UN-DESA], 2015). In South Africa, the attainability of fresh water is considered limited and vulnerable, in terms of availability, quantity and quality (Mhlongo et al., 2018). The limited water supply poses a risk to all the needs of surface water (Singh & Saharan, 2010; Cañedo-Argüelles et al., 2016).

Water scarcity is one of the major environmental issues in developing countries. The increasing demand for water is mostly attributed to population increase and climate change (Lee et al., 2017). The population growth, along with an increased water demand for human activities, such as

industrialisation and urbanisation, is challenging to the sustainability of water resources (Dey & Mishra, 2017). Worldwide, the proportion of urban communities that have access to safe water is much greater than rural communities. Currently, 96% of urban communities have access to safe drinking water, compared with the 84% of rural populations (UN-DESA, 2015). The proportion of the global rural populations without access to safe drinking water has declined by more than half from 38 to 16% since 1990 until 2015 (UN-DESA, 2015). Similarly, when comparing water supply, four out of five people living in urban areas have access to piped drinking water, while only one in three people living in rural areas has access to piped drinking water.

Surface water plays an important role in many biological processes. Surface water, such as rivers and streams, is used for domestic purposes, irrigation for food production, industrial and recreational activities (Barakat et al., 2016; Zhai & Tao, 2017). Rivers and streams act as channels for the transport of water, nutrients, inorganic and organic materials (Ferreira Marmontel et al., 2018). Rivers and streams also support aquatic organisms, such as aquatic macroinvertebrates, algae and fish (Singh & Saxena, 2018). Rivers and streams provide habitats for more than 120,000 species of aquatic macroinvertebrates (Matthews & Bernard, 2015).

2.2 Water resources in South Africa

South Africa is an arid country. It is ranked as the 30th driest country in the world (GreenCape, 2017). The availability of water varies extensively from place to place and over time. The western regions of South Africa are relatively dry with rainfall during the summer, and an average as low as 100 mm (GreenCape, 2017). In contrast, the eastern and south eastern regions receive rainfall throughout the year, with an average rainfall of up to 1,000 mm. The total annual surface runoff is estimated at 43 to 48 km³ (Mwendera & Atyosi, 2018).

Surface water occurs inland permanently or intermittently. Surface water is found in lakes, rivers, and reservoirs (Amouei et al., 2012; Sardar et al., 2013). Lakes form through the natural flow of water that moves under the force of gravity along channels and accumulates in depressions in the earth (Khattak et al., 2012). Lakes are fed by surface water runoff and rivers (Owa, 2014). When water is not trapped in a lake, the lake is only temporary. This may occur when the water flow is fast allowing the water to flow into a river or the water seeps into the ground or it evaporates (Department of Water Affairs [DWA], 2012). Several factors determine the size of a lake. These factors include the origin of the depression where water accumulates, the water regime, river channel stability, water exchange characters, water balance structure, temperature and dissolved load (Lazarova et al., 2011). In contrast to a naturally formed lake, a human-made inland lake is referred to as a reservoir.

Groundwater is fresh water found in the subsurface pore space of soil and rocks along which it travels and fills openings in the rocks. The spaces in the rocks that store and transmit groundwater are referred to as aquifers. Large porous aquifers occur only in a few areas in South Africa (Ponsadailakshmi et al., 2018). However, in the rural and more arid areas, people rely on groundwater as their primary water source (DWA, 2013). In South Africa, it is expected that groundwater use will increase, especially in the western part of the country, where perennial rivers do not occur (GreenCape, 2017).

In South Africa, both mountain and lowland rivers occur. Mountain river water flows rapidly along narrow valleys, while the water in lowland rivers flows slower in wider channels, often with terraced valleys (Khan et al., 2013). In South Africa, there are only a few large rivers. Most rivers are small and can be described as streams. The main rivers in South Africa are the Orange River that drains to the Atlantic Ocean, while the Limpopo River, Incomati River, Maputo River, Tugela River, Olifants River (Limpopo), and Breede River all drain to the Indian Ocean (GreenCape, 2017).

Since cities often develop around a water supply, many South African streams flow through urban areas (Sarkar & Abbasi cited in Nyoh, 2015). Four main stream types are distinguished (Rossouw, 2011). Ephemeral streams are streams that flow for less than a month in a year and only after rain. Intermittent or temporary streams, on the other hand, are streams that flow periodically for more than three months in a year. Semi-permanent streams flow most of the year, but may stop flowing during dry periods. The fourth type of stream is a permanent stream. This type of stream flows indefinitely, but could stop flowing under extreme drought. Most South African streams flow intermittently (Rossouw, 2011).

2.3 Water pollution

Water pollution occurs when harmful substances, such as chemicals or microorganisms, accumulate in surface water bodies making them toxic to humans and/or the environment. According to Olaniran in 1995, water pollution is defined as the presence of excessive amounts of pollutants, which cause the water to be unsuitable for drinking, bathing, cooking or other uses (cited in Owa, 2014). In South Africa, acceptable water resources continue to decline because of resource depletion and pollution. The continual decrease in water resources and their pollution affects social and economic development negatively and is closely linked to the prevalence of poverty, hunger and diseases (Ochieng et al., 2010).

Surface water bodies become polluted through natural processes or through mechanisms of displacement and dispersal related to human activities. However, water degradation is mostly as the result of human activities (Han et al., 2016; Daud et al., 2017; Liu et al., 2017; D'Ugo et al., 2018). Pollutants enter the water environment from two main types of sources. These sources include point sources and non-point sources (Varol & Şen, 2009). A point source is a single, identifiable source of pollution, such as a pipe or a drain. Pollutants from point sources are discharged either into surface water or groundwater through an area that is small relative to the area or volume of the receiving water

body (Tsuzuki, 2015; Trang et al., 2017). For example, industrial wastes are commonly discharged into rivers and the sea in this way. In contrast, non-point sources, often termed 'diffuse' pollution, refers to pollution that occurs over a wide area and cannot easily be attributed to a single source (Khan et al., 2004; Vrebos et al., 2017). Non-point sources are often associated with particular land uses and deposition from the atmosphere, either by precipitation (wet deposition) or by dry fallout (dry deposition). Fertilisers and pesticides from agricultural fields also release contaminants into surface water bodies (Jerome & Pius, 2010; Zia et al., 2013; Hofstra & Vermeulen, 2016; Howladar, 2017). Consequently, the deposition of pollutants in surface water resources threatens human and animal health and their associated ecosystems (Molina-Navarro et al., 2018).

In 2002, there was a study carried out to understand the major causes of pollution in the study area. This study showed that bacterial pollution was high and that the levels of dissolved inorganic nitrogen and dissolved solids were high (Pretorius et al., 2002). Another study showed that areas surrounding the Fonteinspruit stream were plagued by sewer blockages, with the overflow running into the stream (Nyoh, 2015).

2.3.1 Water pollution through natural processes

Natural pollution of water bodies occurs mainly through geological influences and rainfall. The geological composition of areas that surround river drainage basins influences the content of the water in these basins. Rocks in the drainage basins are slowly dissolved by carbonic and sulphuric acids that occur in the atmosphere and absorbed by rain (DWA, 2004). The dissolved rocks increase the sediment load and also alter the acidity of the water running along the drainage basins (DWA, 2004). Heavy metals, such as lead, mercury, zinc, cadmium and arsenic from the geological surroundings, are often deposited with natural sediment in the bottoms of stream channels (Khattak et al., 2012). Many of these heavy metals are dangerous pollutants, for example, mercury contamination of aquatic ecosystems has been known for a long time (Khattak et al., 2012). One of the best-known examples

of mercury toxicity occurred in the coastal town of Minamata on Kyushu island of Japan in the middle of the 20th century when people became seriously ill because of high toxic levels of mercury in the water (Khattak et al., 2012).

Rainfall also contributes to the pollution of surface water bodies. Rainfall pollutes water bodies by depositing the contents of runoff gathered from the vicinity, such as plastics, papers, faeces and sewage, into nearby surface water bodies (Chigor et al., 2013). In addition, runoff may also carry plant debris and sand, silt and clay into rivers and streams, which results in muddy, turbid water. Severe rainfall events lead to excessive erosion and landslides, which may dramatically increase the content of the suspended material in affected rivers and lakes (Palmer et al., 2004). Arsenic may enter surface water bodies through rainfall. The occurrence of arsenic in drinking water is now recognised as a global problem. For example, arsenic in groundwater in Bangladesh affected millions of the poorest people in the world (Khattak et al., 2012).

2.3.2 Water pollution through human activities

A great variety of human activities also contribute to the pollution of surface water bodies. Indiscriminate industrial, household, mining and agricultural waste disposal practices are major contributors to the pollution in water bodies (Owa, 2014). With the ever-increasing world population, the disposing of sewage wastewater has also become a substantial problem. In poorer and developing countries, many townships and municipalities do not have the means to dispose household waste (Chigor et al., 2013). Treatment capabilities for sewage and wastewater are also lacking (Minolfi et al., 2018). As a result, large amounts of sewage-polluted untreated water are discharged into surface water bodies every day, contaminating the water intended for drinking and other uses (Zamxaka et al., 2004; Bodrud-Doza et al., 2016).

Manufacturing industries as well as the mining sector are major contributors to water pollution. These industries are often responsible for point source pollution by discharging chemicals and industrial waste into streams, rivers or the sea. Chemical production by metal processing and smelting has been proven to be a primary source of heavy metal pollution in surface water (Yang et al., 2018). Tie and dye industries produce chemicals, such as zinc sulphate and copper salts, which have devastating effects on aquatic ecosystems when discharged into rivers (Owa, 2014).

Agriculture is another major contributor to the pollution of surface water bodies. Through the application of pesticides and fertilisers and also poor sewage disposal, nutrients enter water bodies, mainly through runoff (Ambani & Annegarn, 2015). Nutrient enriched runoff causes excessive algae growth, mainly through enrichment with nitrogen and phosphorus. The enrichment of excessive amounts of nutrients, known as eutrophication, can lead to low levels of dissolved oxygen in the water, which together with algal bloom growth, blocks much needed light penetration for other aquatic organisms, causing the death of plants, fish and other aquatic animals (Yang et al., 2018).

The mining sector contributes to the degradation of water quality in water bodies in various ways. The impact of mining pollution depends on the type of minerals and chemicals used in the metal extraction processes, climate and life stage of the mine as well as environmental management practices that are in place (Dold, 2014). In mining, large volumes of water are used to process the ore, which generates excessive amounts of chemicals, heavy metals, soil and other waste rock materials. Metals, such as arsenic, cadmium and lead, leach from mining sites into the surroundings and contaminate the environment and surface water bodies in the vicinity of the mines (Minolfi et al., 2018). Besides the heavy metals that leach during the processing of ore, sulphide minerals that are also present in the mining rocks leach from mining sites when exposed to water and oxygen through a process known as acid mine drainage (AMD), which is also referred to as acid rock drainage (Dold, 2014).

AMD has far reaching effects on the environment. The resulting sulphuric acid creates acidic conditions that speed up the leaching of heavy metals from the rocks (Ambani & Annegarn, 2015). Thus, environments surrounding mines often contain elevated concentrations of metals and metalloids (Masindi & Khathutshelo, 2018). A study of groundwater in the mining district of Johannesburg by Naicker et al. in 2003 revealed that the groundwater was acidified and contaminated with elevated concentrations of heavy metals as a result of the oxidation of pyrite contained in the mine tailings near the mines (cited in Ochieng et al., 2010). AMD contamination has also gradually increased around multiple dams, such as the Middelburg, Witbank and Boesmanspruit dam in South Africa. The effect of AMD on the Boesmanspruit dam that supplies potable water to Carolina, Mpumalanga, was studied in 2012. This study showed dam water pH levels as low as 3.7 as well as high concentrations of iron, aluminium, manganese and sulphate, which indicated that the water was toxic (McCarthy & Humphries, 2013).

2.3.3 Water pollution through emerging contaminants

Emerging contaminants (ECs) are synthetic or naturally occurring chemicals that are not commonly monitored in the environment, but have the potential to enter the environment and cause known or suspected adverse ecological and/or human health issues. ECs are consistently being found in surface water, groundwater, municipal wastewater, drinking water and food sources (Blum et al., 2018; Mandaric et al., 2018). It is estimated that more than 80,000 chemicals are produced and used worldwide, with more than 2,000 produced in amounts exceeding 450 tons per year (McKnight et al., 2015). The threat lies in the fact that the environmental and human toxicology of most of these compounds has not yet been studied. Furthermore, many ECs are not or cannot be tested for in municipal water systems (Talib & Randhir, 2017). As a result, ECs may bio-accumulate in the environment and in various aquatic macroinvertebrates and other aquatic organisms, which form part of the aquatic food web (Inostroza et al., 2017).

The major groups of ECs are pharmaceuticals (both over-the-counter-drugs and prescription), pesticides, personal care products (PCPs) and endocrine disrupting compounds (EDCs). Other groups of ECs include industrial chemicals, surfactants and flame retardants. Pharmaceuticals are a group of ECs that are broadly and progressively being used as part of human and veterinary medication (Rodriguez-Narvaez et al., 2017). It is estimated that approximately 3,000 different substances are used as pharmaceuticals, including pain-killers, antibiotics, anti-diabetics, beta blockers, contraceptives, lipid regulators, antidepressants and impotence drugs (Agunbiade & Moodley, 2014). Only a small subset of these ECs has been investigated in environmental studies (Sorensen et al., 2015; Gogoi et al., 2018). The major routes of entry of pharmaceuticals into the water bodies are via human excretion, veterinary use and disposal of unused products (Hamza et al., 2016; Philip et al., 2018). Their presence in surface water bodies has caused estrogenic and endocrine-related effects on humans and aquatic organisms (Richardson & Kimura, 2017; Rodriguez-Narvaez et al., 2017).

Pesticides refer to a wide range of compounds that are typically categorised on the basis of their actions. Nearly 20,000 pesticide products have entered the market since registration began in 1947 (Pintado-Herrera et al., 2017). Pesticides include herbicides, fungicides, insecticides, nematicides and plant growth regulators (McKnight et al., 2015). This class of ECs is extensively used for crop control and management in the agricultural sector, golf course maintenance and landscaping as well as grass management at industrial sites and public recreational areas (Huber et al., 2016; Weber et al., 2018). It is estimated that over 2 million tons of active ingredients are used each year, predominantly in agriculture (Rocha et al., 2018). Pesticides may reach the soil through precipitation, irrigation water and wind (Hamza et al., 2016). These chemicals can be transported from the land surface to surface water bodies or groundwater through runoff and infiltration (Bai et al., 2018); and as effluent from municipal water treatment plants, which gets discharged into the receiving water bodies (Hamza et al., 2016).

PCPs are a group of synthetic chemicals or substances used on a daily basis. These synthetic chemicals include perfumes, soaps, cosmetics, lotions and sunscreens (Montes-Grajales et al., 2017). A variety of chemicals used in the production of PCPs intended for external use, have been described as potentially hormone disrupting substances (Rimayi et al., 2018). Because of their regular and extensive use, PCPs are constantly released into the environment via discharged household wastewater after showering, bathing and cleaning (Pivetta & Gastaldini, 2019). Many PCPs are bioactive and may persist and bio-accumulate in the environment (Hamza et al., 2016; Polidoro et al., 2017; Rimayi et al., 2018).

Endocrine disrupting chemicals (EDCs) have been detected in discharged effluent from industrial and wastewater treatment plants, and have been found in surface water bodies and even in drinking water. EDCs are characterised as artificial chemicals that, when ingested into the body, can either copy or obstruct hormones and thus affect the body's normal functioning (Raghav et al., 2013; Gogoi et al., 2018). EDCs often come from industrial products, such as plasticizers, flame retardants, synthetic hormones, detergents and pesticides, and natural hormones (Dotan et al., 2017; Wooding et al., 2017; Song et al., 2018). In surface water, EDCs can have adverse reproductive effects in aquatic organisms, such as fish species (Le Thi Minah et al., 2016; Gogoi et al., 2018).

ECs may enter surface water bodies through various ways. The sources of ECs include both point sources of pollution, like the discharge of wastewater, and non-point sources of pollution, such as sewer leakages and sewer overflow (Lei et al., 2015; Ebele et al., 2017). Healthcare facilities are major contributors of ECs in surface water through the discharge of wastewater and expired drugs into streams and rivers (Gogoi et al., 2018). Unused pharmaceuticals, disposed of in toilets or drains, as well as partially or un-metabolised medication excretions by humans, have been shown to be a significant source of prescription and non-prescription drug contamination (López-Doval et al., 2017; Wilkinson et al., 2017).

Agriculture and industries are other contributors to the enrichment of surface water bodies with ECs. EC enrichment from agriculture is through runoff from agricultural fields and wildlife animal waste as well as illegal waste discharge. The wastewater effluent from agricultural lands often contains animal manure and fertilisers, which are rich in nutrients, such as nitrogen and phosphorus (Guldhe et al., 2017; Matamoros & Rodríguez, 2017). Hormones and drugs, such as antibiotics, injected into animals or fed to fish also end up in water bodies through wastewater and surface water runoff (Gogoi et al., 2018). A wide range of industrial wastewater, especially from agricultural and food industries, contains a composition of chemically and biologically degradable and non-degradable substances, organic matter and inorganic matter, which can severely deplete dissolved oxygen in an aquatic environment (Guldhe et al., 2017). ECs may also end up in surface water bodies as a result of illegal or poorly managed wastewater effluent, sewer leakages and overflow discharges (Sorensen et al., 2015).

Municipal wastewater is a significant source of ECs as a result of wastewater produced in households, institutions and commercial buildings. Nutrients, such as ammonia, phosphate and other inorganic substances, are drained or flushed with municipal wastewater and may end up in the aquatic environment as part of sewage discharged directly into the receiving water bodies (Gogoi et al., 2018). Leaky sewer and septic systems may further increase the presence of ECs when the wastewater enters the receiving water bodies as part of surface water runoff (Talib & Randhir, 2017).

2.4 Water quality

One of the unique characteristics of water is its excellent dissolving capability. During the hydrological cycle of water, the water may come into contact with a wide range of substances, which may be dissolved to a greater or lesser extent (Department of Water Affairs & Forestry [DWAF], 1998). The type of substances as well as the amount of the substances determines the properties of the water. Oxygen and carbon dioxide are important gasses that dissolve in water (Rossouw, 2011). Inorganic compounds that dissolve in water include sodium chloride and calcium sulphate, while organic

substances include humic acids and carbohydrates (Water Research Commission [WRC], 1998). Besides the dissolved substances found in water, substances that do not dissolve, but remain in suspension as very small suspended or colloidal particles, are also found in water. Such suspended substances, in particular microorganisms, also affect the quality of water. Thus, to evaluate the quality of water, the concentrations of dissolved substances are determined, together with physical and microbiological properties of the water (Lund et al., 2014).

The properties of water may change over time and with location. Such changes may be minute-to-minute and day-to-day, usually brought about by rain and wind that deposit environmental contaminants and debris in the streams (Rossouw, 2011; Lund et al., 2014). Daily variations in the properties of surface water are caused by natural biological cycles and chemical reactions taking place in the water bodies, which may also be affected by the variations of daylight or darkness cycles. Seasonal and hydrological changes are driven by climate change. For example, heavy rainfall could cause increased runoff from agricultural land and urban areas, which in turn speeds up changes in water properties (Lund et al., 2014). Year-to-year water property patterns are caused by changes in land use, land cover and other human-induced activities in the catchment.

Water quality is generally managed through the implementation of water legislations and water quality guidelines that have been developed locally and/or internationally (Nyoh, 2015). Certain criteria for a particular water quality property are used as scientific and technical information to guide the assessment of water properties and to determine the potential effects on aquatic ecosystems as well as fitness for other environmental uses (DWAf, 1996b, 1996c). A range of water quality properties are assessed to indicate the suitability of water resources to sustain and support various uses. These water quality properties include the physical, chemical and microbiological properties.

2.4.1 Physical properties

The physical properties of water largely determine the aesthetic properties of water. Physical water quality properties that are generally assessed are dissolved oxygen (DO), pH, electrical conductivity (EC), turbidity and temperature. The physical properties of water that are mostly determined by senses include touch, sight, odour and taste (Jotwani et al., 2014). Suspended and colloidal material in water is a measure of the clarity and described as the turbidity of the water. Turbidity of water determines the light-transmitting properties of the water and ultimately influences the clarity of the water (Mohsin et al., 2013). Because of the reduced light penetration in turbid water, photosynthesis and the production of dissolved oxygen (DO) are reduced. Aerobic organisms require adequate DO for their respiration, therefore dwindling levels of DO affects the growth and survival of aquatic algae, phytoplankton and other aquatic plants (Stutter & Cains, 2017). As turbidity increases, so does the temperature because suspended particles tend to absorb more heat (WHO–OECD, 2003). Warmer water causes the concentration of DO to decline because warm water holds less DO than cold water (Stutter & Cains, 2017).

When DO levels drop to below 5 mg/L, the functioning and survival of biological communities are threatened, while levels below 2 mg/L may result in the death of most fish and aquatic macroinvertebrates (Diamantini et al., 2018). Therefore, low oxygen levels often indicate that the water has concentrations of organic material. A study, which was conducted across the three major surface water body types (temporary rivers, depression wetlands and semi-permanent dams) in the Eastern Cape Karoo, showed that the high levels of turbidity caused by suspended lime-rich soils and sandstone sediments had detrimental effects on the abundance and diversity of aquatic macroinvertebrates (Mabidi et al., 2017). The changes in turbidity levels mostly affected the larvae of dragonflies and damselflies (Odonota), which are known to be susceptible to changes in water flow and turbidity (Mabidi et al., 2017).

Temperature plays an important biochemical role in water. Temperature regulates the chemical reactions and the solubility of gases and minerals in the water (Ignatius & Rasmussen, 2016). It also influences the metabolic activities of aquatic organisms and productivity of aquatic plants (Barakat et al., 2016). Aquatic organisms can usually adapt to narrow temperature ranges in the environment in which they live. However, changes in the range of temperatures may cause death or migration of aquatic organisms (Karthika et al., 2018). High temperatures can increase the solubility of contaminants in the water and this might further increase the toxicity of these contaminants. In addition, the DO is dependent on the temperature levels. For example, the warmer the water, the less oxygen it can hold. In a study by Rossouw (2011), it was reported that high temperatures during the summer months attributed to increased algal growth in the Mokolo River, which falls within the Limpopo Water Management Area. In contrast, during the winter months, algal growth decreased as a result of the low temperatures (Rossouw, 2011).

Similarly, to temperature, many biological and chemical processes in an aquatic ecosystem are affected by levels of pH. pH provides information on the intensity of the acid or alkaline levels in a solution (Le Thi Minah et al., 2016; Karthika et al., 2018). Factors, such as temperature, concentrations of inorganic and organic ions and biological activity, affect water pH (Lothrop et al., 2018). The results of a study in North Li Shi in the Cheng District of Beijing in 2011, revealed that the release of phosphorus from sediments increased with an increase in water temperature and when the pH was increased from 8 to 10. (Li et al., 2013).

EC is a measure of the capacity of water to conduct electrical current. EC is also directly related to the concentration of salts dissolved in water. Salts and inorganic materials, such as chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium, dissolve in water into positively charged ions and negatively charged ions, which conduct electricity (Karthika et al., 2018). Most of these solids enter the aquatic environment through the dissolution of minerals in surrounding rocks, soil and

decomposing plant material (Rizo-Decelis, et al., 2017; Şener et al., 2017). Surface runoff from urban, industrial and cultivated areas are examples of the types of sources that may contribute to increased concentrations of solids (Dalu et al., 2017).

2.4.2 Chemical properties

The chemical properties of water are determined by measuring the concentrations of mainly four groups of dissolved substances. Metallic substances include arsenic, cadmium, calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc. Metallic substances usually occur in trace amounts in water, i.e. at very low concentrations. Inorganic non-metallic substances include chloride, fluoride, nitrate and sulphate (Water Research Commission [WRC], 1998). Most of the dissolved inorganic substances in water occur as ions. These ions enter a water body from atmospheric rock weathering and runoff (Daud et al., 2017). The other chemical groups are the aggregate group of organic substances and the aggregate inorganic substances.

The amount of inorganic substances is measured by total dissolved solids (TDS) and hardness. TDS is the term used to describe the inorganic salts and small amounts of organic matter present in solution in water. The principal constituents are usually calcium, magnesium, sodium and potassium cations, and carbonate, hydrogen carbonate, chloride, sulphate and nitrate anions. The hardness of water is due to the presence of higher levels of calcium and magnesium minerals in the water (Ignatius & Rasmussen, 2016; Ewaid & Abed, 2017).

Studies show there is not enough direct negative health effects associated with hard water on humans. The evidence relating to epidemiological studies about the potential effects of hard water on the skin is being debated as it does not prove causality (Engebretsen et al., 2017). The study, which used water hardness results data, reported that the skin disease (eczema) was prevalent among Japanese, Nottinghamshire and Spanish children, particularly in areas where the hardness levels of water were

the highest rather than in the areas with the lowest levels. However, it is unknown whether there were any synergistic effects (Engebretsen et al., 2017).

Inorganic nitrogen, phosphate and sulphate are important inorganic non-metallic substances to consider when describing water quality. Inorganic nitrogen, which includes the major forms of inorganic nitrogen; i.e. ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_2^-) and nitrite (NH_3^-), may affect water quality (Rossouw, 2011). Ammonia and ammonium are reduced forms of inorganic nitrogen, which can exist in the air, soil and water as dissolved ions or can be adsorbed from decomposed organic material (DWAf, 1996a). Nitrite is the inorganic intermediate, while nitrate is the end product of oxidation of organic nitrogen and ammonia. Nitrate is the more stable of the two forms and is usually far more abundant in the aquatic environment (Rossouw, 2011). These nutrients enter surface water bodies in animal waste and fertilisers from agricultural and farming environments as well as from municipal sewage in urban areas (Şener et al., 2017).

Phosphate originates from the weathering of rocks and the subsequent leaching of phosphate salts into surface waters. The majority of phosphate enters rivers and streams as effluent from the wastewater and agricultural or residential cultivated land (Rossouw, 2011). Treatment plants, leaking pipes and sewage overflow contribute to the amounts of phosphate in wastewater (Guldhe et al., 2017). Sulphate reaches surface water bodies through the breakdown of carbon-based substances caused by the decay of plants and animal matter as well as weathered soils and sediment (Ignatius & Rasmussen, 2016). Several chemicals, such as ammonium sulphate fertilisers, contain sulphate and thus may be released into surface water resources as runoff from urban areas and agricultural farms (Varol & Davraz cited in Şener et al., 2017). Other sulphate-containing water treatment chemicals, such as aluminium sulphate or copper sulphate, may be discharged into the water as effluent. The enrichment of surface water bodies with phosphate and sulphate could result in speeding up the eutrophication process in the water.

Highly enriched water, favourable temperatures and light may result in algal blooms. An algal bloom is a rapid increase or accumulation in the population of algae in the water, and is often recognised by the discolouration of the water from their pigments. The term algae in an algal bloom refers to many types of aquatic photosynthetic organisms, both macroscopic, multicellular organisms, like seaweed, and microscopic, unicellular organisms, like cyanobacteria. The presence of algal blooms in water limits the amount of dissolved oxygen needed by other aquatic organisms for respiration (Gupta et al., 2017). When the environment can no longer support the increased population of algae, they die and decay. The decomposition of algae by bacteria may lead to the depletion of oxygen, which could result in the suffocation of the fish and other aquatic organisms living in the water (Gupta et al., 2017).

In a study carried out in South Africa in 2015, it revealed that between 2002 and 2012, almost 70% of the 50 largest surface water bodies, including the Gariep dam, Vaal, Sterkfontein, Theewaterskloof and Vanderkloof, among others, were highly eutrophic. Almost 30 of these surface water bodies had a high presence of cyanobacteria blooms (blue-green algae) (Matthews & Bernard, 2015). When the cyanobacteria cell dies, toxins called cyanotoxins are released into the water and these toxins may persist for weeks and even months (Liang et al., 2018). If people eat cyanotoxins-contaminated seafood or inhale the toxins while swimming, it can cause liver damage and skin problems. The presence of blue-green algae degrades the water quality and further this may have an impact on aquatic macroinvertebrates (Matthews & Bernard, 2015; Liang et al., 2018). A study conducted in Lake Chaohu in China showed that the lake was eutrophic and sensitive aquatic macroinvertebrates belonging to the families of ephemeroptera, plecoptera and trichoptera were affected the most by the eutrophic water rather than the pollution tolerant species, such as oligochaeta and chironomidae (Zhang et al., 2018).

Chlorides are highly mobile ions, which can also end up in the aquatic ecosystem. Chlorides are leached from different rocks into soil and water through weathering (Karthika et al., 2018). These

include sodium chloride and potassium chloride, which are extensively used in the production of industrial chemicals, such as caustic soda and fertilisers (WHO–OECD, 2003). Chlorides may enter aquatic ecosystems naturally or through anthropogenic sources, such as runoff containing road de-icing salts, effluent from local industries and discharged irrigation water/runoff containing inorganic fertilisers from farms (Ignatius & Rasmussen, 2016). Increased chloride concentrations can induce a variety of ecological effects on the aquatic ecosystems, such as salinisation (the increase of salt content) in streams. The high concentration levels of chloride in the water may increase electrical conductivity levels in the water. Electrical conductivity and chloride are identified as good indicators of ecological quality (Berger et al., 2017).

The increased salt content in water may affect the aquatic organisms in several different ways, such as increasing physiological stress, affecting osmotic pressure, mortality and reproduction of aquatic plants and animals (Cañedo-Argüelles et al., 2013). Aquatic organisms need to maintain an internal osmotic pressure relative to the environment they live in. This means that if the internal salinity is higher than the external salinity, the organisms must use energy to maintain ions in their bodies and exclude water. If the water is more saline than their internal salinity, they may have to either survive with their higher internal salinity or use energy to rid ions and keep water (Cañedo-Argüelles et al., 2013). Chloride was identified as the most important factor favouring species invasions in nearly 70% of the German rivers and streams investigated (Berger et al., 2017).

Water can contain a variety of different organic compounds of which the main element is carbon. Organic compounds usually do not dissolve as ions but rather go into solution as molecules of the compound. Organic compounds in water include algae and bacterial by-products; carbohydrates and proteins; and synthetic organic compounds, such as pesticides and herbicides (WRC, 1998). Although these compounds are usually present in very low concentrations, they may be harmful even at low concentrations. Because it is not always possible to determine the concentration of each organic

compound in water, an indication of the general organic quality in the water can be obtained by determining the aggregate substances, such as total organic carbon (TOC), chemical oxygen demand (COD) and trihalomethanes (THM) (WRC, 1998).

2.4.3 Microbiological properties

Microbiological properties include indicator organisms such as *Escherichia coli* (*E. coli*) and other bacterial coliforms. Many types of microorganisms live in water and could cause fish, land animals and humans to become ill. Serious diseases, such as cholera, shigellosis and campylobacteriosis, come from microorganisms that live in water (Burgess & Pletschke, 2008; Epele et al., 2018). Waterborne diseases are usually caused by enteric pathogens. Enteric pathogens are mainly transmitted by the faecal-oral route; namely infected people excreting faeces that are carried in faecally contaminated food or water and then ingested by other individuals. However, water also plays a role in the transmission of pathogens, such as opportunistic pathogens, that are normal external body flora and not faecally excreted (Burgess & Pletschke, 2008).

The assessment of microbiological quality of water is usually based on the test of indicator microorganisms being present. An ideal indicator organism:

- Should always be present when the pathogen is present and should be absent in uncontaminated water;
- Should be present in numbers greater than the pathogen it indicates;
- Should have a survival in the environment and resistance to the treatment processes so that it is comparable to that of pathogens;
- Should not be harmful to human health; should be easy to identify and to isolate; and should be suitable for all types of water (Burgess & Pletschke, 2008); and

- Indicator organisms are further influenced by other contaminants that are either dissolved or suspended in the water (Lund et al., 2014; Liao et al., 2018).

One of the categories of indicator microorganisms is coliform bacteria. This group of bacteria is commonly found in the environment, such as water, soil or vegetation as well as the intestinal tract of humans and warm-blooded animals. The presence of coliform bacteria in water is thus an indication of faecal contamination. Coliform bacteria are gram negative, non-sporing, rod shaped organisms, which need organic carbon for growth (Guldhe et al., 2017). This group of bacteria include the group of *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella*, all of which may end up in surface water as part of storm water runoff, leached animal manure and improperly treated sewage (Kora et al., 2017).

The indicator organism *Escherichia coli* is used as an indicator for the presence of faeces of humans and other warm-blooded animals in water (Liao et al., 2018). *E. coli* is a species within the thermo-tolerant coliform group and is generally regarded as the most specific indicator of faecal contamination (Lam et al., 2017). These bacteria are found in the environment, foods and intestines of humans and animals (Garcia et al., 2017). Therefore, *E. coli* is used as an indicator organism of sewage or animal waste contamination. Although most strains of *E. coli* are harmless, the *E. coli* 0157:H7 may cause bloody diarrhoea and infections in the urinary tract and respiratory system (Liao et al., 2018).

2.5 Effects of polluted stream water

2.5.1 Effects of polluted stream water on humans

Polluted stream water poses serious health risks to humans. Although, most of the South African streams are used extensively for recreational and irrigation purposes, homeless people may depend on the stream water for their daily livelihood (Nyoh, 2015). When humans use polluted stream water for recreational activities, such as swimming, they may accidentally ingest the contaminated water and get infected with disease-causing pathogens. Water polluted with pathogenic microorganisms is

responsible for causing infectious diseases, such as cholera, typhoid and dysentery, in humans (Zia et al., 2013; Antonini et al., 2017). More than 2 million people die each year from these diseases that are spread through drinking contaminated water (Rizo-Decelis et al., 2017; Ayandiran et al., 2018). Surveillance data from the United States of America (USA) show that between 2009 and 2010, there were about 24 water-based disease outbreaks related to swimming events and 11 of those outbreaks were attributed to cyanobacterial toxins. Other outbreaks were attributed to microbial pathogens such as *Campylobacter jejuni*, *E. coli* O157:H7, *Shigella sonnei*, *Cryptosporidium* spp., *Giardia intestinalis*, norovirus and avian schistosomes, with the largest outbreak being due to norovirus (Fewtrell & Kay, 2015). Polluted water can also cause other water-based diseases, such as schistosomiasis, which are spread through the ingestion of vegetables irrigated with contaminated water or by eating half-cooked fish (Nyoh, 2015). Polluted water further causes water-related insect vectors, such as mosquitoes, to breed in or near water and thus spread diseases such as dengue fever and malaria (Zia et al., 2013).

2.5.2 Effects of polluted stream water on the aquatic ecosystems

The effects of polluted stream water may cause the aquatic ecosystem to be unable to promote ecological integrity. The ecological integrity is a measure of the health of an aquatic ecosystem to support and maintain an integrated, adaptive community of organisms and their natural habitat (DWAF, 1996a). Polluted stream water may give rise to effects, such as eutrophication, acidification and salinization, all of which may threaten the aquatic organisms and the quality of their aquatic habitat (Alemneh et al., 2017; Berger et al., 2017). These effects may act independently or coexist with other threats, such as climate change, alien species and emerging contaminants (ECs) and human activities, such as land use change, that wield complicated interactive responses and cause synergistic effects (Peng et al., 2015; Milner et al., 2016; Rozman et al., 2018).

The following events are instances of the synergistic effects:

- High temperatures as a result of global warming can speed up the eutrophication process and continue to cause the growth of toxic algal blooms (Tanaka et al., 2016; Trang et al., 2017);
- Water polluted with emerging contaminants may introduce endocrine disruptive diseases to aquatic organisms (Berger et al., 2017); and
- A high salt content in water may affect plants and cause reduced crop yield or crop quality if they are irrigated with this contaminated water (Ding et al., 2016).

Some studies acknowledge that sometimes the effects of polluted water on specific components of ecosystems are often indirect and thus too complex to understand. For example, certain species of fish may disappear because the organisms that are its primary food source might be eliminated due to changes in water quality properties, such as temperature, and not directly because of the health conditions of the aquatic ecosystem (Cheng et al., 2018; Luo et al., 2018; Zhang et al., 2018). The Index of Biological Integrity (IBI) and Qualitative Habitat Evaluation Index (QHEI), which were applied to evaluate the health status of Keum-Ho river in South Korea, showed that the health conditions of Keum-Ho river was poor. The poor conditions of Keum-Ho river were attributed to both chemical contaminants and the modification of the habitat (An et al. cited in Singh & Saxena, 2018). A study in Scotland reported that there were high correlations across 16 aquatic sampling sites, which were grouped into four linked types of land use activities (i.e. semi-natural on the basis of no urban influence, agriculture, urban and peri-urban). The strongest correlation was between the agriculture activities and substantially high levels of dissolved nutrient and chemical concentrations in the Scottish streams (Stutter & Cains, 2017).

2.6 Assessment of water quality

In South Africa, the provision of good quality water and sanitation services to all is underpinned in the Constitution. In Section 24 of the Bill of Rights in the Constitution, it is stated that everyone has the right to an environment that is not harmful to their health or wellbeing (Hatchard, 1994). It is this constitutional command that promoted the promulgation of water legislations, such as the National Water Act (Act No. 36 of 1998) and the Water Services Act (Act No. 108 of 1997). The National Water Act legislates and provides the framework for the way in which water resources must be protected, used, developed, conserved, managed and controlled. The Department of Water and Sanitation, who is the custodian of South Africa's water resources, has thus been mandated to ensure that the quality of water resources remains fit for recognised water uses, and that the aquatic ecosystems are protected and sustained.

Several international documents were used as background and supplementary information for the development of the South African Water Quality Guidelines (DWAF, 1996c). The main international documents were:

- WHO, 1984. Guidelines for Drinking Water Quality. Volume 2: Health Criteria and Other Supporting Information. World Health Organisation, Geneva;
- WHO, 1993. Guidelines for Drinking Water Quality. 2nd Edition, Volume 1; Recommendations. World Health Organisation, Geneva;
- Australian Water Quality Guidelines for Domestic Supplies; and
- Canadian Drinking Water Guidelines.

The South African Water Quality Guidelines that were developed cover several water quality criteria. These water quality criteria are used to guide the water quality assessment of a particular water use type. These criteria are the Target Water Quality Range (TWQR), Chronic Effect Value (CEV) and Acute Effect Value (AEV). The TWQR is the array of concentrations where no measurable negative impacts are expected on the health of aquatic ecosystems. Thus, it is a protective measure to ensure

the continued health of aquatic ecosystems. The CEV is defined as the concentration or level of a constituent at which measurable chronic effects in the aquatic community are expected. If a chronic effect prevails over time and/or frequency, such an effect may lead to the eventual death of organisms and the disappearance of sensitive species from aquatic ecosystems (DWAF, 1996c). The AEV is defined as the concentration or level of a constituent above which acute toxic effects in the aquatic community are expected. If an acute chronic effect persists, even for a short while, or occurs at too high a frequency, it may lead to the death and disappearance of sensitive species or communities from aquatic ecosystems (DWAF, 1996c).

The South African Water Quality Guidelines that were developed comprise of the following seven volumes (DWAF, 1996c):

- Volume 1: South African Water Quality Guidelines Domestic Water Use;
- Volume 2: South African Water Quality Guidelines Recreational Water Use;
- Volume 3: South African Water Quality Guidelines Industrial Water Use;
- Volume 4: South African Water Quality Guidelines Agricultural Water Use: Irrigation;
- Volume 5: South African Water Quality Guidelines Agricultural Water Use: Livestock Watering;
- Volume 6: South African Water Quality Guidelines Agricultural Water Use: Aquaculture;
- Volume 7: South African Water Quality Guidelines Aquatic Ecosystems.

2.6.1 Water quality index

Several indices are often employed to determine the overall quality of water through the calculation of a single value index while taking the measurements of many water quality properties into account. The indices include the US National Sanitation Foundation Water Quality Index (NSFWQI), Florida Stream Water Quality Index (FWQI), British Columbia Water Quality Index (BCWQI), and the Oregon Water Quality Index (OWQI) (Poonam et al., 2013). The Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI) is applied to calculate the overall water quality by

combining water quality data over more than one sampling round (Canadian Council of Ministers of the Environment [CCME], 2017). The CCME-WQI is suitable for grading the quality of water by using the five grades categorisation, which include 'excellent, good, fair, marginal and poor', for the different index score values (CCME, 2017).

2.7 Assessment of emerging contaminants

The vast majority of emerging contaminants (ECs) are currently not assessed or regulated. A number of ECs can be easily dissolved in water and transported through the water cycle with a high potential to be a threat to aquatic organisms and humans. As a result, in recent years, multiple ECs have grown substantially leading to an unknown amount of parent compounds and transformed products being present in wastewater effluent, surface and groundwater and drinking water. Although, researchers have shown an increased interest in monitoring ECs, there is still little agreement on the list of ECs that should be monitored (Rodriguez-Narvaez et al., 2017). This makes the identification and quantification of ECs in water or wastewater to be a major scientific task as it also requires highly sophisticated analytical methodologies, which must be able to detect at levels of nano-grams per litre.

The main analytical tools, which are used in detecting the levels of compounds in nano-grams per litre, include chromatography (either gas or liquid) joined to the mass spectrometry (MS). MS has been associated with significant advantages, such as sensitivity, specificity and the capacity to accurately identify and quantify contaminants in the surface water or wastewater (Inostroza et al., 2017). Other analytical tools include capillary electrophoresis (CE), immuno-analytical techniques (IAT) or microbiological assays (MA). CE is generally considered less sensitive, whilst IAT is highly limited for simultaneous determination of different analytes, and MA is highly dependent on the nature of the sample (Inostroza et al., 2017). In a study that was conducted in the River Holtemme, which stretches over 47 km in the Bode catchment, Saxony-Anhalt in Germany, all 86 analytes belonging to different classes of ECs, such as pesticides, pharmaceuticals and industrial chemicals, were found present

using a 1260 Infinity LC system (Agilent) coupled to a QTrap 6500 MS (ABSciex) with a Turbo V ion source (Inostroza et al., 2017).

Some countries have developed standards to regulate ECs that are found in surface water bodies. In South Africa, there are no standards for ECs, as a result, the country relies on the use of standards developed in other countries. The Water Framework Directive (WFD) published in 2000 is the main European Water Policy Instrument for the protection of European waters and was aimed at achieving good surface water chemical status (European Commission [EC], 2018). Furthermore, Decision 2455/2001 by the European Commission presented the first list of priority substances that were considered to be the most dangerous for the aquatic environment. The Directive on Environmental Quality Standards 2008/105 (known as the Priority Substances Directive; European Commission, 2008) set the quality standards for 33 priority substances and eight other pollutants that are persistent, bio-accumulative and toxic (EC, 2018). For the first time, three pharmaceutical compounds (from oral contraceptives to hormone medications) were included among the regulated substances in EU countries. These substances included the endocrine disruptors such as 17 alpha-ethinylestradiol (EE2), 17 beta-estradiol (E2) and diclofenac (EC, 2018).

2.8 Assessment of ecological water quality

It should be realised that every significant water resource in South Africa should be classified and the resource quality objectives be determined according to the assigned class. The assessment and conservation of the ecological integrity of aquatic ecosystems be legislated (South Africa, National Water Act, 1998, s. 13, ss. 1) This classification system and resource quality objectives must take into consideration the characteristics and quality of the water resource, in-stream and riparian habitat as well as the characteristics and distribution of aquatic biota (South Africa, National Water Act, 1998, s. 13, ss. 3). In this regard, the national River Health Programme (RHP) plays a direct role in the determination of the classes and resource quality objectives for river ecosystems. Ecological

assessments of aquatic ecosystems must be undertaken (South Africa, Environment Conservation Act, 1989, s. 18. 1).

Stressors affecting aquatic ecosystem can lead to several effects occurring. The classification of these effects is dependent on the possible direct and indirect implications they may have on the water resources (Rossouw, 2011). Some stressors directly influence the biota in an adverse way and other stressors may not be direct but they can still adversely affect aquatic ecosystem. An example of an indirect effect is when cattle grazing in or near surface water resources cause increased soil erosion, surface runoff and soil bulk density. A direct effect may involve disposal/discharge of agricultural waste directly into the surface water body, thereby causing poor water quality that may negatively affect the aquatic communities and/or human health (Malan et al., 2018).

Although many studies continue to provide stressor-specific effects and interactions, some acknowledge that the experimental results might not adequately account for what is observed in natural environments. Therefore, complex interactions may limit how far the experiments can resolve the issues (Yang et al., 2018).

2.8.1 Aquatic macroinvertebrates as indicator organisms of ecological water quality

Aquatic macroinvertebrates constitute an integral part of an aquatic ecosystem. These organisms provide essential ecological services, which include cycling nutrients in the water and breaking down organic matter (Zhang et al., 2018). Different species of aquatic macroinvertebrates respond differently to changes in the physical-chemical properties of surface water bodies, both in time and location (Pinna et al., 2017). Changes in water quality, as a result of various contaminants present in water, affect their survival and reproduction (Lockerbie et al., 2016; Luo et al., 2018). Among the advantages, aquatic macroinvertebrates are easy to collect and identify, making them suitable indicator organisms

of ecosystem impairment and water pollution (Dalu et al., 2017; Pinna et al., 2017; Chai & Lha, 2018; Steward et al., 2018).

The distribution of aquatic macroinvertebrates in the aquatic environment is influenced by various factors. In more recent experimental studies on various aquatic macroinvertebrates, multiple influences were accounted for. According to Martinez-Haro et al. (2015), it is difficult to study the identification of single and combined stressors of aquatic macroinvertebrates due to many factors, such as:

1. Hydrological changes – These changes are considered to influence the response of aquatic macroinvertebrate communities. However, in other cases, the presence of contaminants in the water was found to cause an intensified ecological threat (Martinez-Haro et al., 2015).
2. Stream flow patterns – In a similar study, a conclusion was drawn that variations in the flow of the stream had a significant impact on the presence of aquatic macroinvertebrates rather than the contaminants in the water (De Castro-Català et al., 2015).
3. In another study, it was demonstrated that there is a harmful association between chemicals and aquatic macroinvertebrates communities, in spite of stream flow variations. So aquatic macroinvertebrates have shown to be susceptible to organic pollution and sensitive to degradation of their habitat (Kalogianni et al., 2017).
4. Another study demonstrated that pollution from agricultural activities produces the most severe effects in the streams, which eventually affect the distribution of aquatic macroinvertebrates (Johnson et al., 2017).

Several biotic indices and scoring systems, based on the abundance of aquatic macroinvertebrate families, have been developed to indicate the health status of a stream. The use of aquatic macroinvertebrates as the means of measuring the health status of streams has driven various countries, including the USA, Canada, Australia, South Africa and some countries in Europe, to develop simple and rapid techniques that can be used to assess the health status of streams (Nyoh, 2015). Beck's Biotic Index, which was developed for streams in Florida (USA) in 1954, is considered as the first true biotic index. This index was based on the tolerance of aquatic macroinvertebrates to slight organic pollution. Aquatic

macroinvertebrates that were known to be intolerant to organic pollution were classified as “Class I organisms”, and those that were tolerant to moderate organic pollution were classified as “Class II organisms” and thus separated from the rest of the sample. The final index value for a sampling site was calculated by multiplying the number of species of Class I organisms by two, and then adding the number of Class II organisms. A single value was generated and if that value was greater than 10, it was an indication of an unpolluted site. Values between 1 and 6 indicated moderately polluted sites (Ollis, 2005). In the 1960s, Beck’s Biotic Index was modified further and the modified version is known as the Florida Index (FI). The FI is currently used as a metric in the multimetric Rapid Bioassessment Protocols (RBPs) of the US EPA.

Since 1960, improved biotic indices have been developed as an aquatic macroinvertebrate based biotic index. One common aquatic macroinvertebrate based biotic index, which was developed for and applied to river assessments in South Africa, is the South African Scoring System (SASS) (Ollis, 2005). This index, developed in 1994/95 and later modified in 1998 and again in 2002, adapted the Iberian Biological Monitoring Working Party (IBMWP) scoring system that was previously known as the BMWP index (Ollis, 2005). The BMWP was developed and used in the United Kingdom (Ollis, 2005). The SASS is a rapid and inexpensive technique used to demonstrate patterns in changes to the water quality over time. A list of pre-defined taxa (mostly identified as aquatic families, such as the oligochaeta) have been allocated sensitivity “scores” based on their sensitivity to pollution and disturbances (Ollis, 2005). These sensitivity scores range from 1 (extremely pollution-tolerant) to 15 (extremely pollution-sensitive). With Version 4 of the SASS, all biotopes are sampled together. However, with the modified SASS 5 (Version 5), aquatic macroinvertebrate samples are collected separately from three different biotopes, which include stones in and out of current; marginal and aquatic vegetation; and gravel, sand and mud biotopes. The collection is done by kicking and sweeping samples using a net with a 1,000 µm mesh size.

After collection and enumerating the identified aquatic macroinvertebrate family samples for each biotope, three principle indices are calculated. These principle indices include the SASS 5 Score, the Number of

Taxa and Average Score per Taxon (ASPT), are calculated. The SASS 5 Score is the sum of the sensitivity scores for all SASS taxa present, while ASPT is calculated by dividing the SASS 5 Score by the Number of Taxa. The SASS 5 Score and ASPT are generally used together for the analysis and interpretation of SASS data. The SASS is used extensively in other African countries, such as Zimbabwe, Swaziland and Zambia (Ollis, 2005).

Another integral part for the assessment of the health status of the stream's ecosystem is the measurement of aquatic macroinvertebrate habitats. However, other countries use different approaches for their aquatic macroinvertebrate Rapid Bioassessment Protocols (RBPs) for streams and rivers and therefore do not include any form of habitat assessment. The multimetric approach involves the integration of a number of structural and functional attributes of aquatic macroinvertebrate communities, known as metrics, which are combined to form a composite index (Ollis, 2005). For example, the RBPs that was developed by the US Environmental Protection Agency (US EPA) and used widely throughout the USA, such as the Invertebrate Community Index (ICI) and the Benthic Integrated Biotic Index (B-IBI), are based on the multimetric approach to bio-assessment.

In South Africa, the widely used invertebrate habitat assessment system is the Integrated Habitat Assessment System (IHAS, Version 2) that was developed by McMillan in 1998 (cited in Ollis, 2005). This system was developed to help with the interpretation of SASS scores since the quantity and quality of aquatic macroinvertebrate habitats determines the diversity and composition of aquatic macroinvertebrate families per habitat (Dickens & Graham, 2002). However, there was no correlation between the SASS and IHAS scores for measurements taken in different rivers in the southern Western Cape (Ollis, 2005; Ollis et al. cited in Nyoh, 2015).

Another IHAS, named mIHAS, was modified by Nyoh (2015) and used in the Nyoh' study. Contrary to the IHAS developed by Ollis (2005), Nyoh (2015) included the measurements of physico-chemical properties, such as pH and temperature, into the existing IHAS sheet to provide an accurate assessment of the

conditions of the macroinvertebrate habitats. This was done because physical and chemical water quality properties have a direct or indirect impact on aquatic macroinvertebrates as well as their habitats, especially if the physical and chemical constituents are beyond the required limits. After the assessment of physical and chemical properties, the measurements are compared with proposed Aquatic Water Quality Limits for Urban Streams (AWQUS) and the aquatic habitat sites are then allocated specific scores for the water conditions ranging from 5 (Ideal) to 0 (Unacceptable) (Nyoh, 2015).

The Index of Habitat Integrity (IHI) is also another tool applied to assess the severity of modifications on aquatic macroinvertebrate habitats. These modifications include factors like water abstraction, flow modification, bed modification, solid waste disposal and bank erosion (Dallas cited in Nyoh, 2015). To use this field-based assessment tool, the assessor must be very high in confidence regarding their knowledge of the site and catchment.

2.9 Conclusions

Many studies have shown that the quality of surface water resources is deteriorating and this is exacerbated by a water scarcity that is driven by the growing demand for water. Various water-polluting activities take place in water catchments that may compromise the natural ecosystems that support human health and aquatic biodiversity. Pollutants consist of a wide range of substances, which include chemicals derived from the natural environment or chemicals produced through human activities. Although many polluting substance are relatively non-toxic, many are toxic, which may lead to environmental deterioration and when ingested by humans and animals may lead to ill health.

Chapter 3

Materials and Methods

3.1 Introduction

The study was conducted to assess the water quality in the Fonteinspruit stream in Mangaung, Free State. Physical, chemical and microbiological properties of the stream were measured to determine the water quality. In total, 13 water quality properties were assessed in water samples collected at the 12 water sampling sites. These properties included turbidity, electrical conductivity (EC), pH, temperature, dissolved oxygen (DO), chemical oxygen demand (COD), nitrate (NO_3^-), ammonia (NH_3), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), total hardness, *Escherichia coli* (*E. coli*) and total coliforms. The presence of emerging contaminants (ECs) in the stream was also determined. The concentrations of ECs, including pharmaceutical analytes, such as carbamazepine, estrone, estradiol, 17 α ethynylestradiol, and one plasticizer, bisphenol A, as well as herbicide analytes, such as atrazine, metolachlor and terbuthylazine, were measured in water samples collected at each of the water sampling sites in the Fonteinspruit stream.

An ecological quality assessment was also carried out to determine the overall health status of the Fonteinspruit stream. Aquatic macroinvertebrates were used as biological indicators for this bio-assessment as they are affected by the physical, chemical and microbiological properties of the stream (Dickens & Graham, 2002; Kuzmanovic et al., 2017). Aquatic macroinvertebrates were collected over a wide area of the stream to ensure an acceptable representation of the various biotopes. These biotopes included stones-in-current (SIC), and vegetation (VEG) as well as gravel, sand, and mud (GSM).

3.2 Study area

The study was conducted in the Fonteinspruit stream located in the Mangaung municipal area of Bloemfontein. Fonteinspruit stream is approximately 4.5 km in length and drains an area of about 17 km² (Pretorius et al., 2002). During the summer months from October to April, the rainfall is over 80% of the total annual rainfall and it is primarily in the form of nocturnal thunderstorms. The summer water runoff is thus relatively high and most of the discharge is in the form of storm flow (Pretorius et al., 2002). This area is also characterised by rapid, largely uncontrolled, high-density housing developments. The residential sections in this area include Erlich Park, Heidedal, Batho, Bochabela, Phahameng, Hamilton, White City and several informal sections of Mangaung. The Fonteinspruit stream joins the Bloemspruit stream downstream. The Bloemspruit stream then joins the Renosterspruit stream, which finally flows into the Modder River on the outskirts of Bloemfontein (Pretorius et al., 2002).

3.3 Water sampling sites

A total of 12 water sampling sites within the Fonteinspruit stream were identified. These water sampling sites were selected to represent the Fonteinspruit stream and were identified by the prefix “F” and numbers from 1 to 12 to specify the different water sampling sites. Prefix “F” referred to the Fonteinspruit stream and the number 1 indicated the water sampling site furthest upstream in the stream, while the number 12 indicated the water sampling site furthest downstream, with the other water sampling site numbers sequentially in-between. Water sampling site F1 was located close to Rocklands, while water sampling site F12 was located close to the confluence point of the Fonteinspruit stream and Bloemspruit stream. Figure 3.1 shows the water sampling sites in the Fonteinspruit stream as well as some of the settlements in its vicinity.

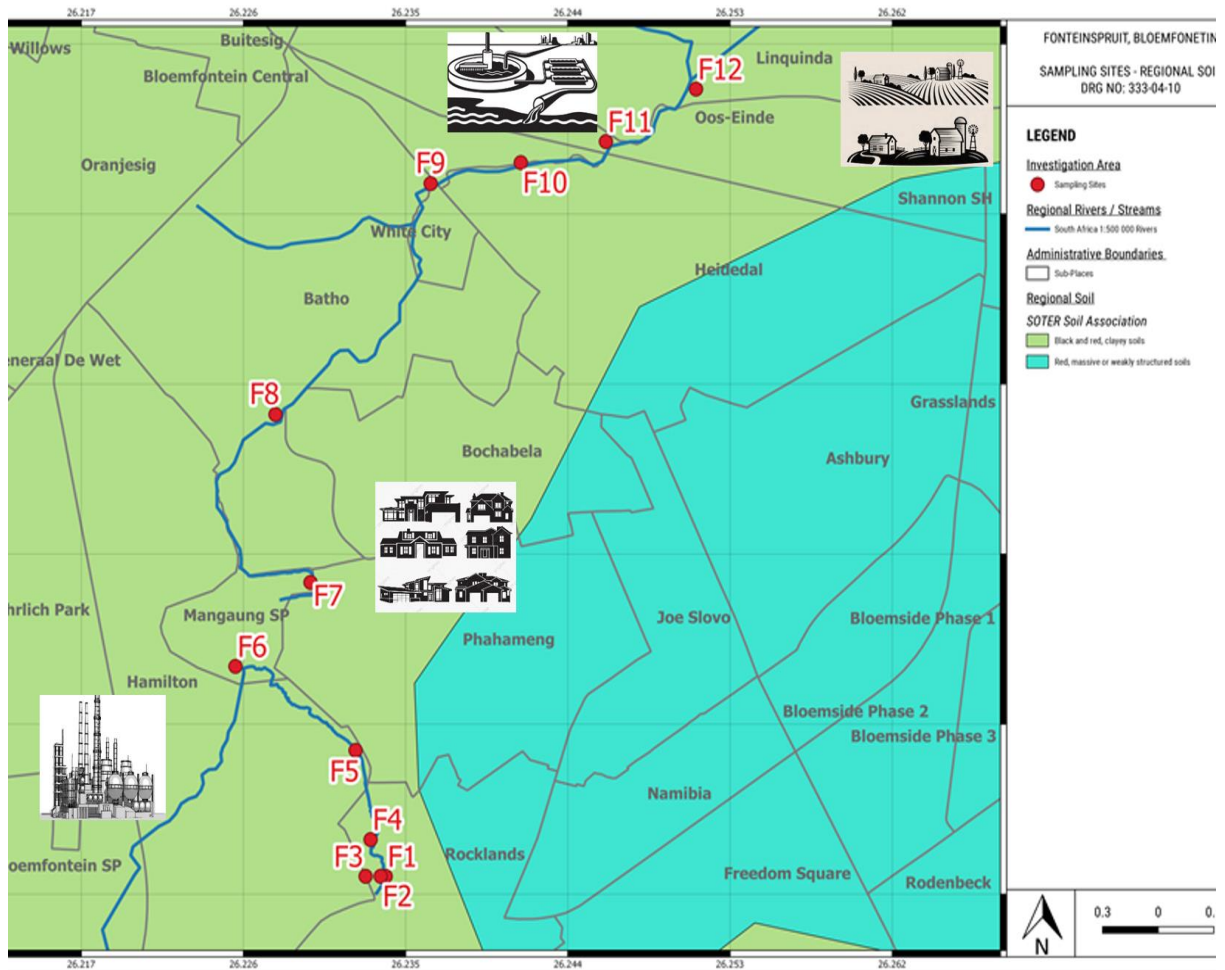


Figure 3.1 Study area of the Fonteinspruit stream and identified water sampling sites surrounded by settlements.

Each of the water sampling sites was chosen because of its potential to contribute to an understanding of the extent of the pollution in the Fonteinspruit stream. For all the water sampling sites, a range of supplementary data was recorded, which included the coordinates and a description of the environmental conditions at the water sampling site as well as the reason why the water sampling site was selected. The GPS coordinates of the water sampling sites were determined with a Garmin GPS 60CSx. Table 3.1 provides the coordinates and the motivation for choosing each of the 12 water sampling sites selected for the project.

3.4 Data collection period

The collection of water samples at the 12 water sampling sites in the Fonteinspruit stream was done as follows:

1. Round 1 of water sampling was conducted on the 29th of June 2018, during the winter season.
2. Round 2 of water sampling was conducted on the 8th of August 2018, during the winter season.
3. Round 3 of water sampling was conducted on the 19th of February 2019, during the summer season.

Table 3.1 Water sampling sites, coordinates, description and motivation for choice of site.

Sampling site	Coordinates	Site description	Motivation for choice of the water sampling site
F1	S 29.16825 E 026.23394	The drainage area that channels the water from Rocklands location into the stream.	The water sampling site was sampled to isolate the impact of pollutants received from Rocklands location (North eastern).
F2	S 29.16812 E 026.23298	The drainage area that channels the water from a different section of Rocklands location into the stream.	The water sampling site was sampled to isolate the impact of pollutants received from Rocklands location (North).
F3	S 29.16640 E 026.23302	The drainage area that channels the water from Hamilton industrial area into the stream.	The water sampling site was sampled to isolate the impact of pollutants received from Hamilton industrial area.
F4	S 29.16632 E 026.23304	The confluence point of water streaming in from the Rocklands sections and Hamilton Industrial area.	The water sampling site was sampled to determine the impact level of pollutants received from the Rocklands sections and Hamilton industrial area.
F5	S 29.11173 E 026.31326	Part of the Fonteinspruit stream adjacent to the cemetery.	The water sampling site was sampled to assess the change in water quality properties of the stream after the confluence of water from the Rocklands sections and Hamilton industrial area.
F6	S 29.15610 E 026.22635	The inflow point of the Blou dam	The water sampling site was sampled to assess the impact of pollutants before the Blou dam receives water from various locations including the drainage areas.

F7	S 29.15269 E 026.23000	The outflow point of the Blou dam	The water sampling site was sampled to assess the impact of pollutants after the Blou dam discharges the outflow into the stream.
F8	S 29.14351 E 026.22787	Part of the Fonteinspruit stream located along the Bochabela area.	The water sampling site was sampled to assess the change in water quality properties of the stream after the discharge of water from the Blou dam.
F9	S 29.13176 E 026.23670	The drainage area that channels the water from Batho location into the stream. The site is adjacent to a car wash facility and a local clinic.	The water sampling site was sampled to isolate the impact of pollutants received from Batho location.
F10	S 29.13033 E 026.24135	Part of the Fonteinspruit stream located adjacent to the Heidedal informal settlements.	The water sampling site was sampled to determine the impact level of pollutants received from Batho location and the informal settlement adjacent to Heidedal.
F11	S 29.06079 E 026.20598	Part of the Fonteinspruit stream located next to the Oos-Einde informal settlements.	The water sampling site was sampled to assess the change in water quality properties along the stream.
F12	S 29.12651 E 026.25139	The water sampling site is located close to the Bloemspruit stream.	The water sampling site was sampled to determine the impact level of pollutants before the confluence of the Fonteinspruit stream and Bloemspruit stream.

3.5 Study design

The study design illustrates the flow of the various phases undertaken in this study. The study comprised of four phases (Figure 3.2). Phase 1 involved scouting and identifying the 12 water sampling sites in the Fonteinspruit stream. Phase 2 involved the collection of water samples at the 12 water sampling sites, while Phase 3 involved the assessment of the water quality properties and the presence of emerging contaminants as well as the assessment of the ecological quality of the Fonteinspruit stream. Water quality was assessed in terms of physical, chemical and microbiological properties in water samples collected at the 12 water sampling sites in the Fonteinspruit stream, over three water sampling rounds. The CCME-WQI was calculated to grade the quality of water using the five grades categorisation, which include 'excellent, good, fair, marginal and poor', for the different index score values (CCME, 2017).

Various groups of emerging contaminants, which included pharmaceuticals, endocrine disruptors, plasticizers and herbicides, were assessed in the water samples collected at each of the 12 water sampling sites during one of the water sampling rounds. For the ecological quality assessment, aquatic macroinvertebrates were assessed in the water samples collected in stones-in-current (SIC), vegetation (VEG), and gravel, sand and mud (GSM) biotopes at the 12 water sampling sites during one of the water sampling rounds. Phase 4 included the analyses of the water quality property measurements and emerging contaminants measurements as well as the ecological quality measurements. Once all the analyses were completed, conclusions about the extent of the pollution in the Fonteinspruit stream could be reached.

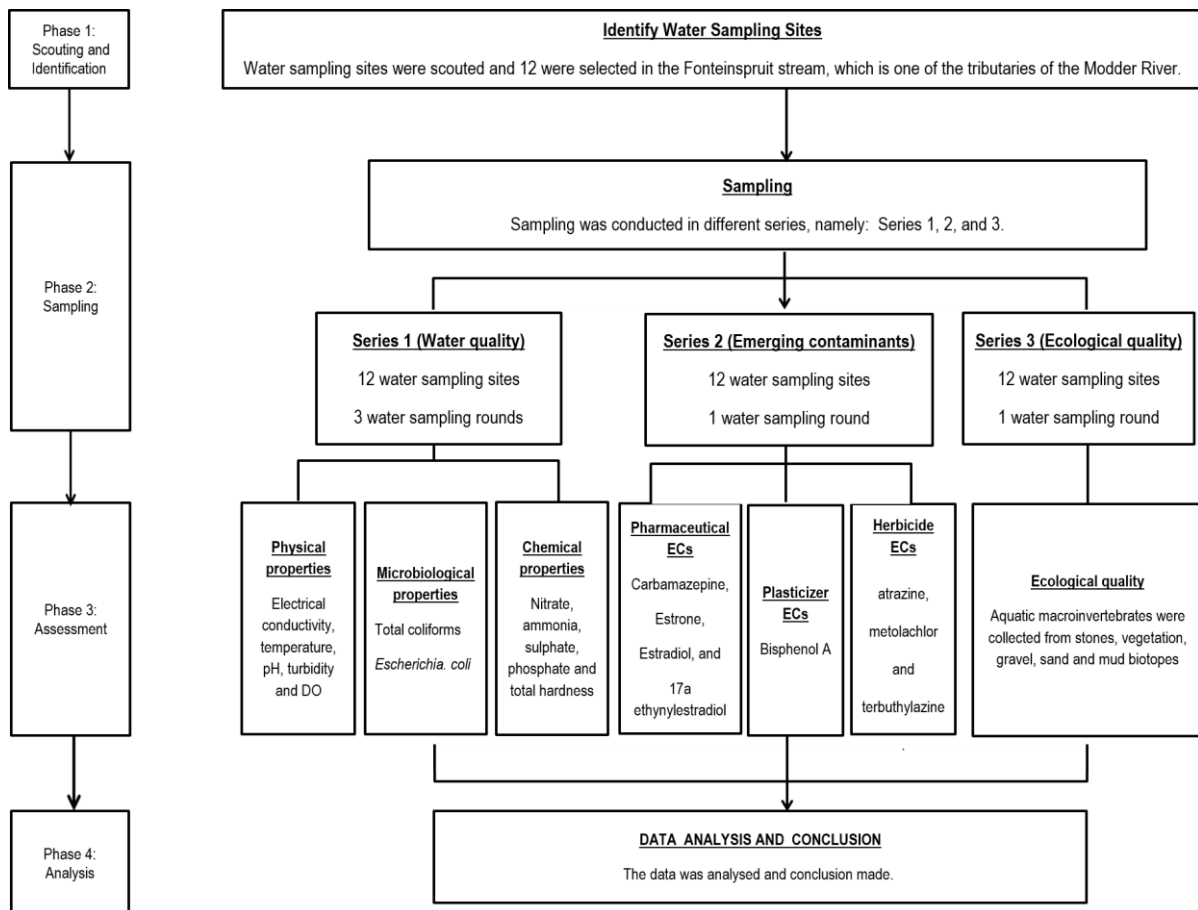


Figure 3.2 Flow diagram of the study design of the project.

3.6 Water quality assessment

For this study, various water sampling and analytical methods were employed to assess the water quality in the Fonteinspruit stream. Some of the measurements were taken directly on-site, whereas other measurements were performed in various laboratories. The measurements of the chemical water properties were performed at the Central University of Technology, Free State, while the measurements of the microbiological water quality properties were performed at the accredited laboratory, Test It Lab, Free State. In contrast, the measurements of most of the physical water quality properties were carried out on-site.

3.6.1 Measurement of physical properties

In this study, the physical water quality properties, namely turbidity, EC, pH, temperature and DO, were measured on-site. The measurements of these physical water quality properties were determined using the HQ40d multi-meter and the portable turbidity meter 2100Q. The HQ40d handheld multi-meter was used to measure EC, pH, temperature and DO, while the 2100Q Portable turbidity meter was used to measure turbidity (Figure 3.3). These apparatuses and probes were calibrated in the laboratory before the measurements were undertaken.



Figure 3.3 Apparatuses used for the measurement of physical water quality properties **a.** HQ40d handheld multi-meter **b.** 2100Q Portable turbidity meter.

Calibration of electrical conductivity, pH and dissolved oxygen probes

Before the initial use of the EC, pH, and DO probes, they were calibrated to ensure the highest level of accuracy in the following manner:

1. The HQ40d handheld multi-meter was switched on and the appropriate probe connected to the probe port for one of the three water quality properties, i.e. EC, pH, and DO.

2. Each probe uses a different type of calibration solution.
3. After each probe was connected to the probe connection port of the HQ40d handheld multi-meter, the probe was then rinsed with deionised water and dried with a lint-free cloth.
4. Each probe was placed in a beaker containing the appropriate calibration solution.
5. For the calibration of EC, the EC standard solution was poured into a beaker, the probe was placed in the beaker and the read-button was pushed to obtain the readings.
6. For pH, the solution cap that contains a storage solution to help preserve the life of the probe was removed and the probe was rinsed with distilled water to ensure the correct readings were obtained.
7. For the calibration of DO, the water-saturated air (100%) calibration procedure was applied.
8. The probe was connected to the meter and calibration button was pushed.
9. The probe was rinsed with deionised water and dried with a lint-free cloth.
10. Thereafter, the reagent water (approximately 6.4 mm) was added to a narrow-neck bottle.
11. The stopper was put in the bottle and the bottle was shaken vigorously for approximately 30 seconds to saturate the entrapped air with water.
12. The contents were allowed up to 30 minutes to equilibrate to room temperature.
13. The probe was put in the bottle and the read button pushed until the reading stabilised to show the standard value.

Calibration of Hach 2100Q turbidity meter

The Hach 2100Q turbidity instrument was calibrated in the laboratory using the calibration standards in the following manner:

1. Four cells containing stable liquid calibration standards, 10 NTU, 20 NTU, 80 NTU and 200 NTU (NTU = nephelometric turbidity units) were shaken vigorously for about 10 seconds.
2. Each cell containing stable liquid calibration standards was allowed to sit for five minutes to allow the bubbles to fade before it was used.
3. Each calibration standard cell was then wiped with a clean cloth before being inserted into the cell compartment of the Hach 2100Q turbidity meter.
4. The orientation mark on the standard cell was aligned with the orientation mark on the cell compartment.

5. After closing the lid of the cell compartment, the read-button was pressed to verify the calibration details recorded in NTU for each liquid calibration standard.

Measurements of Turbidity

Measurements of turbidity were obtained using the turbidity measurement procedure in the following manner:

1. The water taken from a particular water sampling site was poured into a sample cell up to the orientation mark on the sample cell.
2. Thereafter, the sample cell was wiped with a soft, lint-free cloth to remove water spots and fingerprints.
3. The Hach 2100Q turbidity instrument was placed on a flat, sturdy surface and switched on.
4. The sample cell, containing the water sample, was then inserted into the instrument cell compartment and aligned up with the markings on the cell compartment.
5. After closing the lid, the read-button was pressed and the turbidity reading recorded.

Measurements of electrical conductivity, pH, temperature and dissolved oxygen

The measurements of EC, pH, temperature and DO were obtained using the handheld HQ40d multi-meter in the following manner:

1. Specific probes were connected to the HQ40d multi-meter connection probe ports for the measurement of EC, pH, and DO.
2. Each particular probe was then rinsed with deionized water and dried with a lint-free cloth.
3. Each probe was inserted into the water at the identified water sampling site.
4. Thereafter, the specific read-button was pressed and the reading for each of the measured physical water quality properties was recorded.
5. For temperature, the reading was taken from the screen of the handheld HQ40d multi-meter.

3.6.2 Measurement of chemical properties

For chemical properties, water samples were collected in 500 mL plastic bottles and preserved in a cooler-box filled with ice for later laboratory analysis. For COD measurements, the water samples

were analysed at the accredited laboratory, Test It Lab, Free State. The measurements of nitrate, ammonia, sulphate and total hardness were obtained using a DR3900 spectrophotometer instrument (Figure 3.4). The concentration of phosphate was measured with a DRB200 reactor.



Figure 3.4 DR3900 spectrophotometer for the measurement of nitrate, ammonia, sulphate and total hardness.

Measurements of nitrate

Measurements of nitrate were obtained using the DR3900 spectrophotometer instrument in the following manner:

1. The program 355 N, nitrate HR PP was selected from the 'Stored Programs' in the main menu of the DR3900 spectrophotometer.
2. A sample cell was filled with 10 mL of a specific water sample.
3. The contents of one nitraVer 5 nitrate reagent powder pillow were added to the sample cell containing the water sample.
4. A stopper was placed on the sample cell and the sample cell was shaken vigorously for two minutes.
5. The sample solution was allowed about five minutes of reaction time, after which the solution changed to an amber colour.
6. A blank cell was prepared by filling a second sample cell with 10 mL of the water sample.
7. After wiping the blank cell clean, it was inserted into the cell compartment holder.
8. The zero-button of the DR3900 spectrophotometer was then pushed,
9. Once the display showed 0.00 mg/L, the prepared sample cell was cleaned and inserted into the cell compartment holder.

10. The read-button was pushed and the concentration of nitrate was recorded.

Measurements of ammonia

Measurements of ammonia were obtained using the DR3900 spectrophotometer instrument in the following manner:

1. The program 385 N, ammonia, salic was selected from the 'Stored Programs' in the main menu of the DR3900 spectrophotometer.
2. A blank sample was prepared by filling a sample cell with 10 mL of deionized water.
3. The contents of one ammonia salicylate powder pillow were added to the sample cell containing the 10 mL of deionized water.
4. After the stopper was put on the sample cell and then shaken vigorously, the DR3900 spectrophotometer timer was set on three minutes to allow the reaction process to take place.
5. For the prepared sample, a second sample cell was filled with 10 mL of a specific water sample.
6. The contents of one ammonia cyanurate powder pillow were added to the sample cell containing the 10 mL of water sample.
7. After the sample cell was capped, it was shaken for about two minutes to allow the reagent to dissolve.
8. This reaction process took about 15 minutes and the solution changed to a green colour.
9. After wiping the blank sample cell, it was inserted into the cell compartment holder.
10. The zero-button of the DR3900 spectrophotometer was then pushed.
11. Within one minute of the display showing 0.00 mg/L, the prepared sample cell was cleaned and inserted into the cell holder.
12. The read-button was pushed and the concentration of ammonia was recorded.

Measurements of phosphate

Measurements of phosphate were obtained using the DRB200 reactor instrument in the following manner:

1. The DRB200 reactor was switched on and preheated to 150°C.
2. The barcode programme for measuring phosphate was selected from the main menu of the DRB200 reactor instrument.
3. 5.0 mL of a specific water sample was poured into a test vial.

4. The contents of one potassium persulfate powder pillow for phosphate were then added to the test vial containing the water sample.
5. The test vial was then capped and shaken to dissolve the powder.
6. The test vial was then inserted into the reactor and left for 30 minutes so that the content in the vial could react.
7. After the 30 minutes of reaction time had expired, the vial was removed from the reactor and put on the test-tube rack to cool to room temperature.
8. Thereafter 2 mL of 1.54 N sodium hydroxide standard solution were added to the test vial, capped and inverted to mix.
9. The test vial was cleaned with a soft cloth and inserted into the 16 mm cell holder of the DRB200 reactor instrument.
10. The zero-button of the instrument was then pushed and once the display showed 0.00 mg/L, the contents of one phosVer 3 powder pillow were added to the test vial.
11. The test vial was capped and shaken for 20-30 seconds to mix.
12. After waiting two minutes for the reaction time to expire, the phosphate concentration was recorded within eight minutes of the timer going off.

Measurements of sulphate

Measurements of sulphate were obtained using the DR3900 spectrophotometer instrument in the following manner:

1. The program 680 sulphate was selected from the 'Stored Programs' in the main menu of the DR3900 spectrophotometer instrument.
2. A blank sample was prepared by filling a sample cell with 10 mL of a specific water sample.
3. After wiping the blank sample cell, it was inserted into the cell holder.
4. The zero-button of the DR3900 spectrophotometer instrument was pushed so that the display showed 0.00 mg/L.
5. For the prepared sample, the contents of one sulfaVer 4 reagent powder pillow were added to the sample cell containing the 10 mL of water sample.
6. A stopper was placed on the sample cell and shaken vigorously for two minutes to allow the powder to mix.
7. The solution was allowed three minutes for the reaction process to take place and, if sulphate was present, the solution changed to a cloudy colour.

8. Thereafter, the prepared sample cell was wiped and inserted into the cell compartment holder.
9. The read-button was pushed and the concentration of sulphate was recorded.

Measurements of total hardness

Measurements of total hardness were obtained using the DR3900 spectrophotometer instrument in the following manner:

1. The program 225 hardness, Mg was selected from the 'Stored Programs' in the main menu of the DR3900 spectrophotometer instrument.
2. A 100 mL of a specific water sample was poured into a 100 mL graduated mixing cylinder.
3. After a 1.0 mL dropper was used to add 1.0 mL of calcium and magnesium indicator solution into the mixing cylinder, it was capped and inverted several times.
4. Then another 1.0 mL dropper was used to add 1.0 mL of alkali solution for calcium and magnesium, the stopper was put back on the cylinder and again inverted several times.
5. Thereafter, 10 mL of the total solution were poured into each of the three sample cells.
6. For the blank sample, which was the first sample cell, one drop of EDTA solution was added and swirled to mix.
7. The zero-button of the DR3900 spectrophotometer instrument was pushed and the display showed 0.00 mg/L.
8. For the magnesium sample, which was the second sample cell, one drop of EGTA solution was added and swirled to mix.
9. After the prepared sample cell was wiped, it was inserted into the cell compartment holder and the read-button was pushed to obtain the concentration of magnesium.
10. After the concentration of magnesium was recorded, the magnesium program was exited and the program 220 for the concentration measurement of calcium was selected from the 'Stored Programs' in the main menu.
11. Thereafter, the third sample cell for calcium concentration measurement was inserted into the cell compartment holder, the read-button was pushed and the concentration of calcium was recorded.

3.6.3 Measurement of microbiological properties

The total counts for the microbial organisms *E. coli* and coliform bacteria were performed at the accredited laboratory, Test It Lab, Free State. The measurements were obtained using The IDEXX (Colilert18) Quanti-Tray™ method. The IDEXX (Colilert18) Quanti-Tray™ method is a biotechnological detection approach, which uses the multi-well most probable number (MPN). The Colilert18 test uses proprietary Defined Substrate Technology (DST) nutrient indicators θ -nitrophenyl- β -D-galactopyranoside (ONPG) and 4-methylumbelliferyl β -D-glucuronide (MUG) to detect coliforms and *E. coli*. Coliforms use the enzyme called β -galactosidase to metabolise ONPG and change it from colourless to yellow in colour. *E. coli* uses the enzyme called β -glucuronidase to metabolise MUG and the result is a blue fluorescence. The incubation takes place at 37°C for 18 to 22 hours. The MPN is calculated from the number of positive wells. The manufacturer's instructions for the Colilert®-18 were followed in this way:

1. One pack of Colilert 18 medium was added to a 100 mL water sample and was gently shaken to dissolve.
2. The 100 mL reagent mixture was poured into the Quanti-Tray®2000 and sealed in an IDEXX Quanti-Tray Sealer.
3. The sealed trays were then incubated for 18 to 22 hours at 37°C.

The number of colony forming units (cfu) of total coliform bacteria and *E. coli* present in the 100 mL of a water sample was determined using the Quanti-Tray®2000 Most Probable Number (MPN) table in the following manner:

1. For the total coliform bacterial count, the number of large yellow wells was matched against the number of small yellow wells. For example, if after incubation, 14 large wells and 15 small wells were counted, the counted wells were matched together to provide the CFU of 29 as demonstrated in Figure 3.5.
2. For *E. coli* bacterial count, the number of large fluorescent blue wells was matched against the number of small fluorescent blue wells. For example, if after incubation, seven large wells

and nine small well were counted, the counted wells were matched together to provide the CFU of 16 as shown in Figure 3.5.

- The results for both the total coliform bacterial count and *E. coli* bacterial count were then recorded.

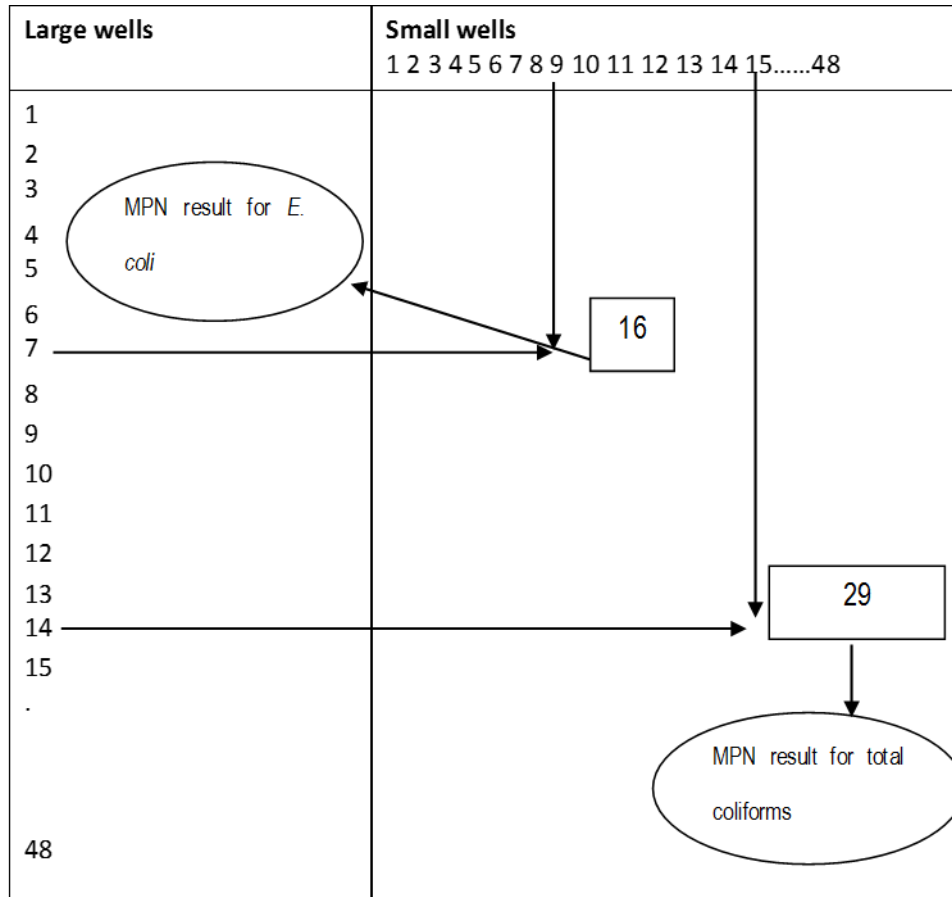


Figure 3.5 Example of the Colilert 97 MPN table used to match the counted large and small wells of *E. coli* and total coliforms.

3.6.4 Statistical analysis of water quality

Microsoft Excel was used for the statistical analyses of the water quality measurements of the respective water quality properties. Summary statistics were calculated for all the water quality properties and included the calculation of the means, ranges and percentage compliance. A one-way analysis of variance (ANOVA) was performed to determine whether any statistically significant differences existed between the means of the three independent water sampling rounds and a one-

way analysis of variance (ANOVA) was performed for each of the water quality properties. In instances where an ANOVA test produced significant results, a Tukey Honest Significant Difference post-hoc test was performed to determine which of the water sampling rounds were significantly different from one another.

3.6.5 Calculation of Water Quality Index

A water quality index was calculated to provide an overall description of the water quality at a particular water sampling site. This index used several water quality measurements to calculate a single value description of water quality. The Canadian Council of Ministers of the Environmental Water Quality Index (CCME-WQI) was selected because measurements from multiple rounds could be used to calculate the index (CCME, 2017). In the calculation of the CCME-WQI, three elements were incorporated: F1, the scope, which referred to the number of parameters not meeting water quality guidelines; F2, the frequency, which is the number of times these guidelines were not met; and F3, the amplitude, which referred to the amount by which the guidelines were not met (CCME, 2017). CCME-WQI was calculated using the following formulas:

1. Calculation of the scope F1, which represents the percentage of parameters that exceeded the standard limit:

$$F_1 = \left(\frac{\text{Number of failed parameters}}{\text{Total number of parameters}} \right) \times 100 \quad (1)$$

2. Calculation of the frequency F2, which represents the percentage of individual tests or measurements that exceeded the standard limit:

$$F_2 = \left(\frac{\text{Number of measurements}}{\text{Total number of measurements}} \right) \times 100 \quad (2)$$

3. Calculation of the amplitude F3, which measures the extent by which failed test values exceed the guideline:

a) An excursion for each failed measurement is calculated in the following manner:

- Where the measurement must not exceed the guideline

$$Excursion_i = \left(\frac{Failed\ measurements}{Limit\ of\ property} \right) - 1 \quad (3)$$

- Where the measurement must not fall below the guideline

$$Excursion_i = \left(\frac{Limit\ of\ property}{Failed\ measurements} \right) - 1 \quad (4)$$

b) The normalised sum of excursion (nse) is calculated as follows:

$$nse = \frac{\sum_{i=1}^n Excursion\ i}{\sum_{j=1}^m Measurements\ j} \quad (5)$$

c) Calculation of F3, which represents the amplitude:

$$F_3 = \left(\frac{nse}{0.01nse + 0.01} \right) \quad (6)$$

After the three elements were obtained, the CCME-WQI was calculated in the following manner:

$$CCME-WQI = 100 - \left(\frac{\sqrt{F_1^2 + F_2^2 + F_3^2}}{1.732} \right) \quad (7)$$

Once a CCME-WQI value had been calculated, the CCME-WQI was used to grade the status of the water. Therefore, after the CCME-WQI value was calculated for a particular water sample of a specific water sampling site, the water sample quality was rated according to the CCME-WQI categorisation schema, which is presented in Table 3.2.

Table 3.2 CCME-WQI categorisation schema (CCME, 2017).

Rank	WQI value	Description
Excellent	≥ 95 – 100	Water quality is protected with a virtual absence of threat or impairment; conditions very close to natural or pristine levels;

Rank	WQI value	Description
Good	$\geq 80 - < 95$	Water quality is protected with only a minor degree of threat or impairment; conditions rarely depart from natural or desirable levels
Fair	$\geq 65 - < 80$	Water quality is usually protected but occasionally threatened or impaired; conditions sometimes depart from natural or desirable levels
Marginal	$\geq 45 - < 65$	Water quality is usually protected but occasionally threatened or impaired; conditions sometimes depart from natural or desirable levels
Poor	$0 - < 45$	Water quality is almost always threatened or impaired; conditions usually depart from natural or desirable levels

3.7 Emerging contaminant assessment

The content of several ECs were assessed in water samples collected at the 12 water sampling sites in the Fonteinspruit stream over a single water sampling round. These included pharmaceutical analytes, such as carbamazepine, estrone, estradiol, 17 α ethynylestradiol, and one plasticizer, bisphenol A, as well as herbicide analytes, such as atrazine, metolachlor and terbuthylazine. The assessment was performed concurrently with the water quality and ecological quality assessments during the third round of water sampling. Water samples were collected using 1L glass bottles at each of the 12 water sampling sites by lowering the bottle approximately 10 cm into the water approximately one metre from the embankment. All water samples were then taken to the University of Free State in Bloemfontein for analysis within 12 hours of sampling.

3.7.1 Emerging contaminant sample preparation

The preparation of water samples was carried out in the following manner:

1. Water samples were poured from the 1L bottle into 500 mL bottles.
2. Internal standards (positive mode: atrazine-D5 and negative mode: bisphenol A-D16) were added to the 500 mL water samples, respectively.

3. The mixed samples were then filtered through glass fiber filters at the flow rate of 5mL/min and concentrated onto methanol conditioned C18 6 mL solid phase extraction cartridges (Strata, Phenomenex).
4. The bound analytes were slowly washed off the dried cartridges using 2 mL methanol followed by 2 mL ethyl acetate.
5. The washed off liquid solvent was dried with a vacuum concentrator (Thermo Scientific Savant Speedvac) until almost dry and then reconstituted in 1 mL H₂O, which contained 10 mM ammonium acetate.

3.7.2 Emerging contaminant sample analysis

For the presence of ECs in the water samples, the water samples were analysed using an AB SCIEX 4000 QTRAP hybrid triple quadrupole/ion trap mass spectrophotometer with a Shimadzu HPLC stack as a front end. All the concentration measurements and processing were performed using Analyst 1.5 (AB SCIEX) software.

3.8 Ecological water quality assessment

For this study, water samples collected at the 12 water sampling sites in the Fonteinspruit stream were assessed to determine the health status of the stream. Aquatic macroinvertebrates were used as indicators for this bio-assessment (Dickens & Graham, 2002). Aquatic macroinvertebrates were collected from the biotopes, such as stones (S), vegetation (VEG) and gravel, sand and mud (GSM) over one water sampling round. The assessment was performed concurrently with the water quality and emerging contaminant assessments during the third round of assessment. Stones biotopes included the stones-in-current and out of current, such as bedrock and any solid material of 2 – 25 cm average size. The vegetation biotopes included all the plant life hanging and growing in and at the edge of the stream. Approximately, two metres of vegetation were sampled. For gravel, sand and mud biotopes, the water sampling was carried out for approximately one minute, depending on the available biotope. The aquatic macroinvertebrates were collected using a soft 2 mm mesh net on a 30 cm

square frame, over a wide area, to ensure that all the biotopes were fully represented. The collection was done in the following manner:

Aquatic macroinvertebrate collection from the stones biotopes

1. The net was placed downstream of the stones to allow the water flow to carry the dislodged aquatic macroinvertebrate into the net.
2. The stones were kicked and turned for approximately two minutes and the net was swept through the disturbed area. Where the stones were difficult to move, the water sampling was then carried out for up to a maximum of five minutes.
3. The collected aquatic macroinvertebrate samples both in and out of current were combined into a single stones biotope sample.

Aquatic macroinvertebrate collection from the vegetation biotopes

1. The net was spread over one or more locations, particularly, where there was vegetation, such as reeds and grass.
2. The net was kept below and swept vigorously through the vegetation in order to allow dislodged aquatic macroinvertebrates to be caught into the net.
3. The collected samples both in and out of current were combined into a single vegetation biotope sample.

Macroinvertebrate collection from the gravel, sand and mud biotopes

1. The gravel, sand and mud biotopes were stirred by shuffling and scraping with the feet.
2. The net was then swept over a wide range of the disturbed area to allow dislodged aquatic macroinvertebrates to be caught in the net.
3. The collected samples both in and out of current were combined into a single gravel, sand and mud (GSM) biotope sample.

3.8.1 Aquatic macroinvertebrate sample preparation and preservation

The aquatic macroinvertebrate samples were prepared and preserved in the following manner:

1. Once the collection of the aquatic macroinvertebrate samples was done, the samples were washed down to the bottom of the net, and the water was allowed to drain out.
2. The samples were put into separate containers clearly marked according to the names of the water sampling site and the sampled biotope.
3. Large obstructing leaves, stones and unwanted debris were removed and then sufficient water to immerse the sample was added to those containers.
4. Samples were placed immediately into 2 L clean lidded containers and preserved in a cooler-box containing ice-packs for later laboratory identification.

3.8.2 Aquatic macroinvertebrate sample analysis

Aquatic macroinvertebrate samples were analysed in the following manner:

1. Aquatic macroinvertebrate samples were drained with clean water in to a tray and allowed to warm up to room temperature.
2. The South African Scoring System version 5 sheet was used to compare the identified aquatic macroinvertebrate families.
3. The sheet contains a list of the different families and their sensitivity scores (SASS score) based on their susceptibility and resistance to pollution conditions.
4. 1 – 5 SASS scores indicate highly tolerant to pollution, 6 – 10 moderately tolerant to pollution and 11 – 15 shows very low tolerance to pollution.
5. The viewing and identification of different aquatic macroinvertebrate families was done for 15 minutes per biotope.
6. In instances where it was difficult to identify the aquatic macroinvertebrate families, the analysis was limited to five minutes. A magnifying lens was used to check the features of the possible identified macroinvertebrate families.
7. The aquatic macroinvertebrate families that were identified were ticked off under the appropriate biotope section before the three biotope columns were combined into a single total column.

3.8.3 Calculation of aquatic macroinvertebrate data

Three main indices were calculated for the 12 water sampling sites in the Fonteinspruit stream. The indices included the number of families identified (= No. Taxa), summing the sensitivity scores of the various aquatic macroinvertebrate families recorded on the SASS 5 scoring sheet (= SASS Score) and dividing the SASS Score by the number of aquatic macroinvertebrate families (= ASPT – Average Score per Taxon).

3.8.4 Interpretation of aquatic macroinvertebrate data

Presently, there is no definitive reference that gives impartial guidance on the interpretation of SASS data. However, the SASS data becomes important when it is assessed together with various factors, such as the habitat quantity, quality and diversity (Dickens & Graham, 2002). For this study, the Biological Bands and Ecological categories for Highveld – lower Ecoregion modelled reference (Dallas, 2007) were used to classify the SASS Score and ASPT values of each of the 12 water sampling sites. This classification outlines the least or unmodified natural habitat sites to the critically modified sites. For example, a water sampling site would fall in Biological Band A if the SASS score is > 124 or ASPT is > 5.6. The Biological Band and Ecological categories for interpreting the SASS data are given in Table 3.3.

Table 3.3 Modelled reference conditions for the Highveld – lower Ecoregion based on SASS 5 and ASPT scores (Dallas, 2007).

SASS score	ASPT	Biological Band/Ecological Categories	Condition
> 124	> 5.6	A	Unimpaired, high diversity of taxa with high sensitivity

SASS score	ASPT	Biological Band/Ecological Categories	Condition
≥ 83 – 124	4.8 – 5.6	B	Slightly impaired, high diversity of taxa, but with few sensitive taxa
≥ 65 – 82	4.6 – 4.8	C	Moderately impaired, moderate diversity of taxa
≥ 52 – 65	4.2 – 4.6	D	Considerably impaired, most tolerant taxa present
≥ 30 – 51	< 4.2	E	Severely impaired, only tolerant taxa present
< 30	< 4.2	F	Critically impaired, few tolerant taxa present

3.9 Conclusion

Water samples were collected to measure the physical, chemical and microbiological water quality properties at the 12 water sampling sites in the Fonteinspruit stream. The physical water quality properties, namely turbidity, EC, pH, temperature and DO, were measured on-site. For chemical properties, water samples were collected in 500 mL plastic bottles and preserved in a cooler-box filled with ice for later laboratory analysis. The total counts for the microbial organisms *E. coli* and coliform bacteria were performed at the accredited laboratory. The Canadian Council of Ministers of the Environmental Water Quality Index (CCME-WQI) was calculated to provide an overall description of the water quality at a particular water sampling site. The assessment of emerging contaminants was performed concurrently with the water quality and ecological quality assessments. For ecological water quality, aquatic macroinvertebrates were used as indicators for this bio-assessment during the third round of assessment.

Chapter 4

Results of Water Quality in the Fonteinspruit Stream

4.1 Introduction

The physical, chemical and microbiological properties of water were determined for the water samples collected at the 12 water sampling sites in the Fonteinspruit stream over three water sampling rounds. A total of 13 water quality properties were assessed, which were electrical conductivity (EC), pH, turbidity, temperature, dissolved oxygen (DO), chemical oxygen demand (COD), ammonia, nitrate, sulphate, phosphate, total hardness and the number of *Escherichia coli* (*E. coli*) and coliform bacteria.

The compliance of the water quality measurements was compared with two different sets of compliance standards. The physical and chemical water quality measurements were compared with the standards of the European Union Surface Water Directives (Environmental Protection Agency [EPA], 2001), while the microbiological water quality measurements were compared with the South African Water Quality Guidelines (second edition). Volume 4: Agricultural Use: Irrigation (DWAF, 1996d).

To determine whether there were any statistically significant differences between the means of the three independent water sampling rounds, one-way analyses of variance (ANOVA) were performed. In the instances where an ANOVA test was significant, a Tukey Honest Significant Difference post-hoc test was performed to determine which of the water sampling rounds differed from the others. Lastly, to determine the overall water quality at a particular water sampling site in the Fonteinspruit stream, the Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI) was calculated (CCME, 2017).

4.2 Results: Physical water quality properties

In this study, six physical water quality properties were measured in the water samples collected at the 12 water sampling sites in the Fonteinspruit stream. These properties were EC, pH, turbidity, temperature, DO and COD.

4.2.1 Electrical conductivity

The measurements of EC revealed relatively large differences amongst water samples in the respective water sampling rounds. For the most, EC measurements were compliant, although two measurements in water sampling Rounds 1 and 2 measurements in water sampling Round 3 exceeded the standard of 1,000 $\mu\text{S}/\text{cm}$ (EPA, 2001). This might be attributed to low flow of the stream. Overall, the EC measurements in water sampling Rounds 1 and 3 were much greater than the EC measurements in water sampling Round 2, with the EC mean for water sampling Round 1 nearly 700 $\mu\text{S}/\text{cm}$ while for water sampling Round 3, it was approximately 400 $\mu\text{S}/\text{cm}$ (Table 4.1). In contrast, the EC mean for water sampling Round 2 was much lower than the other two water sampling rounds, in the order of 5 $\mu\text{S}/\text{cm}$. These EC measurement differences in the water sampling rounds are also reflected in the percentage compliance, where both water sampling Rounds 1 and 3 demonstrated compliance percentages around 80% in comparison to water sampling Round 2 with 100% compliance.

Table 4.1 Electrical conductivity measurements in the three water sampling rounds and summary statistics.

Sample site	EC (limit = $\leq 1,000 \mu\text{S}/\text{cm}$)		
	Round 1	Round 2	Round 3
F1	296.00	5.00	231.00
F2	1,314.00	0.10	1,226.00
F3	1,110.00	6.70	1,252.00
F4	597.00	7.70	827.00

Sample site	EC (limit = $\leq 1,000 \mu\text{S/cm}$)		
	Round 1	Round 2	Round 3
F5	521.00	8.90	510.00
F6	585.00	9.10	155.50
F7	581.00	5.00	149.70
F8	687.00	0.10	317.00
F9	447.00	2.30	347.00
F10	705.00	1.20	38.00
F11	721.00	3.70	3.80
F12	748.00	1.90	4.00
Mean	692.67	4.31	421.75
Median	642.00	4.35	274.00
Range	1018.00	9.03	1248.24
Min	296.00	0.09	3.76
Max	1314.00	9.12	1252.00
SD	277.10	3.28	447.47
% Compliance	83	100	83

When the histogram was prepared for the EC measurements in the three water sampling rounds, the differences amongst the water sampling rounds are clearly discernible. The EC measurements in water sampling Round 1 were the greatest for most of the water sampling sites (10 out of 12 sampling sites), while the EC measurements in water sampling Round 3 were also relatively high (Figure 4.1). In contrast, the bars depicting the exceptionally low EC measurements in water sampling Round 2 are barely visible on the graph. Water sampling sites F2 and F3 recorded the highest measurements, both in water sampling Round 1 and water sampling Round 3.

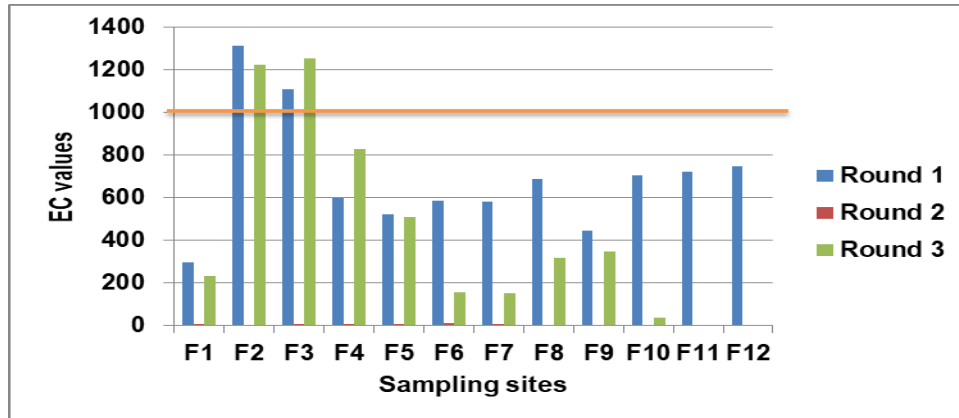


Figure 4.1 Histogram showing the EC measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.2.2 pH

The pH measurements in all three water sampling rounds demonstrated compliance when compared with the standard range of 5.5 – 9.0 (EPA, 2001). The mean pH measurements in the three water sampling rounds showed a relatively narrow range of less than 0.40 (Table 4.2). The mean pH measurements were also relatively close to neutral, although more alkaline for water sampling Rounds 1 and 3.

Table 4.2 The pH measurements in the three water sampling rounds and summary statistics.

Sample site	pH (limit = 5.5–9.0)		
	Round 1	Round 2	Round 3
F1	7.34	6.89	7.40
F2	6.37	8.30	6.73
F3	8.29	7.58	6.13
F4	8.21	7.04	7.51
F5	6.76	6.90	6.62
F6	7.05	8.33	7.33
F7	8.16	7.82	7.41
F8	7.38	6.80	7.09

Sample site	pH (limit = 5.5–9.0)		
	Round 1	Round 2	Round 3
F9	6.89	7.68	7.53
F10	6.95	7.57	7.59
F11	6.81	7.54	6.47
F12	7.05	6.88	6.74
Mean	7.27	7.44	7.05
Median	7.05	7.56	7.21
Range	1.92	1.53	1.46
Min	6.37	6.80	6.13
Max	8.29	8.33	7.59
SD	0.63	0.54	0.49
% Compliance	100	100	100

A histogram was prepared for the pH measurements in the three water sampling rounds to clearly show the differences in measurements amongst the three water sampling rounds. From the histogram, the narrow range of pH measurements around neutral is clearly discernible (Figure 4.2).

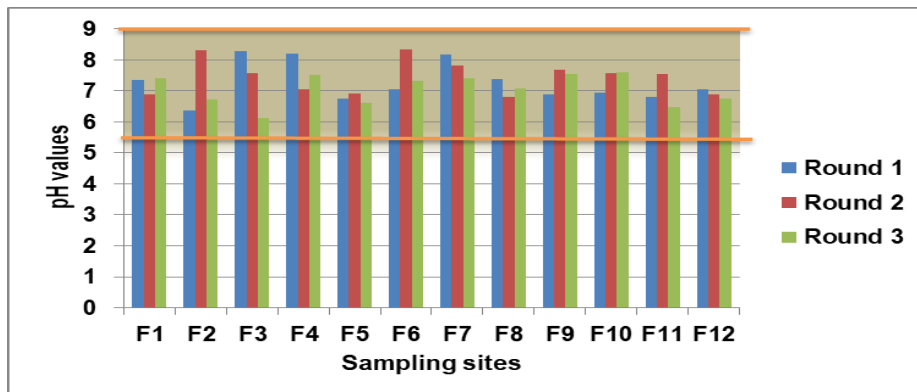


Figure 4.2 Histogram showing the pH measurements at the 12 water sampling sites in the three water sampling rounds. The orange horizontal lines show the compliance range for pH.

4.2.3 Turbidity

The measurements of turbidity in all three water sampling rounds demonstrated substantial variation. All turbidity measurements in water sampling Rounds 1, 2 and 3 showed 0% compliance when compared with the prescribed limit value of 1.0 NTU (EPA, 2001). Water sampling sites F6 and F7 recorded the highest levels of turbidity in all of the water sampling rounds (Table 4.3). This might be attributed to considerable presence of algae and mud at these water sampling sites. The mean turbidity measurements in the three water sampling rounds varied distinctively with water sampling Round 1 recording a mean of less than 20 NTU, water sampling Round 2 a turbidity mean just greater than 25 NTU and water sampling Round 3 a turbidity mean of nearly 40 NTU.

Table 4.3 Turbidity measurements in the three water sampling rounds and summary statistics.

Sample site	Turbidity (limit = ≤ 1.0 NTU)		
	Round 1	Round 2	Round 3
F1	2.54	11.40	8.92
F2	14.90	25.00	45.20
F3	7.97	2.07	22.00
F4	5.15	3.06	71.20
F5	4.62	5.53	6.45
F6	63.10	81.00	125.00
F7	76.60	66.00	66.20
F8	11.10	28.50	27.20
F9	7.04	16.90	6.28
F10	18.80	32.60	23.20
F11	9.01	28.20	29.30
F12	7.46	25.90	34.90
Mean	19.02	27.18	38.82
Median	8.49	25.45	28.25
Range	74.06	78.93	118.72
Min	2.54	2.07	6.28
Max	76.60	81.00	125.00
SD	24.33	24.27	34.42

% Compliance	0	0	0
---------------------	---	---	---

A histogram provides a visual perspective of the turbidity measurements taken in the Fonteinspruit stream. The histogram shows that two adjacent water sampling sites, namely F6 and F7, recorded exceptionally higher turbidity measurements when compared with the other water sampling sites (Figure 4.3). Furthermore, besides the measurements in water sampling Round 3, the turbidity measurement recorded at water sampling sites F3, F4 and F5 were of the lowest in the study.

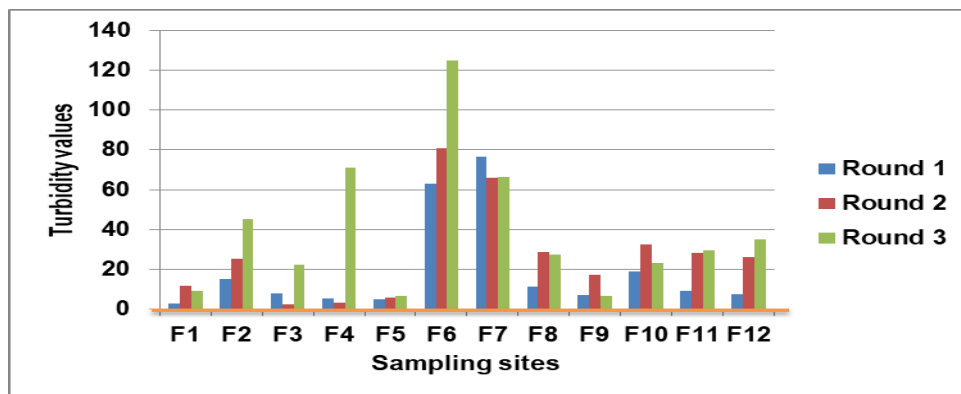


Figure 4.3 Histogram showing the turbidity measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.2.4 Temperature

The temperature measurements in all three water sampling rounds showed 100% compliance when compared with the EU Directives mandatory limit of 25°C (EPA, 2001). All temperatures measured in water sampling Round 3 showed the highest measurements, i.e. above 20°C (Table 4.4). The mean temperatures in water sampling Rounds 1 and 2 were below 15°C, while for water sampling Round 3, the mean temperature was greater than 20°C.

Table 4.4 Temperature measurements in the three water sampling rounds and summary statistics.

Sample site	Temperature (limit = 25°C)		
	Round 1	Round 2	Round 3
F1	11.6	17.3	23.5
F2	15.6	7.1	23.7
F3	11.7	7.6	21.3
F4	11.2	17.0	23.8
F5	7.1	15.3	20.7
F6	13.3	19.1	23.8
F7	12.7	18.7	23.8
F8	13.0	19.2	24.3
F9	11.2	15.9	21.6
F10	11.6	18.5	23.1
F11	12.4	19.3	20.2
F12	11.5	19.5	20.3
Mean	11.91	16.21	22.51
Median	11.65	17.90	23.30
Range	8.50	12.41	4.10
Min	7.10	7.09	20.20
Max	15.60	19.50	24.30
SD	1.96	4.36	1.56
% Compliance	100	100	100

A histogram was constructed to provide a visual perspective of the measurements of temperature in the three water sampling rounds. The histogram clearly shows that all the measurements were below the limit of 25°C (EPA, 2001) (Figure 4.4). It further demonstrates that the recorded temperatures in water sampling Round 3 were substantially warmer than the other two water sampling rounds, i.e. Round 2 followed by Round 1.

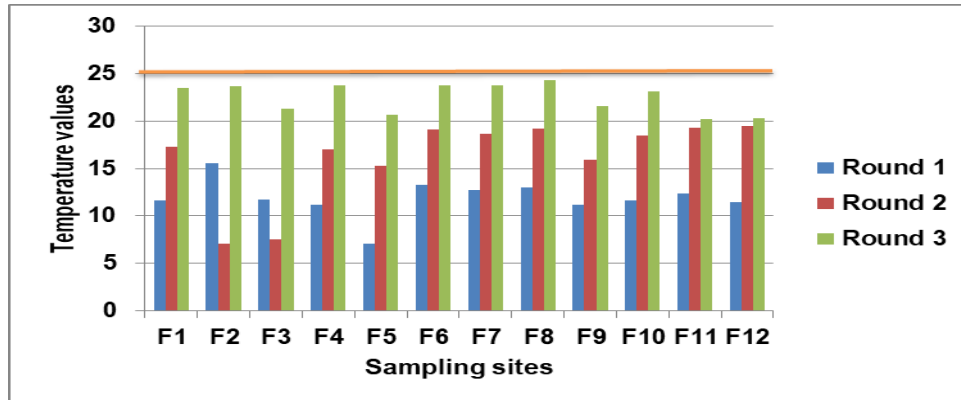


Figure 4.4 Histogram showing the temperature measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.2.5 Dissolved oxygen

The measurements of DO revealed substantial differences amongst the three water sampling rounds. All the DO measurements in water sampling Round 3 were not compliant when compared with the average limit of 6.5 – 9.5 mg/L O₂ (EPA, 2001). In water sampling Rounds 1 and 2, most measurements (66%) were not within the average limit, while in water sampling Round 3, all the DO measurements were below the average limit (Table 4.5). The mean DO measurements in the three water sampling rounds showed a narrow range of less than 4.0 mg/L O₂.

Table 4.5 DO measurements in the three water sampling rounds and summary statistics.

Sample site	DO (limit = 6.5–9.5 mg/L O ₂)		
	Round 1	Round 2	Round 3
F1	5.98	5.04	4.35
F2	7.61	0.13	3.46
F3	8.77	6.73	5.27
F4	6.40	7.71	1.12
F5	7.56	8.91	2.08
F6	14.12	9.12	2.65
F7	8.54	5.34	3.12

Sample site	DO (limit = 6.5–9.5 mg/L O ₂)		
	Round 1	Round 2	Round 3
F8	0.14	0.09	1.22
F9	4.53	2.27	0.79
F10	3.43	1.20	1.44
F11	6.18	3.66	3.19
F12	5.66	1.86	3.14
Mean	6.58	4.34	2.65
Median	6.29	4.35	2.89
Range	13.98	9.03	4.48
Min	0.14	0.09	0.79
Max	14.12	9.12	5.27
SD	3.36	3.29	1.38
% Compliance	33	33	0

The histogram was constructed to discern the pattern of DO measurements in the three water sampling rounds. The histogram shows that at water sampling site F6, the DO measurements taken in water sampling Rounds 1 and 2 were substantially higher than those taken at the other water sampling sites (Figure 4.5). In contrast to F6, the histogram also shows that at water sampling site F8, the DO measurements taken in water sampling Rounds 1 and 2 were low when compared with the other water sampling sites.

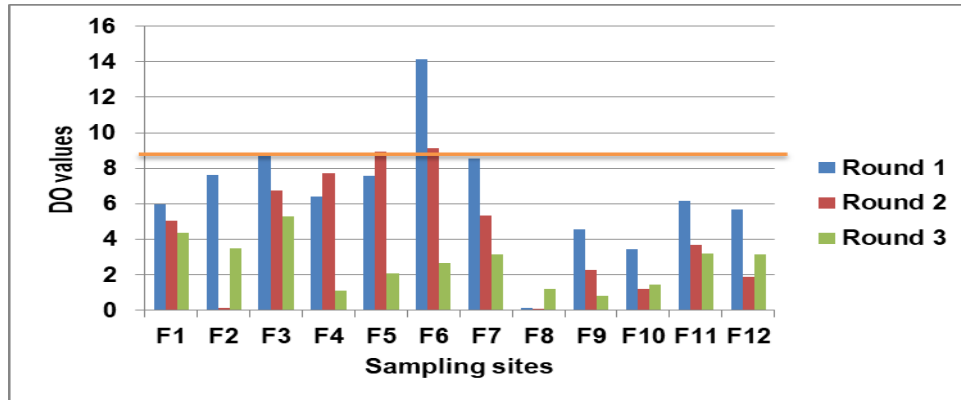


Figure 4.5 Histogram showing the DO measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.2.6 Chemical oxygen demand

The measurements of COD showed comparatively large differences amongst the respective water sampling rounds. Most of the COD measurements in this study were compliant when compared with the limit value of 40 mg/L O₂ (EPA, 2001). In water sampling Round 1, all COD measurements were compliant, while in water sampling Round 2, three measurements exceeded the limit, and in water sampling Round 3, one measurement exceeded the limit. In water sampling Rounds 2 and 3, two measurements were greater than 180 mg/L O₂ (Table 4.6). When considering the mean COD, the mean value in water sampling Round 1 was below 15 mg/L O₂, whereas in water sampling Rounds 2 and 3, the mean COD values were greater than 30 mg/L O₂.

Table 4.6 COD measurements in the three water sampling rounds and summary statistics.

Sample site	COD (limit = ≤ 40 mg/L O ₂)		
	Round 1	Round 2	Round 3
F1	15	16	3
F2	8	12	3
F3	7	4	4
F4	8	11	183

Sample site	COD (limit = ≤ 40 mg/L O ₂)		
	Round 1	Round 2	Round 3
F5	9	22	26
F6	31	28	10
F7	22	112	24
F8	7	56	38
F9	22	199	35
F10	11	37	40
F11	3	33	38
F12	29	32	10
Mean	14.33	46.83	34.50
Median	10.00	30.00	25.00
Range	28.00	195.00	180.00
Min	3.00	4.00	3.00
Max	31.00	199.00	183.00
SD	9.37	55.80	48.99
% Compliance	100	75	91

The histogram was constructed to provide a visual representation of the COD measurements obtained in this study. The histogram shows that compliance was overall relatively high (Figure 4.6). Besides most of the measurements that were compliant, the histogram also shows that three COD measurements taken at water sampling sites F4, F7 and F9 were substantially greater than the other measurements taken in the study (Figure 4.6). In contrast, the COD measurements in water samples collected at water sampling sites F1, F2 and F3 were, overall, the lowest.

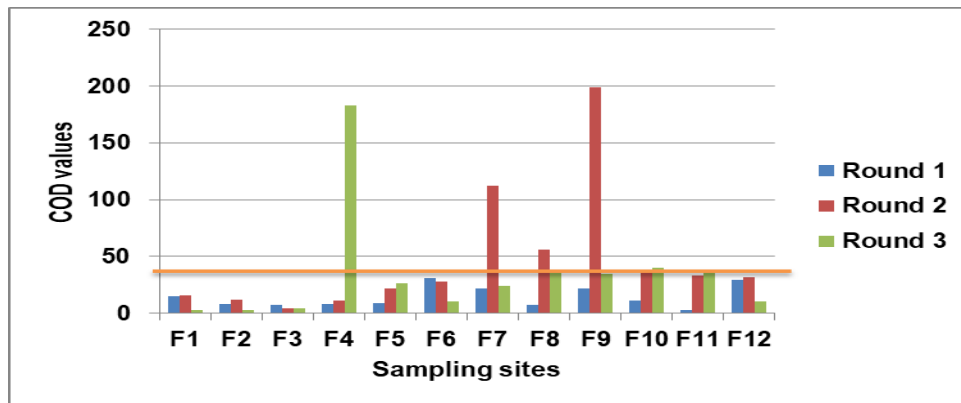


Figure 4.6 Histogram showing the COD measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.3 Results: Chemical water quality properties

Five chemical water quality properties were assessed in the water samples collected at the 12 water sampling sites in the Fonteinspruit stream. The assessed chemical water quality properties were the determination of the concentrations of ammonia, nitrate, sulphate and phosphate as well as the measurement of total hardness.

4.3.1 Ammonia

Only two of the ammonia measurements in water sampling Round 2 exceeded the EU surface water limit of ≤ 1.5 mg/L (EPA, 2001). The mean ammonia concentration was substantially lower in water sampling Round 1 when compared with the other two water sampling rounds, which is also reflected in the recorded maximum readings in the three water sampling rounds (Table 4.7). The maximum readings in water sampling Rounds 2 and 3 were substantially greater than the maximum reading in water sampling Round 1.

Table 4.7 Ammonia measurements in the three water sampling rounds and summary statistics.

Sample site	Ammonia (limit = ≤ 1.5 mg/L)		
	Round 1	Round 2	Round 3
F1	0.00	0.50	1.32
F2	0.00	0.34	0.83
F3	0.01	0.50	0.14
F4	0.02	0.26	0.32
F5	0.01	0.26	0.34
F6	0.01	0.28	0.42
F7	0.00	0.35	0.47
F8	0.00	0.23	0.36
F9	0.01	0.30	0.11
F10	0.00	1.20	1.35
F11	0.01	2.30	0.01
F12	0.00	2.00	0.01
Mean	0.01	0.71	0.47
Median	0.01	0.35	0.35
Range	0.02	2.07	1.34
Min	0.00	0.23	0.01
Max	0.02	2.30	1.35
SD	0.01	0.72	0.46
% Compliance	100	83	100

A histogram was constructed to demonstrate the spread of the ammonia measurements across the water sampling sites. The histogram clearly shows that the two non-compliant ammonia measurements were much greater than the majority of the compliant ammonia measurements (Figure 4.7). The histogram further shows that some of the ammonia measurements at the upstream water sampling sites, namely F1 and F2, and the downstream water sampling site F10, were also relatively high, although compliant.

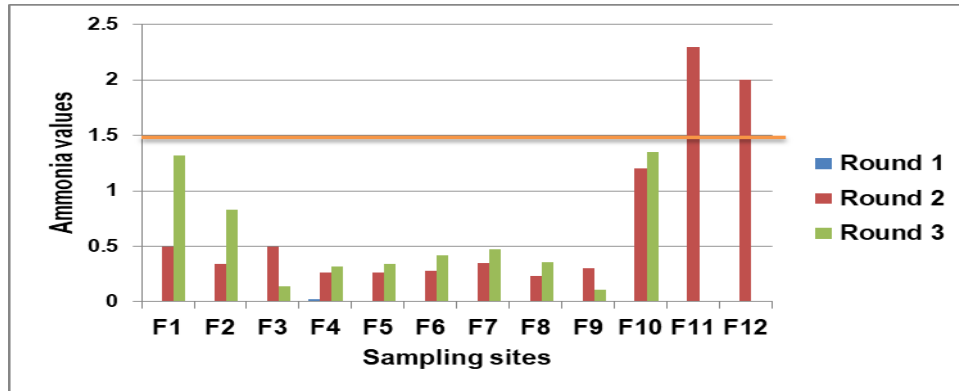


Figure 4.7 Histogram showing the ammonia measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.3.2 Nitrate

In all three water sampling rounds of this study, the measurements for nitrate showed 100% compliance when compared with the EU surface water limit of ≤ 50 mg/L (EPA, 2001). Interestingly, the measurement recorded at water sampling site F6 in water sampling Round 2 was equal to the limit of 50 mg/L and substantially greater than all the other measurements (Table 4.8). This outlier measurement was also responsible for inflating the mean nitrate measurement in water sampling Round 2. If the outlier measurement taken at water sampling site F2 in water sampling Round 2 was ignored, then the mean nitrate value for water sampling Round 2 would have been 3.14 mg/L, which was then marginally greater than the mean nitrate values recorded in water sampling Rounds 1 and 3.

Table 4.8 Nitrate measurements in the three water sampling rounds and summary statistics.

Sample site	Nitrate (limit = ≤ 50 mg/L)		
	Round 1	Round 2	Round 3
F1	1.0	1.3	0.6
F2	1.4	4.2	6.9
F3	3.7	5.4	3.9

Sample site	Nitrate (limit = ≤ 50 mg/L)		
	Round 1	Round 2	Round 3
F4	1.0	4.5	2.0
F5	1.3	1.9	0.6
F6	0.9	50.0	0.2
F7	1.9	9.5	0.1
F8	1.2	2.0	0.2
F9	1.7	0.3	1.3
F10	2.6	1.2	1.0
F11	2.7	2.3	1.7
F12	2.1	2.0	1.4
Mean	1.79	7.05	1.66
Median	1.55	2.15	1.15
Range	2.80	49.70	6.80
Min	0.90	0.30	0.10
Max	3.70	50.00	6.90
SD	0.85	13.75	1.96
% Compliance	100	100	100

A histogram offers a visual perspective of the nitrate measurements taken in this study. The histogram clearly demonstrates the outlier measurement taken at water sampling site F6 (Figure 4.8). Besides the outlier measurement recorded at water sampling site F6, a relatively high nitrate measurement was also recorded at water sampling site F7.

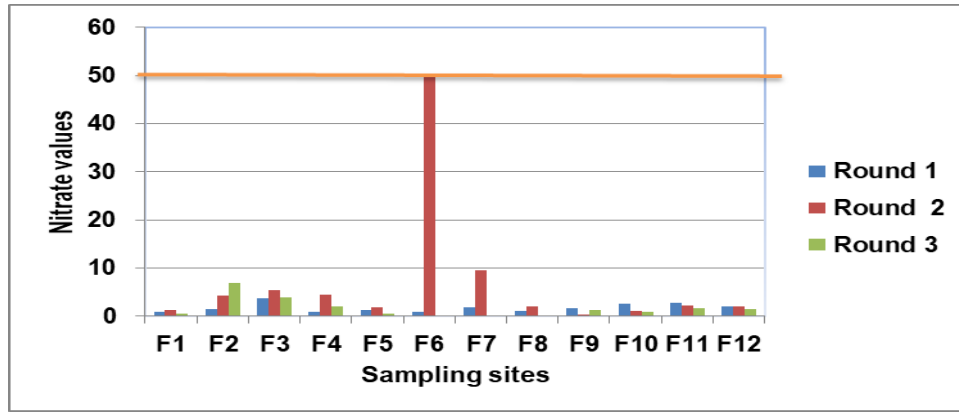


Figure 4.8 Histogram showing the nitrate measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. Orange horizontal line indicates the compliance range.

4.3.3 Sulphate

The measurements recorded for sulphate were all compliant when compared with the limit value of ≤ 200 mg/L (EPA, 2001). Although most of the measurements for sulphate were relatively low, the measurements recorded at water sampling sites F2 and F3, during water sampling Rounds 1 and 3, were substantially higher than all the other measurements (Table 4.9).

Table 4.9 Sulphate measurements in the three water sampling rounds and summary statistics.

Sample site	Sulphate (limit = ≤ 200 mg/L)		
	Round 1	Round 2	Round 3
F1	13	14	9
F2	98	14	87
F3	79	48	93
F4	35	44	41
F5	35	21	25
F6	20	1	8
F7	13	5	9
F8	26	33	19
F9	19	26	20
F10	35	33	28
F11	36	30	29

Sample site	Sulphate (limit = ≤ 200 mg/L)		
	Round 1	Round 2	Round 3
F12	35	40	37
Mean	37.00	25.75	33.75
Median	35.00	28.00	26.50
Range	85.00	47.00	85.00
Min	13.00	1.00	8.00
Max	98.00	48.00	93.00
SD	25.92	15.06	28.34
% Compliance	100	100	100

A histogram provides a visual demonstration of the distribution of sulphate measurements across the water sampling sites. The histogram shows that besides water sampling site F1, the sulphate concentrations at the upstream water sampling sites were relatively high, but decreased towards the midstream water sampling sites, namely F6 and F7. The sulphate concentrations at the downstream water sampling sites demonstrated a steady increase when compared with the water sampling sites F6 and F7 (Figure 4.9).

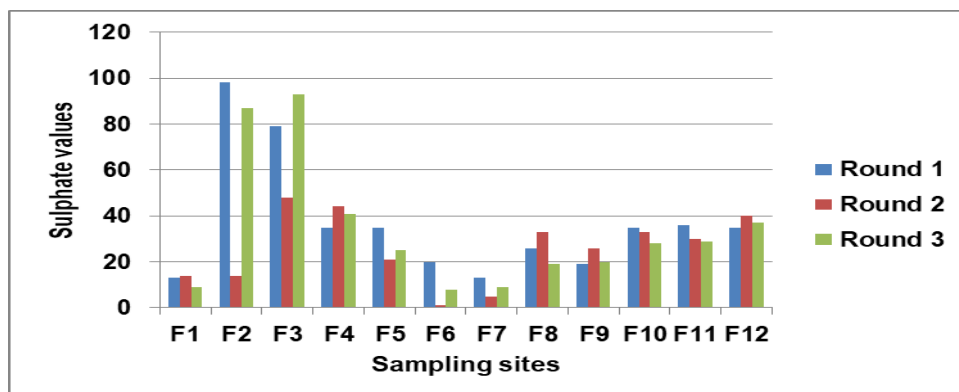


Figure 4.9 Histogram showing the sulphate measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds.

4.3.4 Phosphate

Most of the water quality measurements of phosphate were non-compliant when compared with the EU surface water limit of ≤ 0.7 mg/L (EPA, 2001). Only four phosphate measurements, all recorded in water sampling Round 1, did not exceed the limit (Table 4.10). Overall, the phosphate measurements were substantially higher in water sampling Rounds 2 and 3 when compared with water sampling Round 1. This finding was further supported when viewing the mean phosphate measurements. They were greater by more than a factor of ten in water sampling Rounds 2 and 3 compared with the mean in water sampling Round 1.

Table 4.10 Phosphate measurements in the three water sampling rounds and summary statistics.

Sample site	Phosphate (limit = ≤ 0.7 mg/L)		
	Round 1	Round 2	Round 3
F1	0.58	6.76	26.97
F2	0.62	5.98	24.38
F3	0.50	6.30	26.20
F4	0.92	8.61	0.75
F5	0.68	20.25	0.78
F6	1.11	14.25	13.57
F7	1.54	16.31	7.91
F8	2.89	24.65	15.25
F9	2.28	21.01	17.80
F10	2.18	18.64	15.19
F11	2.52	15.77	1.10
F12	2.64	19.05	2.50
Mean	1.54	14.80	12.70
Median	1.33	16.04	14.38
Range	2.39	18.67	26.22
Min	0.50	5.98	0.75
Max	2.89	24.65	26.97
SD	0.91	6.43	10.04
% Compliance	33	0	0

A histogram demonstrates the relatively low phosphate measurements recorded at all the water sampling sites in water sampling Round 1 in comparison to those measured in water sampling Rounds 2 and 3. The group of downstream water sampling sites, namely F6, F7, F8, F9, F10, F11 and F12, showed relatively high phosphate measurements in both water sampling Rounds 2 and 3 (Figure 4.10). At water sampling sites F1, F2 and F3, the highest phosphate measurements were recorded in water sampling Round 3.

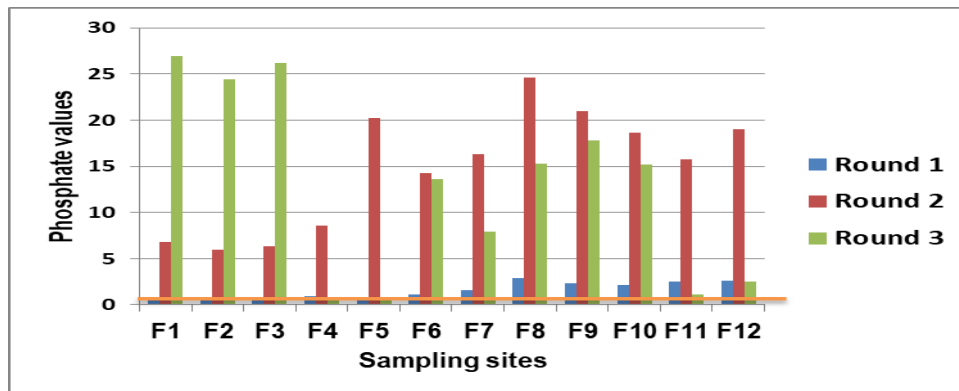


Figure 4.10 Histogram showing the phosphate measurements in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.3.5 Total hardness

All measurements for total hardness in this study were below 50 mg/L CaCO₃. These measurements of hardness were thus 100% compliant when compared with the arbitrary classification of soft water, i.e. 0 to 50 mg/L CaCO₃ (EPA, 2001) (Table 4.11). When comparing the mean values for the total hardness in the three water sampling rounds, the measurements in water sampling Rounds 2 and 3 were relatively low. In contrast, the mean value for total hardness in water sampling Round 1 was substantially higher than the other two water sampling rounds.

Table 4.11 Total Hardness measurements in the three water sampling rounds and summary statistics.

Sample site	Total Hardness (limit = Soft = 0 – 50 mg/L CaCO ₃)		
	Round 1	Round 2	Round 3
F1	31.47	33.45	3.28
F2	14.27	2.60	0.66
F3	10.20	1.44	0.13
F4	18.86	0.30	1.99
F5	27.78	1.99	2.10
F6	13.35	4.60	3.27
F7	14.94	0.44	2.83
F8	12.77	1.81	3.25
F9	14.50	6.92	3.19
F10	24.88	1.85	2.12
F11	25.01	0.43	1.99
F12	23.96	0.61	2.68
Mean	19.33	4.70	2.29
Median	16.90	1.83	2.40
Range	21.27	33.15	3.15
Min	10.20	0.30	0.13
Max	31.47	33.45	3.28
SD	6.97	9.26	1.03
% Compliance	100	100	100

A histogram showing all the hardness measurements was constructed to ascertain if a particular distribution pattern could be discerned. The histogram demonstrates that the total hardness values for all the water sampling sites were much higher in water sampling Round 1, except for water sampling site F1 where high values were recorded for both water sampling Rounds 1 and 2 (Figure 4.11).

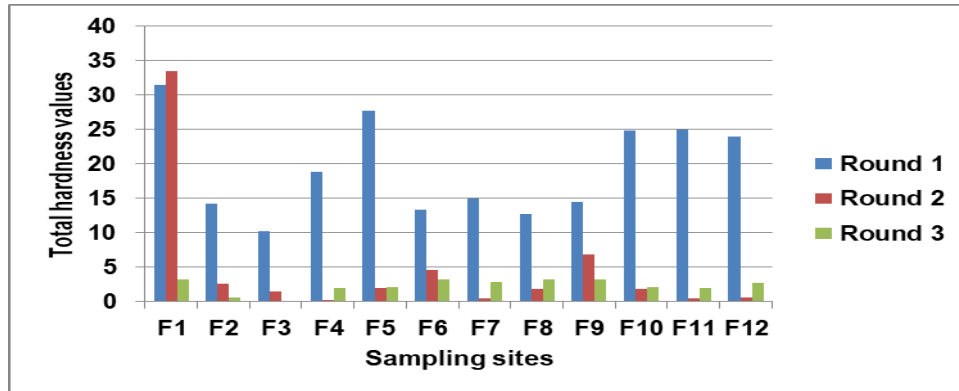


Figure 4.11 Histogram showing the total hardness measurements in water samples at the 12 water sampling sites in the three water sampling rounds.

4.4 Results: Microbiological water quality properties

In this study, two microbiological water quality properties were measured in water samples collected at the 12 identified water sampling sites in the Fonteinspruit stream. These properties were the enumeration of *E. coli* and coliform bacteria. For the compliance of these microbial properties, the measurements were compared with the limits specified by the Department of Water Affairs and Forestry South African Water Quality Guidelines for Agricultural Use: Irrigation (DWAF, 1996d).

4.4.1 *E. coli*

Overall, most of the *E. coli* counts were within the limits specified by the DWAF (DWAF, 1996d) with only eight of the counts exceeding the limits. Thus, the compliance was relatively high in two of the water sampling rounds, with compliance percentages greater than 80% (Table 4.12). The percentage compliance for the water samples collected in water sampling Round 3 was substantially less than the other two water sampling rounds, which can be attributed to the *E. coli* counts of five of the twelve water samples exceeding the limits. Also, the *E. coli* counts determined in all three water samples collected at water sampling site F8 stand out. The *E. coli* counts in the water samples collected in all three water sampling rounds exceeded the 1,000 cfu/100 mL limit specified by the DWAF (DWAF, 1996d) and the limit of the test. Although the true *E. coli* counts cannot be made in some of the water

samples because of the limitation of the test, the mean *E. coli* count in water sampling Round 3 was substantially greater than the other two water sampling rounds when the limit for the test was used in the calculation of the mean.

Table 4.12 *E. coli* measurements in the three water sampling rounds and summary statistics.

Sample site	<i>E. coli</i> (limit = ≤ 1,000 cfu/100 mL)		
	Round 1	Round 2	Round 3
F1	7.5	3.4	700.0
F2	7.5	186.0	>2,419.6
F3	9.8	400.0	1,200.0
F4	4.1	101.0	124.0
F5	3.1	108.0	84.0
F6	4.1	202.0	700.0
F7	42.1	87.0	121.0
F8	>2,419.6	>2,419.6	>2,419.6
F9	419.8	59.0	19.0
F10	472.1	35.0	107.0
F11	509.9	103.0	1,140.0
F12	509.9	1,100.0	>2,419.6
Mean	367.46	400.33	954.48
Median	25.95	105.50	700.00
Range	2,416.50	2,416.20	2,400.60
Min	3.10	3.40	19.00
Max	2,419.60	2,419.60	2,419.60
SD	684.59	702.04	972.32
% Compliance	92	83	58

>2,419.6 = exceeded the limit of the test

The histogram of the *E. coli* counts demonstrates the exceptionally high values at some of the water sampling sites. Water sampling site F8 clearly stands out with exceptionally high *E. coli* counts in all three of the water sampling rounds (Figure 4.12). A number of other water sampling sites in water

sampling Round 3 also stand out with high counts of *E. coli*, although not all counts were non-compliant.

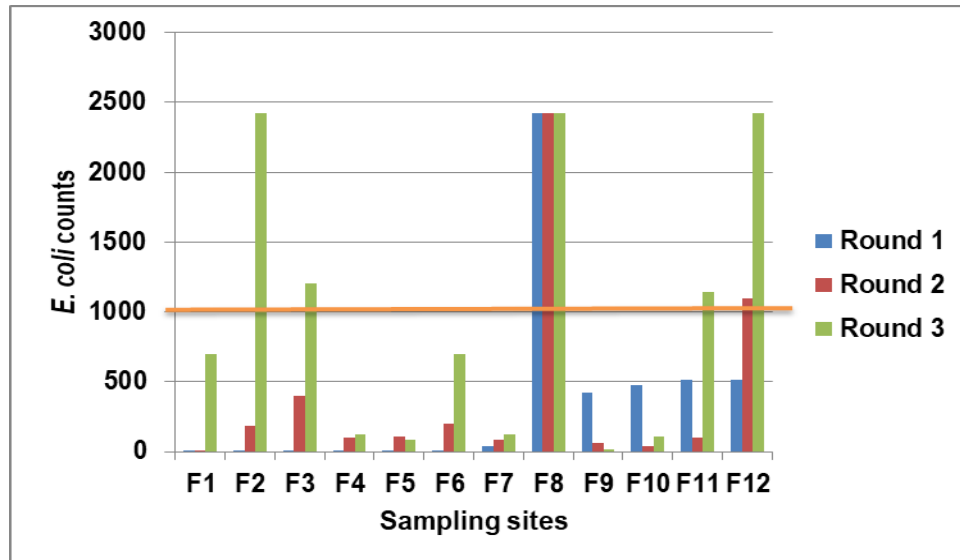


Figure 4.12 Histogram showing the *E. coli* counts in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.4.2 Total coliforms

When viewing the coliform bacterial counts, many more exceeded the limits specified by the DWAF (DWAF, 1996d) when compared with the *E. coli* counts. In particular, exceptionally high coliform bacterial counts were recorded in water sampling Rounds 2 and 3 (Table 4.13). Many of the coliform bacterial counts exceeded the limit of the test, predominantly in water sampling Rounds 2 and 3 (Table 4.13). These high coliform bacterial counts are also reflected in the low percentages of compliance when the measurements were compared with the Target Water Quality Range of 1,000 cfu/100 mL (DWAF, 1996d).

Table 4.13 Total coliforms measurements in the three water sampling rounds and summary statistics.

Sample site	Total coliforms (limit = $\leq 1,000$ cfu/100 mL)		
	Round 1	Round 2	Round 3
F1	3.2	>2,419.6	>2,419.6
F2	39.3	>2,419.6	>2,419.6
F3	24.3	1,021.0	>2,419.6
F4	34.1	1,100.0	500.0
F5	48.7	1,620.0	400.0
F6	36.4	>2,419.6	>2,419.6
F7	78.8	>2,419.6	>2,419.6
F8	>2,419.6	>2,419.6	>2,419.6
F9	816.4	>2,419.6	>2,419.6
F10	980.4	>2,419.6	>2,419.6
F11	1,119.9	>2,419.6	>2,419.6
F12	689.3	>2,419.6	>2,419.6
Mean	524.20	2,126.45	2,091.33
Median	63.75	2,419.60	2,419.60
Range	2,416.40	1,398.60	2,019.60
Min	3.20	1,021.00	400.00
Max	2,419.60	2,419.60	2,419.60
SD	734.06	548.18	766.96
% Compliance	83	0	17

>2,419.6 = exceeded the limit of the test

A histogram was constructed to show the overall picture of the total coliform bacterial counts in the respective water samples collected at the different water sampling sites. The histogram shows excessively high counts of coliform bacteria in the water samples collected from most of the water sampling sites. Furthermore the histogram shows that the water samples collected at water sampling sites F4 and F5 were relatively lower when compared with the water samples collected at the other water sampling sites (Figure 4.13).

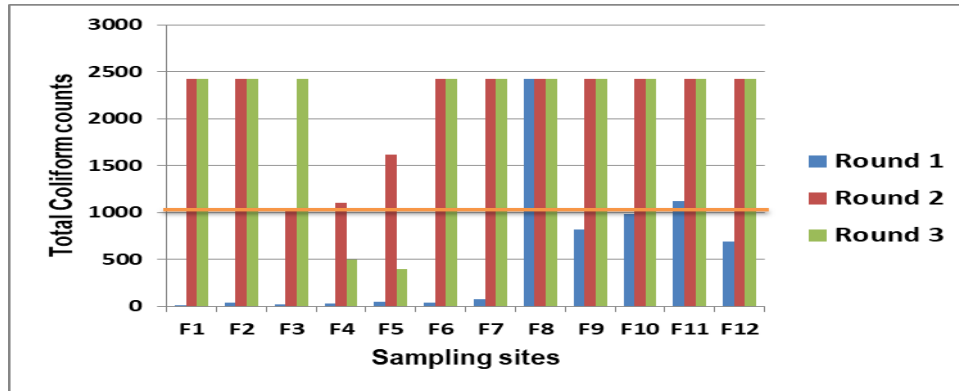


Figure 4.13 Histogram showing the total coliforms counts in water samples collected at the 12 water sampling sites in the three water sampling rounds. The orange horizontal line indicates the compliance range.

4.5 Comparative analysis of the water sampling rounds

One-way ANOVA tests were performed for the respective water quality properties to ascertain if significant differences existed amongst the measurements determined in the different water sampling rounds. Of the 13 ANOVA tests that were performed, seven returned significant differences amongst the water sampling rounds at $\alpha = 0.05$ (Table 4.13). The Tukey Honest Significant Difference post-hoc tests revealed that for temperature all the pairs of the water sampling rounds differed significantly from one another. The remainder of the Tukey tests showed significant differences between two pairs of water sampling rounds, except for DO and ammonia with only one significant pair.

Table 4.14 Statistical comparison results of physical, chemical and microbiological water quality properties in the three water sampling rounds.

Property	SS	MS	f	p	Significant rounds
Physical properties					
EC	2885915.16	1442957.58	15.63	<0.0001	R1 and R2 R2 and R3
pH	0.96	0.48	1.54	0.23	NS
Turbidity	3157.08	1578.54	2.18	0.12	NS

Proper	SS	MS	f	p	Significant rounds
Temperature	682.20	341.10	40.44	< 0.0001	R1 and R2 R1 and R3 R2 and R3
DO	93.01	46.50	5.81	0.00	R1 and R3
COD	6460.22	3230.11	1.73	0.19	NS
Chemical properties					
Ammonia	3.08	1.54	6.26	0.001	R1 and R2
Nitrate	226.95	113.48	1.76	0.19	NS
Sulphate	804.5	402.25	0.71	0.50	NS
Phosphate	1219.25	609.62	12.8	< 0.0001	R1 and R2 R1 and R3
Total hardness	2041.00	1020.50	22.63	< 0.0001	R1 and R2 R1 and R3
Microbiological properties					
<i>E. coli</i>	2611045.36	1305522.68	2.05	0.10	NS
Total coliforms	20097380.50	10048690.25	21.12	< 0.0001	R1 and R2 R1 and R3

SS = sum of squares; MS = mean square; f = f statistic; p = probability; S = significant at $\alpha = 0.05$; NS = non-significant at $\alpha = 0.05$; R1 = sampling Round 1; R2 = sampling Round 2; R3 = sampling Round 3; EC = Electrical conductivity, DO = Dissolved oxygen; COD= Chemical oxygen demand

4.6 Overall water quality of the water samples

CCME-WQIs were calculated for each water sampling site in the Fonteinspruit stream to convey the overall quality of the collected water samples. The CCME-WQI results demonstrated that none of the water samples collected at the 12 water sampling sites in the Fonteinspruit stream were of good quality when compared with the index categorization schema (CCME, 2017). Only one water sampling site had fair water quality, while for the majority of the water sampling sites (58%), the CCME-WQI results revealed that the water quality was marginal and poor for the remainder of the water sampling sites (33%) (Table 4.15).

Table 4.15 Water quality indexes and water quality ranges of water samples collected at the different water sampling sites.

Sampling site	CCME-WQI	Rank	Explanation
F1	53.89	Marginal	Marginal water quality at water sampling site F1 might be attributed to fewer human activities or their impacts had little effect on the water quality.
F2	42.93	Poor	Poor water quality at water sampling site F2 might be attributed to the effects of open disposal of solid waste near this water sampling site.
F3	50.53	Marginal	Marginal water quality at water sampling site F3 might be attributed to an increased flow in this section of the stream that assisted in diluting the pollutants.
F4	47.93	Marginal	Marginal water quality at water sampling site F4 might be attributed to an increased flow in this section of the stream that assisted in diluting the pollutants.
F5	78.71	Fair	Fair water quality at water sampling site F5 might be attributed to the unrestrained presence of domestic animals and illegal dumping sites near this water sampling site.
F6	43.82	Poor	Poor water quality at water sampling site F6 might be attributed to the combined effects of human activities and inflow of pollutants from various drainage areas.
F7	41.51	Poor	Poor water quality at water sampling site F7 might be attributed to the combined effects of an illegal dumping site and human activities near this water sampling site.

Sampling site	CCME-WQI	Rank	Explanation
F8	52.65	Poor	Poor water quality at water sampling site F8 might be attributed to the effects of human activities, such as wastewater from a car-wash facility and car panel-beating workshops near the water sampling site.
F9	46.69	Marginal	Marginal water quality at water sampling site F9 might be attributed to overflow from the manhole and solid waste being discarded into the stream.
F10	47.14	Marginal	Marginal water quality at sampling site F10 might be attributed to overflow from the manhole and solid waste being discarded into the stream.
F11	47.90	Marginal	Marginal water quality at water sampling site F11 might be attributed to the combined effects of overflow from the manhole and waste water from the WWTP.
F12	52.83	Marginal	Marginal water quality at water sampling site F12 might be attributed to the combined effects of overflow from the manhole and waste water from the WWTP.

4.7 Summary

The water quality results of the Fonteinspruit stream clearly indicate that the stream water is of poor quality. This is demonstrated by the CCME-WQIs, which revealed that none of the water samples collected at the 12 water sampling sites are of good water quality. Of the 13 water quality properties assessed at the 12 water sampling sites in the Fonteinspruit stream, only five demonstrated a 100% compliance rate when the measurements were compared with various standard limits of the South African Water Quality guidelines and EU Surface Water Directives. These five water quality properties

included two physical water quality properties, namely pH and temperature, and three chemical water quality properties, namely nitrate, sulphate and total hardness. The majority of the water quality properties (66%) demonstrated relatively low levels of compliance. They included the four physical water quality properties, namely EC, turbidity, DO, COD; two chemical water quality properties, namely ammonia and phosphate as well as two microbiological water quality properties, namely *E. coli* and bacterial counts of coliforms.

For the physical water quality properties, water sample measurements at water sampling sites F2 and F3 were the highest of the EC measurements. Water sample measurements at water sampling sites F6 and F7 were relatively the highest of the turbidity measurements. The DO water sample measurements at water sampling sites F8, F9 and F10 were the lowest and the COD water sample measurements at water sampling sites F7, F8 and F9, were considerably high.

For chemical properties, ammonia water sample concentration measurements at water sampling sites F11 and F12 were the highest, while phosphate water sample concentration measurements at water sampling site F8 were the highest. Water samples collected at water sampling site F8 had the highest counts of *E. coli* and total coliforms. The high levels of these water quality properties may have adverse effects on the aquatic life, animals and people who may eat the food irrigated with the stream water.

Chapter 5

Results of Emerging Contaminants on Water Quality in the Fonteinspruit Stream

5.1 Introduction

Emerging contaminants (ECs), which are synthetic or naturally occurring chemicals or any microorganisms that are not commonly monitored in the environment, were also assessed in this study. For the assessment for the presence of ECs in the Fonteinspruit stream, water samples were collected at the 12 water sampling sites during a single round of water collection. The presence of eight ECs was assessed in the water samples, which included four pharmaceuticals, one plasticizer and three herbicide analytes.

The pharmaceuticals that were assessed were carbamazepine, estrone, estradiol and 17a ethynylestradiol. Carbamazepine is an anticonvulsant and works by decreasing nerve impulses that cause seizures and nerve pain, such as trigeminal neuralgia and diabetic neuropathy. Carbamazepine is also used to treat bipolar disorder (CCME, 2018). Estrone, like estradiol and 17a ethynylestradiol, is an estrogen. These hormones are used to treat postmenopausal woman (European Commission, 2018). The plasticizer assessed in this study was bisphenol A, which is an industrial chemical that is used to make certain plastics and resins (Canadian Environmental Protection Act, 1999).

The three herbicide analytes that were assessed were atrazine, metolachlor and terbuthylazine. The herbicide atrazine is used to prevent pre- and post-emergence of broadleaf weeds in crops, such as maize, sugarcane and turf (CCME, 1999). Metolachlor is an organic compound that is a highly effective herbicide used in grasses (CCME, 1999). A suspension of terbuthylazine concentrate is used to combat the pre- and post-emergence of a broad spectrum of annual weeds (WHO, 2003). To

determine the status of the compliance of the eight ECs that were assessed, their concentrations were compared with appropriate preselected sets of compliance standards.

5.2 Results: Pharmaceutical analytes

5.2.1 Carbamazepine

After determining the concentrations of carbamazepine in the 12 water samples collected at the respective water sampling sites, they were compared with the Canadian Environmental Quality Guidelines of the Canadian Council of Ministers of the Environment (CCME, 2018). Of the 12 concentrations determined for carbamazepine, only one of the concentrations (water sampling site F2) was compliant when compared with the standard of 10 µg/L (CCME, 2018) (Table 5.1). The concentrations of carbamazepine in the water samples collected at water sampling sites F8 and F12 were the highest concentrations of all the concentrations determined. The concentration in the water sample at water sampling site F8 exceeding the standard by approximately 150 µg/L, while the concentration of carbamazepine in the water sample at water sampling site F12 exceeded the standard by more than 90 µg/L.

Table 5.1 Carbamazepine concentration values in water sampling Round 1 and summary statistics.

Sampling site	Carbamazepine (limit ≤ 10 µg/L)
	Round 1
F1	13
F2	9
F3	24
F4	38
F5	28
F6	14
F7	22
F8	161
F9	42

Sampling site	Carbamazepine (limit ≤ 10 µg/L)
	Round 1
F10	46
F11	42
F12	101
Mean	45
Median	33
Range	152
Min	9
Max	161
SD	44
% Compliance	8

The histogram for the concentration values of carbamazepine analyte shows a discernible difference amongst the water samples collected at the water sampling sites in the Fonteinspruit stream. Most concentration values (11 out of 12) of the water samples collected at the respective water sampling sites exceeded the limit. Water samples collected at the majority of the water sampling sites (Figure 5.1) recorded high concentration values, i.e. above 20 µg/L whereas, the water samples at water sampling sites F2 and F6 demonstrated the lowest concentration values of carbamazepine analyte, i.e. below 15 µg/L.

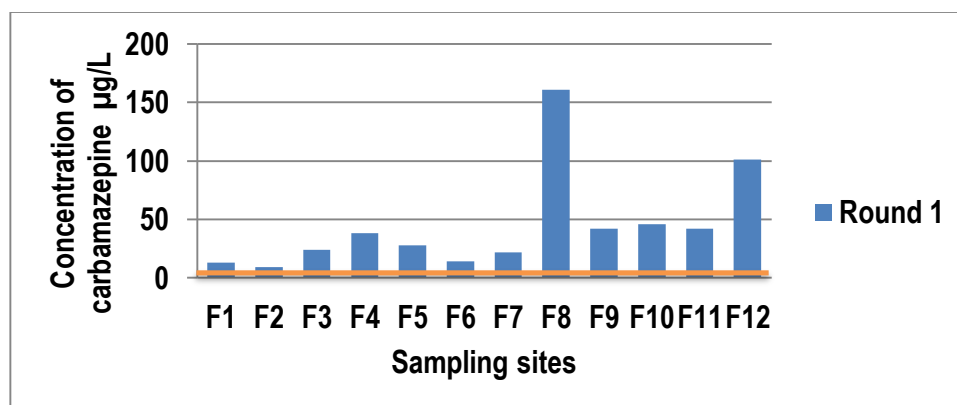


Figure 5.1 Histogram showing the measurements of carbamazepine in water samples collected at the 12 water sampling sites in water sampling Round 1.

5.2.2 Estrone

The concentration values of estrone analyte in all water samples collected at the 12 water sampling sites in the Fonteinspruit stream revealed non-compliance when compared with the standard of ≤ 0.4 ng/L (European Commission, 2018). The two water samples from water sampling sites F9 and F11 showed the highest concentration values, i.e. over 15 ng/L (Table 5.2). The mean estrone concentration value of this water sampling round showed a relatively low range of less than 10 ng/L.

Table 5.2 Estrone concentration values in water sampling Round 1 and summary statistics.

Sampling site	Estrone (limit ≤ 0.4 ng/L)
	Round 1
F1	8
F2	1
F3	1
F4	2
F5	3
F6	1
F7	6
F8	6
F9	19
F10	10
F11	18
F12	1
Mean	6
Median	5
Range	18
Min	1
Max	19
SD	6
% Compliance	0

The histogram was prepared to provide a visual perspective of the concentration values of estrone analyte detected in the Fonteinspruit stream. All the estrone analyte concentration values from the 12

water sampling sites exceeded the 0.4 ng/L limit as highlighted by the orange compliance line. Water sampling sites F9 and F11 showed the highest concentration values of estrone exceeding 15 ng/L (Figure 5.2). In contrast, most of the estrone concentration values (10 out of 12) showed levels below 11 ng/L.

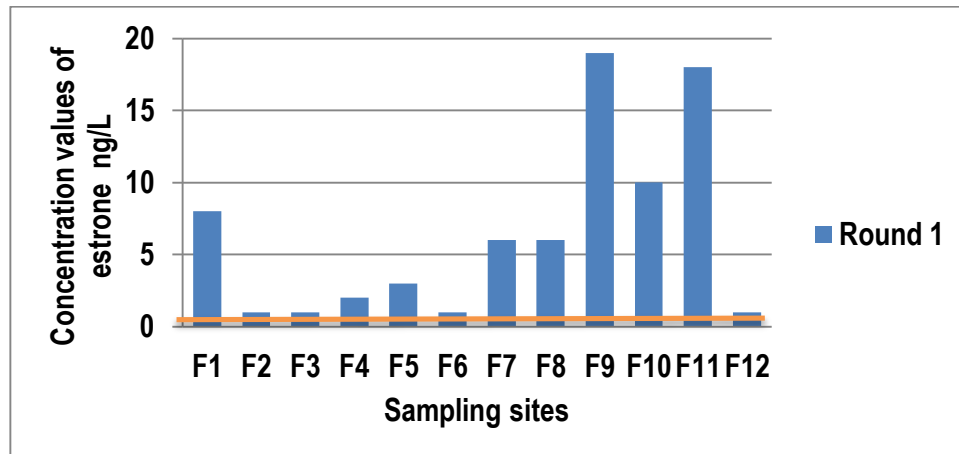


Figure 5.2 Histogram showing the measurements of estrone in water samples collected at the 12 water sampling sites in water sampling Round 1.

5.2.3 Estradiol

The concentration values of estradiol analyte at all 12 water sampling sites showed 100% compliance as the values did not exceed the standard of ≤ 0.4 ng/L (European Commission, 2018). In fact, all the water sampling sites recorded a zero concentration value for estradiol analyte (Table 5.3).

Table 5.3 Estradiol concentration values in water sampling Round 1 and summary statistics.

Sampling site	Estradiol (limit ≤ 0.4 ng/L)
	Round 1
F1	0
F2	0
F3	0

Sampling site	Estradiol (limit ≤ 0.4 ng/L)
	Round 1
F4	0
F5	0
F6	0
F7	0
F8	0
F9	0
F10	0
F11	0
F12	0
Mean	0
Median	0
Range	0
Min	0
Max	0
SD	0
% Compliance	100

5.2.4 17a ethynylestradiol

All 17a ethynylestradiol analyte concentration values revealed non-compliance as the values exceeded the standard of ≤ 0.035 ng/L (European Commission, 2018). The water sampling sites F1, F6 and F7 recorded the highest concentration values, i.e. above 500 ng/L (Table 5.4). The mean 17a ethynylestradiol of this water sampling round was nearly 400 ng/L.

Table 5.4 17a ethynylestradiol concentration values in water sampling Round 1 and summary statistics.

Sampling site	17a ethynylestradiol (limit ≤ 0.035 ng/L)
	Round 1
F1	538
F2	197
F3	458

Sampling site	17a ethynylestradiol (limit ≤ 0.035 ng/L)
	Round 1
F4	496
F5	227
F6	752
F7	920
F8	157
F9	259
F10	221
F11	211
F12	158
Mean	383
Median	243
Range	763
Min	157
Max	920
SD	252
% Compliance	0

The histogram was constructed to discern the pattern of 17a ethynylestradiol analyte concentration values measured at the 12 water sampling sites. The histogram shows that all 12 concentration values of 17a ethynylestradiol analyte exceeded the 0.035 ng/L limit. The concentration values at water sampling sites F2, F8 and F12 were below 200 ng/L when compared with the concentration values of the other water sampling sites. These other water sampling sites ranged from over 200 ng/L to nearly 1 000 ng/L (Figure 5.3).

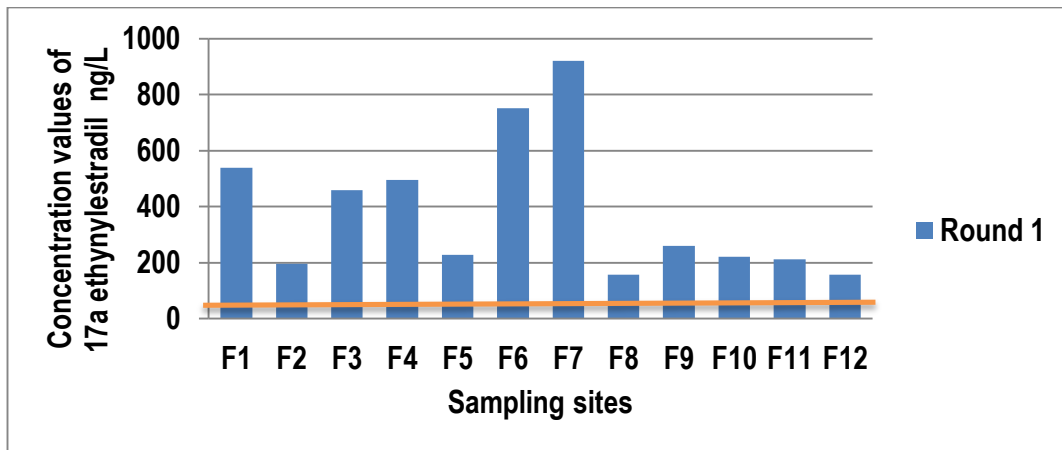


Figure 5.3 Histogram showing the measurements of 17a ethynylestradiol in water samples collected at the 12 water sampling sites in water sampling Round 1.

5.3 Results: Plasticiser analytes

5.3.1 Bisphenol A

The measured concentration values of bisphenol A analyte showed substantially high values at the respective water sampling sites with all exceeding the standard of $\leq 1.4 \mu\text{g/L}$ (Canadian Environmental Protection Act, 1999). The water sampling site F12 showed the highest concentration value, i.e. above $800 \mu\text{g/L}$ (Table 5.5). The mean bisphenol A of this water sampling round was nearly $200 \mu\text{g/L}$.

Table 5.5 Bisphenol A concentration values in water sampling Round 1 and summary statistics.

Sampling site	Bisphenol A (limit $\leq 1.4 \mu\text{g/L}$)
	Round 1
F1	116
F2	87
F3	55
F4	23
F5	27
F6	77
F7	203

Sampling site	Bisphenol A (limit $\leq 1.4 \mu\text{g/L}$)
	Round 1
F8	205
F9	280
F10	338
F11	113
F12	820
Mean	195
Median	115
Range	797
Min	23
Max	820
SD	220
% Compliance	0

The histogram for the bisphenol A analyte concentration values shows non-compliance across all of the 12 water sampling sites in the Fonteinspruit stream. Most water sampling sites (7 out of 12) recorded the highest concentration values, i.e. above 100 $\mu\text{g/L}$. While water sampling sites F2, F3, F4, F5 and F6 showed concentration values below 90 $\mu\text{g/L}$ (Figure 5.4).

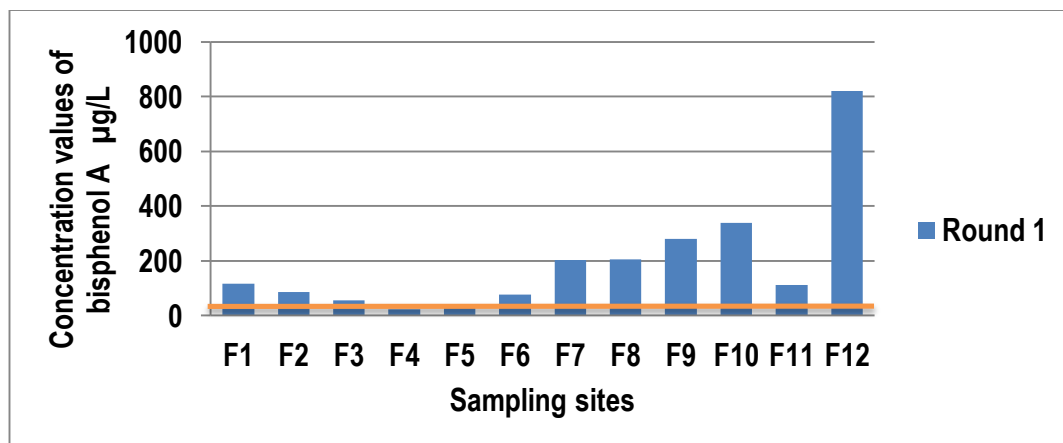


Figure 5.4 Histogram showing the measurements of bisphenol A in water samples collected at the 12 water sampling sites in water sampling Round 1.

5.4 Results: Herbicide analytes

5.4.1 Atrazine

All concentration values of atrazine analyte were non-compliant as the concentration values exceeded the standard of $\leq 1.8 \mu\text{g/L}$ (CCME, 1999). Water sampling site F8 recorded the highest concentration value, i.e. above $250 \mu\text{g/L}$ (Table 5.6). The mean of atrazine was over $50 \mu\text{g/L}$.

Table 5.6 Atrazine concentration values in water sampling Round 1 and summary statistics.

Sampling site	Atrazine (limit $\leq 1.8 \mu\text{g/L}$)
	Round 1
F1	74
F2	7
F3	7
F4	29
F5	37
F6	10
F7	12
F8	257
F9	38
F10	71
F11	46
F12	40
Mean	52
Median	38
Range	250
Min	7
Max	257
SD	68
% Compliance	0

A histogram demonstrates the spread of the atrazine analyte concentration values across the water sampling sites. All concentration values detected in the water samples collected at the 12 water

sampling sites in the Fonteinspruit stream showed no compliance. However, most water sampling sites (11 out of 12) recorded concentration values below 80 µg/L with only water sampling site F8 showing a concentration value of almost 260 µg/L (Figure 5.5).

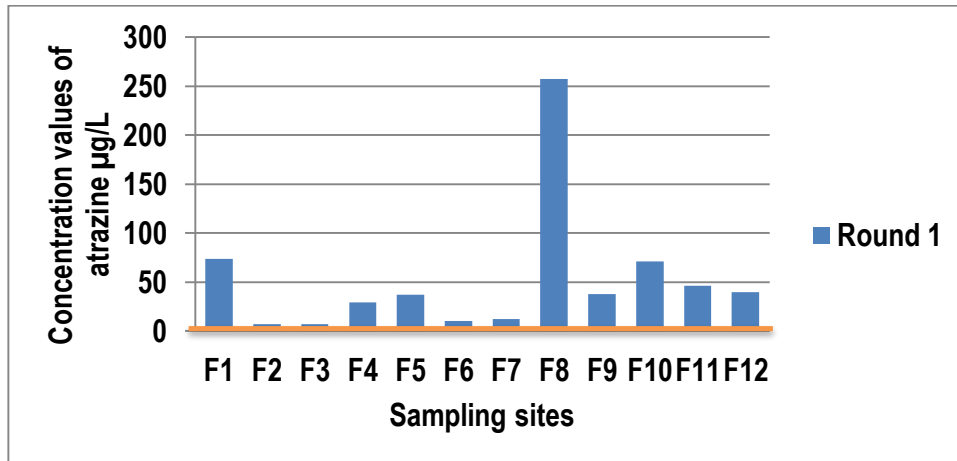


Figure 5.5 Histogram showing the measurements of atrazine in water samples collected at the 12 water sampling sites in water sampling Round 1.

5.4.2 Metolachlor

The concentration values of metolachlor analyte showed substantial differences among the respective water sampling sites in the water sampling round. The two water sampling sites F2 and F3 demonstrated compliance whereas the other ten water sampling sites exceeded the standard of ≤ 7.8 µg/L (CCME, 1999). Water sampling site F8 showed the highest concentration value, i.e. above 200 µg/L (Table 5.7). The mean of metolachlor in the water sampling round was below 50 µg/L.

Table 5.7 Metolachlor concentration values in water sampling Round 1 and summary statistics.

Sampling site	Metolachlor (limit ≤ 7.8 µg/L)
	Round 1
F1	73
F2	4
F3	4

Sampling site	Metolachlor (limit $\leq 7.8 \mu\text{g/L}$)
	Round 1
F4	15
F5	61
F6	8
F7	11
F8	213
F9	34
F10	62
F11	53
F12	39
Mean	48
Median	37
Range	209
Min	4
Max	213
SD	58
% Compliance	17

A histogram of the metolachlor analyte concentration values shows that most water sampling sites (10 out of 12) exceeded the $\leq 7.8 \mu\text{g/L}$ limit. The histogram further demonstrates the outlier concentration value of water sampling site F8. The metolachlor concentration values recorded at water sampling sites F1, F5, F8, F10 and F11 (Figure 5.6) were overall the highest of the concentration values.

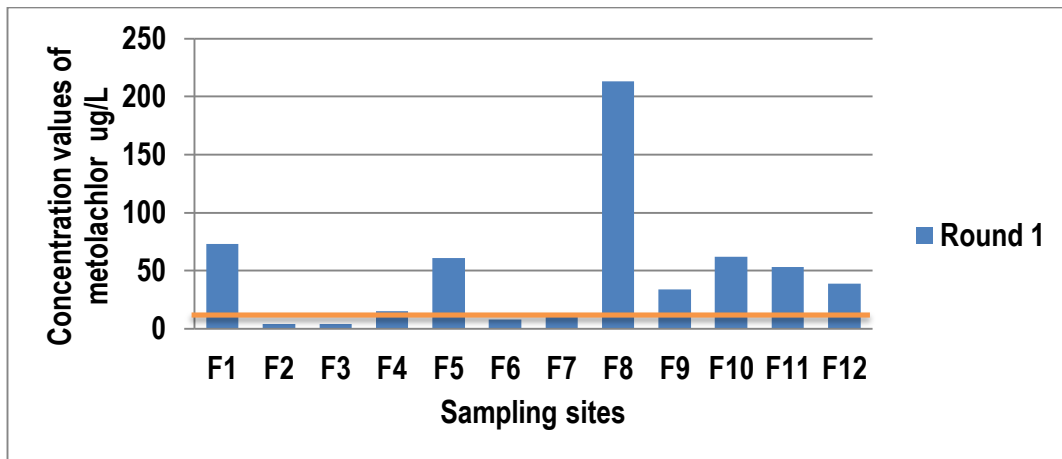


Figure 5.6 Histogram showing the measurements of metolachlor in water samples collected at the 12 water sampling sites in water sampling Round 1.

5.4.3 Terbutylazine

Only one terbutylazine analyte concentration value showed compliance and eleven concentration values were above the standard of ≤ 0.007 mg/L (WHO, 2003). The compliance rate for the water sampling round was below 10%. Water sampling site F8 recorded the highest concentration value of nearly 0.500 mg/L (Table 5.8). The mean of terbutylazine analyte in the water sampling round was above 0.100 mg/L.

Table 5.8 Terbutylazine concentration values in water sampling Round 1 and summary statistics.

Sampling site	Terbutylazine (limit ≤ 0.007 mg/L)
	Round 1
F1	0.069
F2	0.005
F3	0.009
F4	0.069
F5	0.061
F6	0.144
F7	0.183
F8	0.499

Sampling site	Terbuthylazine (limit ≤ 0.007 mg/L)
	Round 1
F9	0.039
F10	0.129
F11	0.178
F12	0.086
Mean	0.122
Median	0.078
Range	0.494
Min	0.005
Max	0.499
SD	0.133
% Compliance	8

The histogram demonstrates the distribution of terbuthylazine analyte concentration values across the water sampling sites. Out of the 12 water sampling sites, only one water sampling site (F2) showed compliance. Of the other eleven water sampling sites which exceeded the 0.007 mg/L limit, F3 was the only water sampling site which recorded a concentration value below 0.010 mg/L. Most water sampling sites recorded terbuthylazine concentration values above 0.030 mg/L (Figure 5.7).

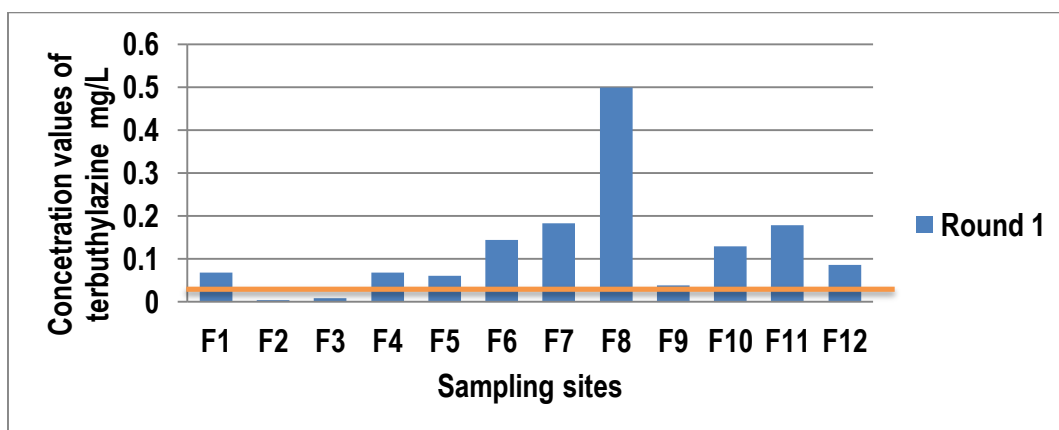


Figure 5.7 Histogram showing the measurements of terbuthylazine in water samples collected at the 12 water sampling sites in water sampling Round 1.

5.5 Summary

Of the eight emerging contaminants measured in water samples collected at the 12 water sampling sites in the Fonteinspruit stream, only one analyte (estradiol) demonstrated 100% compliance rate when compared with a watch-list of substances for Union-wide monitoring set out in Article 8b of Directive 2008/105/EC (European Commission, 2018). About 60% (three out of five) of the pharmaceutical analytes measured at the water sampling sites demonstrated 0% compliance rate. These analytes were estrone, 17 α ethynylestradiol and bisphenol A. Meanwhile, 33% (one out of three) of the herbicide analytes showed 0% compliance rate, namely atrazine. Amongst the 12 water sampling sites assessed for ECs, water sampling site F8 recorded the highest concentration levels of four analytes, namely carbamazepine, atrazine, metolachlor and terbuthylazine. In contrast, water sampling site F2 revealed the lowest concentration levels of four analytes, namely carbamazepine, estrone, atrazine, and metolachlor, which might be attributed to low flow. Overall, the results of the emerging contaminants in the Fonteinspruit stream illustrated that there is a high presence of emerging contaminants in the stream water, which is adding to the deterioration of the stream water.

Chapter 6

Results of Ecological Water Quality in the Fonteinspruit Stream

6.1 Introduction

For this study, the ecological water quality in the Fonteinspruit stream was assessed in order to determine the ecological state of the stream. A total of eight aquatic macroinvertebrate families were found in the various biotopes, such as stones (S), vegetation (VEG) and gravel, sand and mud (GSM), at the 12 macroinvertebrate habitat water sampling sites in one water sampling round, in the autumn season. These aquatic macroinvertebrate family groups belonged to various orders, including annelida, ephemeroptera, odonota, hemiptera, coleopteran and diptera. Two annelida macroinvertebrate families identified in the stream were comprised of the oligochaeta and hirudinea macroinvertebrate families. Baetidae was the only macroinvertebrate family belonging to the order of ephemeroptera that was identified. The two odonota macroinvertebrate families that were found in the Fonteinspruit stream were coenagrionidae and libellulidae. Corixidae, hydrophilidae and chironomidae macroinvertebrate families belonging to the orders hemiptera, coleoptera and diptera, respectively, were also amongst the macroinvertebrate families that were identified in the Fonteinspruit stream.

To determine the range of the degree of impairment and the diversity of aquatic macroinvertebrate families found in the Fonteinspruit stream, the South African Scoring System (SASS) method was applied. The SASS scores and ASPT values for each of the 12 macroinvertebrate habitat water sampling sites were classified using the Biological bands for Highveld Ecoregion modelled reference (Dallas, 2007).

6.2 Results of aquatic macroinvertebrates

For this study, the eight aquatic macroinvertebrate families identified at the 12 macroinvertebrate habitat water sampling sites in the Fonteinspruit stream varied in many respects. Some aquatic macroinvertebrate families were confined to those parts of the stream where chemical and physical conditions were suitable for them. However, due to the flow changes and seasonal changes, other macroinvertebrate families were found along the length of the stream. Table 6.1 provides a description of these aquatic macroinvertebrate families in terms of their structure, behaviour and their natural habitats. This information was obtained from Gerber and Gabriel (2002).

Table 6.1 Description of aquatic macroinvertebrate families found at the 12 macroinvertebrate habitat water sampling sites.

Order	Family	Description
Annelida	Oligochaeta	Known as earthworms, typically with soft, slender and elongated bodies; this family inhabit swamps and muddy areas in the streams.
	Hirudinea	Segmented and flattened worms with rear slightly wider than front; inhabit shallow pools or quiet areas in the stream.
Ephemeroptera	Baetidae	Aquatic insect family with elongated, cylindrical or flattened body and inhabits rocks, plants or coarse sand in moderately flowing streams.
Odonota	Coenagrionidae	Aquatic insect family with slender bodies and leaf-like gills, pointed tips; inhabits the slow-moving or still water areas in the stream
	Libellulidae	Aquatic insects with oval bodies and triangular heads, which breed in still-water; can thrive in water with low dissolved oxygen levels and very slow flowing streams.

Order	Family	Description
Hemiptera	Corixidae	Also known as water boatmen; a family of aquatic insects that are small cigar-shaped and widely found in slow flowing streams or muddy areas in streams.
Coleoptera	Hydrophilidae	Also called water scavenger beetles; have oval bodies with rounded backs; live in muddy patches along the stream and quiet shallow pools or slow edges in streams.
Diptera	Chironomidae	A large and diverse family of flies, commonly known as “non-biting flies” with narrow bodies and long legs. This family inhabits the bottom of the stream and may thrive in low oxygen or heavily polluted habitats.

6.2.1 Aquatic macroinvertebrate families

A number of aquatic macroinvertebrate families were identified at the various macroinvertebrate habitat water sampling sites in the Fonteinspruit stream. Three of the eight aquatic macroinvertebrate families were found in more than 50% of the macroinvertebrate habitat water sampling sites. These aquatic macroinvertebrate families were the oligochaeta, corixidae and chironomidae families of the orders annelida, hemiptera and diptera, respectively (Table 6.2). However, the family of oligochaeta found at 10 of the macroinvertebrate habitat water sampling sites in the Fonteinspruit stream, demonstrated a far greater total number than the other aquatic macroinvertebrate families.

Table 6.2 Aquatic macroinvertebrate families at the 12 water sampling sites.

Sampling site	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	Total number of each family
Taxa													
ANNELIDA													
Oligochaeta	1			1	1	2	3	3	1	4	2	3	21
Hirudinea			1	2				1	1			1	6
EPHEMEROPTERA													
Baetidae		1				2		1	1				5
ODONOTA													
Coenagrionidae		1	2										3
Libellulidae			1										1
HEMIPTERA													
Corixidae				1				1	1	2	1	1	7
COLEOPTERA													
Hydrophilidae					1				1				2
DIPTERA													
Chironomidae				1			1	2	1		1	2	8
Number of families	1	2	3	4	2	2	2	5	6	2	3	4	

To obtain a visual perspective of the number of aquatic macroinvertebrate families found at the 12 macroinvertebrate habitat water sampling sites, a histogram was constructed. A histogram clearly shows that the aquatic macroinvertebrate families found at the macroinvertebrate habitat water sampling sites F4, F8, F9 and F12 were substantially greater than at the other macroinvertebrate habitat water sampling sites. However, the macroinvertebrate habitat water sampling site F9 stood out with the greatest number of aquatic macroinvertebrate families (Figure 6.1). This might be attributed to the pollution tolerant aquatic macroinvertebrate families found at this macroinvertebrate habitat water sampling site.

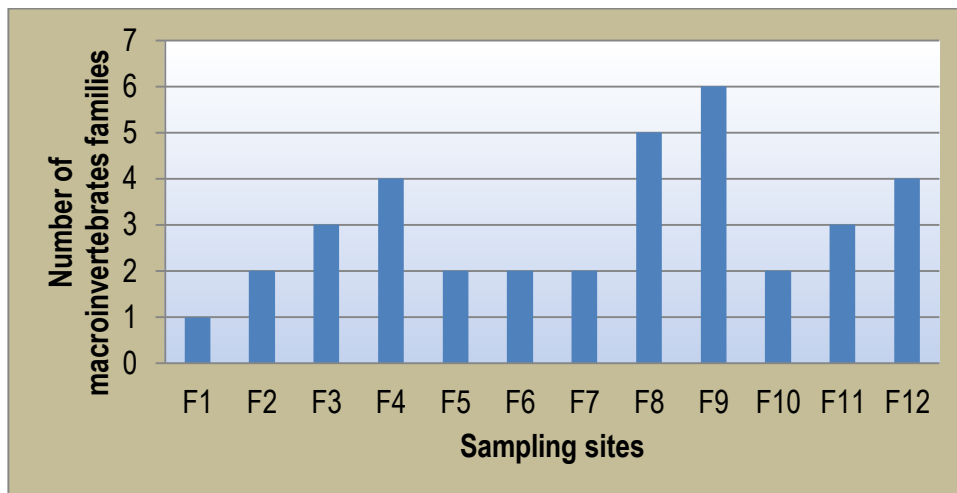


Figure 6.1 Histogram showing the number of macroinvertebrate families at the 12 water sampling sites.

6.2.2 Sensitivity classification of aquatic macroinvertebrate families

The sensitivity levels of the eight aquatic macroinvertebrate families found in the Fonteinsspruit stream were derived from the tolerances to pollution as previously used in the SASS 5 scoring system (Gerber & Gabriel, 2002). All the aquatic macroinvertebrate families found in the Fonteinsspruit stream were apportioned sensitivity scores, with 1–5 scores indicating families that are highly tolerant to pollution. For this study, all the aquatic macroinvertebrate families found at the 12 macroinvertebrate habitat water sampling sites fell below the scale of five (Table 6.3).

Table 6.3 Aquatic macroinvertebrate families with their sensitivity scores.

Observed families	Sensitivity scores
ANNELIDA	
Oligochaeta	1
Hirudinea	3
EPHEMEROPTERA	
Baetidae	4
ODONOTA	
Coenagrionidae	4
Libellulidae	4
HEMIPTERA	
Corixidae	3
COLEOPTERA	
Hydrophilidae	5
DIPTERA	
Chironomidae	2

The number of the aquatic macroinvertebrate families found in the Fonteinspruit stream varied greatly amongst the 12 macroinvertebrate habitat water sampling sites. Water sampling sites F8 and F9 demonstrated the highest number of aquatic macroinvertebrate families (Table 6.4). Although the SASS scores of both of these water sampling sites were the highest amongst the water sampling sites, the mean sensitivity per taxon indicated by ASPT was below three. This indicated that the aquatic macroinvertebrate families found at macroinvertebrate habitat water sampling sites F8 and F9 were highly resistant to pollution conditions.

Table 6.4 Number of taxa, SASS scores and ASPT values at the 12 water sampling sites.

Sampling sites	Number of taxa	SASS score	ASPT
F1	1	1	1
F2	2	8	4
F3	3	11	3.7
F4	4	9	2.3
F5	2	6	3
F6	2	5	2.5
F7	2	3	1.5
F8	5	13	2.6
F9	6	18	3
F10	2	4	2
F11	3	6	3
F12	4	9	2.3
Mean	3	7.8	2.6
Median	2.5	7	2.6
Range	5	17	3
Min	1	1	1
Max	6	18	4
SD	1.5	4.7	0.9

6.2.3 Classification of the pollution condition

The SASS 5 scores and ASPT values at the 12 macroinvertebrate habitat water sampling sites in the Fonteinspruit stream revealed substantially low values. The classification of SASS 5 and ASPT values was done in terms of the Biological bands and Ecological categories for Highveld - lower Ecoregion modelled reference (Dallas, 2007). Overall, the SASS 5 score and ASPT values for each macroinvertebrate habitat water sampling site in the Fonteinspruit stream fell within the E/F Biological bands and Ecological Categories (Table 6.5). This is attributed to the SASS 5 score and ASPT values,

which were below 30 and 4.2 (Dallas, 2007), respectively. Thus, the 12 macroinvertebrate habitat water sampling sites were classified as critically impaired and only a few macroinvertebrate families tolerant to pollution conditions in the stream were present.

Table 6.5 Classification of the SASS 5 score and ASPT values at the 12 water sampling sites.

Sampling site	SASS5 score	ASPT	Bands	Condition*
F1	1	1	F	Critically impaired
F2	8	4	F	Critically impaired
F3	11	3.7	F	Critically impaired
F4	9	2.3	F	Critically impaired
F5	6	3	F	Critically impaired
F6	5	2.5	F	Critically impaired
F7	3	1.5	F	Critically impaired
F8	13	2.6	F	Critically impaired
F9	18	3	F	Critically impaired
F10	4	2	F	Critically impaired
F11	6	3	F	Critically impaired
F12	9	2.3	F	Critically impaired

* = according to Dallas (2007); Black = Class F

A scatter plot was constructed to discern the pattern of the ASPT values at the 12 macroinvertebrate habitat water sampling sites in the Fonteinspruit stream. Figure 6.2 clearly indicates that the spread of the ASPT values for all the macroinvertebrate habitat water sampling sites fell in the E/F Biological Bands/Ecological Categories (Dallas, 2007). This is an indication of a critical degree of impairment at all the macroinvertebrate habitat water sampling sites in the Fonteinspruit stream.

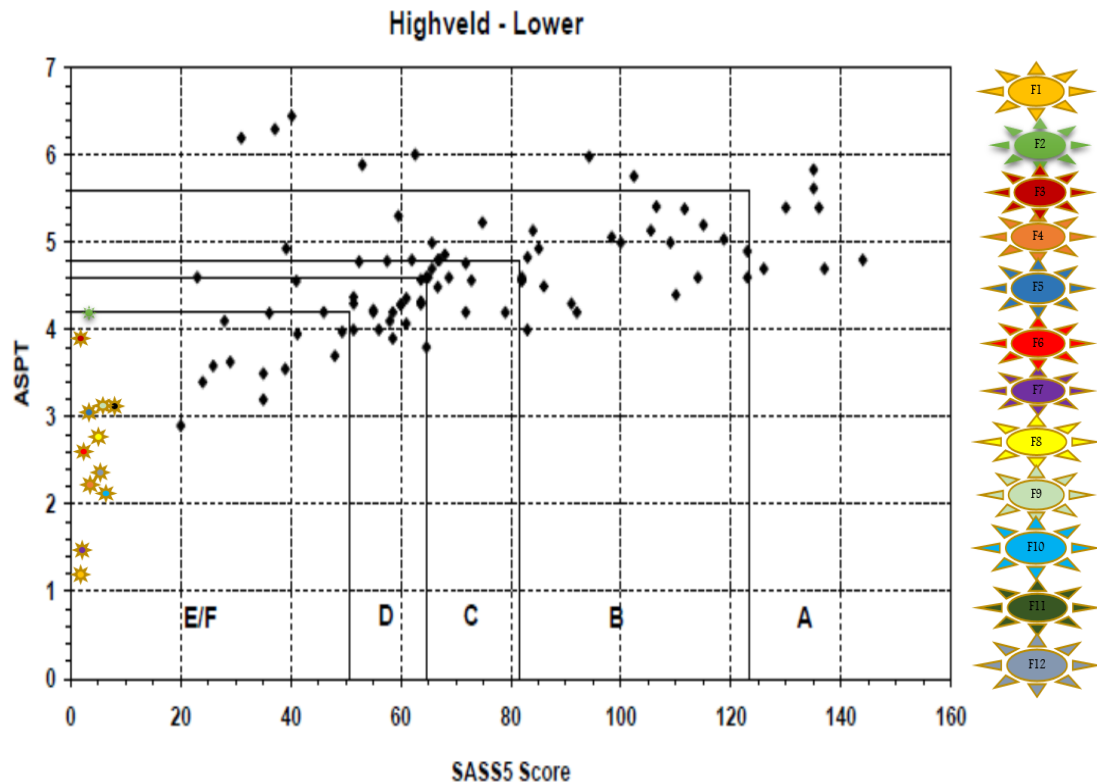


Figure 6.2 Scatter plot of SASS 5 scores at the 12 macroinvertebrate habitat water sampling sites using Biological bands - Highveld lower Ecoregion (Dallas, 2007).

6.3 Summary

For this study, multiple aquatic macroinvertebrate families were found at the various macroinvertebrate habitat water sampling sites in the Fonteinspruit stream. Almost 100% of these identified aquatic macroinvertebrate families were highly tolerant to pollution conditions and this is indicated by their respective tolerance scores (SASS score) as used in the SASS 5 scoring system (Gerber and Gabriel, 2002). Amongst all these aquatic macroinvertebrate families, which are tolerant to pollution conditions, the oligochaeta family, belonging to the order Annelida, was found in more than 80% of the macroinvertebrate habitat water sampling sites. The macroinvertebrate habitat water sampling sites F8 and F9 demonstrated a relatively high number of aquatic macroinvertebrate families with more than five compared with the other macroinvertebrate habitat water sampling sites.

The classification of pollution conditions at macroinvertebrate habitat water sampling sites in the Fonteinspruit stream illustrated that the stream is critically impaired. This is indicated by the SASS scores and ASPT values, which for all the water sampling sites, fell in the E/F categories (Dallas, 2007). Thus, only a few tolerant families were present in the Fonteinspruit stream. The anthropogenic activities, which might have contributed to the impairment of the stream and macroinvertebrate diversity, include solid waste disposal and overflow of untreated sewage as well as urban, industrial and agricultural runoff along water sampling sites F1, F4, F8, F9, F10 and F12. The increased erosion, due to overgrazing by roaming animals near water sampling sites F5, F6 and F7, might have also given rise to this impairment. The increased input of sediment attributed to new settlements, solid waste disposal and the overflow of untreated sewage along the macroinvertebrate habitat water sampling site F10 might have contributed significantly to the impairment of natural habitat and the diversity of aquatic macroinvertebrates.

Chapter 7

Discussion and Conclusion

7.1 Introduction

In this study, the quality of the water in the Fonteinspruit stream was measured at 12 water sampling sites. This stream originates in the city of Bloemfontein and feeds into the Blou dam on the eastern side of the city from where it flows to join the Bloemspruit and Renosterspruit streams, also to the east, before eventually leading into the Modder River on the outskirts of Bloemfontein. The Fonteinspruit stream drains an area of about 17 km², in which a Water Treatment Plant, high-density residential areas, informal settlements, agricultural activities and industrial areas are found. Early in the 21st century, Pretorius et al. (2002) showed that the Fonteinspruit stream was highly polluted with high levels of dissolved inorganic nitrogen, phosphate and dissolved solids in the stream water as well as faecal bacteria, which could mostly be attributed to municipal sewage, industrial and agricultural wastewater flowing into the Fonteinspruit stream. In a later study, Nyoh (2015) confirmed that the Fonteinspruit stream was still heavily polluted and that the extent of the pollution was increasing, mostly because of the Water Treatment Plant effluent and a leaking sewage pipe. This study was, therefore, undertaken to establish the water quality of the Fonteinspruit stream and what the impact was on aquatic organisms living in the stream.

7.2 Water quality in the Fonteinspruit stream

Several categories of water quality properties were measured in this study to obtain an understanding of the extent of the pollution in the water at the 12 water sampling sites in the Fonteinspruit stream. These water quality properties included physical, chemical and microbiological properties. The physical properties analysed were the measurement of electrical conductivity (EC), pH, turbidity, temperature, dissolved oxygen (DO) and chemical oxygen demand (COD); the chemical properties were the measurement of ammonia, nitrate, sulphate, phosphate and total hardness; and for the

microbial properties the bacterial coliforms and *Escherichia coli* (*E. coli*) were enumerated. Because of the increase in emerging contaminants (ECs) in surface water (Lei et al., 2015), the concentrations of three pharmaceutical drugs, namely carbamazepine, estradiol and 17 α ethynylestradiol; one plasticizer, namely bisphenol A; and three herbicides, namely atrazine, metolachlor and terbuthylazine, were also measured in this study.

In this study, the majority of water quality properties, including the ECs, were beyond various standard limits applied in this study. Eight of the thirteen physical, chemical and microbiological water quality properties, namely turbidity, EC, COD, DO, ammonia, phosphate, bacterial coliforms and *E. coli* were greater than the various limits of the EU Surface Water Directives and South African Water Quality Guidelines. All groups of ECs were relatively high when compared with Canadian Environmental Quality Guidelines (CCME, 2018) and Watch-list of substances set in Article 8b of Directives 2008/105/EC (European Commission, 2018). The percentage of compliance rates for both the conventional water quality properties and ECs, particularly for turbidity, phosphate, *E. coli*, bacterial coliforms, carbamazepine, estrone, 17 α ethynylestradiol, bisphenol A, atrazine, metolachlor and terbuthylazine, were below 20%. The low percentage compliance rates could be related to the increased sewage that flows into the stream (Nyoh, 2015).

One of the water quality properties that were of particular concern was turbidity. All measurements of turbidity exceeded the limit of 1.0 NTU (EPA, 2001), with measurements ranging from approximately 2 NTU to more than 120 NTU. These relatively high measurements in turbidity could be attributed to thunderstorms at the time of the study, which caused vast volumes of soil to be washed into the Fonteinspruit stream, thereby increasing the level of turbidity. The occurrence of heavy rain and thunderstorms is generally responsible for changes in the level of turbidity. During 2014, heavy rain caused a dramatic increase in the level of turbidity in 33 water bodies, made up of 9 dams, 13 depression wetlands and 11 rivers, covering the bulk of the Eastern Cape Karoo region (Mabidi et al.,

2017). Heavy sedimentation from suspended lime-rich soils and sandstone sediments caused by the rain was washed into the Eastern Cape Karoo water bodies (Mabidi et al., 2017). These high turbidity levels may have effects on the aquatic macroinvertebrates. Sedimentation, caused the increased turbidity in the surface freshwater systems and larvae of almost all species of dragonflies and damselflies (odonata) are susceptible to changes in turbidity levels. (Mabidi et al., 2017).

Similar to turbidity measurements, phosphate measurements were also of great concern. Phosphate measurements were significantly high for most of the water sampling sites, exceeding the EU surface water limit of ≤ 0.7 mg/L (EPA, 2001), with measurements ranging from approximately 0.9 mg/L to more than 26.8 mg/L. This finding concurred with the Pretorius et al. (2002) study, which revealed that the Fonteinspruit stream was highly concentrated with phosphate. The high concentration levels of phosphate in the stream could strongly be linked to municipal sewage that flows into the stream, particularly at water sampling sites F8 to F12, and water runoff carrying faeces of domestic animals that roam and defecate near the stream (Pretorius et al., 2002). Phosphate can enter rivers and streams as either the constituent of effluent from wastewater treatment plants, leaking pipes and sewage overflow (Guldhe et al., 2017).

High levels of phosphate contribute to the loading of nutrients in the water which may lead to eutrophication. Eutrophication is directly caused by an excessive richness of nutrients, which often comes through runoff from the land into the rivers and streams (Guldhe et al., 2017). Eutrophic water induces the excessive growth of algae bloom, but when the water conditions do not favour the bloom anymore, the algae die and decompose. The decomposition of algae, assisted by bacteria such as toxic cyanobacteria, depletes the oxygen in the water through cellular respiration, thereby affecting other aquatic species (Da Costa et al., 2018).

The presence of *E. coli* and faecal coliforms in the Fonteinspruit stream yielded concerns as well. The bacterial count for both of these faecal pollution indicators exceeded the standard limits of $\leq 1,000$ cfu/100 mL (DWAF, 1996d), with the bacterial counts ranging from 1,100 cfu/100 mL to more than the test limits of $>2,419.6$ cfu/100 mL. The solid waste and mostly uncontrollable faecal pollution that abound throughout the stream area because of sewage overflow, could have contributed to the high count of *E. coli* and coliforms in the water, particularly at water sampling site F8. The primary source of faecal pollution in water sources is often attributed to leakage in sewer pipes (Malla et al., 2018). However, high levels of *E. coli*, and faecal coliforms do not have direct effects on the aquatic macroinvertebrates (Palmer et al. cited in Nyoh, 2015).

High concentration levels of a group of pharmaceutical drugs measured in the Fonteinspruit stream were of particular concern. The high concentration levels of pharmaceutical drugs, including carbamazepine, estrone and 17a ethynylestradiol, exceeded the limits of $10 \mu\text{g/L}$ (CCME, 2018), $\leq 0.4 \text{ ng/L}$ (European Commission, 2018) and $\leq 0.035 \text{ ng/L}$ (European Commission, 2018), respectively. The concentration levels of carbamazepine ranged from approximately $13 \mu\text{g/L}$ to more than $160 \mu\text{g/L}$; estrone concentration levels ranged from 1 ng/L to more than 18 ng/L ; and 17a ethynylestradiol concentration levels ranged from around $150 \mu\text{g/L}$ to more than $900 \mu\text{g/L}$. The occurrence of pharmaceutical drugs in the stream can mostly be attributed to pollution by domestic sewage and industrial effluent as a result of manhole overflow into the stream. For example, municipal wastewater or sewage overflow was considered as one of the principle discharge sources for the emanation of pharmaceutical drugs in rivers in India (Gogoi et al., 2018). Another study revealed that pharmaceuticals occur as a result of sewage being discharged into the receiving surface waters (Oetken et al., 2005).

High concentration levels of pharmaceutical drugs affect aquatic macroinvertebrates. Although the antiepileptic drug carbamazepine showed low acute toxicity to aquatic macroinvertebrates, particularly

the oligochaete, *Lumbriculus variegatus* and snail, *Potamopyrgus antipodarum*, significant and chronic effects on the population and emergence of the non-biting midge, *Chironomus riparius* were reported in the water bodies of Germany (Oetken et al., 2005). Despite the presence of carbamazepine in surface waters, there are still too few studies done on the effects of carbamazepine on aquatic macroinvertebrates (Jarvis et al., 2014). Bhandari et al. (2015) concurred that significant effects of related steroid sex hormones, such as estradiol and 17 α ethinylestradiol, on aquatic macroinvertebrates have not been adequately studied (Bhandari et al., 2015).

In this study, high concentration levels of bisphenol A (BPA) were also recorded at most sites in the Fonteinspruit stream. The bisphenol A concentration levels exceeded the standard of $\leq 1.4 \mu\text{g/L}$ (Canadian Environmental Protection Act, 1999), with concentration levels ranging from approximately 20 $\mu\text{g/L}$ to 800 $\mu\text{g/L}$. The presence of this endocrine disruptor in the water could have originated from the leaching of various household and commercial products, such as sanitary/hygiene towels and plastic packaged food and beverage containers, discarded at the illegal dumping sites near the stream. BPA, primarily used as an intermediate in the production of polycarbonate plastic and epoxy resins, has been reported to leach from various products, including tin cans and food contact items, and the plastic leachate (Mathieu-Denoncourt et al., 2015). During wastewater treatment, BPA is often transported to rivers and streams as part of the effluent. BPA thus settle onto sediment and this is how it affects aquatic macroinvertebrates (Staples et al., 2016). Although no studies could be found which have revealed chronic toxicity of BPA to aquatic macroinvertebrates through direct sediment exposure, one study where the sediment was spiked with BPA in the water bodies of North Carolina, USA, showed that high concentration levels of BPA reduced the diversity of aquatic macroinvertebrates families of oligochaete and midge (Staples et al., 2016).

High concentration levels of a group of herbicides were recorded at most of the water sampling sites in the Fonteinspruit stream. The high concentration levels of herbicides, including atrazine,

metolachlor and terbuthylazine, exceeded the limits of $\leq 1.8 \mu\text{g/L}$ (CCME, 1999), $\leq 7.8 \mu\text{g/L}$ (CCME, 1999) and $\leq 0.007 \text{ mg/L}$ (WHO, 2003), respectively. The concentration levels of atrazine ranged from about $7 \mu\text{g/L}$ to more than $250 \mu\text{g/L}$; metolachlor concentration levels ranged from $8 \mu\text{g/L}$ to more than $200 \mu\text{g/L}$; and the concentration levels of terbuthylazine measurements ranged from approximately 0.060 mg/L to more than 0.400 mg/L . The atrazine, metolachlor and terbuthylazine may have originated from surface water runoff containing herbicides and domestic wastewater from the residential lawns.

Although little is known about the effects of herbicides, such as atrazine, metolachlor and terbuthylazine on aquatic macroinvertebrates, indirect effects on the aquatic ecosystem, such as the death and decay of submerged aquatic plants, have been reported (Ralston-Hooper et al., 2009). The decay of aquatic plants may affect the oxygen-carbon dioxide balance of the water and the levels of pH, which in turn, affect the aquatic macroinvertebrates (Huber et al., 2016). For example, the pH and oxygen decreased in Netherlands water bodies in 1998 after being exposed to a mixture of the herbicides atrazine and metolachlor and the total number of aquatic macroinvertebrates decreased slightly (Hartgers et al., 1998).

7.3 Overall water quality of the Fonteinspruit stream

Several methods are often employed to determine the overall quality of water through the calculation of a single value index, taking the measurements of many water quality properties into account. Some of these indexes, the US National Sanitation Foundation Water Quality Index (NSFWQI), Florida Stream Water Quality Index (FWQI), British Columbia Water Quality Index (BCWQI) and the Oregon Water Quality Index (OWQI), provide a single value that describes the overall quality of water at a particular site using measurements from a single season (Poonam et al., 2013). In contrast, the Canadian Council of Ministers of the Environment Water Quality Index (CCME-WQI) is applied to calculate the overall water quality by combining water quality data from more than one season or water

sampling round (CCME, 2017). The CCME-WQI is suitable for grading the quality of water using the five grades categorisation, which are 'excellent, good, fair, marginal and poor' for different index score values (CCME, 2017). As three water sampling rounds of water quality measurements were undertaken in this study, the CCME-WQI (CCME, 2017) was used to describe the overall water quality at the 12 water sampling sites sampled along the Fonteinspruit stream.

The calculated composite CCME-WQI showed that the water quality was poor at all the water sampling sites in the Fonteinspruit stream, with values of 45 and less. These rather low CCME-WQI (CCME, 2017) values indicate that the water in the Fonteinspruit stream contained contaminants in concentrations greater than the acceptable levels when compared with standard limits of the South African Water Quality Guidelines (DWAF, 1996a) and the European Union Surface Water Directives (Environmental Protection Agency, 2001). In this study, this view is supported by the absence of many sensitive macroinvertebrate families. Aquatic macroinvertebrates and their habitats are often used to determine the state of the water of environmental resources (Ollis, 2005). Many aquatic macroinvertebrates are sensitive to the presence of contaminants in water and cannot survive in polluted waters, therefore by analysing the number of individual and aquatic macroinvertebrate families in water is often used as an indicator of the quality of water (Ollis, 2005).

7.4 Health conditions of Fonteinspruit stream

In this study, the health conditions of water collected at the 12 water sampling sites in the Fonteinspruit stream revealed that the Fonteinspruit stream is significantly impaired. This was demonstrated by the presence of aquatic macroinvertebrate families belonging to orders of annelida, ephemeroptera, odonota, hemiptera, coleopteran and diptera, which are all (eight) classified as highly tolerant to pollution in terms of the SASS 5 scoring system (Gerber & Gabriel, 2002). Although corixidae and chironomidae families of the orders hemiptera and diptera respectively, were found in more than half

of the aquatic habitat water sampling sites, the family of oligochaeta (annelida) was recorded at most of the water sampling sites but with low numbers of families ranging from 1 to 4.

The presence of mainly tolerant aquatic macroinvertebrate families and the absence of sensitive families in the Fonteinspruit stream demonstrated that the Fonteinspruit stream is highly impaired. This outcome concurs with the study in North Carolina, USA, which showed that the presence of only chironomidae and oligochaeta families in the stream signals the stream is poor (Staples et al., 2016). Again, in the study that was done in the Klip River of South Africa, the presence of only chironomidae and oligochaeta families showed that the health conditions of the river was impaired (Nyoh, 2015). Table 7.1 provides an overall summary of conditions at the water sampling sites and human-induced activities affecting the health status of the water sampling sites in the Fonteinspruit stream.

Table 7.1 Overall summary of conditions at the water sampling sites and human-induced activities affecting the health status of the water sampling sites in the Fonteinspruit stream.

Sampling site	Condition*	Human-induced activities
F1	Critically impaired	Overflowing manhole and open disposal of solid waste
F2	Critically impaired	Open disposal of solid waste
F3	Critically impaired	Open disposal of solid waste
F4	Critically impaired	Overflowing manhole and open disposal of solid waste
F5	Critically impaired	Unrestrained presence of domestic animals
F6	Critically impaired	Unrestrained presence of domestic animals and illegal dumping sites
F7	Critically impaired	Unrestrained presence of domestic animals and illegal dumping sites

Sampling site	Condition*	Human-induced activities
F8	Critically impaired	Wastewater from car-wash facility and car panel-beating outlets
F9	Critically impaired	Overflowing manhole and solid waste
F10	Critically impaired	Overflowing manhole and solid waste
F11	Critically impaired	Open disposal of solid waste
F12	Critically impaired	Open disposal of solid waste

7.5 Limitations of the study

This study was limited by lack of available analytic methodologies for the analysis of emerging contaminants. This meant that the number of ECs identified at the 12 water sampling sites in the Fonteispuit stream was limited. No prior study was found on the assessment of emerging contaminants in the Fonteispuit stream. This can be an obstacle in finding a trend of present ECs in the Fonteinspruit stream.

7.6 Suggestion of future studies

The vast majority of emerging contaminants (ECs) are currently not assessed or regulated. In South Africa, there are no standards for ECs, as a result, the country relies on the use of standards developed in other countries. This means that there is limited identification and quantification of ECs in water or wastewater. More scientific studies on the presence of emerging contaminants in surface water bodies and their effects on the aquatic ecosystems could be conducted. This will not only help with the identification of these ECs but incorporate new information and environmental quality guidelines that are periodically released by Canadian Council of Ministers of the Environment CCME (CCME, 1999).

7.7 Recommendations

Interventions, such as the employment of municipal services, including improved waste collection and infrastructural maintenance of sewer systems/network, and strict application of existing regulations and bylaws on human activities, will help improve the water quality in the Fonteinspruit stream.

7.8 Practical applicability

The practical application of this study and the revealed results were postulated in terms of:

1. Waste water management: One of the major concerns in waste water management is the need to find cost-effective and appropriate methods of monitoring pollution. Various water-polluting activities in the Fonteinspruit stream such as leakage or overflow of municipal sewage, particularly along water sampling sites F8 to F12 and discharge of untreated waste water from Waste Water Treatment Plants into the stream affect the water quality in the Fonteinspruit stream. Therefore, the management of waste water could play a crucial role in lowering the adverse impact on natural ecosystems. Innovative measures and available technology are required to mitigate the risk of unregulated discharges including water quality monitoring and this could also increase the accessible quantity of water (Mhlongo et al., 2018). The Department of Water and Sanitation (DWS) could also reinforce its previously implemented incentive-based programme (Blue and Green Drop certification) as a quality measure of the microbiological and chemical qualities of drinking water and effluent (Mhlongo et al., 2018).
2. Waste management: South Africa has a fairly high rate of waste generation compared to other developing countries, with an unequal amount of this waste being generated by the affluent. This contributed to the development of policy and legislation, such as the enactment of the National Environmental Management: Waste Act (NEM: WA) (Act 59 of 2008). The Act seeks

- to regulate waste management and the environment by providing environmentally sound waste management measures for the prevention of pollution and ecological degradation. Central to the implementation of the act is the application of a hierarchy of waste management which focuses on waste avoidance and minimization rather than disposal. There is dire need for improvements in provision of waste services such as waste removal, particularly along water sampling sites F1, F2, F3, F6 and F7. As compliance and enforcement in SA have been empowered through 'Green', as well as 'Blue Scorpions', further resources need to be allocated to ensuring that the policy and legislation is translated into the regulatory framework.
3. Health education: More health education is needed and should be imparted in communities and industrial areas (Hamilton areas). This will help create the awareness on the importance of waste management and healthcare waste management at various facilities and establishments, particularly near water sampling site F8 where Batho clinic is located.
 4. Use of pesticides: Pesticides are an important group of emerging contaminants in surface water bodies. This is due to their pesticidal actions which include herbicides, fungicides, insecticides, nematicides, and plant growth regulators, and others, and their presence in surface water bodies has generated significant concerns regarding the risk of estrogenic and other adverse effects on humans. Since these compounds are designed for external usage, they are easily released into aquatic environments. The frequent use of pesticides for agricultural purposes must be closely monitored so that the adverse impact caused by the pesticides on the runoff is limited.

7.9 Conclusion

The results of this study revealed that the overall water quality in the Fonteinspruit stream was poor. The poor quality of the Fonteinspruit stream water could be mainly attributed to human activities along the embankments of the stream. These activities included extensive littering and open disposal of

solid waste as well as municipal and industrial wastewater that overflowed into the stream. Domestic wastewater from informal trading establishments, such as the car-wash facility; unrestrained presence of domestic animals near the stream; and other land use activities, such as informal agriculture and informal backyard industries (car panel-beating), seem to have directly and indirectly contributed to the poor water quality in the Fonteinspruit stream.

The constant use of the Fonteinspruit stream water can have adverse effects on human health and aquatic life. The residents in the area use the water in the stream for various purposes, such as swimming, fishing and cultural activities (ritual body cleansing), and animals also drink the stream water. The exposure of residents to poor water quality through contact or inhalation of contaminants when swimming may result in skin rashes, throat, ear infections, irritation of eyes and mucous membrane (DWAF, 1996a) while animals may also contract diseases from drinking the polluted water (Nyoh, 2015). The loading of nutrients, such as phosphate, in the stream may further cause the stream to be more eutrophic. Eutrophic stream water may lead to the rapid growth of toxic algal blooms and this may lead to oxygen depletion for the other aquatic organisms that need oxygen for respiration (Inostroza et al., 2017).

Reference List

- Agunbiade, F.O. and Moodley, B. (2014). Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa, *Environmental Monitoring and Assessment*, 186(11): 7273–7291. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1007/s10661-014-3926-z>].
- Alemneh, T., Ambelu, A., Bahrndorff, S., Mereta, S.T., Pertoldi, C. and Zaitchik, B.F. (2017). Modeling the impact of highland settlements on ecological disturbance of streams in Choke Mountain Catchment: Macroinvertebrate assemblages and water quality, *Ecological Indicators*, 73: 452–459. [Accessed on: 24 July 2018, from doi: <https://doi.org/10.1016/j.ecolind.2016.10.019>].
- Ambani, A.E. and Annegarn, H. (2015). A reduction in mining and industrial effluents in the Blesbokspruit Ramsar Wetland, South Africa: Has the quality of the surface water in the wetland improved?, *Water SA*, 41(5): 648–659. [Accessed on: 09 February 2018, from doi: <https://doi.org/10.4314/wsa.v41i5.08>].
- Amouei, A., Miranzadeh M.B., Shahandeh, Z., Taheri, T., Asgharnia, H.A., Akbarpour, S. and Mokari, B. (2012). A Study on the Microbial Quality of Drinking Water in Rural Areas of Mazandaran Province in North of Iran (2011), *Journal of Environmental Protection*, 3(07): 605–609. [Accessed on: 09 February 2018, from doi: <https://doi.org/10.4236/jep.2012.37073>].
- Antonini, K., Langer, M., Farid, A., and Walter, U. (2017). SWEET CubeSat – Water detection and water quality monitoring for the 21st century, *Acta Astronautica*, 140: 10–17. [Accessed on: 10 February 2018, from doi: <https://doi.org/10.1016/j.actaastro.2017.07.046>].
- Ayandiran, T.A., Fawole, O.O. and Dahunsi, S.O. (2018). Water quality assessment of bitumen polluted Oluwa River, South-Western Nigeria, *Water Resources and Industry*, 19: 13–24. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.wri.2017.12.002>].
- Bai, X., Lutz, A., Carroll, R., Keteles, K., Dahlin, K., Murphy, M. and Nguyen, D. (2018). Occurrence, distribution, and seasonality of emerging contaminants in urban watersheds, *Chemosphere*, 200: 133–142. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.chemosphere.2018.02.106>].
- Barakat, A., El Baghdadi, M., Rais, J., Aghezzaf, B. and Slassi, M. (2016). Assessment of spatial and seasonal water quality variation of Oum Er Rbia River (Morocco) using multivariate statistical techniques, *International Soil and Water Conservation Research*, 4(4): 284–292. [Accessed on: 10 February 2018, from doi: <https://doi.org/10.1016/j.iswcr.2016.11.002>].
- Berger, E., Haase, P., Kuemmerlen, M., Leps, M., Schafer, B.R. and Sundermann, A. (2017). Water quality variables and pollution sources shaping stream macroinvertebrate communities, *Science of the Total Environment*, 587–588: 1–10. [Accessed on: 10 February 2018, from doi: <https://doi.org/10.1016/j.scitotenv.2017.02.031>].
- Bhandari, R.K., Deem, S.L., Holliday, D.K., Jandegian, C.M., Kassotis, C.D., Nagel, S.C., Tillitt, D.E., vom Saal, F.S and Rosenfeld, C.S. (2015). Effects of the environmental estrogenic contaminants

- bisphenol A and 17 α -ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species, *General and Comparative Endocrinology*, 214: 195–219. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ygcen.2014.09.014>].
- Blum, K.M., Andersson, P.L., Ahrens, L., Wiberg, K., and Haglund, P. (2018). Persistence, mobility and bioavailability of emerging organic contaminants discharged from sewage treatment plants, *Science of the Total Environment*, 612: 1532–1542. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.09.006>].
- Bodrud-Doza, M., Towfiqul Islam, A.R.M., Ahmed, F., Das, S., Saha, N. and Rahman, M.S. (2016). Characterization of groundwater quality using water evaluation indices, multivariate statistics and geostatistics in central Bangladesh, *Water Science*, 30(1): 19–40. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.wsj.2016.05.001>].
- Burgess, J.E. and Pletschke, B.I. (2008). Hydrolytic enzymes in sewage sludge treatment: A mini-review, *Water SA*, 34(3): 343–349. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.4314/wsa.v34i3.180627>].
- Canadian Council of Ministers of the Environment (CCME). (1999). *Canadian Water Quality Guidelines for the Protection of Aquatic Life - Atrazine*: 1–4. Available at: <<http://ceqg-rcqe.ccme.ca/download/en/144>>.
- Canadian Council of Ministers of the Environment (CCME). (2017). *CCME Water Quality Index User's Manual 2017 Update, Canadian Water Quality Guidelines for the Protection of Aquatic Life*: 1–5. Available at: <https://www.ccme.ca/files/Resources/water/water_quality/WQI%20Manual%20EN.pdf>.
- Canadian Council of Ministers of the Environment (CCME). (2018). *Canadian Water Quality Guidelines for the Protection of Aquatic Life: Carbamazepine*: 1–8. Available at: <<http://ceqg-rcqe.ccme.ca/download/en/358>>.
- Canada. (1999). Canadian Environmental Protection Act, 1999. *Federal Environmental Quality Guidelines Bisphenol A*. Canada. Available at: <<https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/federal-environmental-quality-guidelines-bisphenol-a.html>>.
- Cañedo-Argüelles, M., Kefford, B.J., Piscart, C., Prat, N., Schäfer, R.B. and Schulz, C. (2013). Salinisation of rivers: An urgent ecological issue, *Environmental Pollution*, 173: 157–167. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.envpol.2012.10.011>].
- Cañedo-Argüelles, M., Sala, M., Peixoto, G., Prat, N., Faria, M., Soares, A.M.V.M., Barata, C. and Kefford, B. (2016). Can salinity trigger cascade effects on streams? A mesocosm approach, *Science of the Total Environment*, 540: 3–10. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2015.03.039>].
- Chai, L.H. and Lha, D. (2018). A new approach of deriving indicators and comprehensive measure for ecological environmental quality assessment, *Ecological Indicators*, 85: 716–728. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ecolind.2017.11.039>].

- Cheng, X., Chen, L., Sun, R., and Kong, P. (2018). Land use changes and socio-economic development strongly deteriorate river ecosystem health in one of the largest basins in China, *Science of the Total Environment*, 616–617: 376–385. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.10.316>].
- Chigor, V.N., Sibanda, T. and Okoh, A.I. (2013). Studies on the bacteriological qualities of the Buffalo River and three source water dams along its course in the Eastern Cape Province of South Africa, *Environmental Science and Pollution Research*, 20(6): 4125–4136. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1007/s11356-012-1348-4>].
- Da Costa, J.A., de Souza, J.P., Teixeira, A.P., Nabout, J.C. and Carneiro, F.M. (2018). Eutrophication in aquatic ecosystems: a scientometric study, *Acta Limnologica Brasiliensia*, 30. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1590/s2179-975x3016>].
- Dallas, H.F. (2007). River Health Programme: South African Scoring System (SASS) Data Interpretation Guidelines, *Institute of Natural Resources and Department of Water Affairs and Forestry*: 1–85. Available at: <<http://www.dwa.gov.za/iwqs/rhp/methods/SASSInterpretationGuidelines>>.
- Dalu, T., Wasserman, R.J., Tonkin, J.D., Mwedzi, T., Magoro, M.L. and Weyl, O.L. (2017). Water or sediment? Partitioning the role of water column and sediment chemistry as drivers of macroinvertebrate communities in an austral South African stream, *Science of the Total Environment*, 607–608: 317–325. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.scitotenv.2017.06.267>].
- Daud, M.K., Nafees, M., Ali, S., Rizwan, M., Bajwa, R.A., Shakoor, M.B., Arshad, M.U., Chatha, S.A.S., Deeba, F., Murad, W., Malook, I. and Zhu, S.J. (2017). Drinking Water Quality Status and Contamination in Pakistan, *BioMed Research International*: 1–18. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1155/2017/7908183>].
- De Castro-Català, N., Muñoz, I., Armendáriz, L., Campos, B., Barceló, D., López-Doval, J. and Pérez, S., Petrovic, M., Picó, Y. and Riera, J.L. (2015). Invertebrate community responses to emerging water pollutants in Iberian river basins, *Science of the Total Environment*, 503–504: 142–150. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.scitotenv.2014.06.110>].
- Department of Water Affairs and Forestry (DWAf). (1996a) *South African Water Quality Guidelines (second edition). Volume 1: Domestic Use*. Pretoria, South Africa: Government Printer. Available at: <http://www.dwa.gov.za/iwqs/wq_guide/Pol_saWQguideFRESH_vol1_Domesticuse.PDF>
- Department of Water Affairs and Forestry (DWAf) (1996b). *South African Water Quality Guidelines. (second edition). Volume 2: Recreational Use*. Pretoria, South Africa: Government Printer. Available at: <http://www.dwa.gov.za/Documents/Other/RMP/RWUM/RWU_GP6.pdf>.
- Department of Water Affairs and Forestry (DWAf). (1996c). *South African Water Quality Guidelines. (second edition). Volume 4: Recreational Use*. Pretoria: Government Printer. Available at: <http://www.dwa.gov.za/Documents/Other/RMP/RWUM/RWU_GP6.pdf>.
- Department of Water Affairs and Forestry (DWAf) (1996d). *South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems*. Pretoria, South Africa: Government Printer. Available at:

- <http://www.dwa.gov.za/iwqs/wq_guide/Pol_saWQguideFRESHAquaticecosystemsvol7.pdf>.
- Department of Water Affairs (DWA) (2004). *National Water Resource Strategy, First Edition*. Pretoria, South Africa.
- Department of Water Affairs (DWA) (2012). The Annual National State of Water Resources Report October 2011 to September 2012. Pretoria, South Africa. Available at: <<http://www.dwa.gov.za/Groundwater/documents/2011-12%20Annual%20National%20State%20of%20Water%20Resources%20report.pdf>>
- Department of Water Affairs (DWA) (2013). Strategic overview of the water resources of South Africa, *Water SA*.
- Dey, P. and Mishra, A. (2017). Separating the impacts of climate change and human activities on streamflow: A review of methodologies and critical assumptions, *Journal of Hydrology*, 548: 278–290. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.jhydrol.2017.03.014>].
- Diamantini, E., Lutz, S.R., Mallucci, S., Majone, B., Merz, R. and Bellin, A. (2018). Driver detection of water quality trends in three large European river basins, *Science of the Total Environment*, 612: 49–62. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.08.172>].
- Dickens, C. and Graham, P.M. (2002). The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers, *African Journal of Aquatic Science*, 27(1): 1–10. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.2989/16085914.2002.9626569>].
- Ding, J., Jiang, Y., Liu, Q., Hou, Z., Liao, J., Fu, L. and Peng, Q. (2016). Influences of the land use pattern on water quality in low-order streams of the Dongjiang River basin, China: A multi-scale analysis, *Science of the Total Environment*, 551–552(19): 205–216. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.scitotenv.2016.01.162>].
- Dold, B. (2014). Evolution of acid mine drainage formation in sulphidic mine tailings, *Minerals*. 4(3): 621–641. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.3390/min4030621>].
- Dotan, P., Yeshayahu, M., Odeh, W., Gordon-Kirsch, N., Groisman, L., Al-Khateeb, N., Rabbo, A.A., Tal, A. and Arnon, S. (2017). Endocrine disrupting compounds in streams in Israel and the Palestinian West Bank: Implications for transboundary basin management, *Journal of Environmental Management*, 204: 355–364. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.jenvman.2017.09.017>].
- D'Ugo, E., Marcheggiani, S., D'Angelo, A.M., Caciolli, S., Puccinelli, C., Giuseppetti, R., Marcoaldi, R., Romanelli, C. and Mancini, L. (2018). Microbiological water quality in the medical device industry in Italy, *Microchemical Journal*, 136: 293–299. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.microc.2016.12.012>].
- Ebele, A.J., Abdallah, M.A. and Harrad, S. (2017). Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment, *Emerging Contaminants*, 3(1): 1–16. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.emcon.2016.12.004>].
- Engelbrechtsen, K.A., Bager, P., Wohlfahrt, J., Skov, L., Zachariae, C., Nybo Andersen, A., Melbye, M.

- and Thyssen, J.P. (2017). Prevalence of atopic dermatitis in infants by domestic water hardness and season of birth: Cohort study, *Journal of Allergy and Clinical Immunology*, 139(5): 1568–1574.e1. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.jaci.2016.11.021>].
- Environmental Protection Agency (EPA), Ireland. (2001). *Parameters of Water Quality: Interpretation and Standard*, 35(12): 715–718. Full document available at: (https://www.epa.ie/pubs/advice/water/quality/Water_Quality.pdf)
- Epele, L.B., Manzo, L.M., Grech, M.G., Macchi, P., Claverie, A.Ñ., Lagomarsino, L., and Miserendino, M.L. (2018). Disentangling natural and anthropogenic influences on Patagonian pond water quality, *Science of the Total Environment*, 613–614: 866–876. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.09.147>].
- European Commission. (2018). Commission Implementing Decision (EU) 2018/840 of 5 June 2018 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council and repealing Commission Implementing Decision (EU) 2015/495 (notified under document C(2018) 3362), *Official Journal of the European Union*, 141(June): 9–12. Available at: <<https://op.europa.eu/en/publication-detail/-/publication/06ece275-6a22-11e8-9483-01aa75ed71a1/language-en>>.
- Ewaid, S.H. and Abed, S.A. (2017). Water quality index for Al-Gharraf River, southern Iraq, *The Egyptian Journal of Aquatic Research*, 43(2): 117–122. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.ejar.2017.03.001>].
- Ferreira Marmontel, C.V., Lucas-Borja, M.E., Rodrigues, V.A. and Zema, D.A. (2018). Effects of land use and sampling distance on water quality in tropical headwater springs (Pimenta creek, São Paulo State, Brazil), *Science of the Total Environment*, 622–623: 690–701. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.12.011>].
- Fewtrell, L. and Kay, D. (2015). Recreational Water and Infection: A Review of Recent Findings, *Current Environmental Health Reports*, 2(1): 85–94. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1007/s40572-014-0036-6>].
- Garcia, C., Amengual, A., Santaner, V.H. and Zamora, A. (2017). Losing water in temporary streams on a Mediterranean island: Effects of climate and land-cover changes, *Global and Planetary Change*, 148: 139–152. [Accessed on: 16 February 2018, from doi: <https://doi.org/10.1016/j.gloplacha.2016.11.010>].
- Gerber, A. and Gabriel, M.J.M. (2002). *Aquatic Invertebrates of South African Rivers: Field Guide*. Pretoria: Government Printer. Available at: <http://www.dwa.gov.za/iwqs/biomon/aquabugsal/Aquatic_Invertebrates_of_South_African_Rivers_Field_Guide_en.pdf>.
- Gogoi, A., Mazumder, P., Tyagi, V.K., Chaminda, G.G.T., An, A.K. and Kumar, M. (2018). Occurrence and fate of emerging contaminants in water environment: A review, *Groundwater for Sustainable Development*, 6(2018): 169–180. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.gsd.2017.12.009>].
- Grassi, M., Kaykioğlu, G., Belgiorno, V. and Lofrano, G. (2012). Removal of Emerging Contaminants

- from Water and Wastewater by Adsorption Process. In: Lofrano, G. (eds). *Emerging Compounds Removal from Wastewater*. SpringerBriefs in Molecular Science. Dordrecht: Springer: 15–37. [Accessed on: 14 January 2019, from doi: https://doi.org/10.1007/978-94-007-3916-1_2].
- GreenCape (2017). Water 2017 Market Intelligence Report, Cape Town: GreenCape. Available at: <https://www.greencape.co.za/assets/Uploads/GreenCape-Water-MIR-2017-electronic-FINAL-v1.pdf>.
- Guldhe, A., Kumari, S., Ramanna, L., Ramsundar, P., Singh, P., Rawat, I. and Bux, F. (2017). Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation, *Journal of Environmental Management*, 203: 299–315. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.jenvman.2017.08.012>].
- Gupta, N., Pandey, P. and Hussain, J. (2017). Effect of physicochemical and biological parameters on the quality of river water of Narmada, Madhya Pradesh, India, *Water Science*, 31(1): 11–23. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.wsj.2017.03.002>].
- Hamza, R.A., Lorhemen, O.T. and Tay, J.H. (2016). Occurrence, impacts and removal of emerging substances of concern from wastewater, *Environmental Technology and Innovation*, 5: 161–175. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.eti.2016.02.003>].
- Han, D., Currell, M.J. and Cao, G. (2016). Deep challenges for China's war on water pollution, *Environmental Pollution*, 218: 1222–1233. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.envpol.2016.08.078>].
- Hartgers, E.M., Aalderink, G.H., van den Brink, P.J., Gylstra, R., Wiegman, J.W.F. and Brock, C.M. (1998). Ecotoxicological threshold levels of a mixture of herbicides (atrazine, diuron and metolachlor) in freshwater microcosms, *Aquatic Ecology*, 32: 135-152. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1023/A:1009968112009>].
- Hatchard, J. (1994). The Constitution of the Republic of South Africa. *Journal of African Law*, 38(1): 70-77. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1017/S0021855300011499>].
- Hofstra, N. and Vermeulen, L.C. (2016). Impacts of population growth, urbanisation and sanitation changes on global human *Cryptosporidium* emissions to surface water, *International Journal of Hygiene and Environmental Health*, 219(7): 599–605. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.ijheh.2016.06.005>].
- Howladar, M.F. (2017). An assessment of surface water chemistry with its possible sources of pollution around the Barapukuria Thermal Power Plant impacted area, Dinajpur, Bangladesh, *Groundwater for Sustainable Development*, 5: 38–48. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.gsd.2017.03.004>].
- Huber, C., Preis, M., Harvey, P.J., Grosse, S., Letzel, T. and Schröder, P. (2016). Emerging pollutants and plants – Metabolic activation of diclofenac by peroxidases, *Chemosphere*, 146: 435–441. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.chemosphere.2015.12.059>].
- Ignatius, A.R. and Rasmussen, T.C. (2016). Small reservoir effects on headwater water quality in the

- rural-urban fringe, Georgia Piedmont, USA, *Journal of Hydrology: Regional Studies*, 8: 145–161. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.ejrh.2016.08.005>].
- Inostroza, P.A., Massei, R., Wild, R., Krauss, M. and Brack, W. (2017). Chemical activity and distribution of emerging pollutants: Insights from a multi-compartment analysis of a freshwater system, *Environmental Pollution*, 231: 339–347. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.envpol.2017.08.015>].
- Jarvis, A.L., Bernot, M.J. and Bernot, R.J. (2014). The effects of the psychiatric drug carbamazepine on freshwater invertebrate communities and ecosystem dynamics, *Science of the Total Environment*, 496: 461–470. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.scitotenv.2014.07.084>].
- Jerome, C. and Pius, A. (2010). Evaluation of water quality index and its impact on the quality of life in an industrial area in Bangalore, South India, *American Journal of Scientific and Industrial Research*, 1(3): 595–603. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.5251/ajsir.2010.1.3.595.603>].
- Johnson, R.K., Angeler, D.G., Hallstan, S., Sandin, L. and McKie, B.G. (2017). Decomposing multiple pressure effects on invertebrate assemblages of boreal streams, *Ecological Indicators*, 77: 293–303. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.ecolind.2017.02.020>].
- Jotwani, J., Jain, B. and Malik, S. (2014). Assessment of Water Quality of Groundwater and Municipal supply of Bhopal City, *Journal of Chemical, Biological and Physical Sciences*, 4(1): 759–765. Available at: <www.jcbssc.org> E-ISSN: 2249-1929
- Kalogianni, E., Vourka, A., Karaouzas, I., Vardakas, L., Laschou, S. and Skoulikidis, N.T. (2017). Combined effects of water stress and pollution on macroinvertebrate and fish assemblages in a Mediterranean intermittent river, *Science of the Total Environment*, 603–604: 639–650. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.scitotenv.2017.06.078>].
- Karthika, I.N., Thara, K. and Dheenadayalan, M.S. (2018). Physico-chemical study of the ground water quality at selected locations in Periyakulam, Theni district, Tamilnadu, India, *Materials Today: Proceedings*, 5(1): 422–428. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.matpr.2017.11.101>].
- Khan, A.A., Paterson, R. and Khan, H. (2004). Modification and application of the Canadian Council of Ministers of the Environment Water Quality Index (CCME WQI) for the communication of drinking water quality data in Newfoundland and Labrador, *Water Quality Research Journal*, 39(3): 285–293. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.2166/wqrj.2004.039>].
- Khan, S., Shahnaz, M., Jehan, N., Rehman, S., Shah, M.T. and Din, I. (2013). Drinking water quality and human health risk in Charsadda district, Pakistan, *Journal of Cleaner Production*, 60: 93–101. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.jclepro.2012.02.016>].
- Khattak, M.A., Ahmed, N., Qazi, M.A., Izhar, A., Ilyas, S., Chaudhry, M.N., Khan, M.S.A., Iqbal N. and Waheed, T. (2012). Evaluation of ground water quality for irrigation and drinking purposes of the areas adjacent to Hudiera Industrial Drain, Lahore, Pakistan, *Pakistan Journal of Agricultural Sciences*, 49(4): 549–556. Available at: <<http://pakjas.com.pk/papers%5C2101.pdf>>

- Kora, A.J., Rastogi, L., Kumar, S.J. and Jagatap, B.N. (2017). Physico-chemical and bacteriological screening of Hussain Sagar lake: An urban wetland, *Water Science*, 31(1): 24–33. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.wsj.2017.03.003>].
- Kuzmanovic, M., Dolédec, S., de Castro-Catala, N., Ginebreda, A., Sabater, S., Muñoz, I. and Barceló, D. (2017). Environmental stressors as a driver of the trait composition of benthic macroinvertebrate assemblages in polluted Iberian rivers, *Environmental Research*, 156: 485–493. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.envres.2017.03.054>].
- Lam, S., Cunsolo, A., Sawatzky, A., Ford, J. and Harper, S.L. (2017). How does the media portray drinking water security in Indigenous communities in Canada? An analysis of Canadian newspaper coverage from 2000-2015, *BMC Public Health*, 17(282): 1–14. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1186/s12889-017-4164-4>].
- Lazarova, V., Emsellem, Y., Paille, J., Glucina, K. and Gislette, P. (2011). Water quality management of aquifer recharge using advanced tools, *Water Science and Technology*, 64(5): 1161–1168. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/wst.2011.418>].
- Lee, M., Kim, M., Kim, Y. and Han, M. (2017). Consideration of rainwater quality parameters for drinking purposes: A case study in rural Vietnam, *Journal of Environmental Management*, 200: 400–406. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.jenvman.2017.05.072>].
- Lei, M., Zhang, L., Lei, J., Zong, L., Li, J., Wu, Z. and Wang, Z. (2015). Overview of emerging contaminants and associated human health effects, *BioMed Research International*, 2015. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1155/2015/404796>].
- Le Thi Minh, T., Nguyen Phuoc, D., Dinh Quoc, T., Ngo, H.H. and Do Hong Lan, C. (2016). Presence of e-EDCs in surface water and effluents of pollution sources in Sai Gon and Dong Nai river basin, *Sustainable Environment Research*, 26(1): 20–27. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.serj.2015.09.001>].
- Liang, L., Lal, R., Wu, W., Ridoutt, B.G., Du, Z., Li, L., Feng, D., Wang, L., Peng, P., Hang, S. and Zhao, G. (2018). The water footprint and validity analysis of ecological engineering in North Beijing, China, *Journal of Cleaner Production Journal*, 172: 1899–1909. doi: <https://doi.org/10.1016/j.jclepro.2017.11.251>.
- Liao, H., Sarver, E. and Krometis, L.H. (2018). Interactive effects of water quality, physical habitat, and watershed anthropogenic activities on stream ecosystem health, *Water Research*, 130: 69–78. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.watres.2017.11.065>].
- Li, H., Liu, L., Li, M. and Zhang, X. (2013). Effects of pH, temperature, dissolved oxygen, and flow rate on phosphorus release processes at the sediment and water interface in storm sewer, *Journal of Analytical Methods in Chemistry*, 2013. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1155/2013/104316>].
- Liu, G., Zhang, Y., Knibbe, W., Feng, C., Liu, W., Medema, G. and van der Meer, W. (2017). Potential impacts of changing supply-water quality on drinking water distribution: A review, *Water Research*, 116: 135–148. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.watres.2017.03.031>].

- Lockerbie, E.M., Shannon, L.J. and Jarre, A. (2016) The use of ecological, fishing and environmental indicators in support of decision making in southern Benguela fisheries, *Ecological Indicators*, 69: 473–487. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.ecolind.2016.04.035>].
- López-Doval, J.C., Montagner, C.C., de Albuquerque, A.F., Moschini-Carlos, V.M., Umbuzeiro, G.A. and Pompêo, M.L. (2017). Nutrients, emerging pollutants and pesticides in a tropical urban reservoir: Spatial distributions and risk assessment, *Science of the Total Environment*, 575: 1307–1324. [Accessed on: 15 June 2018, from doi: <https://doi.org/10.1016/j.scitotenv.2016.09.210>].
- Lothrop, N., Bright, K.R., Sexton, J., Pearce-Walker, J., Reynolds, K.A. and Verhougstraete, M.P. (2018). Optimal strategies for monitoring irrigation water quality, *Agricultural Water Management*, 199: 86–92. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/agwat.2017.12.018>].
- Lund, A., McMillan, J., Kelly, R., Jabbarzadeh, S., Mead, D.G., Burkot, T.R., Kitron, U. and Vazquez-Prokopec, G.M. (2014). Long term impacts of combined sewer overflow remediation on water quality and population dynamics of *Culex quinquefasciatus*, the main urban West Nile virus vector in Atlanta, GA, *Environmental Research*, 129: 20–26. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.envres.2013.12.008>].
- Luo, K., Hu, X., He, Q., Wu, Z., Cheng, H., Hu, Z. and Mazumder, A. (2018). Impacts of rapid urbanization on the water quality and macroinvertebrate communities of streams: A case study in Liangjiang New Area, China, *Science of the Total Environment*, 621: 1601–1614. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.10.068>].
- Mabidi, A., Bird, M.S. and Perissinotto, R. (2017). Distribution and diversity of aquatic macroinvertebrate assemblages in a semi-arid region earmarked for shale gas exploration (Eastern Cape Karoo, South Africa), *PLOS ONE*, 12(6): 1–27. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1371/journal.pone.0178559>].
- Malan, J.C., Flint, N., Jackson, E.L., Irving, A.D. and Swain, D.L. (2018). Off stream watering points for cattle: Protecting riparian ecosystems and improving water quality? *Agriculture, Ecosystems & Environment*, 256: 144–152. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.sgee.2018.01.013>].
- Malla, B., Shrestha, R.G., Tandukar, S., Bhandari, D., Inoue, D., Sei, K., Tanaka, Y., Sherchand, J.B. and Haramoto, E. (2018). Identification of human and animal fecal contamination in drinking water sources in the Kathmandu Valley, Nepal, using host-associated *Bacteroidales* Quantitative PCR Assays, *Water*, 10(12): 1796. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.3390/w10121796>].
- Mandarić, L., Mor, J., Sabater, S. and Petrović, M. (2018). Impact of urban chemical pollution on water quality in small, rural and effluent-dominated Mediterranean streams and rivers, *Science of the Total Environment*, 613–614: 763–772. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.09.128>].

- Martinez-Haro, M., Beiras, R., Bellas, J., Capela, R., Coelho, J.P., Lopes, I., Moreira-Santos, M., Reis-Henriques, A.M., Ribeiro, R., Santos, M.M. and Marques, J.C. (2015). A review on the ecological quality status assessment in aquatic systems using community based indicators and ecotoxicological tools: what might be the added value of their combination? *Ecological Indicators*, 48: 8–16. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ecolind.2014.07.024>].
- Masindi, V. and Khathutshelo, L.M. (2018). Environmental Contamination by Heavy Metals. In: Saleh, H.E. and Aglan, R. (eds). *Heavy Metals*. London: IntechOpen. [Accessed on: 14 January 2019, from doi: [10.5772/intechopen.76082](https://doi.org/10.5772/intechopen.76082)].
- Matamoros, V. and Rodríguez, Y. (2017). Influence of seasonality and vegetation on the attenuation of emerging contaminants in wastewater effluent-dominated streams. A preliminary study, *Chemosphere*, 186: 269–277. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.chemosphere.2017.07.157>].
- Mathieu-Denoncourt, J., Wallace, S.J., de Solla, S.R. and Langlois, V.S. (2015). Plasticizer endocrine disruption: Highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species, *General and Comparative Endocrinology*, 219: 74–88. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ygcen.2014.11.003>].
- Matthews, M. W. and Bernard, S. (2015). Eutrophication and cyanobacteria in South Africa's standing water bodies: A view from space, *South African Journal of Science*, 111(5/6): 1–8. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.17159/sajs.2015/20140193>].
- McCarthy, T.S. and Humphries, M.S. (2013). Contamination of the water supply to the town of Carolina, Mpumalanga, January 2012, *South African Journal of Science*, 109(9/10): 1–10. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1590/sajs.2013/20120112>].
- McKnight, U.S., Rasmussen, J.J., Kronvang, B., Binning, P.J. and Bjerg, P.L. (2015). Sources, occurrence and predicted aquatic impact of legacy and contemporary pesticides in streams, *Environmental Pollution*, 200: 64–76. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.envpol.2015.02.015>].
- Mhlongo, S., Mativenga, P. and Marnewick, A. (2018). Water quality in a mining and water-stressed region, *Journal of Cleaner Production*, 171: 446–456. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.jciepro.2017.10.030>].
- Milner, A.M., Woodward, A., Freilich, J.E., Black, R.W. and Resh, V.H. (2016). Detecting significant change in stream benthic macroinvertebrate communities in wilderness areas, *Ecological Indicators*, 60: 524–537. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ecolind.2015.07.025>].
- Minolfi, G., Albanese, S., Lima, A., Tarvainen, T., Fortelli, A. and De Vivo, B. (2018). A regional approach to the environmental risk assessment - Human health risk assessment case study in the Campania region, *Journal of Geochemical Exploration*, 184(B): 400–416. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.gexplo.2016.12.010>].
- Mohsin, M., Safdar, S., Asghar, F. and Jamal, F. (2013). Assessment of drinking water quality and its

- impact on residents health in Bahawalpur City, *International Journal of Humanities and Social Science*, 3(15): 114–128. Available at: <http://www.ijhssnet.com/journals/Vol_3_No_15_August_2013/14.pdf>.
- Molina-Navarro, E., Andersen, H.E., Nielsen, A., Thodsen, H. and Trolle, D. (2018). Quantifying the combined effects of land use and climate changes on stream flow and nutrient loads: A modelling approach in the Odense Fjord catchment (Denmark), *Science of the Total Environment*, 621: 253–264. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.11.251>].
- Montes-Grajales, D., Fennix-Agudelo, M. and Miranda-Castro, W. (2017). Occurrence of personal care products as emerging chemicals of concern in water resources: A review, *Science of the Total Environment*, 595: 601–614. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.03.286>].
- Mwendera, E. and Atyosi, Y. (2018). A Review of Water Storage for Socio-Economic Development in South Africa, *Journal of Water Resource and Protection*, 10(3): 266–286. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.4236/jwarp.2018.103016>].
- Nyoh, B. G. (2015). Water Quality of the Bloemspruit stream in Mangaung, Free State Province. Magister Technologiae thesis, Central University of Technology, Department of Life Sciences, Bloemfontein. Available at: <<http://ir.cut.ac.za/bitstream/handle/11462/1409/Nyoh%2C%20Belle%20Gladys.pdf?sequence=1&isAllowed=y>>.
- Ochieng, G.M., Seanego, E.S. and Nkwonta, O.I. (2010). Impacts of mining on water resources in South Africa: A review, *Scientific Research and Essays*, 5(22): 3351–3357. Available at: <<http://www.academicjournals.org/SRE>>.
- Oetken, M., Nentwig, G., Löffler, D., Ternes, T. and Oehlmann, J. (2005). Effects of pharmaceuticals on aquatic invertebrates. Part I. The antiepileptic drug Carbamazepine, *Archives of Environmental Contamination and Toxicology*, 49(3): 353–361. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1007/s00244-004-0211-0>].
- Ollis, D.J. (2005). Rapid bioassessment of the ecological integrity of the Lourens, Palmiet and Hout Bay rivers (south Western Cape, South Africa) using aquatic macroinvertebrates. Master of Sciences thesis, University of Stellenbosch, Stellenbosch. Available at: <<https://scholar.sun.ac.za/handle/10019.1/20937>>.
- Owa, F.W. (2014). Water pollution: Sources, effects, control and management, *Mediterranean Journal of Social Science*, 4(8): 1–6. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.5901/mjss.2013.v4n8p65>].
- Palmer, T., Berold, R. and Muller, N. (2004). Environmental water quality in water resources management. *Water Research Commission Report No. TT 217/04*, Pretoria, South Africa: Water Research Commission. Available at: <<http://www.wrc.org.za/wp-content/uploads/mdocs/TT217-04.pdf>>
- Pasternak, G., Greenman, J. and Ieropoulos, I. (2017). Self-powered, autonomous Biological Oxygen

- Demand biosensor for online water quality monitoring, *Sensors and Actuators B: Chemical*, 244: 815–822. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.snb.2017.01.019>].
- Peng, X., Shi, D., Guo, H., Jiang, D., Wang, S., Li, Y. and Ding, W. (2015). Effect of urbanisation on the water retention function in the Three Gorges Reservoir Area, China, *Catena*, 133: 241–249. doi: <https://doi.org/10.1016/j.catena.2015.05.021>].
- Philip, J.M., Aravind, U.K. and Aravindakumar, C.T. (2018). Emerging contaminants in Indian environmental matrices – A review, *Chemosphere*, 190: 307–326. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.chemosphere.2017.09.120>].
- Pinna, M., Janzen, S., Franco, A., Specchia, V. and Marini, G. (2017). Role of habitats and sampling techniques on macroinvertebrate descriptors and ecological indicators: An experiment in a protected Mediterranean lagoon, *Ecological Indicators*, 83: 495–503. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ecolind.2017.08.022>].
- Pintado-Herrera, M.G., Wang, C., Lu, J., Chang, Y., Chen, W., Li, X. and Lara-martin, P.A. (2017). Distribution, mass inventories, and ecological risk assessment of legacy and emerging contaminants in sediments from the Pearl River Estuary in China, *Journal of Hazardous Materials*, 323: 128–138. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.jhazmat.2016.02.046>].
- Pivetta, G.G. and do Carmo Cauduro Gastaldini, M. (2019). Presence of emerging contaminants in urban water bodies in southern Brazil, *Journal of Water and Health*, 17(2): 329–337. [Accessed on: 18 November 2019, from doi: <https://doi.org/10.2166/wh.2019.092>].
- Polidoro, B.A., Comeros-Raynal, M.T., Cahill, T. and Clement, C. (2017). Land-based sources of marine pollution: Pesticides, PAHs and phthalates in coastal stream water, and heavy metals in coastal stream sediments in American Samoa, *Marine Pollution Bulletin*, 116(1–2): 501–507. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.marpolbul.2016.12.058>].
- Ponsadailakshmi, S., Sankari, S.G., Prasanna, S.M. and Madhurambal, G. (2018). Evaluation of water quality suitability for drinking using drinking water quality index in Nagapattinam district, Tamil Nadu in Southern India, *Groundwater for Sustainable Development*, 6: 43–49. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.gsd.2017.10.005>].
- Poonam, T., Tanushree, B. and Sukalyan, C. (2013). Water Quality Indices – Important Tools for Water Quality Assessment: A Review, *International Journal of Advances in Chemistry*, 1(1):15–28. Available at: <https://airccse.com/ijac/papers/1115ijac02.pdf>.
- Pretorius, E., de villiers, G.D.T. and Viljoen, M.F. (2002). 'The Integration of Environmental, Physical and Socio-Economic Issues for the Effective Management of Water Quality in a Developing Community'. In: *Proceedings of the WISA Biennial Conference of the Water Institute of Southern Africa (WISA), International Convention Centre, Durban, 19-23 May 2002*: 1–9. Available at: <https://wisa.org.za/document/page/6/?document-year=2002&document-conference=wisa2002>.
- Raghav, M., Eden, S., Mitchell, K. and Witte, B. (2013). Contaminants of Emerging Concern in Water, *Arroyo 2013*. Available at: <https://repository.arizona.edu/handle/10150/325905>.

- Ralston-Hooper, K., Hardy, J., Hahn, L., Ochoa-Acuña, H., Lee, L.S., Mollenhauer, R. and Sepúlveda, M.S. (2009). Acute and chronic toxicity of atrazine and its metabolites deethylatrazine and deisopropylatrazine on aquatic organisms, *Ecotoxicology*, 18(7): 899–905. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1007/s10646-009-0351-0>].
- Richardson, S.D. and Kimura, S.Y. (2017). Emerging environmental contaminants: Challenges facing our next generation and potential engineering solutions, *Environmental Technology & Innovation*, 8: 40–56. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.eti.2017.04.002>].
- Rimayi, C., Odusanya, D., Weiss, J.M., de Boer, J. and Chimuka, L. (2018). Contaminants of emerging concern in the Hartbeespoort Dam catchment and the uMngeni River estuary 2016 pollution incident, South Africa, *Science of the Total Environment*, 627: 1008–1017. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2018.01.263>].
- Rizo-Decelis, L.D., Pardo-Igúzquiza, E. and Andreo, B. (2017). Spatial prediction of water quality variables along a main river channel, in presence of pollution hotspots, *Science of the Total Environment*, 605–606: 276–290. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.06.145>].
- Rocha, A.C., Camacho, C., Eljarrat, E., Peris, A., Aminot, Y., Readman, J.W., Boti, V., Nannou, C., Marques, A., Nunes, M.L. and Almeida, C.M. (2018). Bioaccumulation of persistent and emerging pollutants in wild sea urchin *Paracentrotus lividus*, *Environmental Research*, 161: 354–363. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.envres.2017.11.029>].
- Rodriguez-Narvaez, O.M., Peralta-Hernandez, J.M., Goonetilleke, A. and Bandala, E.R. (2017). Treatment technologies for emerging contaminants in water: A review, *Chemical Engineering Journal*, 323: 361–380. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.cej.2017.04.106>].
- Rossouw, L. (2011). Determining the water quality ecological reserve for non-perennial rivers: a prototype environmental water assessment methodology. PhD thesis, University of the Free State, Department of Natural Science and Agriculture, Bloemfontein. Available at: <http://scholar.ufs.ac.za:8080/xmlui/handle/11660/1679>.
- Rozman, M., Acuña, V. and Petrović, M. (2018). Effects of chronic pollution and water flow intermittency on stream biofilms biodegradation capacity, *Environmental Pollution*, 233: 1131–1137. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.envpol.2017.10.019>].
- Sardar, K., Ali, S., Hameed, S., Afzal, S., Fatima, S., Shakoor, M.B., Bharwana, S.A and Tauqeer, H.M. (2013). Heavy Metals Contamination and what are the Impacts on Living Organisms, *Greener Journal of Environmental Management and Public Safety*, 2(4): 172–179. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.15580/GJEMPS.2013.4.060413652>].
- Şener, Ş., Şener, E. and Davraz, A. (2017). Evaluation of water quality using water quality index (WQI) method and GIS in Aksu River (SW-Turkey), *Science of the Total Environment*, 585: 131–144. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.01.102>].
- Singh, P. and Saharan, J.P. (2010). Elemental Analysis of Satluj River Water Using EDXRF, *Nature and Science*, 8(3): 24–28. Available at: <http://free-journal.umm.ac.id/download-pdf-journal-216->

-elemental-analysis-of-satluj-river-water-using-edxrf.pdf>.

- Singh, P.K. and Saxena, S. (2018). Towards developing a river health index, *Ecological Indicators*, 85: 999–1011. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ecolind.2017.11.059>].
- Song, X., Wen, Y., Wang, Y., Adeel, M. and Yang, Y. (2018). Environmental risk assessment of the emerging EDCs contaminants from rural soil and aqueous sources: Analytical and modelling approaches, *Chemosphere*, 198: 546–555. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.chemosphere.2018.01.060>].
- Sorensen, J.P.R., Lapworth, D.J., Nkhuwa, D.C.W., Stuart, M.E., Gooddy, D.C., Bell, R.A., Chirwa, M., Kabika, J., Liemisa, M., Chibesa, M. and Pedley, S. (2015). Emerging contaminants in urban groundwater sources in Africa, *Water Research*, 72: 51–63. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.watres.2014.08.002>].
- South Africa. 1989. Environment Conservation Act 73 of 1989. Available at: <https://www.gov.za/documents/environment-conservation-act-24-mar-2015-1507>.
- South Africa. 1998. National Water Act 36 of 1998. Available at: <https://www.gov.za/documents/national-water-act>.
- Staples, C., Mihaich, E., Ortego, L., Caspers, N., Klečka, G., Woelz, J. and Hentges, S. (2016). Characterizing the effects of bisphenol A on sediment-dwelling benthic organisms, *Environmental Toxicology and Chemistry*, 35(3): 652–659. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1002/etc.3217>].
- Steward, A.L., Negus, P., Marshall, J.C., Clifford, S.E. and Dent, C. (2018). Assessing the ecological health of rivers when they are dry, *Ecological Indicators*, 85: 537–547. doi: <https://doi.org/10.1016/j.ecolind.2017.10.053>.
- Stutter, M.I. and Cains, J. (2017). Changes in aquatic microbial responses to C-substrates with stream water and sediment quality related to land use pressures, *Chemosphere*, 184: 548–558. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.chemosphere.2017.06.009>].
- Talib, A. and Randhir, T.O. (2017). Managing emerging contaminants in watersheds: Need for comprehensive, systems-based strategies, *Sustainability of Water Quality and Ecology*, 9–10: 1–8. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.swage.2016.05.002>].
- Tanaka, M.O., de Souza, A.L.T., Moschini, L.E. and de Oliveira, A.K. (2016). Influence of watershed land use and riparian characteristics on biological indicators of stream water quality in southeastern Brazil, *Agriculture, Ecosystems & Environment*. 216: 333–339. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.agee.2015.10.016>].
- Trang, N.T.T., Shrestha, S., Shrestha, M., Datta, A. and Kawasaki, A. (2017). Evaluating the impacts of climate and land-use change on the hydrology and nutrient yield in a transboundary river basin: A case study in the 3S River Basin (Sekong, Sesan, and Srepok), *Science of the Total Environment*, 576: 586–598. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2016.10.138>].

- Tsuzuki, Y. (2015). Relationships between pollutant discharge and water quality in the rivers from “better” to “worse” water quality, *Ecological Indicators*, 52: 256–269. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.ecolind.2014.12.001>].
- United Nations Department of Economic and Social Affairs (UN-DESA) (2015). *The Millennium Development Goals Report 2015*, New York: United Nations: 52–61. Available at: <[https://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20\(July%2015\).pdf](https://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20(July%2015).pdf)>.
- Van der Hoven, C., Ubomba-Jaswa, E., van der Merwe, B., Loubser, M. and Abia, A.L.K. (2017). The impact of various land uses on the microbial and physicochemical quality of surface water bodies in developing countries: Prioritisation of water resources management areas, *Environmental Nanotechnology, Monitoring & Management*, 8: 280–289. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.enmm.2017.10.006>].
- Varol, M. and Şen, B. (2009). Assessment of surface water quality using multivariate statistical techniques: A case study of Behrimaz Stream, Turkey, *Environmental Monitoring and Assessment*, 159(1–4): 543–553. doi: <https://doi.org/10.1007/s10661-00650-6>.
- Vrebos, D., Beauchard, O. and Meire, P. (2017). The impact of land use and spatial mediated processes on the water quality in a river system, *Science of the Total Environment*, 601–602: 365–373. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.05.217>].
- Weber, G., Christmann, N., Thiery, A., Martens, D. and Kubiniok, J. (2018). Pesticides in agricultural headwater streams in southwestern Germany and effects on macroinvertebrate populations, *Science of the Total Environment*, 619–620: 638–648. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.11.155>].
- Water Research Commission (1998). *Quality of domestic water supplies Volume 1: Assessment Guide (2nd edn)*. Pretoria. Available at: <<https://wedc-knowledge.lboro.ac.uk/details.html?id=16390>>
- Wilkinson, J., Hooda, P.S., Barker, J., Barton, S. and Swinden, J. (2017). Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field, *Environmental Pollution*, 231: 954–970. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.envpol.2017.08.032>].
- Wooding, M., Rohwer, E.R. and Naudé, Y. (2017). Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: A disposable sorptive sampler with comprehensive gas chromatography – Time-of-flight mass spectrometry and large volume injection with ultra-high performance liquid chromatography-tandem mass spectrometry, *Journal of Chromatography A*, 1496: 122–132. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.chroma.2017.03.057>].
- World Health Organization and the Organisation for Economic Co-operation and Development (2003). *Assessing microbial safety of drinking water: Improving approaches and methods*. London: IWA Publishing. Available at: <https://www.who.int/water_sanitation_health/dwq/9241546301full.pdf>
- World Health Organization (WHO) (2003). *Terbutylazine in drinking-water: background document for*

- preparation of WHO guidelines for drinking-water quality. In: Guidelines for Drinking-water Quality Volume 1. Geneva: World Health Organization (WHO/SDE/WSH/03.04/63): 1–8. Available at: <<https://pdf4pro.com/view/terbuthylazine-tba-in-drinking-water-44900e.html>>.
- WWAP (UNESCO World Water Assessment Programme) (2019). *The United Nations World Water Development Report 2019: Leaving No One Behind*. Paris, UNESCO. Available at: <<https://en.unesco.org/themes/water-security/wwap/wwdr/2019>>.
- Yang, X., Warren, R., He, Y., Ye, J., Li, Q. and Wang, G. (2018). Impacts of climate change on TN load and its control in a River Basin with complex pollution sources, *Science of the Total Environment*, 615: 1155–1163. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.09.288>].
- Zamxaka, M., Pironcheva, G. and Muyima, N.Y.O. (2004). Microbiological and physico-chemical assessment of the quality of domestic water sources in selected rural communities of the Eastern Cape Province, South Africa, *Water SA*, 30(3): 333–340. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.4314/wsa.v30i3.5081>].
- Zhai, R. and Tao, F. (2017). Contributions of climate change and human activities to runoff change in seven typical catchments across China, *Science of the Total Environment*, 605–606: 219–229. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.06.210>].
- Zhang, M., Wang, S., Fu, B., Gao, G. and Shen, Q. (2018). Ecological effects and potential risks of the water diversion project in the Heihe River Basin, *Science of the Total Environment*, 619–620: 794–803. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2017.11.037>].
- Zhang, Y., Cheng, L., Tolonen, K.E., Yin, H., Gao, J., Zhang, Z., Li, K. and Cai, Y. (2018). Substrate degradation and nutrient enrichment structuring macroinvertebrate assemblages in agriculturally dominated Lake Chaohu Basins, China, *Science of the Total Environment*, 627: 57–66. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.scitotenv.2018.01.232>].
- Zia, H., Harris, N.R., Merrett, G.V., Rivers, M. and Coles, N. (2013). The impact of agricultural activities on water quality: A case for collaborative catchment-scale management using integrated wireless sensor networks, *Computers and Electronics in Agriculture*, 96: 126–138. [Accessed on: 14 January 2019, from doi: <https://doi.org/10.1016/j.compag.2103.05.001>].